Bailey's
TEXTBOOK OF HISTOLOGY

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REVISED BY

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PREFACE TO FOURTEENTH EDITION

Unusual advances in our knowledge of microscopic structure have been made in recent years. These have been based largely on improved techniques in electron microscopy and histochemistry and in further progress in cell physiology as studied by tissue culture. Advances in these fields make it imperative to correct previous misinterpretations of structure. They also encourage an expansion in the amount of detail given in a textbook of histology. In the present revision, a definite effort has been made to limit the text to the needs of first year students in histology rather than to produce a source book for teachers and specialists. Likewise, the references at the end of each chapter have been restricted in number. Besides being selected for their excellence *per se*, these references have been chosen, not primarily from the point of view of priority of discovery, but because they contain other important references.

One of the most important outgrowths from recent studies has been the evolution of a better understanding of the correlation of structure and function. Therefore it is appropriate that the present edition places an increased emphasis on the physiological significance of structure.

I wish to express my indebtedness to those who contributed to previous revisions. Following the death of Professor Bailey, Professors Strong and Elwyn revised the VII edition (1925) and a part of the VIII edition (1932). Professors R. L. Carpenter, C. M. Goss, and A. E. Severinghaus participated with Professor P. E. Smith and myself in the revisions of the VIII, IX and X editions. The text still contains valuable contributions made by them.

Professor Philip E. Smith has had a particularly important influence on the nature of the book. He served as editor of the IX and X editions, and as coauthor of the XI, XII, and XIII editions. His contributions of material plus his sound editorial judgment played an important rôle in whatever success the textbook achieved during editions VIII to XIII inclusive. After reaching the retirement age for teaching and after leaving Columbia University, Dr. Smith decided to withdraw from further textbook revisions. Words cannot express how much he has been missed. Needless to say, a considerable amount of material which he contributed previously is still present in this edition.

It is a pleasure to state that Professor Dorothy D. Johnson, who assisted with the XIII edition, has taken a greater part in the present revision. She has assumed the primary responsibility for Chapters 15, 16, 17, and 21. She has also made valuable constructive suggestions for most of the other chapters.

I am greatly indebted to Mr. Robert Demarest for the new drawings which appear in this edition. I am also indebted to Mr. Carl Kellner (now retired) for the drawings which appeared first in editions IX to XIII inclusive.

Many helpful suggestions have been made by my colleagues at Columbia and by histologists from other schools. Among these I wish particularly to thank Professor Burton Baker and Professor Thomas Hunt.

Last, but not least, I wish to express appreciation for the splendid cooperation of the publishers. They have performed their work with efficiency and with a sympathetic and patient understanding of the problems which beset an editor.

WILFRED M. COPENHAVER

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INTRODUCTION

All living organisms consist of minute elements which are called cells. These cells are the smallest structural units possessing those properties which we commonly associate with life. They are able to nourish themselves, to grow, to respond to stimuli, and to reproduce. Some organisms, the protozoa, consist of one cell only; the higher types, metazoa, may consist of infinite numbers of cells varying greatly in structural characteristics. Each of these multicellular organisms starts its existence as a single cell, the fertilized ovum, which by a process of proliferation gives rise to the adult body. At first the cells of the developing embryo are similar in shape and structure. As growth continues, differentiation takes place leading to the formation of groups of specialized cells, each group differing in structure from the others, each group adapted to subserve one or more specific functions. These specialized groups form the *tissues* of the adult body. At a very early period the cells of the embryo become separated from each other by the formation of intercellular substance, which may be the result of cellular secretion or may represent actual modifications of cellular substance. In some of the tissues this intercellular material assumes enormous proportions. Thus the adult body is composed of cells and intercellular material, all elements so interrelated as to form a normally functioning machine.

Histology in a restricted sense is the study of the tissues of the body, but since the tissues are composed of cells and their products, a knowledge of the structure and activities of the cell must necessarily form the basis of histology. The first two chapters of the book are therefore given to a discussion of the cell, the first of these dealing with the cell after fixation and the second with the living cell. Each of these chapters obviously supplements the other. Physiology is stressed in both. Succeeding these, the structure of the tissues is presented. This is followed by the microscopic anatomy of the various organs.

While histology is a structural science and serves to complete the anatomical knowledge gained from dissection, its intimate relation to physiology and pathology must be emphasized. The cell is not only a unit of structure but also of physiological activity. The formation of the specialized tissues is the structural expression of a physiological division of labor. The structures seen under the microscope assume a meaning only in the light of their functional significance. Thus the structure of muscles and glands can only be studied by constant reference to contraction and secretion. Normal physiological processes are associated with normal structure, abnormal processes are usually expressed in the altered structure and relationship of the cells and intercellular substance. A thorough knowledge of normal histology is essential for the understanding of the altered structure seen in the various conditions of disease.

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I

THE CELL

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The introduction has stated that the chief objective of anatomical studies is the accumulation of structural knowledge which will enable us to understand the functioning of living tissues. The dissections of Gross Anatomy, and the more minute structural analysis of Microscopic Anatomy have in turn made important contributions to this end. With the deeper mysteries of function still unsolved, we now are seeing an enormous expansion of the youngest of the anatomical sciences, Cell Anatomy or Cytology. Thus is being fulfilled the prophetic statement of the eminent physiologist, Verworn, who wrote in his Allgemeine Physiologie, many years ago, "It is to the cell that the study of every bodily function sooner or later drives us".

Neither the term "cell" nor the "cell concept" will be new to readers of this text. The cell is the minimal structural unit of protoplasm in animal or plant organization which can carry on all the vital functions. It is important to emphasize the fact that it does carry on all of the vital functions. Failing to do this, it could not live. It is, therefore, a physiological unit. It is also a structural unit. We cannot improve upon the simple and useful definition of Leydig that "a cell is a mass of protoplasm containing a nucleus". Earlier writers, including Leydig, used the term protoplasm to mean the substance of the cell body. Cytology now employs the term protoplasm to denote the entire living substance of the cell. This includes the cell body (the cytosome) and the nucleus which lies in it. The substance of the cytosome is called cytoplasm; the substance of the nucleus is karyoplasm or nucleoplasm.

METHODS OF STUDY

Our present knowledge of cell structure has been gained from a variety of methods of study. These methods fall logically into two groups, (1) methods employed with the living cell, (2) methods employed with dead cells (fixed or preserved). Some of the special methods employed in studies on the living cell will be treated separately in the second chapter but it should be emphasized that no single method should be used to the exclusion of all others. Studies on the living cell and those on preserved material yield supplementary data, and by different approach, each corroborates or questions the other's findings.

Preparation of Material. Some types of cells can be satisfactorily studied by placing them directly on slides for staining and for microscopic observation, (e.g., Wright's stained blood smears). However, for most cytological work it is necessary to cut tissues into thin, translucent slices only a few microns thick. Sectioning is done on a mechanical instru-

ment, the microtome, and is facilitated by freezing the tissue or by embedding it in a supporting medium such as paraffin or celloidin. A brief outline of some of the technical procedures will be given to aid the student in the interpretation of slides prepared for his study. Those who wish more details on technique should refer to the books listed at the end of the chapter.

In the *paraffin method*, the first step in the preparation of material is known as *fixation*. Numerous chemicals and their mixtures are used as fixatives (formalin, alcohol, Bouin's fluid, Zenker's fluid, etc.). Fixation preserves tissue, stops post-mortem change, begins a hardening which facilitates sectioning, and affects the affinity of certain tissue elements for certain dyes. In the process of fixation, proteins are precipitated and rendered insoluble; lipids and carbohydrates may or may not be preserved, depending on the nature of the fixative. For example, fats are removed from tissues immersed in alcohols. Therefore, it is necessary to use different technical procedures to study all of the various constituents of a cell.

Sectioning by the paraffin method requires that the tissue be thoroughly infiltrated with paraffin. Since paraffin will not mix with water, the former will not penetrate into tissues until the latter is removed. *Dehydration* is accomplished by passing the tissues through a series of graded alcohols, up to absolute. Now, since paraffin is also insoluble in alcohol, the latter must be replaced by an agent miscible with both alcohol and paraffin—xylol, cedar wood oil, or carbon bisulphide. These agents render the tissue translucent and therefore this step in technique is known as *clearing*. From the clearing agent, the tissue is placed in melted paraffin in an oven to replace the clearing agent with paraffin. Next, the tissue is embedded in paraffin by allowing the latter to harden and then the material is ready for sectioning and for subsequent staining. Figure 1 is a photomicrograph of cells prepared by this method.

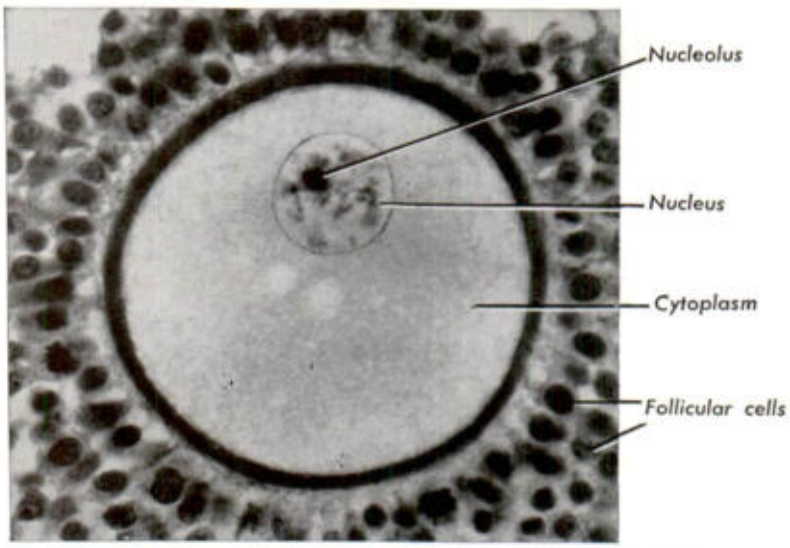

FIG. 1. Human ovum with some of the surrounding follicular cells. The thick membrane around the ovum is the zona pellucida, not the plasma membrane. Photomicrograph. ×600.

Even the brief outline given above must have made it obvious that structures seen in sections may be altered by chemical fixation, by dehydration and by the temperature of the paraffin oven. Again and again, the description of features in the fixed cell has brought forth the objection that they are not true features of the living cell, but artifacts of technique. The answer to such objections must always be the consistent repetition of the findings by different technical procedures.

The *freezing-drying technique* (Gersh, 1932; Hoerr, 1936) seems in some instances to cause less alteration of the living tissue than the standard methods. In this technique, fresh tissue is preserved by placing it in a liquid such as isopentane, chilled to about $-170°C$ with liquid nitrogen. The frozen tissue is dehydrated in a vacuum and brought to melted paraffin for infiltration and embedding without previous chemical fixation and dehydration in alcohols. This method is particularly useful for studying the localization of certain enzymes which are destroyed by the standard methods.

In *frozen section technique* (not to be confused with the freezing-drying method just outlined), a piece of tissue is placed directly on the stage of a special microtome equipped with an outlet for compressed carbon dioxide gas which cools the stage and freezes the tissue sufficiently for the cutting of sections. This method is widely used in clinical work for sectioning biopsy material when speed is important. In cytological work, the freezing method is particularly useful for studying the lipid content of cells because it avoids the use of fat-solvent dehydrating and clearing agents. The method may be used for either fresh or fixed material; in the former case, it is useful for studying cell enzymes which are destroyed by chemical fixation. Limitations of this method include the difficulties in cutting sections sufficiently thin and in keeping them in serial order.

The *celloidin technique* has many similarities to the paraffin method. The former avoids the heat of a paraffin oven and gives less shrinkage. It is particularly useful for cutting large objects (e.g., brain stem) and for hard and brittle objects (cartilage, tendon). Again, there is a disadvantage in that sections cannot be cut as thin as by the paraffin method.

The list of chemicals used for *staining* is even longer than that used for fixation. In one of the commonly used methods, the sections are stained first with hematoxylin and then with eosin. With this combination, nuclei are generally colored blue or purple and the cytoplasm is generally stained pink. Students should not make the mistake of thinking that nuclei should always be colored blue; for example, nuclei can be stained red with azocarmine and cytoplasm can be colored a bright green by a dye known as fast green.

Most stains are classified as acids or bases. Actually they are neutral salts having both acidic and basic radicals. When the coloring property is in the acid radical of the neutral salt, the stain is spoken of as an acid dye, and the tissues which stain with the dye are called acidophilic. Eosin is an acid dye with such general usage that the terms eosinophilic and acidophilic are often used synonymously. In some cases, it is clear that the basophilic substances which attract the basic dyes are themselves acids, as in the staining of nucleic acids with methylene blue, but the explanation is not always this simple. It has long been realized that special methods and stains are frequently necessary to demonstrate different structures but the nature of many of the reactions has been poorly understood.

Histochemical methods for the study of

chemically-recognizable substances within tissues began many years ago with the iodine test for starch. Nevertheless, progress in this field was slow until the publication of Lison's *Histochimie animale* in 1936. Since that time, numerous techniques have been developed for the identification and localization of chemical substances at the cellular level. Methods of particular interest include Caspersson's use of spectrophotometry for nucleic acids, Brachet's method for ribonucleic acid and Gomori's method for phosphatase. In applying spectrophotometry to cytology, ultraviolet light is used in conjunction with a quartz lens optical system because biological substances absorb transmitted light more strongly in the ultraviolet and infrared regions of the spectrum. Brachet's method uses an enzyme, ribonuclease, which selectively removes the ribonucleic acid. When any material stains with a basic dye such as pyronine or toluidine blue in an ordinary section and then becomes unstainable after a section has been treated with pure ribonuclease it may be concluded that the material is ribonucleic acid. A similar principle is used in the histochemical study of glycogen; in this case, the control slides are treated with saliva where the enzyme ptyalin removes the glycogen. For further details on histochemical methods, reference should be made to the publications listed at the end of the chapter.

Other methods for studying cell structure include microincineration and ultracentrifugation. By means of microincineration, microscopic sections are reduced to ash on a quartz slide. The amounts and distribution of inorganic components of the cell are demonstrated by this method. With the perfection of the Beams ultracentrifuge, which develops a centrifugal force of 500,000 times the force of gravity, components of the cell can be stratified according to their specific gravity. This method is useful for the separation of cell constituents and for the intact displacement of structures (see mitochondria and Golgi apparatus).

The Microscope. The usefulness of a microscope is dependent not merely upon its ability to magnify but upon its ability to resolve detail. Beyond certain limits of magnification no new details are observable. The useful magnification of an ordinary microscope is only about 1500×. The resolving power of a lens is its capacity to give clear images of points close together. It is measured as the least distance between two points which can be seen as two instead of one. With the ordinary light microscope, an oil immersion objective with a 1.40 numerical aperture resolves about 0.2 micron (i.e., about 2000 Ångstrom units). The resolving power is governed by the numerical aperture or light gathering capacity of the objective lens and by the wave-length of light. By using ultraviolet light and quartz lenses, the resolving power and effective magnification can be increased somewhat, but the main use of the ultraviolet microscope is for absorption spectrophotometry in histochemical studies.

The chief advance in increasing resolving power and magnification has been made with the *electron microscope* which uses electron beams in place of light, and electromagnetic or electrostatic fields in place of lenses. The final image is visualized on a fluorescent screen and recorded on a photographic plate. The wave-length of a stream of high velocity electrons is so short that the resolving power of an electron microscope is about 20 Å or even 10 Å (0.001 micron). With this amount of resolution the electron microscope can be used profitably at very high magnifications. In common

practice, the image is photographed at 5000 to 20,000× and the negative is enlarged about 6× when the photographic print is made, thus giving magnifications to about 120,000. With the best equipment and with careful attention to all technical procedures it is possible to use even greater magnifications. One of the limitations in electron microscope work is the necessity of having extremely thin sections of 0.1 micron or less on account of the low penetration of the electrons. Another disadvantage stems from the fact that the tissues cannot be mounted on slides in a preserving medium as in other methods of technique but they must be placed in a high vacuum in a dried state. This may produce artifacts and it prohibits the study of living cells.

The *phase microscope* is a modification of the light microscope particularly useful for the study of unstained cells, either living or fixed. Cellular components of unstained cells usually appear indistinct with the ordinary microscope because they are fairly transparent and produce very little change in the intensity of transmitted light. On the other hand, the different protoplasmic constituents produce phase changes because they vary in thickness and refractive index. The phase microscope converts phase variations into intensity variations and thereby enables the eye to detect more contrast between different structures.

CHEMICAL AND PHYSICAL PROPERTIES OF PROTOPLASM

The complexity of the living substance, protoplasm, is responsible for the above mentioned difficulties in analyzing it. Biochemists have revealed that it is composed of proteins, carbohydrates, fats and inorganic salts associated in a watery medium. But chemical analysis, like the cytologist's descriptions, is after all an analysis of dead cells. The secrets of living function lie in the associations and combinations of the elements during life, and these the biochemist has not been able to discover. Protoplasm has the physical properties of a colloidal mass. The medium of dispersion is water, so that protoplasm is a hydrosol or a hydrogel. It is a semifluid or viscid substance, whose consistency varies in different cells or in the same cell under different conditions of physiological activity. It may change from a condition of greater fluidity, a state of sol, to a more viscous gel state which may even approach the state of a solid. The nuclear protoplasm is ordinarily more of a gel, the cytoplasmic more sol in character.

Protoplasm contains simple proteins (albumins, globulins, etc.); conjugated proteins or those in which a simple protein is combined with another substance (nucleoproteins, phosphoproteins, etc.); and derived proteins such as proteoses, peptones and polypeptides. Details on the type of proteins, carbohydrates and lipids found in protoplasm belong to the field of biochemistry rather than to histology. Nevertheless, one cannot draw definite boundaries around the different branches of science which deal with living substance. Histologists are interested particularly in the cellular localization of chemical substances, especially when the substances can be related to observable structures. Nucleic acids and some of the enzymes are interesting in this respect.

Nucleic acids are complex structures consisting of polymers of nucleotides in which each nucleotide contains a pentose sugar combined with phosphoric acid on the one hand and with a nitrogenous substance, pyrimidine or purine, on the other. On the basis of the type of pentose sugar, the several nucleotides

fall into two main groups: (1) desoxyribonucleic acids (frequently abbreviated as DNA); and (2) ribonucleic acids (RNA). The nucleic acids combine with protein bases, protamine or histone, to form nucleoproteins.

Desoxyribonucleic acid is found in nuclei, more specifically in the chromosomes of the nuclei and thus it is important in heredity (see under mitosis). The quantity of DNA in the nuclei of different cells of any given species is relatively constant with the exceptions of mature germ cells which have a reduced (haploid) number of chromosomes and certain other cells which may have a multiple (polyploid) number of chromosomes (some liver cells, etc.). Naturally there must be an increase in chromosomal DNA prior to chromosome division at mitosis; otherwise the DNA of the daughter cells would soon be depleted. Although the amount of DNA in the chromosomes of different cells is relatively constant, the amount of DNA-associated protein varies greatly. The latter is usually high in cells which have high metabolic activity in their cytoplasm (e.g., liver and kidney cells).

Ribonucleic acids are found both in the nucleus and in the cytoplasm, particularly in the latter. The total amount per cell varies for different tissues and for the same cell type at different times. It is usually abundant in the nucleoli and in the cytoplasm of cells which are most active in synthesizing proteins. Thus, it is abundant in the cytoplasm of enzyme secreting cells (chromophilic substance), in nerve cells (Nissl substance), in embryonic cells during the growth period, and in regenerating cells of the adult organism (e.g., regenerating liver cells, myeloblasts of blood formation, etc.).

It has been noted earlier in the chapter that nucleic acids have characteristic absorption spectra in the ultraviolet region by which they can be distinguished from other substances. The Feulgen reaction (obtained by treating sections with an acid followed by leucofuchsin) is particularly useful for distinguishing DNA from RNA and from other basophilic substances. Reference has already been made to the fact that the identity of RNA can be confirmed by the use of a specific enzyme, ribonuclease (see Fig. 113). Although basophilia in itself is not a specific test for nucleic acids (other acids in the protoplasm will attract basic dyes), it is true that many basophilic structures contain nucleic acids. It may be pointed out again that the nucleic acids occur in combination with proteins as nucleoproteins. The nucleoprotein reaction seen in sections stained with both basic and acidic dyes will vary with the proportion of the different substances present. For example, the chromatin of the nucleus is very basophilic by reason of its high proportion of nucleic acid whereas the nucleoprotein of the nucleolus is often acidophilic by reason of its proportion of certain basic proteins.

Enzymes are important constituents of the protoplasm of all cells. They function as biological catalysts in oxidations, reductions, hydrolyses, phosphorylations, etc. Some of the enzymes are localized in particular structures, e.g., cytochrome oxidase in mitochondria. Others, such as the enzymes hydrolyzing dipeptides, seem to be uniformly dispersed through the cytoplasm. Although a number of cellular enzymes can be visualized under the microscope by histochemical methods, this number is only a fraction of the many known to exist. Enzymes which have received particular attention in histochemical studies include alkaline phosphatase, succinic dehydrogenase, cytochrome oxidase and cholinesterase. Alkaline phos-

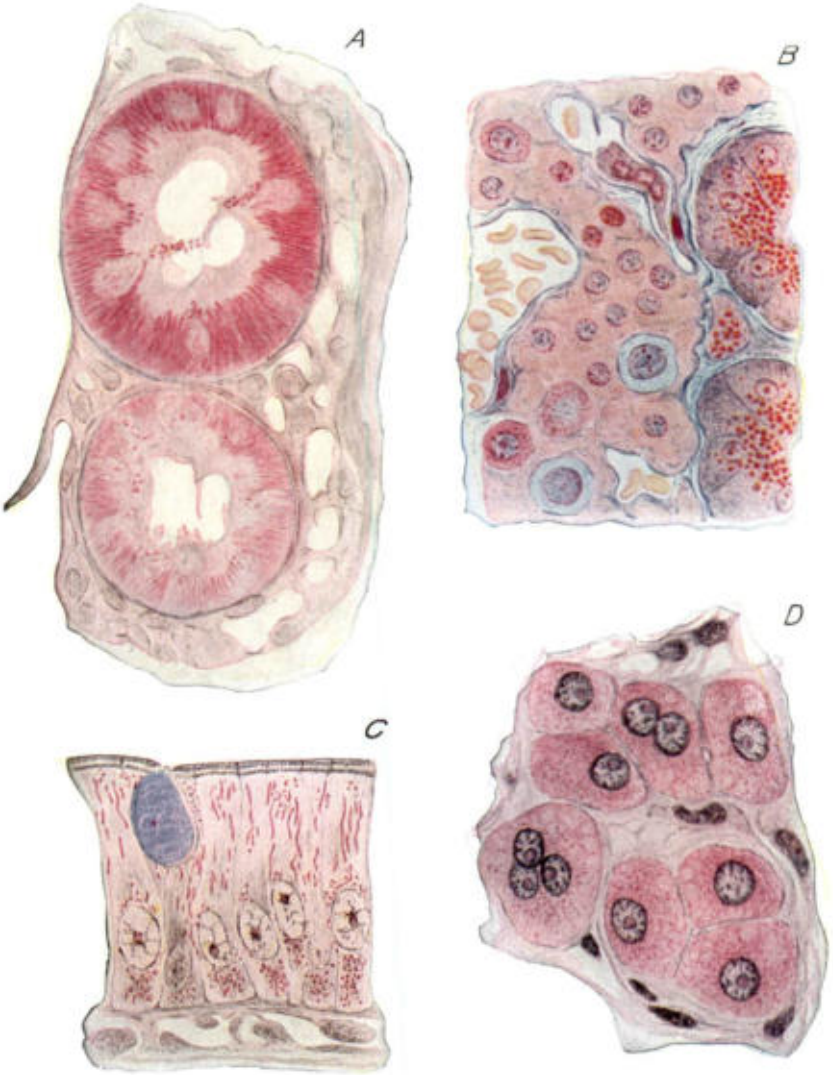

FIG. 2. *A*, Kidney of mouse. Contrast the prominence of parallel lined mitochondria in the proximal convoluted tubule above with the distal convoluted below. Regaud, Altmann acid fuchsin; *B*, Island of Langerhans from human pancreas, bordered at right by acinar cells which show chromophilic substance and apical secretion granules. In the island tissue are many "B" cells (orange), three red granular "A" cells, and two blue "D" cells. A polymorphonuclear leucocyte was caught in the upper sinusoid. Helly, modified Masson. *C*, Intestinal epithelium of cat, showing one goblet cell among absorbing cells. Note polarized mitochondria, and the striated cell border. Champy, modified Masson. *D*, Liver cells from rhesus monkey filled with evenly distributed glycogen granules. One cell has 2 nuclei, another 3, which is not uncommon. Biopsy, alcohol, Best's carmine. Camera lucida drawings. (Preparations by Dr. A. E. Severinghaus.)

phatase is found in the brush border of the proximal convoluted tubule cells of the kidney, in the striated border of the columnar absorbing cells of the intestine, in the nuclei of many cells (particularly endothelium), in ossification centers, etc. Cytochrome oxidase is an enzyme which functions in collaboration with cytochrome c in the series of reactions involved in oxidations.

STRUCTURAL AND FUNCTIONAL ORGANIZATION OF THE CELL

Cells vary greatly in size, shape and structure as shown in Figures 1 and 2. These variations are adaptations for the different functions which the cells perform in different tissues and organs and they will be considered in more detail in succeeding chapters. Regardless of specialization, most cells retain a number of features in common (see Fig. 3). These general characteristics form the subject for the present discussion.

THE NUCLEUS

Structure of the Nucleus. The nucleus is contained within a thin but definite membrane which is often thicker than the plasma membrane surrounding the cytoplasm. With the electron microscope, the nuclear membrane appears as a double layer or envelope (Fig. 3). In places, the two layers fuse and openings or pores appear which may facilitate exchange between nucleus and cytoplasm. The intranuclear protoplasm, the nucleoplasm, is usually more viscous than the cytoplasm although it is sufficiently fluid to give plasticity to the nucleus under varied conditions. In rounded or cuboidal cells, the nucleus usually assumes a globular shape. In tall columnar or spindle-shaped cells, the nucleus is usually elongated with its long axis corresponding to that of the cell (Fig. 4, *A*). In cells whose cytoplasm becomes filled with inclusions, as in mucous secreting and fat cells, the nucleus is often flattened against the cell wall (Fig. 4, *B*, *C*). It usually reverts to a more globular form after the cytoplasmic inclusions have been extruded.

In some cells the nucleus becomes lobated as in the neutrophilic leucocytes, or the megakaryocytes. An extreme form of lobation is shown in Figure 4, *D*. Cells with a lobed nucleus are often, although not always, highly differentiated cells which have lost the ability to divide by mitosis, (e.g., polymorphonuclear leucocytes). The lobed nucleus is usually polyploid, that is, it contains a multiple of the normal diploid number of chromosomes.

Although a cell usually has only one nucleus, some types of cells often have two or more. The parietal cells of the stomach glands frequently have two nuclei, as do also liver cells and the large surface cells of bladder epithelium. Osteoclasts of the bone usually have a large number of nuclei, five or more.

Within the nucleus there are irregularly arranged granules which were originally named chromatin by Flemming because they stained brilliantly with basic coal tar dyes. Slightly acidophilic, pale staining strands of material can often be seen connecting the chromatin granules. These are sometimes spoken of as the linin fibers. The vast majority of nuclei also contain one or more well demarcated bodies known as *nucleoli* or plasmosomes. They frequently take the acid or plasma dyes and they are not to be confused with small, angular clumps of chromatin which are sometimes spoken of as false nucleoli. All of the structures are embedded in a hyaline fluid, the nuclear sap, or the ground substance of the nucleoplasm.

Barr and his associates have shown that a sex difference can be observed in

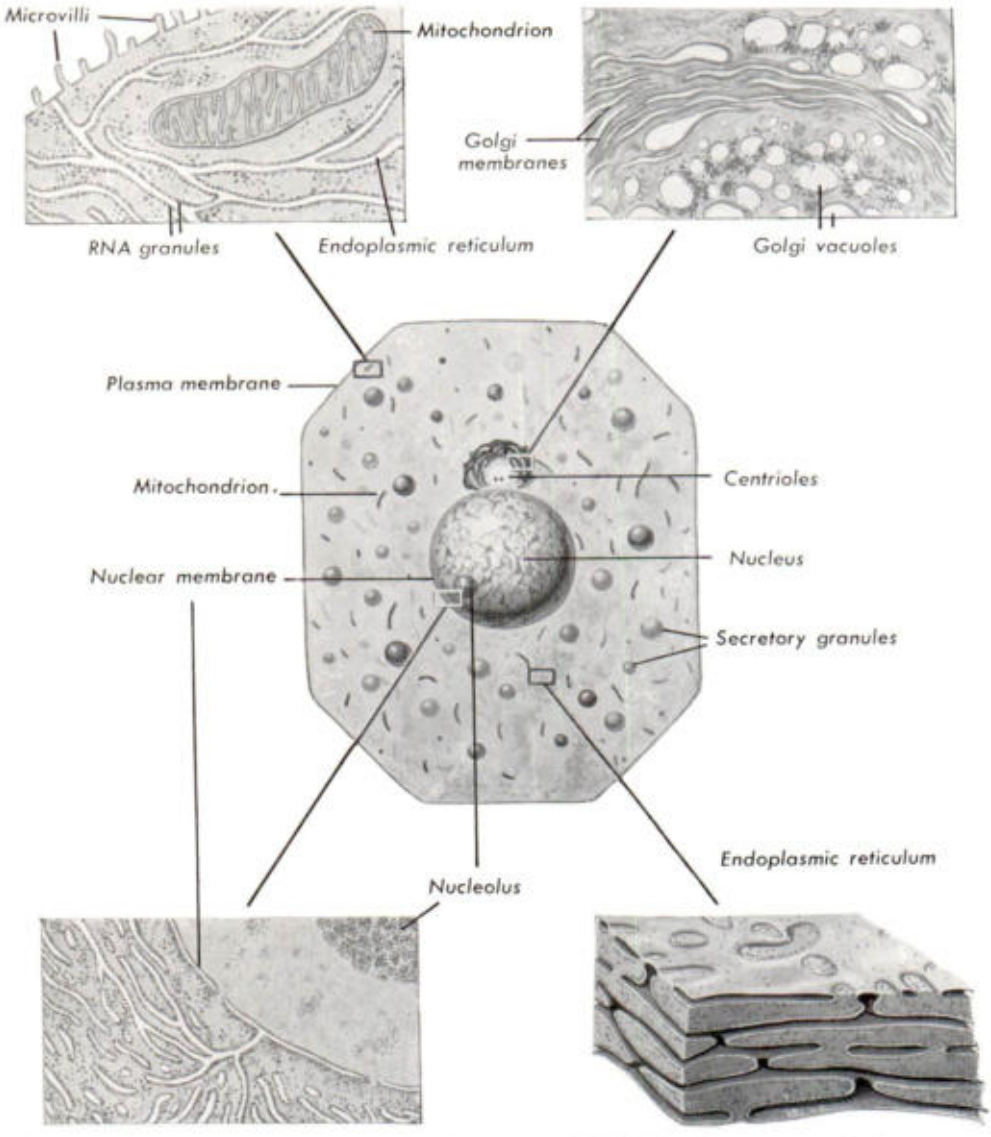

FIG. 3. Schematic representation of a cell as seen with the light microscope and enlargements showing the fine structure of some of the cell constituents as seen under the electron microscope. The endoplasmic reticulum in the lower right quadrant of the figure is shown in three dimensions; other structures are in section. (Diagrams of fine structure are based on studies by Porter, Palade, Fawcett, Novikoff, and others.)

the nuclei of a number of tissues during the interphase (intermitotic) stage. The difference has been described particularly for epidermis and for nerve cells (Fig. 113), where there is a distinct chromatin particle associated with the nucleolus in females. This nucleolar satellite is rudimentary or absent in males, ap-

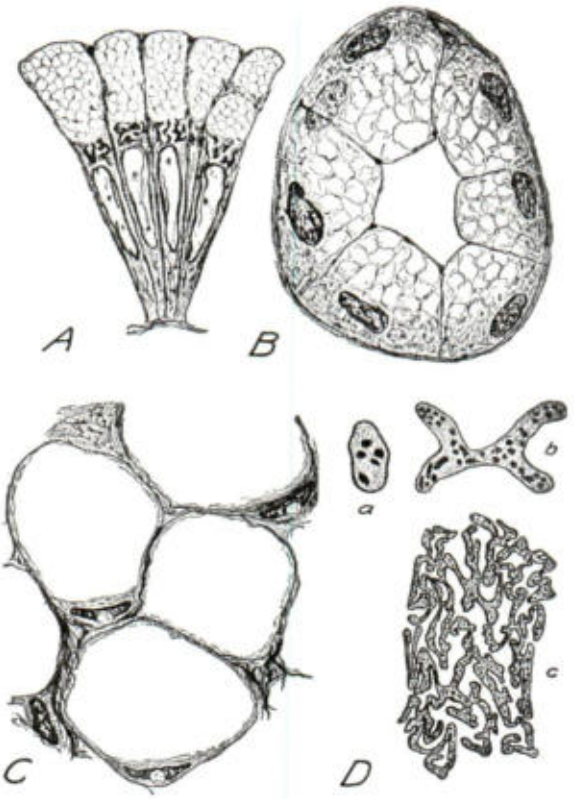

FIG. 4. Nuclear shape and position in various cells. *A*, Surface mucous cells of stomach epithelium with elongated nuclei lying in the long axis of the tall columnar cells. Mucous droplets occupy the apices of the cells. The Golgi apparatus, enclosing clear channels of stainable mucus, lies just above the nucleus; *B*, mucous gland cells from human tongue with apical mucus compressing the nucleus at the broad base of the cells; *C*, fat cells with cytoplasm and nucleus compressed at cell periphery by enclosed fat (here removed by fixation); *D*, cells from insect spinning gland. *a*, Young nucleus; *b*, beginning of nuclear branching; *c*, labyrinthine nucleus in the cytoplasm of mature active cell. (*A*, *B*, *C*, camera lucida; *D*, redrawn from Vorhies, after Wilson.)

parently due to the fact that males possess less sex chromatin.

Our understanding of the nuclear structures described above has been clarified by studies on the composition of chromosomes. The chromosomes become distinct during cell division, whereas they generally cannot be seen as distinct entities during the period between cell divisions (the interphase stage). Each chromosome is composed of one or more polypeptide chains running the length of the chromosomes. The special substance in a chromosome is desoxyribonucleic acid (DNA). This is combined with a basic protein known as histone and a non-basic, tryptophane-containing protein (the latter is also known as the structural protein because it seems to be essential for maintaining the structural relationships within the chromosome). Chromosomes may also contain a small amount of the ribose type of nucleic acid (RNA) but the latter is chiefly a constituent of the nucleolus and of the cytoplasm.

In the section on nucleic acids, it was noted that the nuclei from cells of different tissues contain a similar and constant amount of DNA during the interphase or intermitotic stage. When a cell prepares to divide (prophase stage), its chromosomes are said to become "charged" with DNA. At this time, the structural components of each chromosome become closely aggregated, partly from dehydration and partly from the fact that each chromosome becomes contracted and tightly coiled until it appears as a compact cylindrical thread. Opinions differ as to whether the DNA is increased out of proportion to the DNA-associated proteins at this time (see under mitosis). At any rate, the increase in DNA is sufficient to give each of the two daughter sets of chromosomes an amount equal to that held by the parent set before division. In the final or telophase stage of division (after each chromosome has been equally divided during the metaphase and anaphase stages) each chromosome passes through changes which are the reverse of those that occur during prophase. The structural components appear to be pushed apart through hydration and unspiralling until each chromosome assumes its interphase condition where it can no longer be distinguished as a unit but only as chromatin granules with occasional connecting strands.

In some cases, at least, the nucleolus is associated with a particular region of a particular chromosome. It disappears during prophase and reappears during telophase. During the latter stage, a particular region of chromatin produces a substance rich in basic proteins which accumulate along with the ribose type of nucleic acids to form the bulk of the nucleolus. The nucleolus will exhibit a variable basophilia and acidophilia in different cells and at different times depending on the relative proportions of ribonucleic acid and basic protein.

Function of the Nucleus. The importance of the nucleus in heredity has been established through breeding experiments and through cytological study. It is known that the "genes," which are the actual determiners of inherited characters, lie in definite organization in the nuclear chromosomes. It is also known that the nucleus is important for cell division. Some of the earliest evidence on the latter point was obtained by simple cutting experiments wherein unicellular organisms were bisected so that only one part retained a nucleus. The same type of experiment demonstrated the importance of the nucleus in relation to the general metabolic processes of the cell. It was found that without the nucleus, the constructive phases of metabolism (anabolism) soon cease. Therefore, it is clear that the nucleus exercises some control over the cytoplasm. There are many unsolved problems on the details of how this control is effected but obviously it must be through some type of exchange through the nuclear membrane during the periods between cell division. In this respect, it is interesting to note that some cells of high activity and specialization, such as the cells of the spinning glands of insects, have a labyrinthine lobulation of the nucleus which increases the surface of contact between the nucleus and cytoplasm (Fig. 4, *D*).

Furthermore, the nuclear membrane always disappears at the time of cell division, allowing an intermingling of nuclear and cytoplasmic materials. In multicellular animals, we have no way of estimating the importance of this fact upon the vitality of the cell. We can, however, infer it. In Protozoa, through the reorganization of the nucleus and resorption of portions of it by the cytoplasm (endomixis in paramecium) a sluggish, inactive cell near death is again rapidly stepped up to a state of high vitality and activity.

In recent years an interesting concept has gained ground that the nuclei and their nucleoli play an important role in relation to the synthesis of cytoplasmic proteins. It has been noted above that chromosomes contain a considerable amount of desoxyribonucleoprotein and that particular regions of the chromosomes produce proteins rich in diamino acids which accumulate in the nucleolus during the telophase stage of mitosis. Some of these proteins apparently take part in the formation of ribonucleoproteins within the nucleolus and according to Caspersson they also "diffuse toward the nuclear membrane on the outside of which an intensive production of ribonucleotides takes place. At the same time, the amount of cytoplasmic proteins increases." Thus, some of the chromosomal material directs the metabolism of RNA and the synthesis of cytoplasmic proteins during cell growth. A reversed process may occur during the early stages of mitosis—that is, nucleotides may be transferred from the cytoplasm to the nucleus.

In correlation with the metabolic control ascribed to nucleoli it is interesting to note that nucleoli are small (or absent) in cells which are not actively forming proteins (e.g., mature leucocytes) and generally large in cells which are actively synthesizing proteins (e.g., nerve cells which must replenish their proteins during activity, cells of regenerating tissues, embryonic cells, etc.).

THE CYTOPLASM

Cytoplasm contains a number of formed bodies embedded in a substance which appears translucent and homogeneous in the living cell. This substance has been given a variety of names such as ground cytoplasm, basic or fundamental cytoplasm, hyaloplasm. The formed bodies embedded in the cytoplasm are often divided into two groups: *organoids* (organelles) when they are composed of living, differentiated cytoplasm and *inclusion bodies* when they are metabolic or ingested substances which are temporary constituents. The first group includes (1) central bodies, (2) Golgi apparatus, (3) mitochondria, (4) chromophilic material, and (5) fibrils. The second group includes (1) yolk, (2) fat and carbohydrate (glycogen) deposits, (3) secretion granules, and (4) pigment granules.

Central Body, Cell Center. The central body usually lies close to the nucleus although its position varies somewhat in different cell types (Fig. 5). It is probably present in all mammalian cells, but is often difficult to demonstrate in some types of cells. It has been observed in living cells and it can be stained by iron hematoxylin in fixed preparations. Although it is most distinct during cell division, it can be demonstrated during the intermitotic stage. Its most constant feature is the presence of one or two sharply staining granules, the *centrioles*. The cytoplasm surrounding the centrioles often appears more condensed than that in other parts of the cell. The terminology for this region, like the structure itself, is poorly defined. Some authors use the terms *centrosome* and *centrosphere* for the more homogeneous cytoplasm immediately surrounding the centrioles and the term *astrosphere* for the more peripheral region which assumes the appearance of radiating, fibrilla-like structures during mitosis. Other authors use these terms synonymously for the entire region surrounding the centrioles.

As will be indicated later, the central body has been clearly associated with the process of mitotic cell division. It has, however, a probable wider physiological significance, as may be surmised from its frequent association with the basal granules of motile cilia and flagella, and the further fact that it often forms a center about which such other cyto-

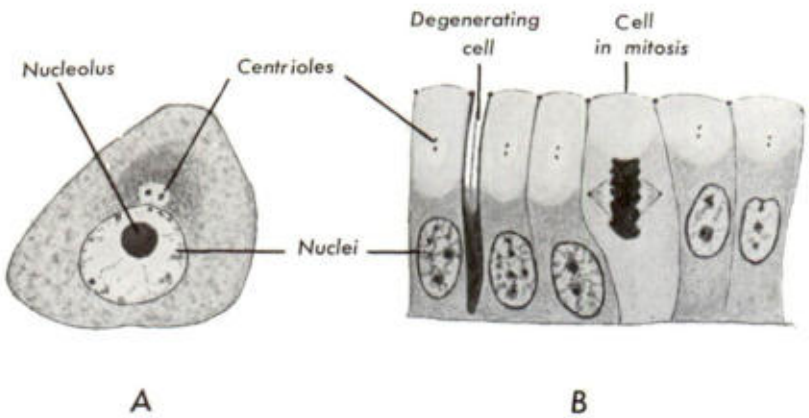

FIG. 5. *A*, Central body in an interstitial cell of human testis. *B*, Centrioles in columnar epithelial cells of human stomach. (*A*, Redrawn after Petersen; *B*, redrawn after Zimmerman.)

plasmic organoids as the Golgi apparatus are polarized.

The Golgi Apparatus. Perhaps no structure of the cytoplasm has aroused more controversy than the *Apparato reticolare interno* which Golgi first described in 1898 in nerve cells. Following the announcement of this discovery, there was the first rush of investigators to discover a Golgi apparatus in all cells. Some of these attempts failed at first and then it was claimed that the Golgi apparatus was not universally present in all cells, and that it was probably a technical artifact in those cells in which it could be demonstrated. Later, it was reported in cells where first, because of technical difficulties, it could not be found. It has now been seen also in living cells. Furthermore, work with the ultracentrifuge has supported the idea that the Golgi apparatus is a cytoplasmic organoid of definite form. Such centrifugation actually displaces the Golgi material intact in the living cell.

There has been considerable controversy concerning the form of the Golgi apparatus as seen with the light microscope. It has been described at various times as consisting of a reticular network, a series of canals, a collection of vacuoles, or a collection of small spheres. Experiments with a particular bearing on this point have shown that the appearance of the Golgi apparatus in a given cell type can be varied at will from a collection of small spheres to expanded vesicles by varying the methods of technique (Fig. 6, Elftman, 1953). The Golgi apparatus can be demonstrated by silver or osmic acid impregnation, but only after fixatives which avoid fat solvents. It is frequently described as being composed of lipoproteins although it is probably mostly lipoid. It usually occupies a fairly constant position in the cell, frequently at the same pole of the nucleus that the central bodies occupy. In epithelial cells which border on an enclosed space, the Golgi apparatus almost invariably lies between the nucleus and the free surface border of the cell (Fig. 9).

In electron micrographs, the Golgi region exhibits a variable appearance. It consists of a collection of double membranes, large vacuoles, small vesicles and granules (Figs. 3, 7, and 8). The small vesicles are intimately associated with the double membranes or flattened sacs and sometimes show continuity with them (Fig. 7). Sections of postosmicated

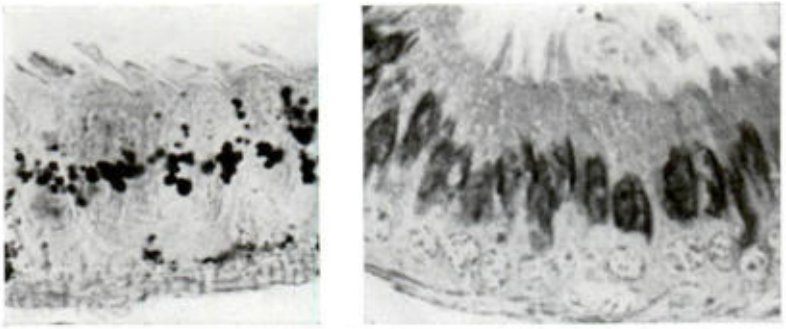

FIG. 6. Photomicrographs of epithelial cells of mouse epididymis showing the Golgi apparatus blackened by OsO_4. Other parts of the cell are stained only faintly. At the left, the Golgi apparatus is in the form of spheroids, while at the right it exhibits the more expanded form produced by longer subjection to OsO_4. ×1000. (Courtesy of Dr. H. Elftman.)

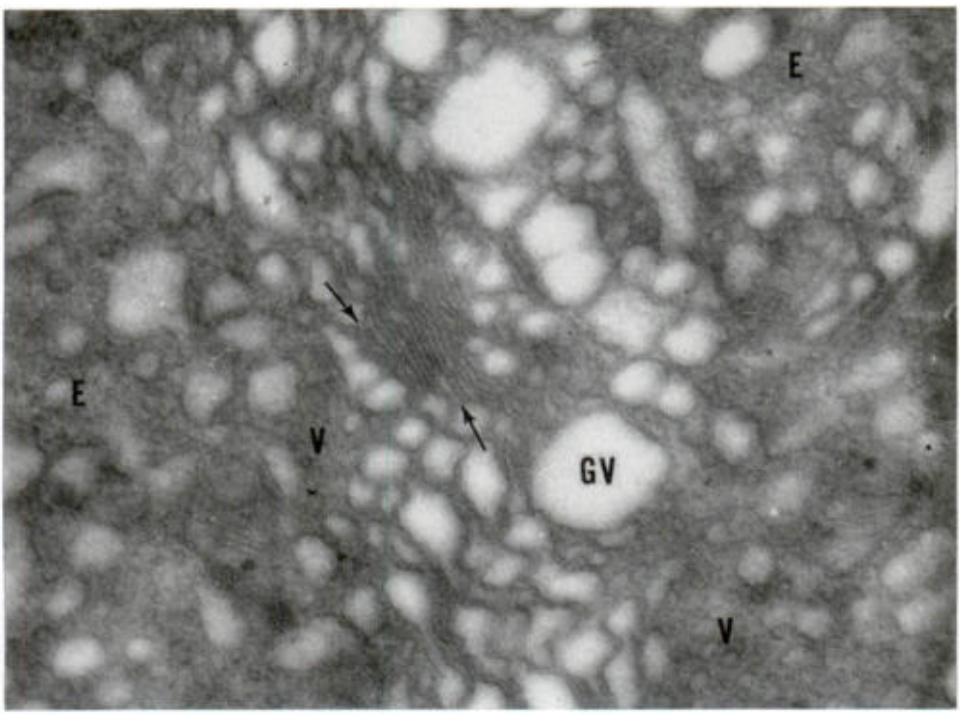

FIG. 7. Electron micrograph of a portion of the Golgi complex from an epithelial cell of the epididymis. Golgi membranes are seen just above the center of the figure. Arrows point to regions where paired membranes become continuous with Golgi vacuoles. E, endoplasmic reticulum; GV, Golgi vacuoles; V, Golgi vesicles. Approximately × 49,000. (Courtesy of Dalton and Felix, J. Biophys. and Biochem. Cytol. vol. 2.)

material (OsO_4 for 20 to 40 hours after fixation) often show a reduction of OsO_4 within and at the margins of the large vacuoles (Fig. 8). In some cases, the small vesicles also reduce the OsO_4. The OsO_4 reduction in these areas gives the blackened Golgi apparatus seen with the light microscope.

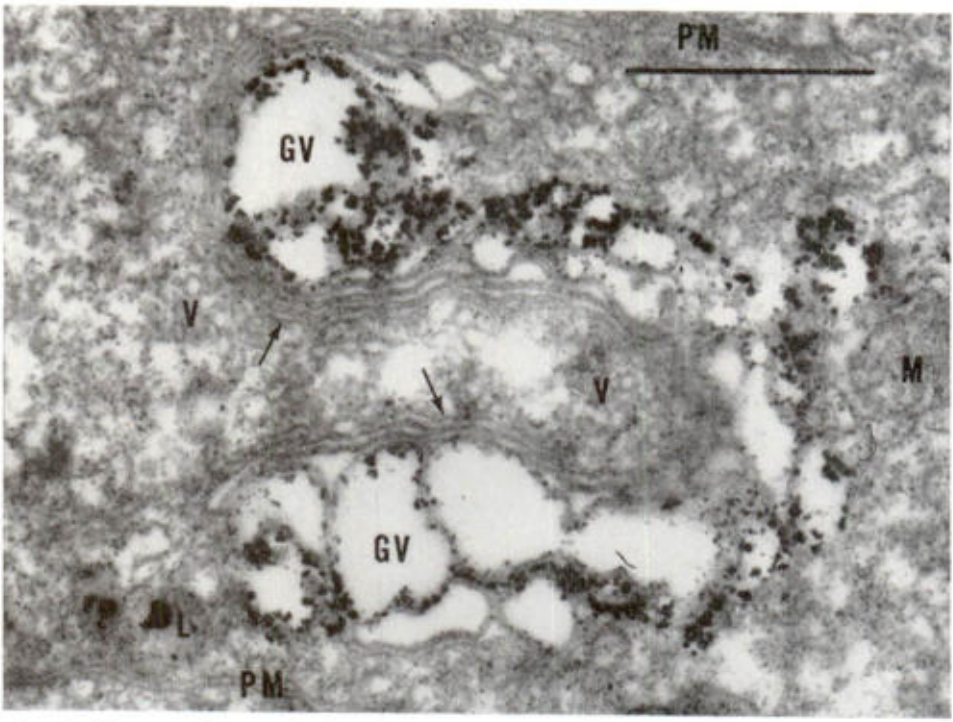

FIG. 8. Electron micrograph of a portion of the Golgi complex from material post-osmicated for 20 hours. Arrows point to double membranes of Golgi complex. Reduction of the OsO_4 has occurred particularly within and around the Golgi vacuoles. GV, Golgi vacuoles; M, mitochondrion; PM, plasma membrane; V, Golgi vesicles. Approximately × 34,000. (Courtesy of Dalton and Felix, J. Biophys. and Biochem. Cytol., vol. 2.)

FUNCTION OF THE GOLGI APPARATUS. It is impossible to speak with finality regarding the function of the Golgi apparatus, but there is some evidence relating it to secretory activity. That the apparatus increases in size during the elaboration of secretory substances is well shown in the thyroid gland where physiological and chemical analyses can be used to check the cytological data (Fig. 9). It is unlikely that the material of the apparatus itself is converted into the secretory product, but it may be capable of inducing secretory elaboration. Secretory granules undoubtedly originate from particles too small to be seen, so that their origin remains a conjecture. However, in many cases, the first and smallest granules that can be seen are found in the region of the Golgi apparatus (Fig. 223).

There is some evidence that the Golgi apparatus has the ability to adsorb various substances. For example, intravital dyes which are concentrated by certain cells often appear first in the region of the Golgi apparatus (trypan blue in liver cells, Figs. 10 and 11). A quotation from Kirkman and Severinghaus (1938) has particular interest in relation to the theory that the Golgi material may act as a condensation membrane. They state: "A great deal of work strongly suggests that the Golgi apparatus neither synthesizes secretory substances nor is transformed directly into them; but it

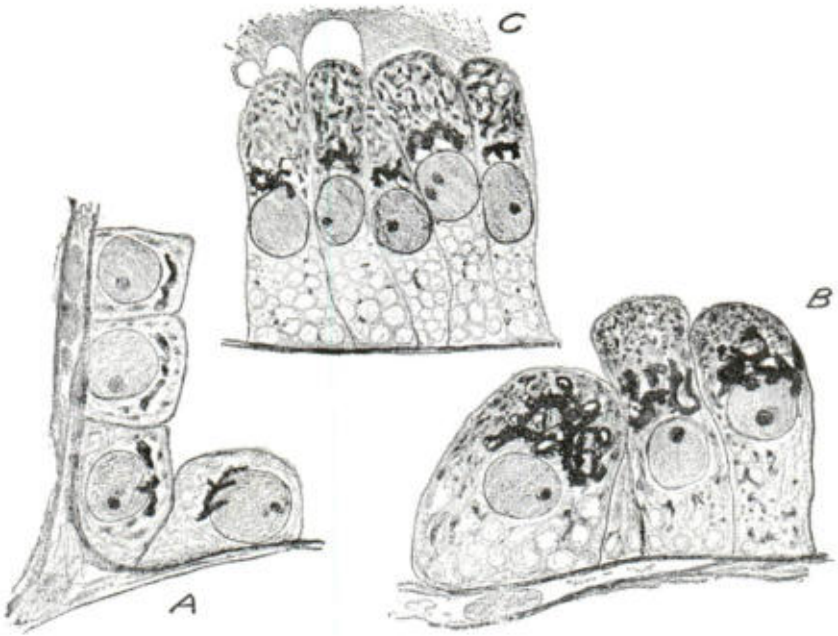

FIG. 9. *A*, Thyroid epithelium from normal sheep; *B*, cells from same thyroid after marked activation with hypophyseal hormone. The greatly hypertrophied Golgi apparatus (black) remains apical in the heightened cells. *C*, moderately activated thyroid cells from rhesus monkey. Central colloid dep'etion active. Golgi apparatus, still apical, not enlarged in the tall cells, but apical mitochondria are increased, and basal colloid vacuoles are prominent in the cells. Nassonov-Kolatschev preparations. Camera lucida. (Preparations by Dr. A. E. Severinghaus.)

acts as a condensation membrane for the concentration, into droplets or granules, of products elaborated elsewhere and diffused into the cytoplasm. These elaborated products may be lipoids, yolk, bile constituents, enzymes, hormones, or almost any other formed substances".

Mitochondria. The mitochondria are cytoplasmic components which may be present in the form of granules, rods or filaments. They are present in all cells, and their identity is firmly established, for they can be seen in both living and fixed protoplasm. Although in the living cell they have been seen to change from one form to another, they nevertheless maintain a striking regularity of form and position (polarization) in many cells (see columnar absorbing cells of the intestinal epithelium, Fig. 2, *C*). In the living cell they may be stained specifically with Janus green B. In fixed preparations they remain visible only after fixatives which omit fat solvents, and they may then be stained rather specifically by a number of special methods. From their reaction to chemical reagents, they have been classified as lipoproteins.

Electron microscope studies show that mitochondria are bounded by a double membrane and that the substance (matrix) within each mitochondrion is partially subdivided by a series of parallel ridges or cristae which protrude inwards from the inner membrane (Figs. 3 and 12).

FUNCTIONS OF MITOCHONDRIA. Our knowledge of the functions of mitochondria has been advanced through a

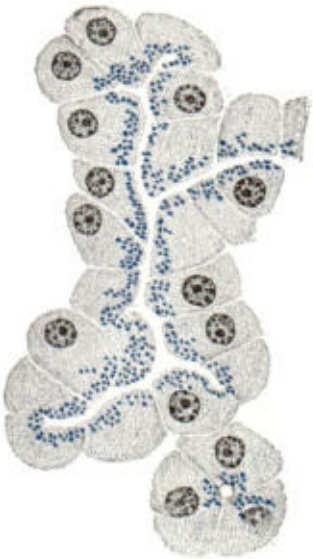

FIG. 10. Granules of intraperitoneally injected trypan blue first appearing in liver cells in region occupied by the Golgi apparatus. See Figure 11. (After Nassonov.)

combination of methods for isolating them and studying their enzyme reactions *in vitro*. When tissues are treated in a way to destroy the cellular membranes and then centrifuged at different speeds (differential centrifugation) it is possible to isolate nuclei, mitochondria, and submicroscopic particles known as microsomes. Mitochondria which have been separated from the other cellular elements can carry out *in vitro* oxidative processes which indicate that they contain succinic dehydrogenase and cytochrome c oxidase. In fact, the mitochondrial fraction catalyzes the whole series of reactions of the Krebs citric acid cycle. Therefore, they apparently contain the bulk of the enzymes which constitute the cyclophorase system (see review by Green, 1952). Furthermore, it has been shown that tissues with an abundance of mitochondria, such as liver and kidney, have a higher cyclophorase activity whereas tissues with relatively few mitochondria, such as white skeletal muscle, have a lower cyclophorase activity. There is also an increase in the number of cristae per mitochondrion in tissues with high activity.

Fibrillae. Definite fibrillae are characteristic and prominent structures in muscle and nerve cells. They are easily demonstrated in fixed materials and they are readily seen in living muscle fibers. Their presence in living nerve cells has been confirmed although they are less distinct than in muscle fibers. Some other cells have characteristic fibers which can be brought out by certain methods of fixation. Thus in the "prickle-cells" of the Malpighian layer of the epidermis, the cytoplasm contains fine fibrils, *tono-*

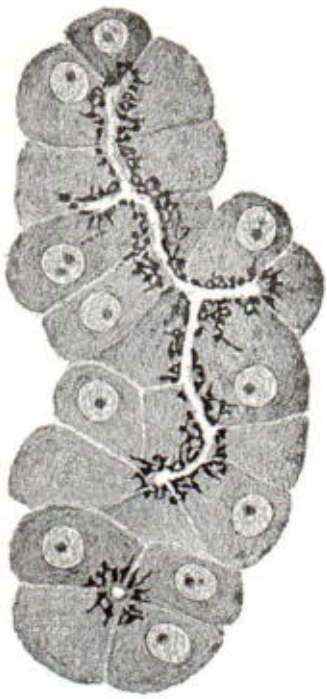

FIG. 11. Golgi apparatus (black) in the liver cells of Lacerta vivipara. Compare with Figure 10. (After Nassonov.)

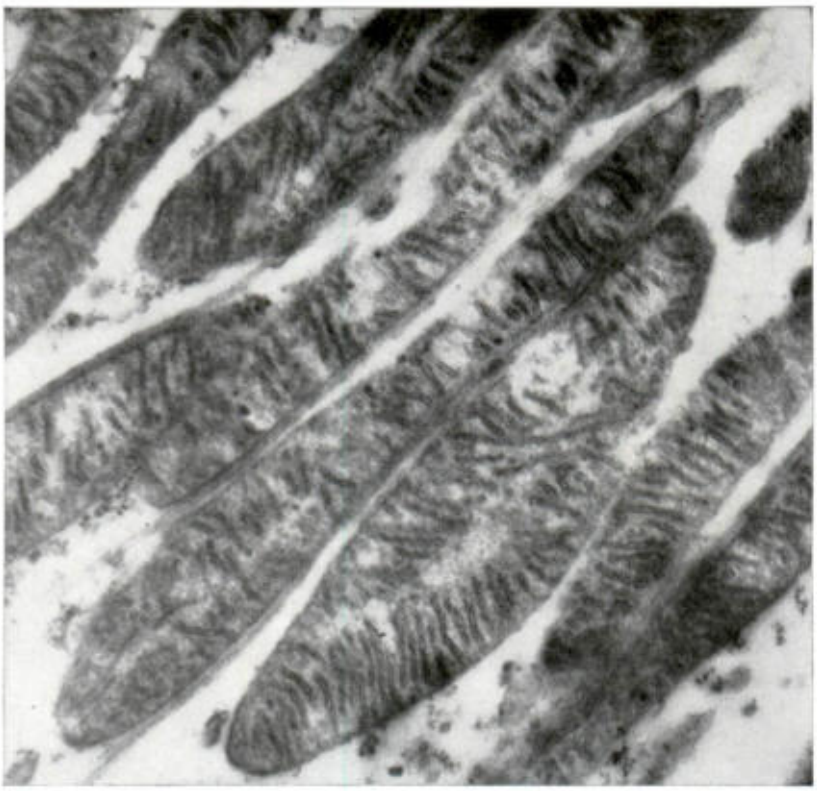

FIG. 12. Electron micrograph of several mitochondria from the proximal convoluted tubule of a rat kidney fixed in buffered OsO_4. Note that each mitochondrion is covered by a membrane from which there are projections (cristae) into the interior. Compare with Figure 2 for the appearance of kidney mitochondria under the light microscope. ×35,600. (Courtesy of Dr. G. E. Palade.)

fibrils. These contain sulfhydryl groups which probably function as precursors for the keratin of the surface layers of the epidermis.

Chromophilic Substance. (*Ergastoplasm, Endoplasmic Reticulum and RNA Granules, Microsome Component*). This substance was named *chromophilic* because it stains similarly to chromatin with basic dyes. In serozymogenic cells of the digestive system, it received the additional name of ergastoplasm (Gr. *ergastēr*, a workman, + *plasma*, plasm) because it was thought to be working in the production of secretory granules. In these cells, it is found chiefly in the basal region of the cell (Fig. 2, *B*). In nerve cells it is more widely distributed and was named after Nissl who was the first to describe it for nerve cells.

Electron micrographs of the chromophilic regions show two structural components: (1) *endoplasmic reticulum* (anastomosing flattened vesicles, *cisternae*), and (2) fine *RNA granules* (Figs. 3 and 13). The endoplasmic reticulum was first identified in the endoplasmic region of fibroblasts grown in tissue culture by Porter, Claude and Fullam in 1945. Since that time it has been found in all animal cells with the exception of adult mammalian erythrocytes (Palade, Porter, and

FIG. 13. Electron micrograph of a portion of the cytoplasm of a rat liver cell fixed in buffered OsO_4. The largest structures in the field are mitochondria which have been cut transversely and obliquely. The slender, canalicular-like structures compose the endoplasmic reticulum. Small granules are seen on the surface of the endoplasmic reticulum and the intervening cytoplasm. ×31,400. (Courtesy of Dr. G. E. Palade; see also Mellors and Selbey, Cancer Research, 1953.)

others.) Furthermore, it has been shown that the cisternae are not confined to the deeper or endoplasmic region of the cytoplasm but that they extend from the outer layer of the nuclear envelope to the plasma membrane at the cell surface (Fig. 3). It should also be noted that the cisternae are not restricted to chromophilic areas but that some of them extend through regions which were formerly considered as hyaloplasm by the light microscopists.

The arrangement of the submicroscopic components and their relative proportions differ for different types of cells. In general, they are prominent with an abundance of RNA granules in regions which appear chromophilic under the light microscope (e.g., ergastoplasm of serozymogenic cells and Nissl bodies of nerve cells). It seems well established that the fine granules are composed of RNA and that they play an active role in the synthesis of cytoplasmic proteins.

By differential centrifugation of homogenized cells, small granules have been

isolated which are known as *microsomes*. Their average size is approximately the same as the diameter of the strands of the endoplasmic reticulum. It has been shown that the microsomes obtained by centrifugation are fragments of endoplasmic reticulum with adherent RNA granules.

Plasma Membrane. The covering at the cell surface consists of a semipermeable membrane, without which no vital activity of the cell could proceed. In it are bound all the problems of osmosis, imbibition and permeability. In some cases, the cell wall is composed of two parts, an inner or plasma membrane which is a modified surface film of cytoplasm and an outer cell membrane with a supporting function. Microdissection studies have shown that the outer membranes are not essential for life whereas the plasma membranes are. In most animal cells, the plasma membrane seems to be the sole covering. In some tissues (chromophobes of anterior hypophysis) even the plasma membrane is so thin that it can be demonstrated only with difficulty.

The plasma membrane is apparently composed of a continuous film of lipoid molecules covered on both the inner and outer surfaces by a layer of adsorbed proteins. The protein layers consist mostly of long polypeptide chains oriented parallel with the surface. They are probably responsible for most of the elasticity and mechanical strength of the plasma membrane.

Cytoplasmic Inclusions. The cytoplasm of the cell may contain numerous inclusions of substances which are usually in the nature of raw food materials or the stored products of the cell's metabolic activity. Thus deposits of proteins, fats and carbohydrates are characteristic features in certain cells. The storage of glycogen by cells of the liver is perhaps the outstanding example of food storage (Fig. 2, *D*). Other cells may contain pigment granules, as do certain epithelial cells. The distribution and character of pigment inclusions become a feature of importance in some pathological conditions which the student of medicine later meets. Yolk granules and secretory granules are also products of the cell's activity. Certain bodies of varying size, unknown origin and structure frequently occur in many cells especially in association with virus infections of the organism. These are called "inclusion bodies" by pathologists and have a diagnostic significance.

FUNCTIONAL ATTRIBUTES OF PROTOPLASM

Protoplasm exhibits among others the following vital properties: (1) *metabolic activities for vegetative existence*—that is, all of the properties of food absorption, assimilation, etc., which are necessary for the existence of the cell; (2) *special properties* such as conduction, contraction, etc.; (3) *growth and reproduction*. These arbitrary headings are not sharply separated but overlap each other.

Metabolism. Used in its broadest sense metabolism may include all the transformations of energy and matter of which protoplasm is capable. Thus considered, it is naturally the most important property of living substance and all other attributes become manifestations of its metabolic activity. Life, in fact, consists of, or is synonymous with, this uninterrupted series of chemical reactions, which we may call metabolism. When they cease, the living substance dies. Metabolic reactions are grouped into two general classes; those through which substances are built up (synthesized) are *anabolic*, those through which substances are broken down (disintegrated) are *katabolic*. With anabolism energy is stored, with katabolism energy is re-

leased. The degree of metabolic activity is variable, and protoplasm may be active at one stage and relatively inactive or resting at another.

The main chemical processes of metabolism are those of hydrolysis and dehydrolysis, of oxidation and reduction. Hydrolysis and oxidation are usually associated with katabolic processes and result in the liberation of energy which living substance requires for its activity. Dehydrolysis and reduction are anabolic processes in which more complex substances, even protoplasm itself, are synthesized with the resultant storage of energy. The endless chain of chemical reactions in living processes may be emphasized by pointing out that anabolic processes can never occur without the utilization of energy. In animal metabolism this energy is released by simultaneous katabolic processes. The splitting of fat into glycerine and fatty acids, of glycogen into glucose, of proteins into amino acids, are examples of hydrolytic reaction, while the reverse synthetic reactions are phenomena of dehydrolysis. It is interesting to note that reactions involving elimination of water (dehydrolysis) can take place in living substance in a medium which itself may contain more than 80 per cent of water.

A remarkable feature of metabolism is the great speed of its chemical processes. Almost all the reactions known to chemistry are represented, and many of these can be made to take place in the laboratory, but usually at an exceedingly slower rate. The explanation of the rapidity of chemical reactions in living substance is the presence of numerous *enzymes* which act as catalysts. These catalysts accelerate a chemical process without being destroyed. They are usually specific in their action. Thus a lipase will be concerned with the hydrolysis of a certain fat, a protease with a protein and an amylase with a carbohydrate. Enzymes themselves are colloidal substances, nondiffusible, and hence localized. Some of them have been identified with specific protoplasmic structures (see mitochondria).

Special Functions. All protoplasm has the ability to react or respond to stimuli, a property which is sometimes spoken of as irritability. This presupposes that living substance is sensitive to receive the stimulus, that it has the power to conduct it, and to react with an integrated response. In unicellular organisms, the simple automatic responses are spoken of as tropisms. In higher multicellular organisms, sensitivity, conduction, integration and movement become separated and are specific functions of differentiated cells. Thus nervous tissue is particularly specialized for the reception and conduction of stimuli and muscle tissue is particularly specialized for contraction.

Growth and Reproduction. Growth results from the chemical synthesis of new protoplasm from primary raw materials which have been assimilated. In so far as the amount of protoplasm is thereby increased, there is in this resultant process of growth the fundamental basis of multiplication or reproduction.

It may be profitable to recall the historical statement of Virchow that all cells arise from the division of preexisting cells. The truth of this statement is the foundation of embryology which realizes that all the cells of the individual body arise from the single fertilized egg cell. It is also the foundation of genetics and evolution which realizes that all "life is an unbroken series of cell-divisions that extend backward from our own day throughout the entire past history of life. . . . It is a continuum, a never-ending stream of protoplasm in the form of cells, maintained by assimilation, growth and

division. The individual is but a passing eddy in the flow which vanishes and leaves no trace, while the general stream of life goes forward".

It is indeed an abrupt transition from these poetic lines of E. B. Wilson to a prosaic description of cell division. Two types of cell division have been observed, direct and indirect. Direct cell division (amitosis) occurs by simple constriction of the nucleus and cytoplasm. Although this process has been observed in some plants, in some Protozoa and in some mammalian tissues during degenerative changes, it is relatively rare in normal mammalian tissues. The usual process of cell division is indirect (mitosis) whereby there is an exact division and distribution of chromatin to the resulting daughter cells.

Mitosis (Karyokinesis) (Figs. 14 and 15). The process of mitosis is composed of a series of continuous changes which may be conveniently divided into four main phases or stages. These are the prophase, metaphase, anaphase and telophase. The prophase involves the preparation of the chromatin for division by condensation of chromosomes; the metaphase, the splitting and separation of the chromosomes; the anaphase, the migration of the chromosomes and the formation of the two daughter nuclei; the telophase, the final reconstruction of the daughter nuclei and the division of the cytoplasm.

1. PROPHASE. In the section on nuclear structure, it was pointed out that it is usually impossible to recognize chromosomes as entities during the intermitotic portion of a cell cycle. When a cell prepares to divide, the chromatin granules begin to stain more deeply and line up in long thread-like structures which can be identified as chromosomes. An intertwining, "ball of yarn" appearance gave rise to an erroneous concept held for many years that the chromosomes are all arranged end to end in a long continuous spireme at this time.

The chromosomes of the early prophase stage often show a series of denser chromatic regions (chromomeres) on a "back-bone" thread known as the chromonema. Each chromomere supposedly contains one or more genes, the units of hereditary factors. The chemical composition of chromosomes has been mentioned earlier; that is, they consist chiefly of desoxyribonucleic acid (DNA), a basic protein, histone, and a non-basic, tryptophane-containing protein.

During the latter part of the prophase stage, the chromosomes become shorter and more compact. With this change, they become more obviously separated from each other and their number can readily be determined in species such as the round worm, Ascaris, which has only four chromosomes. This is, of course, more difficult in man where there are 48 chromosomes (or 46, according to some recent studies). Factors which contribute to the chromosomal changes during the late prophase include spiralling, dehydration and possible changes in the DNA/protein ratio. Spiralling becomes so pronounced that each chromosome appears as a cylindrical thread in which the individual coils can be recognized only by special studies. Dehydration apparently contributes to the compactness and density of the chromosome structure. A breakdown of some of the DNA-associated proteins may be another factor as outlined by Caspersson from his ultraviolet absorption studies of the nucleus at different stages of the cycle. On the other hand, Ris (using a method developed by Pollister and Ris, 1947) finds no evidence for changes in the DNA/protein ratio during these stages. He finds an increase in the total mass of the chromosomes

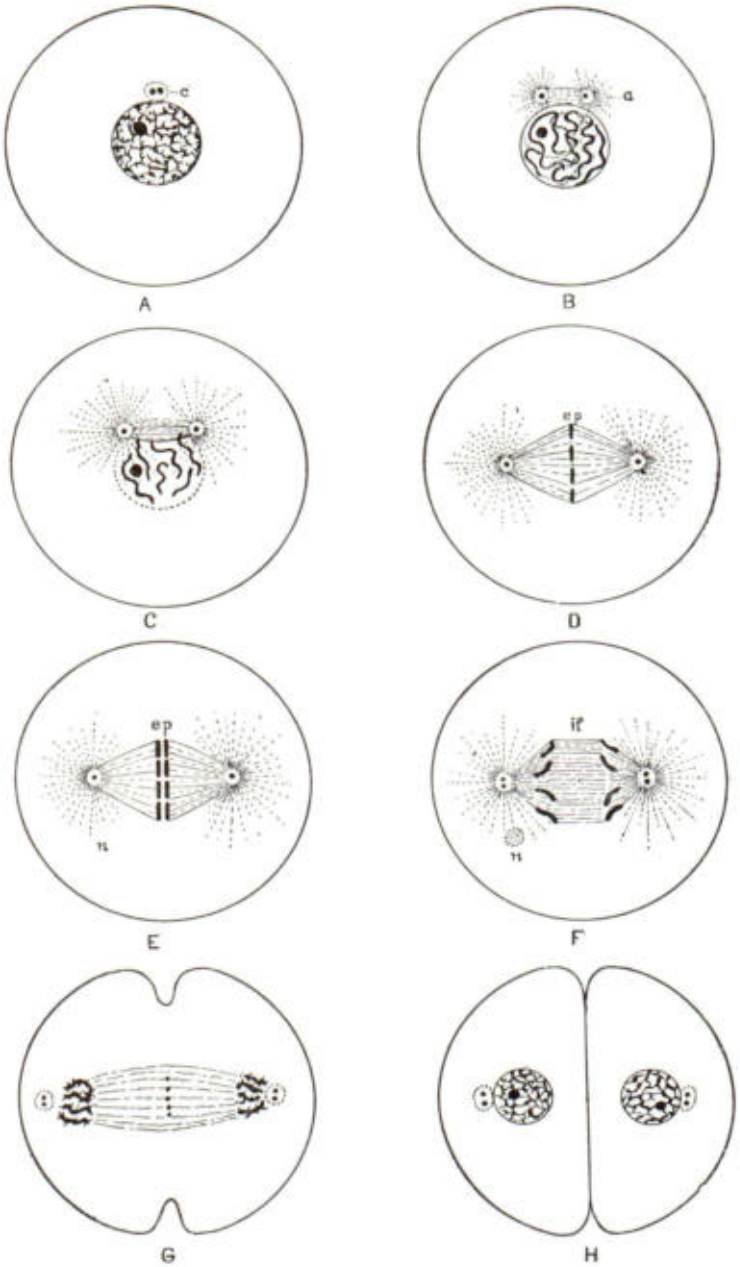

FIG. 14. Diagram of successive phases of mitosis. *A*, *B*, *C*, prophase; *D*, *E*, metaphase; *F*, anaphase; *G*, *H*, telophase; *a*, achromatic spindle; *c*, centrosome; *ep*, equatorial plate of chromosomes *if*, interzonal fibers; *n*, nucleolus. (After Wilson.)

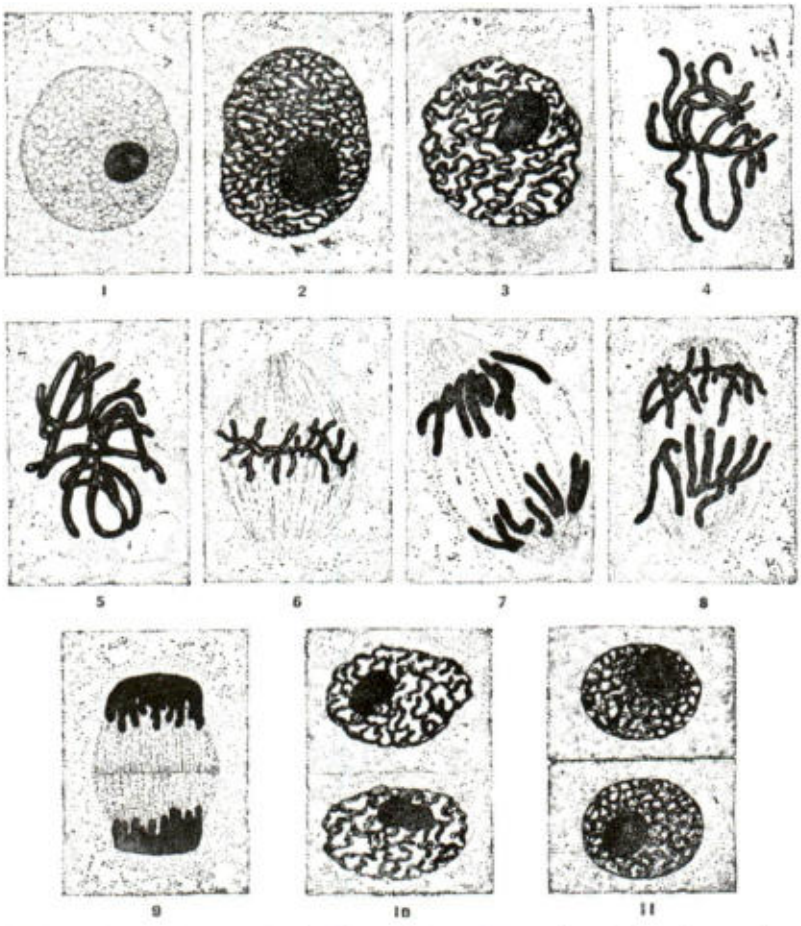

FIG. 15. Mitosis in growing root tip of onion. *1*, Vegetative nucleus; *2*, *3*, early prophase, showing long, intertwining chromsomes; *4*, *5*, late prophase; *6*, metaphase (equatorial plate); *7*, *8*, anaphases; *9*, telophase showing formation of cell plate; *10*, late telophase; *11*, daughter cells.

with a doubling both in nucleic acids and proteins at metaphase.

During the latter part of the prophase, there are two additional changes in the nucleus: the nuclear membrane disappears, thus allowing continuity of nuclear and cytoplasmic substances; and the nucleoli disappear. The ribonucleoproteins of the latter are apparently incorporated into the nucleoli-associated chromosomes.

While the changes described above are occurring in the nucleus, there are changes in the cytoplasm in connection with the central body. The centriole, if single, divides into two. However, in most cells which are dividing actively, the centriole is already double (diplosome) having undergone division during the final stages of the preceding mitosis. The centrioles, each surrounded by a centrosphere, move away from each other but remain connected by fine protoplasmic fibrils which form a spindle-shaped structure known as the central or achromatic spindle. The entire achromatic figure consisting of

centrioles, centrospheres, astral rays and central spindle, constitutes the amphiaster. Towards the end of the prophase the centrioles have moved apart to such an extent that they occupy opposite poles of the cell.

2. METAPHASE. The chromosomes, typically V, I, J or U-shaped, now arrange themselves around the central spindle in a plane exactly midway between the two centrosomes. When viewed laterally they appear in the nature of an irregular, chromatic plate (equatorial plate). By this time it becomes obvious that each chromosome is split longitudinally into two exact halves. It is uncertain when the actual splitting begins but apparently each chromosome is already composed of two closely spiralled filaments (chromatids) in the late prophase stage.

3. ANAPHASE. During this stage, the daughter chromosomes become separated from each other and approach their respective centrosomes near which they become grouped. One half of each split chromosome goes to one pole, the other half to the other pole of the cell. Thus two daughter groups of chromosomes are formed which are in all respects identical. It is often at this time that each centriole divides into a diplosome, preparatory for the next mitosis.

4. TELOPHASE. In this final stage, the daughter nuclei are reconstructed and cytoplasmic division is accomplished with the formation of two daughter cells. The chromosomal changes are the reverse of those taking place during the prophase. Each chromosome changes from a short, compact cylindrical thread to a long, loosely spiralled thread by loss of its tightly coiled condition which it had assumed during prophase. The structures within each chromosome appear to be pushed apart either by hydration, or by increased formation of proteins around the gene loci (Caspersson, 1950), or by both of these factors. Outlines of the chromosomes usually disappear leaving scattered chromatin granules connected by pale staining strands.

Other changes during telophase include the formation of nucleoli, the formation of a nuclear membrane and the division of cytoplasm (Figs. 14 and 15). The nucleoli develop at nucleolar organizing centers of particular chromosomes, forming from substances metabolized under the influence of the desoxyribonucleoproteins of the chromosomes. The nucleolar substances, as described earlier, are chiefly basic proteins and ribonucleic acids (RNA).

General Considerations of Mitosis. The time required for mitosis varies in different cells, but it is usually accomplished in less than one hour. The preparatory stages of the prophase take up most of the time, the middle and end phases occur very rapidly.

The chief feature of mitosis is the reproduction of gene material or the reproduction of the desoxyribonucleoproteins of the chromosomes. It has been pointed out earlier that DNA is fairly constant in the nuclei of all cells of a species, except in haploid or polyploid nuclei which have atypical numbers of chromosomes. Immediately after chromosomal division, each daughter nucleus has the same amount of DNA as the nucleus of the parent cell had. On the other hand, the mass of the nucleus and cytoplasm of each daughter cell at late telophase is only about half that of the parent cell (Figs. 14 and 15). In other words, there is no increase in total cell mass during mitosis. During the intermitotic phase, the daughter cells grow to reach the size of the parent cells. As stated by Caspersson (1950), "the process of mitosis is dominated by redistribution of the proteins in the nucleus. . . . The interphase picture is dominated by augmenta-

tion of the protein masses of the cell body."

The constancy of the number of chromosomes has suggested the possibility that the chromosomes retain their individuality even during the intermitotic stage. In the mitosis of many cells (insects, Lepidosiren, salamander) the various chromosomes can be distinguished from each other by differences in size and shape. In these animals it is interesting that at each division the same figure appears; the size, shape and even the special arrangement of the chromosomes are always the same. It was noted by Boveri many years ago that chromosomes may even be recognized with certainty during the intermitotic phases of the early development of the egg of Ascaris. Since that time, evidence has accumulated for the persistence of chromosomal integrity during the interphase stage in other species. In this respect, it is noteworthy that thread-like structures, apparently chromosomes, have been obtained from interphase nuclei by differential centrifugation (Claude and Potter, 1943). From all of these facts and from breeding experiments it has become established that the chromosomes are intimately associated with the phenomena of heredity. Chromatin is apparently the chief hereditary substance of the cell. If this is true, the chromosomes, or rather the various chromomeres constituting a chromosome, must differ from each other in regard to hereditary value.

GENERAL CONSIDERATIONS

Cell form and Cell size. The cells of the animal body show a wide variation in size and form, coincident with their adaptation to perform a diversity of specific functions. Few of the tissue cells which have acquired a fixed location in the body are able to maintain the typical spheroidal form. They differentiate under different mechanical stresses and pressures. Thus, they become polyhedral, columnar, flat (pavement), fusiform or spindle-shaped, and they may retain a smooth contour or send out numerous processes. The nerve cell with its processes sometimes several feet in length is perhaps the most aberrant type. The laws which control or limit cell size as well as body size are not in the least understood. Some groups of animals have larger cells than do others, but it does not follow that small animals have small cells and large animals large cells. The size of the individual is in general determined by the number of its cells, not by their size. For example, Boveri long since found that the cells of human giants and human dwarfs were no different in size from those of normal individuals.

In rare instances, mutant races of giant individuals have arisen whose cells are giant cells. In these cells the nuclei are of double size, and the cytoplasm has grown likewise until it is twice the normal size. This indicates that there is in any normal cell a size ratio between nucleus and cytoplasm to which the cell rigidly adheres. In fact, R. Hertwig has theorized that it is the departure from this ratio in the growth process of the cell which results in cell division. In recent years some pathologists and experimentalists have given particular attention to variations in the nuclear cytoplasmic (karyoplasmic) ratio of cells in certain tissues, attempting to correlate a variation from the normal with a pathological or changed physiological condition.

Polarity. Aside from the interesting phenomena of polarity in development and regeneration which individuals as well as their cells show, the student of histology will soon see that many cells have a definite structural polarity. A definite invariable arrangement of the

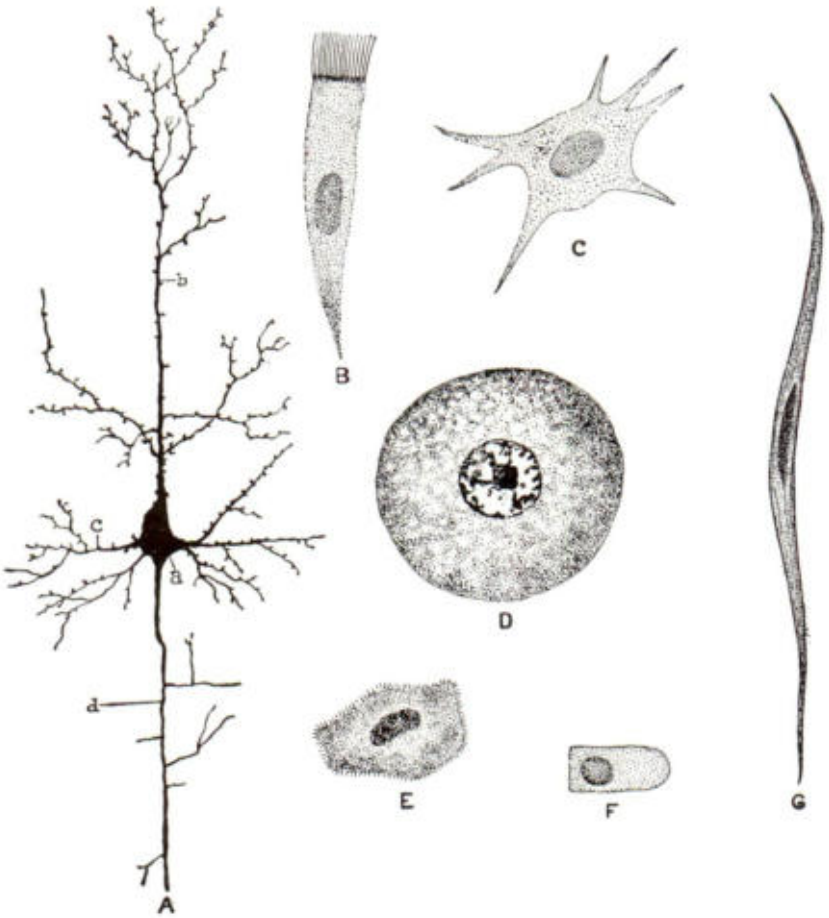

FIG. 16. Shapes of various types of cells. *A*, Nerve cell from human cortex; *B*, ciliated epithelial cell from trachea; *C*, connective tissue cell from rabbit; *D*, human ovum; *E*, epithelial cell from human epidermis; *F*, columnar epithelial cell; *G*, smooth muscle cell.

cell structures is the result. Thus the nucleus occupies a position in the cell, in respect to which the centrioles, mitochondria and Golgi apparatus will be definitely polarized. Such polarity is especially prominent in columnar epithelial cells and in many secretory cells, and in these the nucleus tends to occupy the basal or proximal region of the cell while the centrioles and Golgi apparatus are found toward the distal or free margin of the cell (Figs. 2, *C* and 9).

CYTOMORPHOSIS, NECROSIS

The duration of life of cells shows great variation. The nerve cells which become differentiated very early and lose the power of mitosis, live as long as the body as a whole. This is probably true of some other cells as well, but most of the cells live a much shorter time. There is a constant replacement of dying and dead cells through the mitosis of others. Such mitoses are common in the connective tissue cells and especially in the active

secretory cells. All cells, even the most long lived, show after a certain time the signs of senescence and old age. The various metabolic activities become reduced in intensity, mitosis is infrequent or entirely absent. Finally metabolism and function cease altogether; the cell is dead. Thus growth and differentiation are followed by disintegration; the activities of life lead inevitably to death. Minot has characterized as cytomorphosis all the structural changes which take place during the life of a cell. He recognizes three stages: embryonal, differentiated and degenerative. At first the cells are unspecialized and divide rapidly. With subsequent mitoses, changes in form and structure take place and the cell is differentiated in a more or less specific manner. After variable periods of specialized functioning the cell is worn out and degenerative changes appear which lead to the death of the cell. Progressive development leads to old age and death.

We may sometimes follow the progressive stages of disintegration of a dying cell (necrobiosis), but at other times death (necrosis) may be swift and attended subsequently with disintegrative alteration of cellular structure. Such local death occurs normally in the body, or may be the result of influences which cause disease, such as trauma and inflammation. Morphologically, necrotic tissue can be recognized by the altered structure of the cells and intercellular substance. Coagulation of proteins takes place in the cytoplasm, the latter appearing flocculated or in the nature of a fibrinoid reticulum. The cells appear fused by the obliteration of cellular boundaries. Or the cell may liquefy, swell and finally burst (cytolysis), the liquefaction being often preceded by the appearance of numerous fatty granules. These proteolytic processes are in many cases due to intracellular enzymes liberated after the death of the cell (autolysis). The nucleus, likewise, shows various forms of structural disintegration. The chromatin may contract into a dense, deeply staining irregular mass (pyknosis); fragment into a number of small pieces with obliteration of nuclear boundary (karyorrhexis); or gradually disappear, as evidenced by the loss of its staining capacity (karyolysis, chromatolysis).

This brief introduction to the structures and functions of the cell is intended to serve as a background for a further elaboration of particular cellular features as they occur in the various differentiated tissues which are described in subsequent chapters.

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II

THE LIVING CELL

When fixed and stained preparations of tissues and organs are prepared, they are more or less permanent and may be observed again and again under the microscope. Living tissues, on the other hand, are usually more difficult to prepare and then are valuable for a short time only. It is important, nevertheless, for anyone undertaking the study of microscopic anatomy to have a point of view regarding the living cell, the way in which it differs from the fixed cell and the methods by which it can be studied. The greatest value in the observation of the cell in its living state, is that the barriers between physiology and anatomy are bridged; function and morphology may be studied at the same time.

The very nature of the living cell, the delicacy of its structure and of the equilibrium between it and its environment, make it a difficult object to study. The early biologists overcame this difficulty by seeking out lower forms which could be made available for experimentation. Much valuable information was obtained in this way, but it could not be applied directly to the cells of higher animals, particularly man.

METHODS OF PREPARATION

The first human cells studied in the living condition were blood cells. They were easy to obtain and could be brought under the highest powers of the microscope while still surrounded by their natural environment, the plasma. Due to the fluid nature of the blood, a thin film can be made between a cover glass and slide, where, if the edges are sealed to prevent evaporation and the stage of the microscope heated to body temperature, the conditions inside the body are approximated. With this type of preparation the larger divisions of the white blood cells were recognized and their ameboid and phagocytic activity observed. This method is still one of the best for a study of blood cells as well as for an introduction to active living human cells. A renewed stimulus has been given to this field by the development of the phase microscope which makes it possible to observe more contrast between the different constituents of unstained cells.

The cells of organized tissues could not be made available by this simple procedure. They occur in masses too thick for examination by transmitted light, so that some method had to be devised to separate them from their natural environment. Cells have been obtained from the cut surfaces of organs and tissues, mixed with an appropriate salt solution and mounted in the same manner as the thin film of blood. The spleen and lymph nodes have been studied in this way with valuable results. The drawbacks to the method are that the specific cells are mixed with blood and connective tissue elements, the

relationship of one cell to another is upset, and many cells are injured.

Subcutaneous connective tissue can be studied quite effectively by teasing very small pieces with needles until a thin spread has been drawn out on a slide. The fibers are present in their normal arrangement while the cells, although a few are destroyed by the teasing, are in reasonably good condition. Muscle and nerve fibers are studied frequently in teased preparations. Attempts have been made to cut thin slices of the fresh organs or tissues, but this has not proved satisfactory because of the mechanical difficulties involved.

Tissue Culture. A great step in the progress of the study of living cells was made with the discovery of a technique for their prolonged preservation outside the body. This has been variously called tissue culture, cultivation *in vitro* and explantation. Fragments of tissue one or two mm. in diameter are placed in a physiological medium, sealed to prevent evaporation and kept at a temperature normal for the animal from which the material was taken. An absolute necessity is asepsis or the prevention of infection with bacteria because a medium which promotes the growth of cells also favors the multiplication of bacteria. In order to make the cells available for observation under the microscope, the cultures are placed in thin glass vessels or in hanging drops on a cover glass mounted over a hollow slide.

MEDIA. Two types of media are in general use, solid and liquid. A solid medium contains a fibrin clot. The one in common use is blood plasma which has been clotted by the addition of tissue juice. Blood is obtained from the circulating stream of an experimental animal and the plasma separated from the cells in the centrifuge. Clotting is prevented during preparation either by chilling or by the use of the anticoagulant, heparin. In the original experiments of Harrison on embryonic nerve cells from a tadpole, clotted lymph from an adult frog was used. The plasma of blood has replaced lymph largely because it is more readily available. The clot is a very important part of the medium since it offers a mechanical support for the migration of the cells.

Liquid media such as Ringer's solution, Locke's solution, blood serum or even physiological saline have given excellent results. The most universally accepted at the present time is Tyrode's solution, a mixture of the chlorides of sodium, potassium, calcium and magnesium with sodium phosphate and bicarbonate added as buffers against changes in acidity.

GROWTH PROMOTING SUBSTANCES. The tissues of embryos continue to grow for a time in plasma or saline alone, but their growth is more vigorous and more constant if a growth promoting agent is added. The most generally employed substance of this kind is embryo extract. In its preparation, embryos are crushed or chopped in an equal quantity of Tyrode's solution and the clear fluid obtained by centrifugation. Other substances, such as the products of partial digestion of protein, the extracts of adult bone marrow and anterior pituitary have growth stimulating properties but they are not as effective on most normal tissues as the embryo juice.

SOURCES OF MATERIAL. As stated above, embryonic cells were employed in the original experiments which introduced tissue culture. They have continued to be the most frequently used since that time. Adult tissues have been cultivated but the results generally have not equalled those obtained from embryonic material. Migration and growth occur from explants of nearly all organs but in many cases the growth is composed of connective tissue rather than of the specific cells of the organ. In a later section, reference

will be made to some of the more successful results obtained on adult tissues. Tissue culture was recognized at an early date as a method of value for the study of cancer. The cells from both malignant and benign tumors have been grown for long periods. Many institutions have strains of carcinoma and sarcoma cells taken from the laboratory animals as well as from man which they keep for experimental purposes just as the bacteriologist keeps his various strains of bacteria.

STRUCTURE OF LIVING CELLS

Fibroblasts. In order to give a picture of the structure of a living cell for comparison with one which has been fixed (Fig. 17) we have selected a fibroblast, grown in tissue culture, as it represents a type of cell which has undergone but little specialization. In the living condition, it is colorless and transparent; the structures within it are seen only because they have a refractive power different from their surroundings. The cell itself is spindle-shaped or flat depending on the confinement or stress put upon it by its environment. Isolated cells are flat, somewhat elongated and have pointed processes which vary in size and radiate out in different directions. The nucleus is round or oval in shape but may be distorted into irregularities when forced to accommodate itself to unusual foldings of the

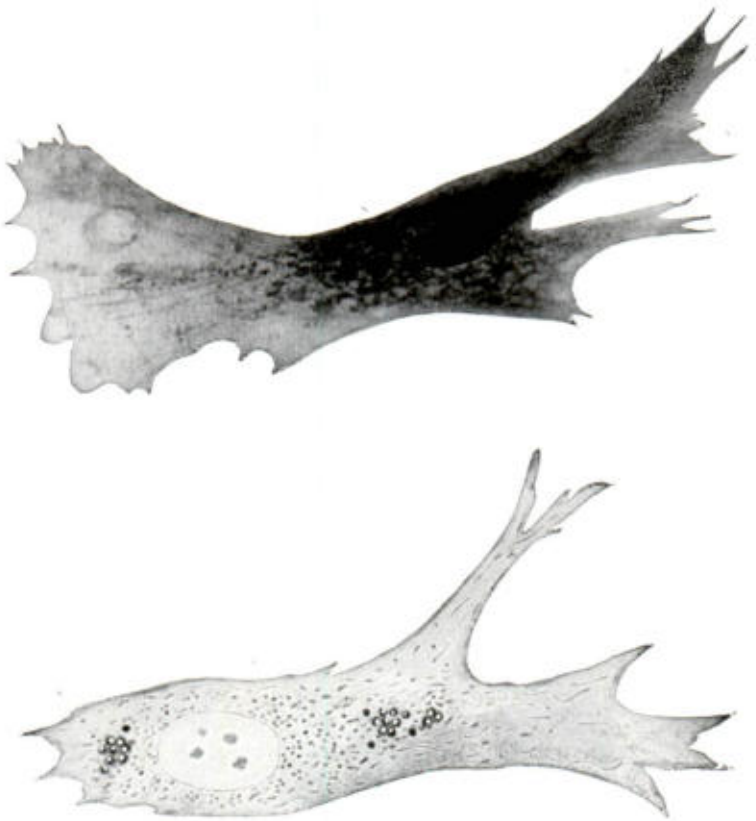

FIG. 17. Fibroblasts from tissue culture of embryonic heart. *Above*, after fixation in Zenker-osmic and stained with iron hematoxylin; *below*, drawing of a living unstained cell. (Courtesy of Dr. C. M. Goss.)

cell. It is a vesicle or bag of clear fluid with a visible membrane, but with none of the granular or net-like chromatin seen in the fixed cell. The only structures seen in the nucleus are the nucleoli, which may be one to four in number.

The cytoplasm is composed of a crystal clear matrix or ground substance in which the various cytoplasmic constituents are embedded. Of these bodies, the most striking and obvious are the fat droplets. In fresh cultures they are few and cluster into small knots at either end of the nucleus; they increase in number with the age of the culture until they practically fill the cell in degenerating cultures. Although they may not be the same size in different cells, they are remarkably uniform within the same cell. Due to their oily nature, they are highly refractive and are often the only structures singled out by the unpracticed eye. Smaller than these and more generally scattered throughout the cytoplasm are varying numbers of granules. They are discrete and well-outlined, but less refractive than the oil globules. They seem to be composed principally of proteins. Mingled with the granules but much more abundant are shadowy structures whose index of refraction is so nearly that of the ground substance that they are imperfectly defined. They can be seen clearly only with the best high-powered lenses. They are the mitochondria.

ACTIVITY OF LIVING CELLS

Motility. Certain activities of the cell which can be followed only by inference in fixed material show up very clearly in the living condition. Movement is the most obvious example. The simplest form of movement and that exhibited to some extent by all cells which have been studied adequately, is a shifting about of the elements within the cytoplasm. This is often very slight and so slow that it is frequently overlooked unless a special apparatus, the microcinema, is employed. There are local currents within the cytoplasm which cause the granules and mitochondria to move back and forth for varying distances. It is particularly well-shown in the actively growing fibroblasts and macrophages of tissue cultures. This activity must not be confused with the more violent type called Brownian movement; the latter occurs very rarely in healthy cells. Another practically universal type of movement is a changing of shape. Fibroblasts send out a process here and withdraw another there until they grow and wander away from the original piece of the culture. The macrophages have actual pseudopodia, which they keep in constant motion, in a fashion reminiscent of the appendages of certain marine invertebrates. In these cells the activity amounts to genuine ameboid movement, for, although they usually do not move far, they are capable of a fairly rapid locomotion. The cells in which this ameboid movement is most marked are the leucocytes (see Chap. VII).

Phagocytosis. Actively ameboid cells usually have the power of phagocytosis. In the case of the more rapidly moving cells such as neutrophilic leucocytes the engulfment is brought about by the passage of the cell over the particle to be absorbed. This can be observed in a thin film preparation made by mixing a suspension of bacteria with a drop of blood. The bacteria settle to the glass surface and remain motionless until the leucocyte passes part way over them. Suddenly they begin to move in unison with the interior of the cell and soon appear in small vacuoles which are carried about with the granules in the currents of the cytoplasm. Cells with less rapid locomotion, such as the histiocytes, come in contact with the material to be engulfed by sending out pseudopodia, which adhere to the débris

and surround it either by drawing it toward the cell or expanding the pseudopodium.

The ingestion of droplets of fluid by cells in tissue culture was described by Lewis in 1937 as *pinocytosis* (Gr. *pinein*, to drink). A similar process apparently occurs *in vivo* and the term pinocytosis is now in more general usage (see capillary endothelium, Chapter XII).

Muscular Contraction. The contraction of all three types of muscle has been observed in tissue culture. No nerves need be present for contraction. In smooth muscle there is a rather slow shortening of the entire cell and a widening of its central part. The entire cytoplasm enters into the movement flowing towards the central part of the cell. Skeletal and cardiac muscle have been observed to contract both with and without the presence of striations. In the embryonic cells a part of the cell may twitch or the entire cytoplasm may suddenly move toward a central area in the cell. In the differentiated fibers, the contraction may involve the entire mass at once but more frequently it travels as a wave down the long axis of the cell.

EXPERIMENTAL METHODS

A large amount of information has been obtained by the simple observation of living cells, but many of the fundamental concepts relating structure and function would have been mere inference without the aid of experimental investigations. Only a few of the methods which are applied more or less directly to living cells will be mentioned.

Vital Staining. Coloring of parts of the living cells by vital or supravital staining has been widely practiced. In vital staining, dyes are injected into the living animal so that the activity of certain cells can be brought out by their selective absorption of the coloring matter. The outstanding example in this field of investigation has been the identification of a macrophage system or reticuloendothelial system. When trypan blue is injected into an experimental animal, accumulations of the dye are found in vacuoles in the macrophages of the loose connective tissue, the reticular cells of the spleen, lymph nodes and bone marrow and in the stellate cells of Kupffer in the liver. This method groups together widely dispersed groups of morphologically different cells on the basis of their ability to phagocytose foreign particles.

A variation of vital staining is used in embryonic tissue grafting. An amphibian larva is stained blue if immersed in a solution of Nile blue sulphate. Primordia of the organs or tissues transplanted from the colored animals to normal hosts retain the dye for many days and mark the rôle of the group of cells in subsequent development.

Supravital Staining. Supravital staining consists in adding dyes to the medium of cells already removed from the organism. When trypan blue is placed on a tissue culture, the macrophages take it up in abundance. Small phagocytic cells have been marked in this way and their subsequent development into epithelioid and giant cells followed. Janus green has a specific affinity for the mitochondria and has been used to identify the latter in the cells of all the organs and tissues of the body in fresh films, spreads or scraped preparations.

The supravital method has been used extensively in the study of blood cells by means of the dyes neutral red and Janus green. It must be emphasized that the cells under observation are alive. Both the stains just mentioned are relatively nontoxic in proper concentration, although Janus green is much more injurious than neutral red. If the dye is too weak it does not stain and if too strong it

kills the cells. The process which brings about the coloring of the cells is quite different with these two dyes. Janus green seems to form an intimate union with the actual substance of the mitochondria. After the latter have been colored by the dye, they can be bleached by such a procedure as altering the acidity of the medium but when normal conditions are restored, the color returns. When the cell dies the color fades out from the mitochondria completely. If the concentration of the dye is too great, the nucleus stains, but as this kills the cell all coloration soon disappears.

Neutral red acts in two ways, as a supravital dye and as a stain for dead tissues. In the living cell it is accumulated on the granules, probably by adsorption, or it is concentrated in the fluid of vacuoles. It has a stimulating effect on the vacuolar apparatus of the cell because the size and number of vacuoles increase with long exposure to the dye. The size of granules, such as those of polymorphonuclear leucocytes, does not change. When the cell is dead, there is an actual staining of the nucleus. Since the living nucleus always remains colorless, staining of the nucleus may be taken as an indication of cell death.

Microdissection. Microdissection has been made possible by the designing of an instrument which moves very fine glass needles with such precision that cells can be dissected under the highest powers of the microscope. Several standardized micromanipulators are now on the market, available for this work. The glass needles are made in a very small flame and may be drawn to points which are too small for the resolving power of the best microscopes. The material to be dissected is placed in a hanging drop on a cover glass which is placed over a moist chamber designed to prevent evaporation. Pipettes may be mounted on the manipulator also, by means of which fluids can be injected directly into the cell. The diameter of the opening in these pipettes may be as small as half a micron (0.0005 mm.).

Experiments with the microneedles have made our concept of the physical nature of the cell much clearer. Protoplasm in general has been shown to be a viscous fluid, its viscosity varying in different cells. The granules, vacuoles and mitochondria have been moved about within the cell, showing that there is no internal structure except in highly specialized cells. The nucleus is a bag of fluid which may be pushed about from one part of the cell to another and be indented by the pressure of the needle. The fluid has been withdrawn from the nucleus with a pipette, leaving a wrinkled membrane, and phenol red has been injected into it showing the fluid nature of its contents and its lack of internal structure. When the cell membrane is torn the cell dies. The disintegration of the membrane spreads very rapidly around the cell; the cytoplasm coagulates while the nucleus, granules and mitochondria become much more refractive. The same wave of death and coagulation occurs after the puncture or tearing of the nucleus.

The relationship of one cell to another has also been clarified by this method. The disintegration which follows rupture of the nucleus does not spread from one cell to another unless there is actual protoplasmic continuity between them. This is clearly demonstrated by injury to the nucleus of one of the daughter cells after mitosis. If the strand of cytoplasm between them has not been broken, the other daughter cell dies. If they have become entirely separated, the other cell remains alive. The fibroblastic outgrowth in tissue cultures has been called a syncytium. Injury to the nuclei of the cells has shown, however, that the coagulation and death spread only to the region

directly surrounding each nucleus; one cell is killed and the disintegration does not spread. The same has been found true of cardiac muscle in cultures, although here units of two nuclei (binucleate cells) are frequent. The entire mass is not in continuity as would be the case with a syncytium. Skeletal muscle, on the other hand is made up of units containing large numbers of nuclei. Injury to one causes coagulation of the entire fiber.

The difference between the physiology of the cell membrane and the internal protoplasm has been studied by the injection of solutions into the interior of the cell with a micropipette. It has been shown that the toxic action of certain substances is mediated through their action on the membrane, for much greater concentrations can be borne within the cell than are tolerated in the surrounding medium. Others, that have little effect on the intact cell, cause havoc when injected into it. The hydrogen-ion concentration of living protoplasm has been determined by the injection of colored indicators into both the cytoplasm and the nucleus. Other determinations of the pH have been made electrically by the insertion of microelectrodes.

Cinematograph. Another technique which has been used for rather specialized investigations is the cinematograph, taken through the objectives of a microscope. It is useful not only to obtain permanent records of cell activity but also as an experimental aid in the analysis of movement too slow or too fast to be appreciated by the unaided eye. When the exposures are taken at intervals of several seconds and thrown on the screen at the usual speed, the photographed processes are speeded up more than a hundred times. In such a film, the movements of the macrophages, which are scarcely appreciated by direct observation, become definite activities and the proliferation and gradual spreading of the growth of fibroblasts comes to have some logical continuity. In the division of the cell by mitosis, the shifting of the nucleus during the prophase, the rounded blebs or pseudopodia which are sent out and withdrawn from every part of the surface of the cell and the violent agitation just before the chromosomes separate are all aspects which cannot be appreciated by any other means. Within the resting cell, the shifting of the granules and mitochondria is beautifully demonstrated by these speeded films.

In order to reverse the process and slow activities too quick for the eye, the exposures are taken three or four times as rapidly as they are to be shown on the screen. The movement of cilia has been studied by this method. The contraction of cardiac muscle, differentiated in tissue cultures, slowed to one-fourth the actual speed, offers an opportunity for one to study the relative importance of the different components of a fiber. A small part of the cell may contract independently of the remainder, or one cell may take up a rhythm of its own quite independent of the neighboring muscle. The contraction may involve the entire cell at once, but more frequently it progresses down the fiber as a wave.

Tissue Culture. The application of tissue culture to the study of experimental morphology will be the principal topic of the next few paragraphs. The physiology of the cell, its growth and metabolism have been studied extensively by this method but the reader is referred to the general treatises listed in the bibliography for an account of this work.

The term 'tissue culture' suggests a growth of the tissues and this property of the cells was emphasized in a modification of the technique introduced by Carrel. The specialized functions of the cells were suppressed in favor of rapid mitotic

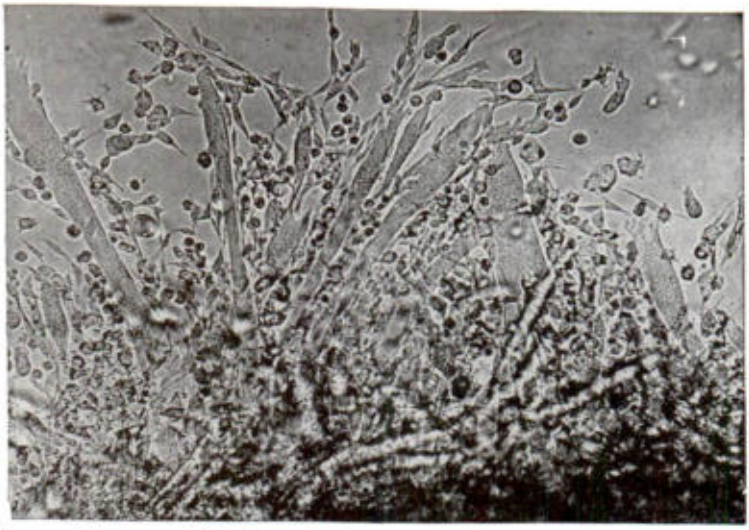

FIG. 18. Tissue culture of skeletal muscle from an embryo rat showing the development of several well-defined muscle bands. ×115. (After Parker, Methods of Tissue Culture.)

division. Other methods were devised which removed the emphasis on growth and permitted the cells to develop some of their specialized structure and function. An antagonism between growth and differentiation has been recognized for some time, but it has received particular attention in some work with tissue cultures (see Bloom '37). Since the differentiation of cells is of more immediate interest to histologists than growth, the following paragraphs will stress the specialized development of a few tissues and organs. For further details the reader is referred to Parker, 1950.

CONNECTIVE TISSUES. The two commonest cells of the connective tissues, fibroblasts and macrophages, have been studied extensively. Although the term fibroblast is frequently used as a synonym for connective tissue cell (see Chap. V), evidence has been accumulating that examples from different sources are not alike. The cells which migrate out from embryonic mesenchyme, adult connective tissues, the perichondrium and the periosteum have a morphological resemblance to each other, particularly if kept under cultural conditions which stimulate proliferation. Even under these conditions of emphasis on growth, however, the cells from different sources may be recognized by their type of outgrowth, rapidity of migration, compactness of arrangement, etc. Fibroblasts from the same organ at different ages as well as fibroblasts from different organs at the same age, may have characteristics which will identify them.

The most famous strain of fibroblasts is that which Carrel and his staff at the Rockefeller Institute in New York City had under continuous cultivation for about three decades. It is described in the scientific literature as a pure culture of fibroblasts from the heart of a chick embryo but it is popularly called "the 28-year-old heart". It preserved its individual characteristics with remarkably little variation and afforded material for innumerable experimental investigations.

The individual characteristics of a strain of fibroblasts may be brought out

still more clearly if they are cultivated under conditions which do not stimulate growth. Fischer and Parker cultured a strain of cells from chick perichondrium for months under conditions of growth. They were called fibroblasts because of their resemblance to the cells of loose connective tissue. After some weeks in a medium without growth stimulation, however, a cartilaginous matrix was formed. Töró obtained pure cultures of epithelium and fibroblasts from the lung. After a period of cultivation, he transplanted them into the lens capsule of a chick eye and they formed cartilage.

The phagocytic activity of the macrophages has been studied by Hetherington with trypan blue. They take up the dye in cultures as they do in the body of a vitally stained animal. The origin of these cells is uncertain. Macrophages and fibroblasts may not preserve their identity. The transformation of fibroblasts into macrophages and of macrophages into fibroblasts has been described. Maximow observed the transformation of the lymphocytes and monocytes of the guinea pig into macrophages and into fibroblasts. The transformation of epithelial cells into macrophages has been described also.

The development of the intercellular components of connective tissue takes place in cultures. The formation of reticular (argyrophile), elastic and collagenous fibers is well established. The formation of cartilage from pure strains of fibroblast-like cells has been mentioned. Bone has been formed in explants of the primordia of bones from the embryo. The formation of bone takes place in explants of the perichondrium of the earlier cartilaginous skeleton but experiments with older perichondrium and periosteum have not been as successful.

EPITHELIAL TISSUES. The cells derived from epithelium have a characteristic growth response which is different from that of fibroblasts. They form thin sheets, one layer of cells thick, or send out compact knobs and sprouts. They remain joined to each other and have but little intercellular space, a behavior which is characteristic of them in the body. The difference in type and rapidity of growth has made it possible to isolate the connective tissue elements from the epithelium in order to obtain so-called pure cultures of each.

EPIDERMIS. Epidermis in tissue culture may grow out into a layer which surrounds the underlying connective tissue as a capsule or it may form internal masses or islands which are surrounded by the connective tissue. In either case the cells can carry on the process of keratinization much as they do in the body. Hair follicles have been cultured but growth is doubtful.

DIGESTIVE SYSTEM. Epithelium of the intestine has a marked tendency to surround the connective tissue explanted with it to form an outer surface layer. Such cultures have been called 'intestinal organisms.' The epithelial cells digest the fibrin in cultures with solid medium causing a liquefaction which makes continuous cultivation difficult. Liver epithelium has been carried for months in pure culture. It has a characteristic morphological appearance. The cells retain their glycogen for a time, but lose it gradually and seem to have no glycogenic power. Fat droplets are a constant and prominent feature, and do not signify degeneration as they do in other types of cells in culture. Tooth germs in tissue culture continue the process of dentin formation and calcified dentin is formed.

RESPIRATORY SYSTEM. In cultures of lung tissue from the embryo, the bronchial epithelium develops ciliated cells which continue their vibratory activity for several weeks. The smooth muscle of the bronchi also continues its rhythmic

activity in the cultures. The connective tissue stroma and epithelium can sometimes be separated into pure cultures. Mention has been made of the formation of cartilage when these two pure strains are implanted into the lens capsule.

NERVE FIBERS AND NERVE CELLS. The question whether or not nerve fibers were outgrowths from nerve cells was conclusively answered by Harrison in 1908. He took pieces from the neural tube of the frog embryo before the neuroblasts had processes, and cultured them in hanging drops. The embryonic cells grew processes, some at a rate as rapid as 56 μ an hour. The terminal ends of the growing fibers formed swellings with active pseudopodia which moved along the fibrin network of the culture medium (Fig. 19).

Adult sympathetic ganglion cells will divide and form processes in tissue cultures (Murray and Stout). The ganglion cells were secured from patients from which part of the sympathetic chain was removed because of hypertension. Extended processes were formed which if severed would be regenerated from the cell body. Adult mammalian nerve cells from other locations have not been shown to divide.

BONE. In a similar way pieces of the limb bud of the chick embryo have formed cartilage, deposited calcium salts and finally differentiated true bone.

BLOOD CELLS. Tissue culture has been of great importance in the study of blood cells. It has been found that the granular leucocytes have no developmental powers; they soon die and disintegrate. The monocytes multiply by mitosis and develop into phagocytic cells like the tissue macrophages or clasmatocytes and even into multinucleated giant cells and epithelioid cells. It has been reported that they may become fibroblasts and lay down connective tissue fibrils. Lymphocytes obtained from the thoracic duct multiply by mitosis, develop into cells similar to monocytes and, like the latter, eventually pass through the same series of changes which terminates with the fibroblast. The formation of myelocytes from lymphocytes has been described also. This is strong evidence that the monocyte and lymphocyte, even the histiocyte and perhaps the fibroblast and myeloblast, are functional stages of the same cell.

MUSCLE. Smooth muscle from various sources survives in culture. It continues its rhythmic contraction but growth seems difficult to obtain. Skeletal fibers from embryos of various ages and from adult rats have been cultivated success-

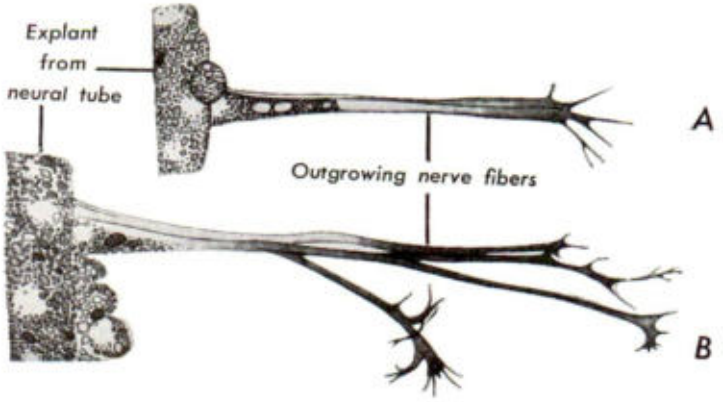

FIG. 19. Outgrowth of nerve fibers from pieces of embryonic frog neural tube grown in tissue culture. *A*, after 25 hours; *B*, after 34 hours. (From v. Mollendorff's Handb., after Harrison.)

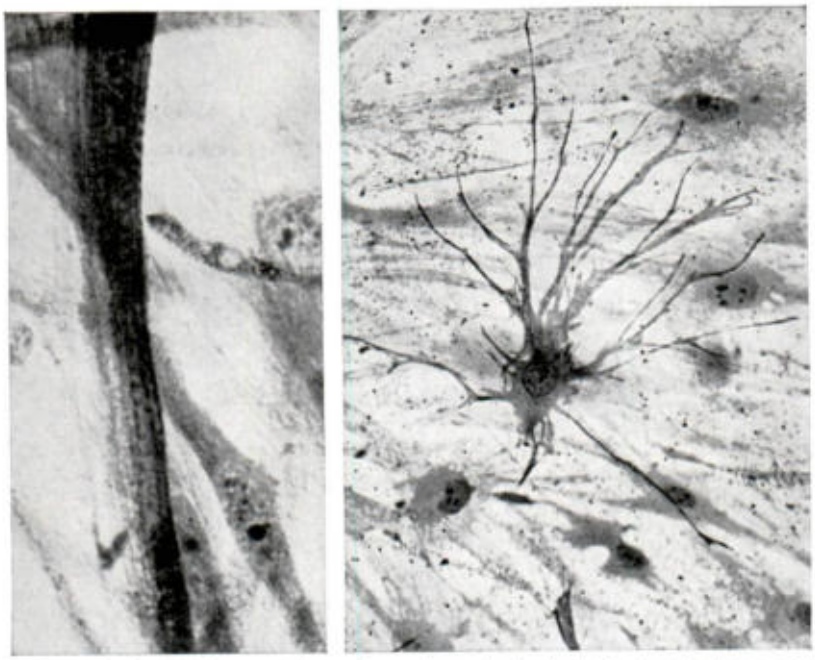

FIG. 20. Growth of adult human tissues in culture. *Left*, skeletal muscle fibers, *right*, sympathetic ganglion cell. (Courtesy of Dr. Margaret Murray, after Pogogeff and Murray, 1946 and Murray and Stout, 1947.)

fully. They will multiply and form elongated elements with one or several nuclei. Cross striations, frequently of a transitory nature, will form and the fibers will contract. When embryonic heart tissue is cultured in a medium which promotes rapid growth, the fibroblasts multiply rapidly but the muscle does not grow. The fibers will contract, however, long after they have lost any structural resemblance to muscle. In a culture with slow growth, the heart muscle proliferates but at a slower rate than the fibroblasts. In the course of two months the cross striated structure may become almost fully differentiated.

OBSERVATION OF CELLS OF LIVING ANIMALS

The descriptions up to this point have dealt with the surviving cells after their removal from the body, but cells have been observed by various methods within the living organism. The earliest attempts were made on the vascular system, the blood cells circulating in the tadpole's tail fin, in the tongue, foot web and mesentery of the adult frog, or in the wing of the bat or the mesentery and omentum of mammals. The transparency of the tail fin of the tadpole makes the latter particularly advantageous for the observation of many kinds of cells. It has been used to study the outgrowth of the nerve fiber and it has given beautiful results with the formation of special sensory nerve endings and with the formation of the myelin sheath of nerve fibers. The growth of blood vessels and lymphatics and the activity of the endothelium, connective tissue cells and phagocytes have been exhaustively worked out.

Mammalian material has been made available for similar observation, by the perfection of a technique for inserting a transparent window in the rabbit's ear.

Through it, the growth of new blood vessels and lymphatics, the activity of capillaries, the opening and closing of vascular anastomoses, the behavior of the phagocytic cells of connective tissue and the growth and resorption of other tissues have been worked out and are described in an extensive series of papers by E. R. and E. L. Clark and their collaborators.

Crucial observations of the vascular mechanism of the spleen have been made by Knisely using a new method of illumination. The important innovation in his method is the transmission and concentration of light by means of a fused quartz rod. The method should be equally useful with other organs and tissues.

An interesting and promising method for microscopic observation of any of the organs or tissues of the living animal has been made by the adaptation of vertical illumination to biological work. With this technique it is unnecessary for the object to be sufficiently thin or transparent to permit transillumination. The light is brought down through the objectives of the microscope. So far it has been used principally for the study of the minute circulation of various organs, but it has given some new concepts of the part played by the cytoplasmic granules of gland cells during secretion.

Vertical illumination has been used as the basis for an application of fluorescence to the study of the living animal. Fluorescence is the property possessed by certain substances of changing the light with which they are irradiated (the incident light) into a light of a different color (the induced light). Solutions with marked fluorescent properties are injected into the animal and ultraviolet light (the best inducing light) is passed through the vertical illuminator to the organ under observation. When the fluorescent fluid is taken up by the blood plasma, the latter becomes equivalent to a circulating source of light in which the corpuscles show as dark objects and other cells become visible either by their absorption or reflection of the light. When the fluorescent substance is taken up by particular cells, they show brilliantly in the color of the induced light, or certain parts of the cell may become luminous and others remain dark.

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III

GENERAL FEATURES OF VERTEBRATE DEVELOPMENT

MORPHOGENESIS

The vertebrates, to which man and other mammals belong, may be defined as triploblastic (three layered), metameric (segmented), celomic (body cavity), bilaterally symmetrical animals possessing at some stage a notochord, a dorsal hollow nerve cord and pharyngeal clefts.

A brief analysis of the developmental processes which result in a body form having these distinctive characteristics will give the student a skeleton outline of the plan of organization of the human body. More detailed information rightly belongs in a textbook of developmental anatomy or of vertebrate embryology.

Normal development begins with a sperm-fertilized egg, a single cell in which the fusion of a male with a female pronucleus has occurred. This cell divides into daughter cells, which continue to divide and adhere, thereby forming a multicellular mass. Although the earlier cell divisions are primarily cleavages of the original egg-cell substance, the process is early associated with actual growth, so that a rapid increase in mass results.

During the early divisions there is little difference among the accumulating cells. Before long, however, the cells, even though not greatly dissimilar in appearance, begin to be arranged in three distinct layers which have been referred to as the three fundamental *germ layers*. Because of their position in the developing embryo they are known as *ectoderm*, *mesoderm* and *entoderm*, terms which mean technically outer, middle and inner skins.

The cells of each of the three germ layers divide, differentiate and group themselves into specialized tissues, which in turn are organized into organs and organ systems. Tissues from different germ layers, although they retain their individuality, frequently associate in the formation of an organ. Through embryological studies we can now name with assurance (except for rare exceptions) the original germ layer from which each and every cell of the fully differentiated body has come. The process whereby undifferentiated cells of a germ layer develop into the specialized cells of tissues is termed *histogenesis*. In terms of principal tissue components the outer epithelia and the nervous system have developed from the ectoderm; the lungs, the gut epithelium and its derivatives from the entoderm; the blood, skeleton and muscles, as well as the organs of excretion and reproduction from the mesoderm. A more complete list of derivatives will be found at the close of this section.

Several of the most prominent and diagnostic features of the vertebrate are

determined in the histogenesis of the mesoderm. Among these are bilateral symmetry, metamerism, and the formation of the celom, commonly called the body cavity. The mesodermal cells first appear bilaterally in a dorsolateral position between the ectodermal and entodermal layers. From this mass of cells a definite tissue layer organizes which pushes its way ventrally between the ectoderm and entoderm of the embryo. On each side of the hollow nerve tube, which has previously organized from ectoderm, the mesodermal cells form a series of compact segments, frequently referred to as mesodermal somites. Later each pair of somites develops a vertebra with its processes and the muscles related to it. Thus the axial skeleton retains an internal segmented structure, although the subsequent relation of the muscle and skin may obscure any external evidence of segmentation.

The ventral extension of mesoderm splits into two sheets. One adheres to the ectoderm and is known as *somatic mesoderm*. Together with the ectoderm it is referred to as the *somatopleure*. The other associates with the entoderm and is called *splanchnic mesoderm*. Together with the entoderm it forms the *splanchnopleure*. The somatic and splanchnic layers of mesoderm enclose a cavity, the *celom*, which becomes the body cavity of the embryo. In later development this cavity gives rise through partitioning to the pericardial, the pleural and the abdominal cavity. It follows, therefore, that the pericardial, pleural and peritoneal membranes which form the walls of these cavities are all of mesodermal origin. The epithelium of these internal surfaces is likewise mesodermal and is called *mesothelium*.

Certain mesodermal elements organize to form the complex system of blood and lymph channels which ramify throughout the body. Their internal cavities are likewise lined with epithelial cells of mesodermal origin, but these cells have been specially designated as *endothelium*. This somewhat confusing term does not imply the origin of these cells from the entodermal germ layer.

The ectoderm thickens and forms a groove which extends along the middorsal line of the embryo. The groove deepens and its walls rise to meet dorsally, forming an ectodermal tube beneath the surface ectoderm. This tube differentiates into the brain and spinal cord, in which the cavities remain as the brain ventricles and spinal canal. The repeated pairs of cranial and spinal nerves which arise from the original tube again follow for the most part the plan of bilateral symmetry and of a segmented organization of the body.

The inner tube of cells, the entoderm, forms the epithelium of the gut. At intervals along its length important organs are derived as, for example, the lungs, liver and pancreas. In the final development of all of these structures the mesoderm is prominently associated and forms their connective tissue, muscle and blood and lymph vessels.

The adult tissues are not composed of cells only. At a very early stage of differentiation, the cells become separated from each other by the formation of intercellular substance which may be the result of cellular secretion, or may represent actual modification of cellular protoplasm. Thus each tissue is an aggregate of cells with its own characteristic intercellular substance, frequently called a *matrix*. In some tissues the intercellular material is scanty in amount, and the cells are in close contact with one another (epithelium, muscle). In other tissues the intercellular substance may assume large proportions and may contain highly differentiated elements of important

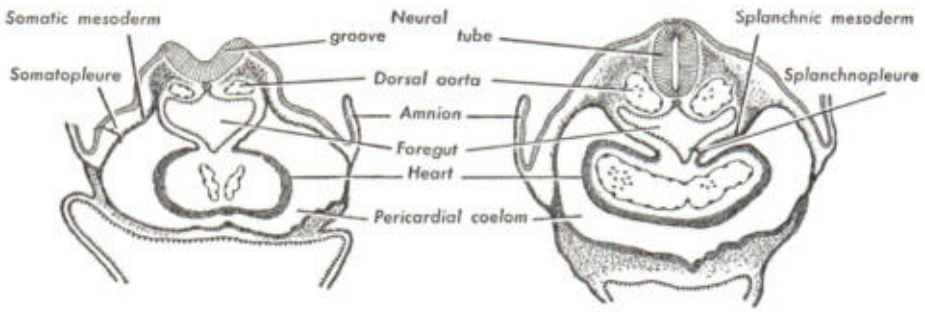

FIG. 21. Section through a seven somite human embryo (left) and a ten somite human embryo (right). (Redrawn after Patten.)

physiological significance (bone, cartilage, connective tissue, blood).

The adult tissues are usually classified into four main groups: (1) epithelial tissue; (2) connective tissue with its derivative, blood and lymph; (3) muscle tissue; (4) nerve tissue.

Of these, epithelium and connective tissue may be regarded as the more elementary tissues. Muscle and nerve are the most highly specialized tissues. Blood is a unique tissue closely related to the connective tissues, in which the cells are suspended and move freely in a fluid intercellular substance.

A number of tissues are, as a rule, intimately associated with each other in forming the various organs and organ systems of the body.

The student of histology learns the finer organization of the body structures largely from prepared slices of tissue mounted upon glass slides. Here he concentrates his attention upon the more solid remnants of its substance. He is likely to pass lightly over the important fact that the body tissues contain large proportions of fluid which are not evident in the preparations. He may also fail to grasp the fact that the vital, physiological reactions depend upon fluid media, and that fluid filled cavities and fluid bathed surface membranes are indispensable in the organization. On numerous occasions one is impressed with the unique manner in which the necessary surface areas are increased by folds, by invaginations, and by evaginations. An alert attention to the presence of these developmental processes in the makeup of the various organ systems will repay with a better understanding and finer appreciation of the manner in which the tissues have organized to serve their functions.

GERM LAYER DERIVATIVES

The various tissue derivatives from the primary germ layers are as follows:

Ectoderm. (1) Epithelium of skin and the hair, nails, sebaceous and mammary glands, and sweat glands, including the associated smooth muscle fibers (myoepithelial cells).

(2) Epithelium of mouth and anus, of glands opening into mouth, taste buds, and enamel of teeth.

(3) Epithelium of nose and of glands and cavities connected with nose.

(4) Epithelium of external auditory canal and of membranous labyrinth.

(5) Epithelium of anterior surface of cornea, of conjunctiva, of lacrimal gland, of pars nervosa, and pars ciliaris and pars iridica retinae, also muscle tissue of sphincter and dilator pupillae, and the crystalline lens.

(6) Epithelium of the penile urethra.

(7) The medulla of the suprarenal

glands, the pineal gland, and all of the lobes of the pituitary gland.

(8) All nerve cells, part of the neuroglia, and linings of the central canal and brain ventricles.

Entoderm. (1) Epithelium of the digestive tract (excepting mouth and its glands and anus), and of glands connected with digestive tract including liver, gall bladder and pancreas.

(2) Epithelium of respiratory tract (except nostrils) and of its glands.

(3) Epithelium of bladder (except the trigonum), of female urethra, of vaginal vestibule and glands of Bartholin, of prostatic portion of male urethra, prostatic glands and glands of Cowper.

(4) Epithelium of tympanum and of Eustachian tube.

(5) Thyroid and parathyroid glands, and reticulum of thymus, and Hassall's corpuscles.

Mesoderm. (1) All the connective or supporting tissues except a part of the neuroglia.

(2) Lymphatic organs (except Hassall's corpuscles and reticulum of the thymus).

(3) Blood cells and bone marrow.

(4) Skeletal, cardiac and smooth muscle (with the exception of smooth muscle of sweat glands and of the pupillary muscles of the eye).

(5) Endothelium lining blood vessels and lymphatics.

(6) Mesothelium lining serous membranes—pleura, pericardium, peritoneum.

(7) Epithelium of genito-urinary system with the exception of the urethra and a large part of the bladder.

(8) The suprarenal cortex.

IV

EPITHELIUM

The surface of the body and of the tubes or passages leading to the exterior and, with a few exceptions, the surfaces of the various cavities in the body, are lined by cells which are closely approximated to each other and thus have but a small amount of intercellular substance. This lining cellular layer is called *epithelium*. It has a free surface and it rests upon a stratum of connective tissue. The layer of epithelial cells frequently develops growths into the underlying tissue. These show special structural differentiation and form glands. They usually contain cavities and the cavities are lined by epithelium (exocrine glands, p. 305). Other ingrowths, however, form deeply lying cords or aggregations of cells which do not have free surfaces (some endocrine glands). Examples are the cortex of the adrenal gland, the anterior hypophysis and the parathyroid gland. The liver is intermediate in type. The cells of the liver cords have free surfaces but these surfaces, the bile canaliculi, are minute.

It should also be kept in mind that all free surfaces are not lined by epithelium, for example, synovial membranes and bursae. In other locations the epithelial layer becomes specially differentiated to receive sensory stimuli (neuro-epithelium).

Epithelium is not subjected to identical or even similar functional demands in different locations. In some locations, as on the surface of the body, it is subjected to abrasion, attrition and drying and thus serves, to a high degree, a protective function. In other less exposed locations, as in the body cavities, it is subjected to but little attrition and is covered with a fluid film. There it forms smooth surfaces which glide over each other, whereas in other locations, it serves not only as a protection, but absorbs and also secretes mucus (intestine). In some places it is adapted to great changes in its surface area (urinary bladder). Correlated with these regional differences in functional requirements is a variation in the shape of the epithelial cells, in the number of layers of cells which form the epithelium and in the physical and structural characteristics of the individual cells and in their attachment to each other. Everywhere, however, epithelium has the characteristic of being composed primarily of cells between which is a minute amount of intercellular material. In this respect it differs from the connective and supporting tissues in which the cellular component is relatively small and the intercellular substance predominant. Like connective tissue, however, it has a marked capacity for regeneration and repair after injury.

Shape and Arrangement of Cells. The height of epithelial cells varies from a very flat type (squamous) to a tall rod-like (columnar) cell (Fig. 22). Forms

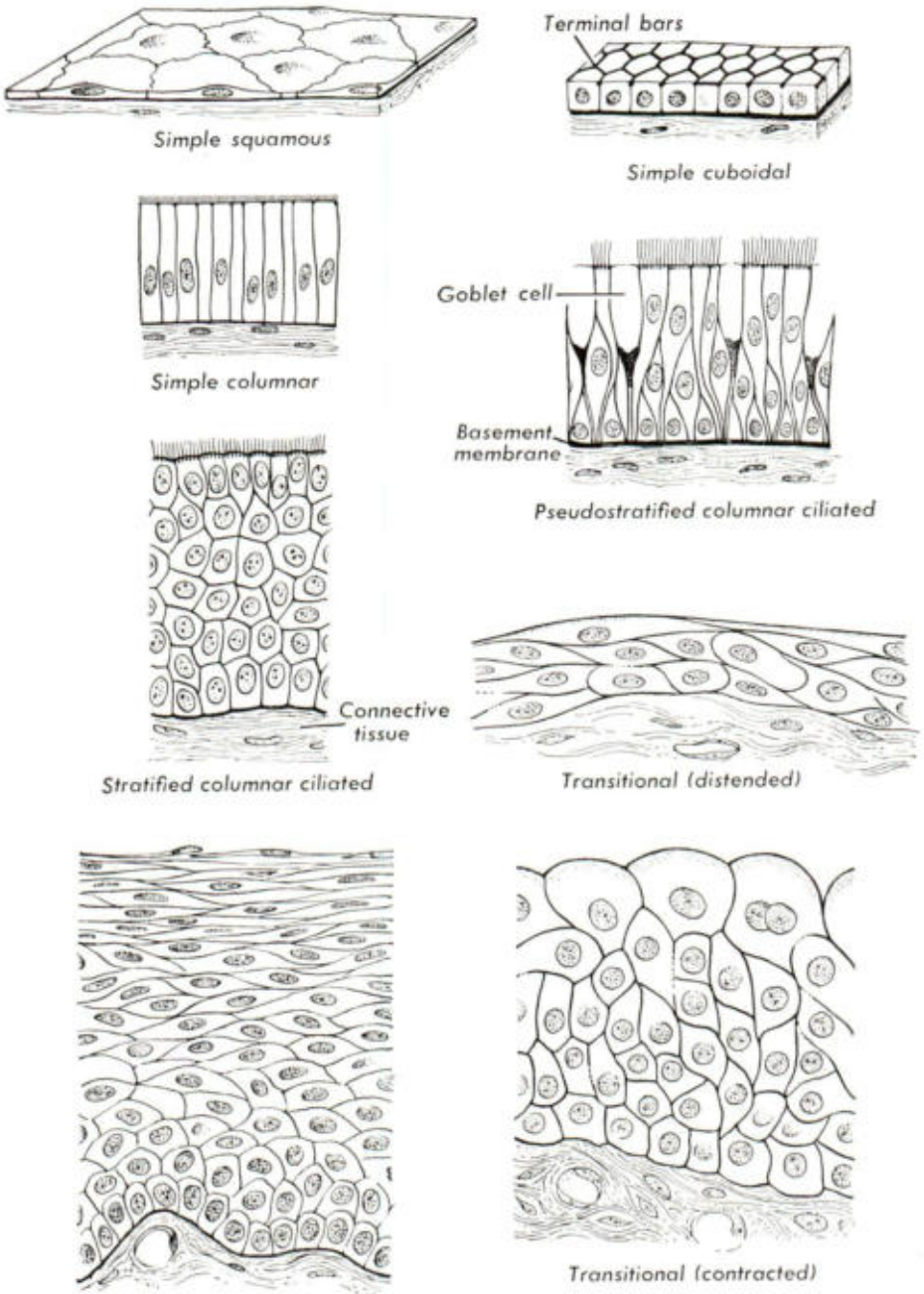

FIG. 22. Schematic representation of the various morphological types of epithelium. In each case, the epithelium is shown with some of the underlying connective tissue.

intermediate between these two types are cuboidal. Not infrequently various gradations are seen in the height of cuboidal cells so that there are low cuboidal or high cuboidal, the latter being equally well characterized as a low columnar. When cells line a small tubule, as the alveolus of a gland, they are pyramidal in shape.

An epithelial layer may be one or many cells in thickness (Fig. 22). When it is composed of but a single layer it is called a *simple* epithelium; when two or more cells in thickness, it is *stratified*. In the latter type it is obvious that only the basal cells rest on the underlying modified connective tissue membrane—the *basement membrane*—and only the uppermost cells have a free surface.

In certain locations, the nuclei of the epithelial cells lie at various levels giving a stratified appearance. Study reveals, however, that all of the cells reach the basement membrane, although not all reach the surface. This is characteristic of the epithelium lining the ducts or passages of the respiratory and male reproductive systems. Since it is not truly stratified it is designated as *pseudostratified* (Fig. 22). In this type of epithelium the cells which reach the surface are columnar in shape. In the urinary system, particularly in the urinary bladder, the number of layers and the shape of cells vary with the degree of distension of the organ. The shape of these cells also has certain characteristic features and this type of epithelium is given a special name, *transitional* epithelium (Fig. 22). It is actually a stratified epithelium.

Attachment between Cells. Epithelial cells are held together by a thin layer of viscous substance known as *intercellular cement*. This substance "stains" black with silver nitrate, (reduces the silver), particularly in simple squamous epithelium (Fig. 23). It stains

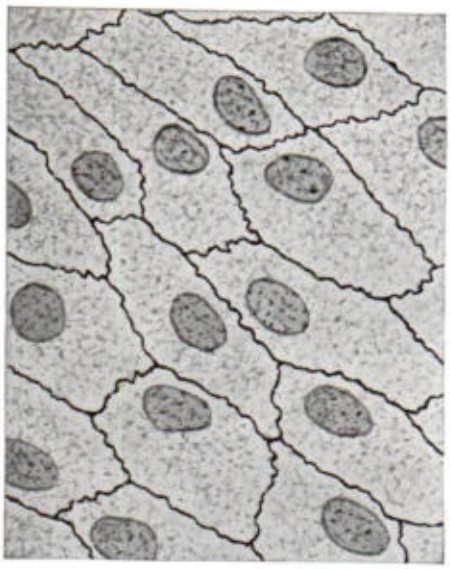

FIG. 23. Surface view of mesothelium of mesentery treated with silver nitrate. The cells are sharply outlined by the silver deposit. ×550.

by the periodic acid-Schiff technique in some of the stratified epithelia indicating that it is a mucoid substance. In columnar types of epithelium the intercellular cement is particularly dense around the apical ends of the cells where it forms *terminal bars*, so named because they stain heavily with certain dyes and give the appearance of bar-like lines (Fig. 22).

In some locations, particularly in the deeper layers of stratified squamous epithelia, the cells are brought into particularly intimate contact at intervals along their margins. With the light microscope, these points of adhesion give the appearance of protoplasmic continuity between cells, and they have been called "intercellular bridges." With the electron microscope it can be seen that short, blunt processes of the adjacent cells make contact, and at each point of contact there is a thickening of the two opposing cell membranes. These are known as *desmosomes*. Fine fibrils, *tonofibrils*, within the

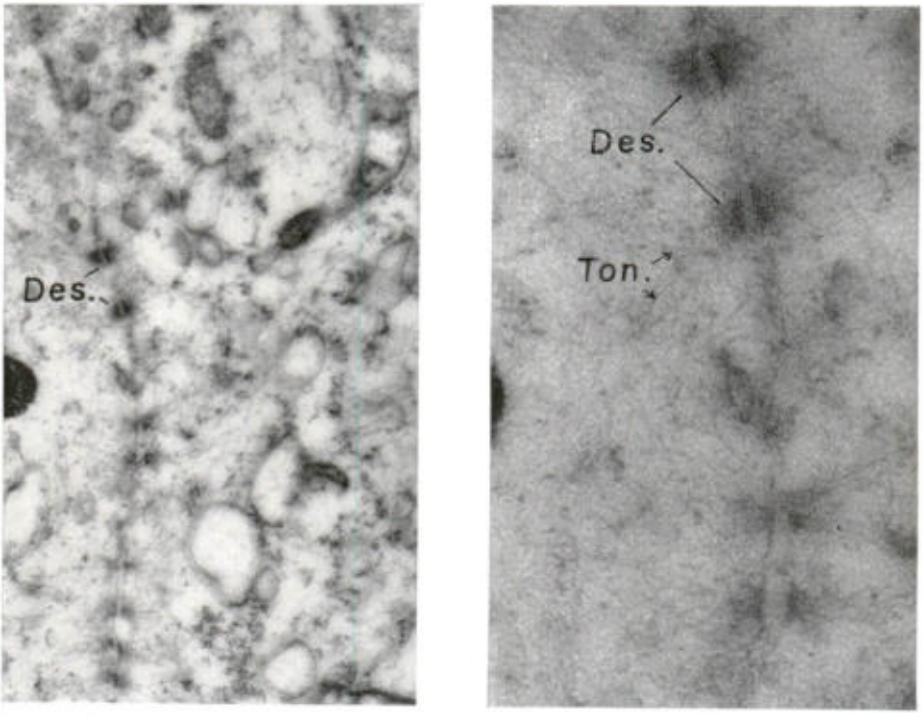

FIG. 24. Electron micrographs showing attachment between epithelial cells of epidermis of salamander. *Des, desmosomes*, dense regions where the cells make intimate contact; *Ton, tonofibrils* of the cytoplasm converge at the desmosomes × 19,800, on left; × 60,000 on right. (Courtesy of Dr. G. D. Pappas.)

cytoplasm converge at the desmosomes but do not pass from one cell to another (Fig. 24).

Another form of attachment between cells is an arrangement of alternating ridges and grooves interlocking with those of adjacent cells. These can be observed with the light microscope in suitably stained preparations of kidney tubules. With the electron microscope, interlocking junctions between epithelial cells have been observed in other locations (Fig. 298).

Modifications of Cytoplasm at the Free Surface. The cytoplasm of the superficial or free surface of epithelium often shows modifications. The simplest modification is a condensation of the cytoplasm so that it stains somewhat more deeply than does the remainder. Such a condensation is evident at the free surface of the cells of transitional epithelium and has also been described in the simple squamous type. The cells of intestinal epithelium show a pronounced surface modification. They have a border of relatively uniform thickness which appears vertically striated under the light microscope. With the electron microscope, it is found that the *striated border* consists of fine protoplasmic extensions which greatly increase the surface area at the apical end of the cell. This modification is correlated with an absorptive function. A somewhat thicker and more irregular border, the *brush border*, is found at the apical ends of the cells in the proximal convoluted tubules of the kid-

ney. Epithelial cells in many other locations have smaller, irregular projections from the free surface. These are known as *microvilli*. The surface cells of the epithelium lining the respiratory passages (pseudostratified) and many of the cells of the epithelium lining the uterine tubes (simple columnar epithelium) have motile cilia. Parts of the male reproductive tract have epithelial cells with nonmotile cilia (cytoplasmic processes known as stereocilia).

Basement Membrane. A basement membrane (membrana propria) is a sheet of variable thickness and distinctness interposed between epithelium and the subjacent connective tissue. One of its components is a condensed, gel-like layer of the ground substance of connective tissue (see p. 66). This gel-like layer is continuous with the ground substance in the underlying connective tissue. The other component is delicate reticular fibers which are embedded in the condensed ground substance and which, also, are continuous with reticular fibers in the subjacent connective tissue. In some locations (beneath, for instance, stratified squamous epithelium and the simple columnar epithelium of the intestine) a basement membrane is barely distinguishable after the commonly used histological stains. In other locations (trachea) it is very evident (Fig. 27*B*). In fetal and young animals basement membranes tend to be much thinner than in adults. The cells of the overlying epithelium may have short processes which penetrate into the basement membrane thus giving a firm attachment; in other places the epithelial cells seem merely to be cemented to the membrane. Basement membranes serve as a limiting substratum to the epithelium and serve, also, in the attachment of epithelium to the underlying connective tissue.

Connective tissue cells (fibroblasts) may or may not be present in a basement membrane.

Blood Vessels. With few exceptions, epithelium is entirely avascular. Nutritive materials and oxygen enter it by diffusion through the cells and the intercellular substance. Capillaries are present in the epithelium of the stria vascularis of the internal ear.

SIMPLE EPITHELIA

1. Simple Squamous Epithelium. Simple squamous epithelium (*pavement epithelium*) consists of flat scale-like or plate-like cells. The edges of the cells are usually serrated, but may be smooth. The nucleus, situated in the center of the cell, is spherical or ovoid causing a bulging of the cytoplasm. On surface view the cells appear as a delicate mosaic which can be demonstrated especially well by the precipitation of silver in the intercellular cement (Fig. 23). In profile the cells are spindle-shaped, thinner at the ends than at the center where the nucleus is located.

Simple squamous epithelium is widely distributed. (1) It lines the peritoneal, pleural and pericardial cavities (mesothelium); the heart and all blood and lymph vessels (endothelium); (2) the membranous labyrinth of the internal ear, portions of the uriniferous tubule and of the rete testis. Thyroid epithelium becomes squamous with distension of the follicles.

Endothelium and *mesothelium* form cellular membranes which have the same histological structure as simple squamous epithelia in other locations but they have some developmental and functional differences. Although some of the other epithelia develop from mesoderm (for example, the epithelium of the uriniferous tubules), endothelium and mesothelium arise from mesoderm and mesenchyme very early and are developmentally related to primitive connective tissue. The regeneration of mesothelium is not

limited, as in other epithelia, to a replacement from neighboring epithelial cells. When the epithelium of the skin or the intestinal tract is denuded, regeneration takes place by mitosis or rearrangement of the adjacent epithelial cells. The epithelial cells are always replaced by cells of the same tissue. It seems probable, however, that when mesothelium of the body cavities is destroyed, new cells are formed from the underlying connective tissue. Mesothelial cells can also change into fibroblasts. It is not so highly specialized as other epithelia, appearing in fact to retain the multipotency of mesenchyme. For this reason mesothelium is sometimes not included under epithelia. It should be considered as an epithelium with special potencies. It lines the serous cavities (peritoneal, pleural, pericardial) and together with a thin underlying stratum of connective tissue forms the peritoneum, pleura, and pericardium. Endothelium lines the blood and lymph vessels and together with a stratum of underlying connective tissue forms their intima. Although arising embryologically from the mesenchyme it can regenerate later only from existing endothelial cells. It probably retains the potency of forming fibroblasts.

MESENCHYMAL EPITHELIUM. The surface of structures enclosing certain cavities (subarachnoid and subdural cavities, the chambers of the eye and the perilymphatic spaces of the ear) are lined by mesenchymal epithelium. The structure of this epithelium is similar to that of

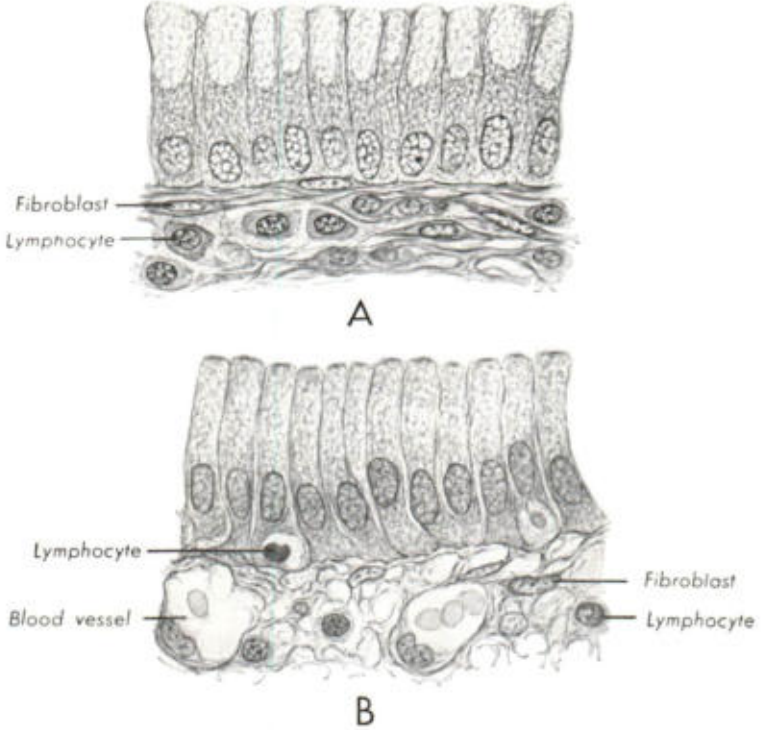

FIG. 25. Simple columnar epithelium. *A*, Epithelium of stomach of rhesus monkey. The apical ends of the cells are clear due to the mucin having been dissolved. *B*, Epithelium of human gall bladder. ×1065.

mesothelium. The cells are plate-like and the serrations at their edges interdigitate. In the cement substance between the cells, silver can be deposited. Such preparations are indistinguishable from similarly treated mesothelium. Mesenchymal epithelium is not included with mesothelium largely on embryological grounds, although it may have different potencies, also.

JOINTS AND BURSAE. The surface of structures enclosing the cavities of joints and bursae do not have an epithelial lining. The enclosing membranes are formed of collagenous fibers, among and on the surface of which are fibroblasts. The surface fibroblasts are flattened but do not form a continuous membrane.

2. Simple Columnar Epithelium. Simple columnar epithelium (Fig. 25, *A*, *B*) in its various modifications represents the chief secretory tissue of the body. It forms membranes consisting of a single layer of tall prismatic cells resting on a continuous basement membrane. The height of the cells varies considerably in different membranes, and the term *cuboidal* epithelium is applied when the height and thickness of the cells are about equal (isodiametric). All transitions from low cuboidal to high columnar types of cells are encountered. The tubules or acini of most glands are lined by epithelium the cells of which are pyramidal or conical in shape, the broad base resting on the basement membrane, the apex facing the narrow lumen. This *pyramidal*

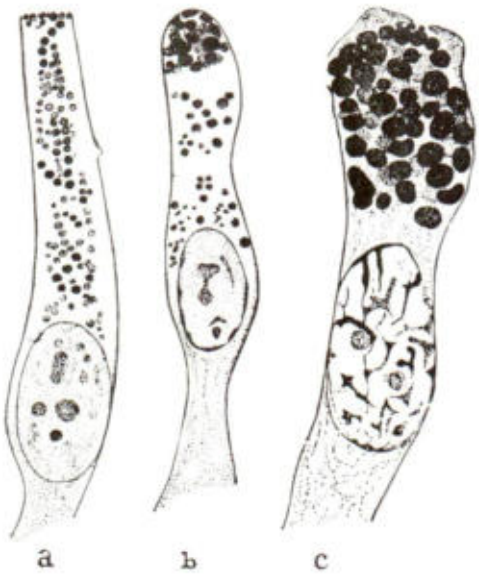

FIG. 26. Stages in the formation of a goblet (mucous) cell in the intestine of the salamander (Heidenhain). *a*, Early stage with numerous fine mucin granules in cytoplasm of cell; *b*, increase in size of mucin granules and their aggregation near the free surface; *c*, fully formed goblet cell.

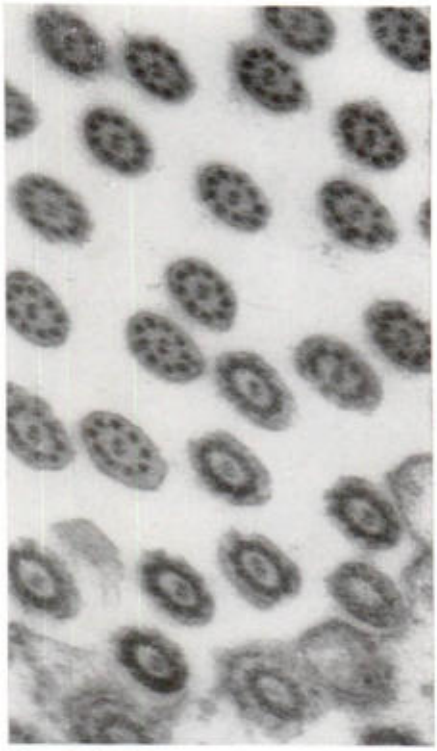

FIG. 27*A*. Electron micrograph of cilia cut transversely. Note that each cilium is covered with a membrane and that each contains 11 filaments (a pair of central ones and 9 double peripheral ones. × 44,000. (Courtesy of Dr. D. W. Fawcett).

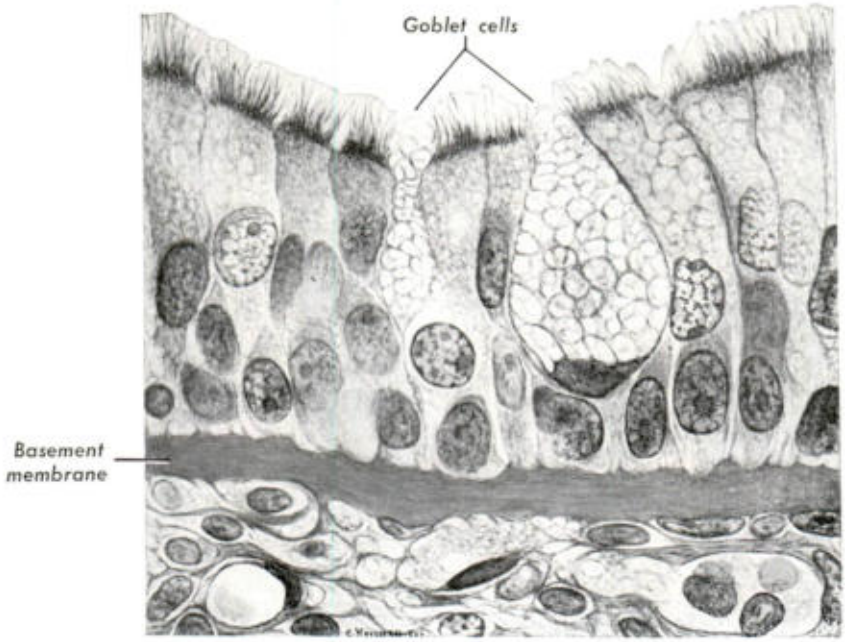

FIG. 27*B*. Pseudostratified columnar ciliated epithelium from the trachea.

or *glandular* epithelium (Fig. 225) is a purely mechanical modification of columnar or cuboidal cells due to their arrangement around a small lumen. The nucleus is oval and usually placed near the base in the columnar cells; it is spherical and centrally placed in the cuboidal. In the basal perinuclear portion of the cytoplasm, there are numerous mitochondria, particularly in the secreting cells (Fig. 286). The distal portion may contain various inclusions in the form of granules or droplets (zymogen, mucin, etc.). The cytoplasmic constitution varies greatly under different conditions of cellular activity. A striated border may be absent as in most glandular epithelium or be very prominent as in the high columnar epithelium of the small intestine (Fig. 2, *C*). The terminal cement bars are usually distinct (Fig. 22).

A common and important variation of simple columnar epithelium is seen in the form of *goblet cells* (Figs. 2, 26). They are found in varying numbers in the intestinal and respiratory tracts, and really constitute unicellular mucous glands. They become modified from the typical columnar cell by the accumulation of mucin droplets which increase in number and size and finally form an ovoid glairy mass (Fig. 26). The cell becomes swollen and the nucleus, surrounded by a small amount of cytoplasm, is pressed toward the base of the cell. The rest of the cytoplasm is greatly displaced by the large amount of mucin. On further expansion the cell membrane ruptures with subsequent escape of the mucin. Within the cell, the mucin (or more precisely its precursor mucinogen) is usually unstained in ordinary preparations. After discharging its secretion, the cell repeats its cycle of secretory activity.

Certain epithelial cells become specialized to serve as sensory cells for the reception of external stimuli (*neuroepithelium*). They are usually tall columnar cells,

FIG. 28. Isolated cells from pseudostratified ciliated epithelium of trachea. Two goblet cells are shown. (After Schaffer.)

often carrying on their free surface a number of nonmotile hair-like processes. They are in intimate connection with nerve endings and are described in the chapters on the nervous system and sense organs.

Pseudostratified Epithelium (Figs. 27*B*, 28). In this type of epithelium, the nuclei lie at different levels, giving it a stratified appearance. All of the cells reach the basement membrane but not all of them extend to the free surface. Those which do reach the surface are columnar with one or more thin processes which extend to the basement membrane. These processes are difficult to see even in very thin and specially stained preparations. Between these slender processes are ovoid or spindle-shaped cells which also reach the basement membrane. This type of epithelium usually has either cilia or stereocilia. It occurs mainly as the lining of the passages of the respiratory and the male reproductive systems.

STRATIFIED EPITHELIA

1. Stratified Squamous Epithelium. Stratified squamous epithelium (Figs. 22, 29) is the main protective epithelium of the body and consists of many cell layers. The number of layers varies considerably in different places but the shape and arrangement of the cells are quite characteristic. The deepest layer which rests on a hardly distinguishable basement membrane, is formed by small, regularly placed columnar or prismatic cells which in sections of the tissue appear as a distinct row. Delicate protoplasmic extensions extend from the bases of the cells and anchor them to the underlying connective tissue. Nearer to the surface, the cells become irregularly cubical or polyhedral in shape and are usually larger than the cells of the basal layers. As the free surface is approached, the cells are more flattened and at the surface are squamous. The deeper cells of the basal and polyhedral layers (stratum germinativum) are young soft cells with large nuclei rich in chromatin. The cytoplasm is finely granular and somewhat basophilic due to its content of RNA. The "intercellular bridges" are prominent, giving the cell a prickly appearance (prickle cells, Fig. 29). With special technique delicate fibrils are demonstrated within the cell and

its processes. Mitosis is frequently observed in these layers. The extent of the changes in the superficial (surface) cells varies with the location and environment of the stratified squamous epithelium. The epidermis, for instance, is subjected to more attrition and drying than is the epithelium of the mouth, pharynx and esophagus. Its surface cells are nonnucleated, scale-like and keratinized. The epithelium in the mouth, pharynx and esophagus, on the other hand, usually is not keratinized, its surface cells being flattened and nucleated.

The process of keratinization in the epidermis, so important for bodily protection, is probably dependent on the insufficient nutrition of the upper cell layers. The deeper cells are in closer relation to the underlying capillaries which supply the nutritive substances that maintain the epithelial cells. These cells show normal protoplasmic structure and are able to divide mitotically. As more cells are formed the basal cells are pressed upward. The dead scale-like surface cells are constantly cast off to be replaced by cells from the deeper strata. In man this is a slow and continuous process; in some of the lower vertebrates (snakes) there is a periodic shedding of the whole superficial layer of the epidermis.

FIG. 29. Stratified squamous epithelium from esophagus of monkey.

Stratified squamous epithelium covers the entire surface of the body and the orifices of cavities opening upon it. It lines the mucous membranes of the mouth, pharynx, esophagus, portions of the larynx, external auditory canal and conjunctiva, vagina, vestibule and labia majora, and portions of the urethra.

2. Stratified Columnar and Stratified Cuboidal Epithelium. A *stratified columnar epithelium* is comparatively rare. It consists of columnar surface cells which rest on several layers of irregular cubical cells. Stratified columnar epithelium is found chiefly where a stratified squamous adjoins a pseudostratified columnar type as for instance at the juncture of the oropharynx with the nasopharynx and with the larynx (Fig. 310). It is said to occur also in part of the penile portion of the male urethra.

In a few locations a stratified epithelium has surface cells which are definitely cuboidal, thus forming a *stratified cuboidal epithelium*. Examples are the ducts of sweat glands (Fig. 209, *B*) and the lining of the antra of ovarian follicles (Fig. 375, *B*). The sebaceous glands are composed of cells which are cuboidal in shape and several layers in thickness (Fig. 216). Although there is no free inner surface it more closely approximates a stratified cuboidal than any other type of epithelium.

The epithelium of seminiferous tubules is a highly specialized type (Fig. 344).

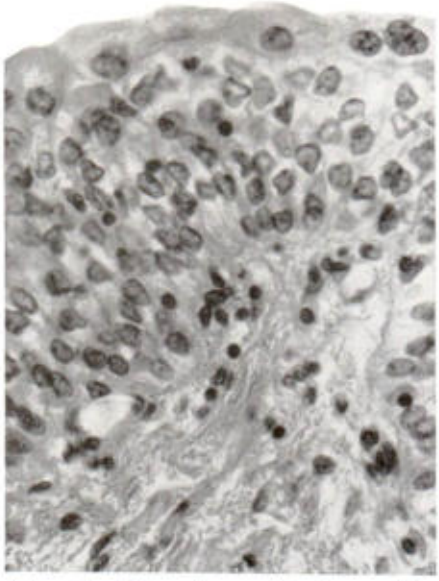

FIG. 30. Photomicrograph of transitional epithelium from human ureter. Note that some of the surface epithelial cells contain two nuclei and that the junction between epithelium and underlying connective tissue is not as clearly defined as in other types of epithelium. × 525.

Functionally it is cytogenic; structurally it probably approximates a stratified cuboidal type more closely than any other although certain of its cells (cells of Sertoli) are elongated and extend from the basement membrane to the lumen. The shape of the surface cells varies greatly with stages of growth and differentiation.

3. Transitional Epithelium (Fig. 30). All epithelial cells are soft and to a certain extent plastic, being able to adjust themselves to influences which increase or decrease the general surface area. These properties are especially striking in the transitional epithelium of the urinary passages and bladder. In the dilated bladder the epithelium consists of two or three layers of cells. The superficial cells are large low cuboidal plates, the lower ones smaller and irregularly cubical. In the contracted bladder the epithelium becomes five or six layered. The surface cells are large and cuboidal with a superficial layer of cytoplasm which often appears condensed and darker staining. These cells have characteristic convex free surfaces and facet-like indentations in their under surfaces. The lower cells have rearranged themselves owing to a reduction of the surface area. They overlap each other and have assumed a flask or pear-shaped form. Some of them fit into the facets of the superficial cells. There is no distinguishable basement membrane and the division between epithelium and connective tissue is extremely irregular.

Transitional epithelium is found only in the urinary system—pelvis of kidney, ureter, bladder and a portion of the urethra.

EPITHELIAL REPAIR

The response of epithelium in the healing of wounds in mammals has been more extensively studied in the epidermis than in other types of epithelium. Extensive work reviewed by Arey, 1936, has clarified our knowledge of the process.

Both epithelium and the underlying connective tissue are injured in abrasions and most other wounds and both have a capacity for cell division and for repair, in contrast to more highly differentiated tissue such as nerve cells and skeletal muscle fibers. The response of the epidermis depends somewhat on the size of the injured surface but there is always an enlargement and migration of the deeper cells near the wound. These exhibit a sort of ameboid movement, with the formation of tongue-like processes which grow over the denuded area or, if there is a scab, grow under it

and help to absorb it. Mitoses are decreased for the first few (4–5) days but soon thereafter exceed the normal rate. The superficial layers of the epidermis form a syncytial layer which also migrates over the wound but it is later cast off as the cells of the deeper zone become transformed into the definitive cell types characteristic of the various layers of the epidermis. In wound repair, as in tissue culture (p. 37), epithelium exhibits the characteristic of forming a cellular layer on a surface.

MEMBRANES

Epithelium assumes its full significance only when considered in conjunction with the underlying connective tissue. In combination with the underlying tissue it forms membranes of varying toughness and thickness, as exemplified by the skin and peritoneum. In conjunction with a stratum of connective tissue it forms the mucosa of the gastro-intestinal, respiratory and genitourinary systems. In general, the connective tissue lying immediately beneath the epithelium is extremely fine but closely woven. This passes gradually into a stratum of coarser closely-woven fibers. The connective tissue membrane thus formed permits a variable amount of stretching depending on the location, but prevents an excessive expansion which might separate the epithelial cells. The denseness of the connective tissue varies with the membranes formed, thus in the mucosa it is not nearly as dense as it is in the skin.

The connective tissue which with the epithelium forms a membrane, changes with no abrupt transition into a looser stratum, which attaches the membrane to the underlying structures and usually permits a movement over them. This underlying zone of looser tissue is well exemplified by the subcutaneous fascia, the submucosa and a thin stratum of subperitoneal tissue.

Serous Membranes. Serous membranes line the *peritoneal*, *pleural* and *pericardial* cavities. A serous membrane consists of mesothelium and an underlying layer of delicate fibroelastic tissue. It should be noted that serous membranes line closed cavities and they do not contain glands. They are moistened by a thin fluid similar to lymph.

Mucous Membranes. Mucous membranes (mucosae) line all of those cavities and canals of the body which connect with the exterior; that is, they line the alimentary tract, the respiratory passages and the genitourinary tract. Although differing in details, the mucous membranes of these various locations all have a similarity in the general plan of their structure. The essential parts are (1) surface epithelium, (2) basement membrane, and (3) a stratum of connective tissue, the lamina propria.

Although the name, mucous membrane, suggests that mucous glands are present, this is not always the case. Both mucous and serous glands are present in the mucous membrane of the alimentary and respiratory tracts but no glands are present in the mucous membrane of most of the genitourinary tract.

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V

THE CONNECTIVE TISSUES

The various types of adult connective tissue, in contrast to epithelium, have relatively few cells and a large amount of intercellular substance. The proportion of cells and intercellular substance shows considerable variation, however. In some types, especially the loose connective tissue, cells are quite numerous; in other types they are few in number and the tissue is composed almost entirely of closely packed fibers. The type and the arrangement of the fibers, and the nature of the substance in which they are embedded (ground substance) furnish the basis for the subdivision of *adult connective tissue* into three main groups, *connective tissue proper*, *cartilage* and *bone*. In connective tissue proper the intercellular substance is soft; in cartilage it is firm yet flexible and may be readily cut; in bone it is rigid due to the deposition of inorganic salts in the matrix.

Connective tissue in the embryo has quite a different structure and consistency from that of the adult. Some knowledge of the development of connective tissue is important, for in wound healing the stages of repair have considerable similarity to the embryonal developmental stages.

- A. Embryonal connective tissue
  - Mesenchyme
  - Mucous
- B. Adult connective tissue
  - Connective tissue proper
    - General
      - Loose—Irregularly arranged—Areolar
      - Dense
        - Irregularly arranged
        - Regularly arranged
          - Collagenous
          - Elastic
    - Special
      - Reticular
      - Adipose
      - Pigmented
  - Cartilage
    - Hyaline
    - White fibrous
    - Elastic
  - Bone

Classification. Since connective tissue in the embryo has a structure very different from the adult, it is placed in

a separate major category, *embryonal connective tissue*.

Adult connective tissue can be classified into different types because of differences: (1) in the relative proportions of the kinds of fibers; (2) in the compactness and in the arrangement of the fibers; (3) in the matrix; and (4) in the types of cells. There are frequently gradations between these types, however, so that the connective tissue in some locations cannot be strictly classified in a certain division. A classification is useful however, in calling attention to differences that occur in the connective tissue as well as in organizing the study of this tissue.

EMBRYONAL CONNECTIVE TISSUE

Most of the connective tissues arise from mesoderm. (Exceptions to this are: neuroglia which arises from neural ectoderm; reticulum of thymus which arises from pharyngeal entoderm; chromatophores and some cartilages of the head which arise from the neural crest.)

That part of the mesoderm which gives origin to connective tissue (and to smooth muscle as well) forms first an embryonal type of connective tissue known as *mesenchyme*. This tissue consists of a network of branching cells in the meshes of which is a homogeneous intercellular fluid (Fig. 31). The processes of a cell appear to anastomose with those of other cells although actual protoplasmic continuity is questionable. The cells behave as independent units in tissue culture, showing ameboid movement, detaching themselves from each other and readily again attaching themselves to other cells. Some of the cells in mesenchyme do not branch or have only short processes (pseudopodia). These are wandering cells.

FIG. 31. Mesenchyme from the subcutaneous tissue of a three and one-half day chick embryo. Two cells are undergoing mitosis. (After Szymonowicz.)

With further development, changes occur in mesenchyme which cause it to assume more and more the characteristics of the adult tissue. In that which is developing into the connective tissue proper delicate wavy primitive fibers appear between the branching cells, and the fluid matrix changes in consistency becoming viscid due to the presence of mucoprotein. This developmentally more mature tissue is *mucous connective tissue* which is widely distributed in the embryo. The fibers later in development become collagenous. Elastic fibers appear relatively late.

The umbilical cord is a classical example of mucous connective tissue and the term *Wharton's jelly* is frequently applied to it in that location. The number of fibers in the cord will increase during pregnancy but the umbilical cord has a short life span and an adult type of connective tissue does not form in it.

ADULT CONNECTIVE TISSUE

Loose Connective Tissue

Loose or *areolar connective tissue* is very widely distributed in the human body, a fact of considerable physiological significance. It forms the superficial and most of the deep fascia; it forms a part of the framework (stroma) of most of the organs; it surrounds the blood vessels and nerves and fills in any otherwise unoccupied spaces. It frequently, or indeed usually, contains fat cells. When these are in great abundance the tissue is designated as adipose or fat tissue. A special name, panniculus adiposus, is frequently applied to superficial fascia which is heavily laden with fat cells.

Areolar or loose connective tissue as with all the connective tissues, consists of cells and fibers (Fig. 32), and a ground substance. The tissue is soft and displaceable, and is translucent in the fresh state.

Connective Tissue Cells. The cells of areolar connective tissue have been objects of intensive histological and experimental study, and the problem of the origin, structure and function of some of them is as yet only partially solved. By means of various methods, the following cell types have been distinguished and found to be more or less constant inhabitants of loose connective tissue, *fibroblasts* or fixed connective tissue cells, *histiocytes* or *macrophages*, *mast cells*, *plasma cells*, and *wandering cells from the blood*. Ordinary preparations give but a poor picture of the detail of the two constantly occurring cell types, *fibroblasts* and *macrophages*. However, recourse to special technique and especially to experimental methods such as vital staining and tissue culture (Chapter II) has yielded important and illuminating data in regard to these two cell types and in regard to other cells which occur less constantly.

(a) The *fibroblasts* (Figs. 17, 32, 33) are one of the two most numerous cell types of loose connective tissue, the other being histiocytes. They are large, flat, branching cells with extensive processes which may touch the processes of other fibroblasts. The cell membrane is extremely delicate and difficult to see. The nucleus is oval or spherical and stains lightly except when shrunken, as in ordinary preparations, when it will stain deeply. The cytoplasm stains very lightly and is homogeneous or finely granular. Fibroblasts are present in all connective tissue except reticular although they may be modified in shape. They are the only ones present in very dense connective tissue such as tendon. As their name suggests, they are responsible for the formation of fibers. Following tissue injury, these cells enlarge, their cytoplasm becomes more basophilic, and they become especially active in forming new fibers. Some investigators prefer to confine the term fibroblast to the relatively active stage of the cell (during development or after injury) and use the term *fibrocyte* for the relatively inactive cell of normal adult connective tissue.

(b) The *histiocytes* or *macrophages*, (Figs. 32, 33) are found in all loose connective tissues. The clasmatocytes, pyrrhol cells, adventitial cells, resting wandering cells, etc., of various investigators have been found to be identical with these histiocytes. They are irregularly-shaped cells with processes which usually are short and blunt but occasionally are long and slender. The nucleus is often somewhat smaller and darker staining than that of the fibroblast. In the resting cell, the cytoplasm, like that of the fibroblast, stains lightly

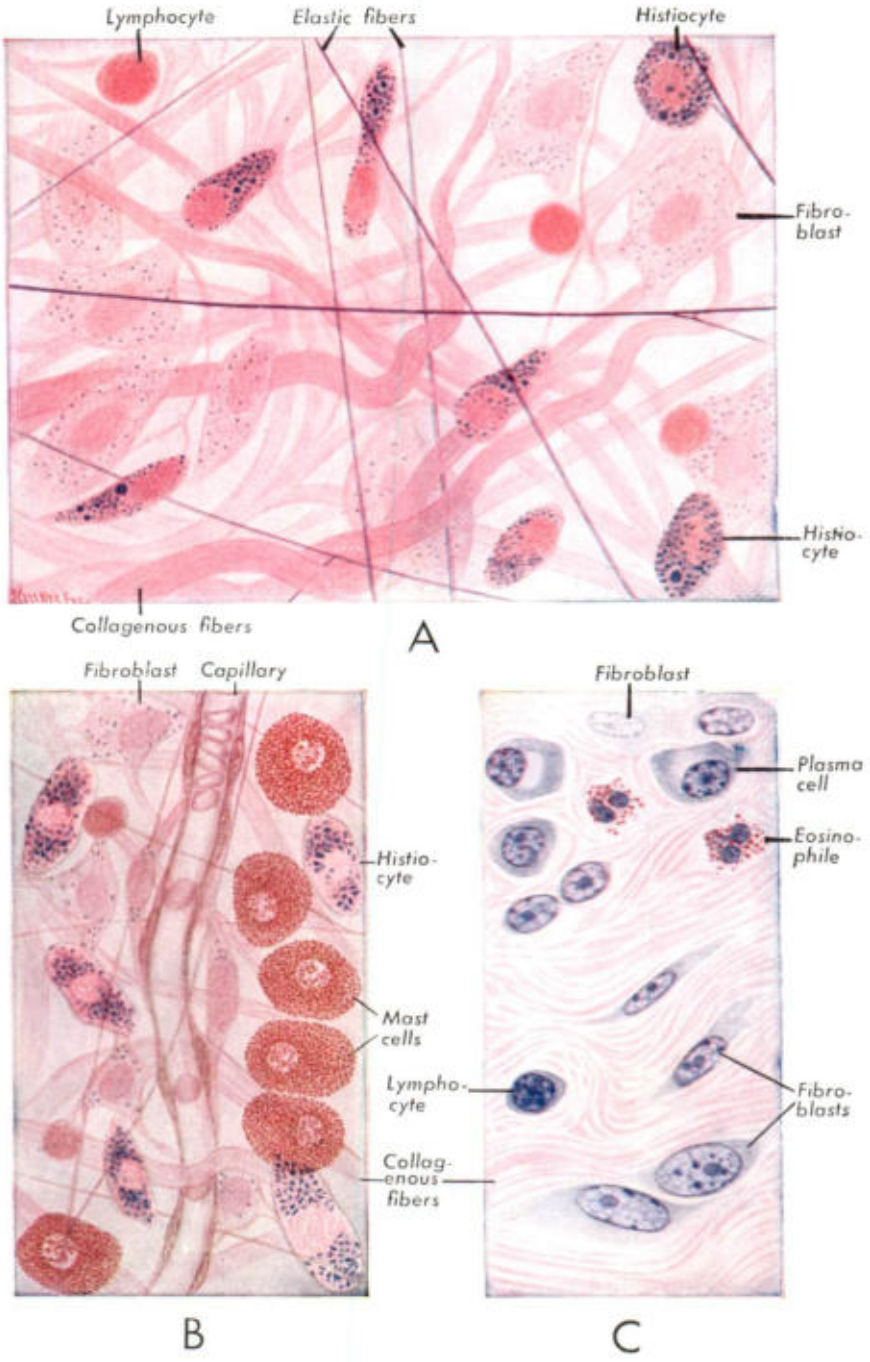
but may contain some granules and vacuoles. The cells have been intensively studied by vital staining and special histological technique. They readily take up trypan blue, colloidal carbon, etc., and store these in vacuoles. Probably quiescent ordinarily, they show active ameboid movement and great phagocytic capacity under abnormal conditions such as inflammation.

Macrophages or histiocytes are widely distributed in the body. They are in loose connective tissue and, therefore, are present in all fascia. They also occur in the connective tissue which is present in organs although the proportion of them to fibroblasts may vary considerably in different regions of an organ. Certain cells in specific locations, e.g., in the sinusoids of the liver, in lymphoid organs and in bone marrow, are also phagocytic. Together with the histiocytes of loose connective tissue, they form the "macrophage system" or "reticulo-endothelial system" which is discussed in more detail on page 75.

(c) The *plasma cells* (Figs. 32, 33) are comparatively rare in most connective tissue, though more abundant in the alimentary mucous membrane and great omentum. In certain pathological conditions their number is greatly increased. They are smaller than the histiocytes, of ovoid or irregular shape, with a basic staining homogeneous cytoplasm. The nucleus is small and eccentrically placed. The chromatin is in the form of deep staining large granules which are adherent to the nuclear membrane and are so regularly arranged as to give a 'cart-wheel' appearance. It is characteristic of these cells to have an unstained or lightly stained area at the side of the nucleus where the cytoplasm is most abundant. They are derived from lymphocyte-like cells. There is evidence that the plasma cell is active in forming antibodies.

(d) *Mast cells* (Fig. 32) occur in varying numbers in most loose connective tissue, being especially numerous along the course of the blood vessels. They are large round or ovoid cells with pale-staining nuclei and coarse cytoplasmic granules which stain with certain basic dyes such as toluidine blue. They are often known as histogenous mast cells to distinguish them from the hematogenous mast cells (basophilic leucocytes of the blood) which they closely resemble. The mast cell granules of some species, including man, are soluble in water and consequently are seldom preserved and stained in ordinary preparations. Their origin is not clear and their function has been conjectural until recently. Evidence now indicates that they contain *heparin*, an anticoagulant, and *histamine*. The granules of mast cells stain metachromatically as does heparin, though this is not conclusive evidence that the two are associated, for other cells and substances also stain metachromatically. Quantitative studies, how-

FIG. 32, *A*. Spread of subcutaneous areolar connective tissue from a rat which had received several intraperitoneal injections of trypan blue over a period of two weeks. The animal was autopsied one week after the last injection and the spread made immediately. After fixation in Bouin's fluid, it was stained with resorcin-fuchsin for elastic fibers and with azocarmine to show cells and collagenous fibers. All of the blue color shown in histiocytes and fibroblasts represents the trypan blue which was taken in by the cells preceding autopsy. No trypan blue is present in the nuclei although a few vacuoles may appear to be within the nuclei when they are in the overlying cytoplasm. ×650. *B*. Subcutaneous spread from the same trypan blue rat as above, stained with neutral red for mast cells. ×650. *C*. A section through the areolar connective tissue of the submucosa of the colon. The field shown is directly beneath the muscularis mucosae. Rhesus monkey. Hematoxylin and eosin-azure. ×1250.


Lymphocyte
Elastic fibers
Histiocyte
Fibro-
blast
Histio-
cyte
Collagenous fibers
A
Fibroblast
Capillary
Histio-
cyte
Mast
cells
Lympho-
cyte
Collag-
enous
fibers
B
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Plasma
cell
Eosino-
phile
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blasts
C

FIGURE 32

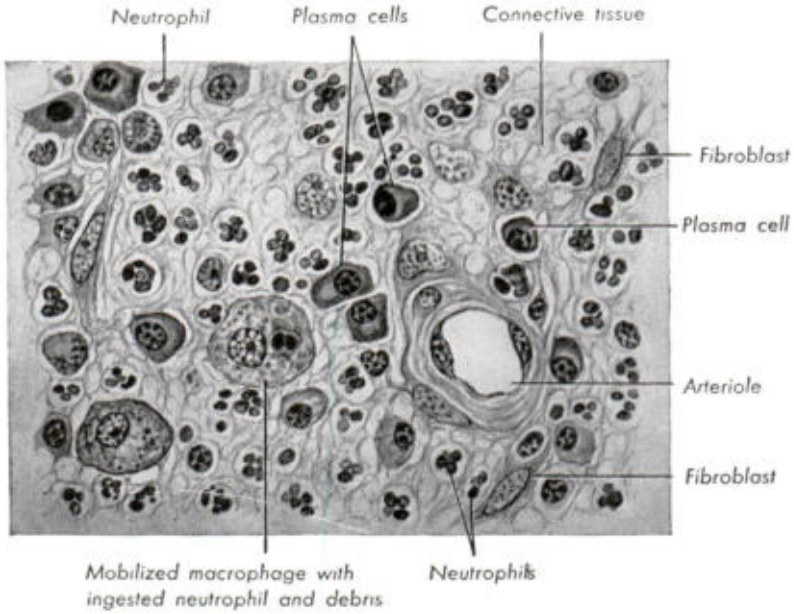


FIG. 33. Section of an intratesticular abscess excised thirty days after the initial infection. An intermediate zone of the abscess is figured. Central to the area shown in the figure is a region where neutrophiles predominate; peripherally is an extensive fibrosis. Hematoxylin and eosin-azure II. ×753.

ever, show that tissues and organs in which mast cells are most numerous contain more heparin than do structures containing but few mast cells (Holmgren and Wilander).

(e) Besides the cells enumerated, temporary visitors from the blood and lymph stream are seen in varying numbers. These may include lymphocytes, as well as eosinophilic and neutrophilic leucocytes.

Connective Tissue Fibers. Three types of fibers occur in adult connective tissue. Each of these types is present in loose connective tissue and thus a knowledge of the fibers in this type of tissue gives an understanding of the fibrillar elements of all adult connective tissue.

(a) The *white* or *collagenous* fibers (Fig. 32) vary in size and are in reality bundles of extremely fine fibrils (fibrillae). The fibrillae lie parallel to one another giving the fiber a longitudinally striated appearance, and are united by a small amount of cement substance. The fibrillae do not branch. The fibers or bundles, on the other hand, branch and anastomose. The course of the fibers may be straight, but is usually wavy. White fibers are soft and flexible but so slightly elastic as to be practically nonextensible.

Chemically they consist of an albuminous substance known as collagen which stains easily with most acid dyes, and which on boiling yields gelatin. The fibers shrink slightly in hot water and swell enormously in dilute acids or alkalis. When immersed in the latter the interfibrillar cement substance is dissolved and the fiber breaks up into the constituent fibrillae. The fibers are rapidly digested by gastric juice, but are not affected by alkaline solutions of trypsin. The latter hence furnishes a

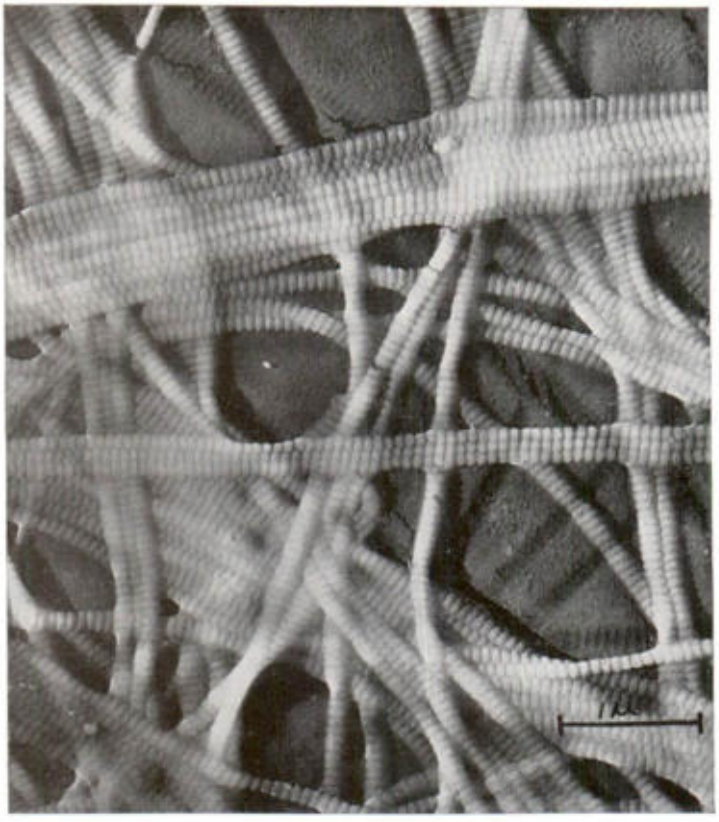


FIG. 34. Electron micrograph of collagen fibrils from the adult human dermis. Chromium shadowed. ×19,300. (Courtesy of Drs. Gross and Schmitt.)

good method for isolating collagenous fibers.

Studies of the fibrils of collagenous tissue with the electron microscope have contributed much information concerning them. Their diameter varies considerably but is about 1000 Ångström units (approximately 0.1μ) in adult human corium. Newly formed fibrils are smaller than old ones. The fibrils show periodic cross-bandings which have an average interval of 640 Å in mature fibrils (Fig. 34). The cross-banding is correlated with the arrangement of long polypeptide chains within the fiber. From studies with the electron microscope and from x-ray diffraction methods, it has been concluded that the fundamental molecule of collagen is approximately 2600–3000 Å in length and 15 Å in width. This substance is known as *tropocollagen*. Particles of tropocollagen unite side by side in a staggered fashion to form collagen with an average periodicity of 640 Å.

(b) *Reticular* fibers are small branching fibers which frequently form a net-like supporting framework or reticulum. Their caliber is so small that they are masked by surrounding structures in ordinary stained preparations, but they blacken intensely after silver impregnation (Bielchowsky's method) while other fibers are colored yellow or brown. Because

they impregnate with silver, reticular fibers are frequently designated as *argyrophilic* fibers.

Reticular fibers are often continuous with collagenous fibers. At various points, they unite forming bundles which almost immediately assume the tinctorial characteristics of collagenous fibers. Chemically, reticular fibers appear to be similar to collagenous fibers although they are more resistant to peptic digestion. Their chief difference from collagenous fibers appears to be their smaller diameter and their greater tendency to form a network (reticulum). In electron micrographs, the reticular fibers have a periodic cross-banding similar to collagenous fibers.

In most areolar tissue, reticular fibers are relatively few in number. In some locations as in glandular organs (Fig. 302) and between muscle fibers (Figs. 95, 103) they are numerous.

In lymphoid organs and in red bone marrow, reticular fibers are associated with a special type of cell, *reticular cell*, the two elements (reticular fibers and reticular cells) forming a type of tissue, *reticular tissue* (p. 71). In other locations reticular fibers have the same relationship to fibroblasts as do collagenous and elastic fibers.

(c) The *elastic* fibers (Fig. 32) are apparently homogeneous, highly refractive fibers which are as a rule thinner than the white fibers but may reach a diameter of 10–12μ in certain places. They branch and anastomose freely, forming networks. The smaller fibers are round in cross section, the larger are flat or polygonal. They are highly elastic. When seen in large masses (elastic ligaments) in the fresh state, they have a distinctly yellow appearance.

Chemically they consist of *elastin* which has a remarkable resistance to most agents. It is not affected by hot or cold water, by dilute acids or alkalis or by gastric juice. It is, however, rapidly digested by pancreatin.

Elastic fibers are best demonstrated in the fresh condition by immersing the tissue in dilute acid solutions. The collagenous fibers swell and become transparent. The elastic fibers are then seen as highly refractive shining threads. The elastic fibers react but poorly to most stains, but are colored deeply by certain specific dyes such as orcein and resorcin-fuchsin (Fig. 32).

Origin of the Connective Tissue Fibers. Many extensive studies on the origin of connective tissue fibers have been reported. According to one view, the fibers arise within the fibroblasts (intracellular) and pass into the ground substance. According to another view, the fibers form outside of, but close to the fibroblasts (extracellular). The best evidence favors the latter view. Maximow demonstrated that in tissue cultures the first fibrils appear outside the cells in the form of a network which is argyrophilic (Fig. 35). The first fibrils can become transformed into broader, less argyrophilic, typical collagenous fibers. Stearns studied the formation of fibrils in the living animal by the use of transparent chambers placed in the ears of rabbits. In some cases, globules of material were seen to be extruded from the fibroblasts and fibrils formed around these globules.

Electron microscope studies have made particularly significant contributions to our understanding of fibril formation. The first fibrils arise in extremely close contact with the outer surface of the cell, probably by a polymerization of material formed by the cell (Fig. 36). The earliest fibrils are more slender than mature fibrils and they have been called "unit fibrils."

The first-formed fibrils run in all

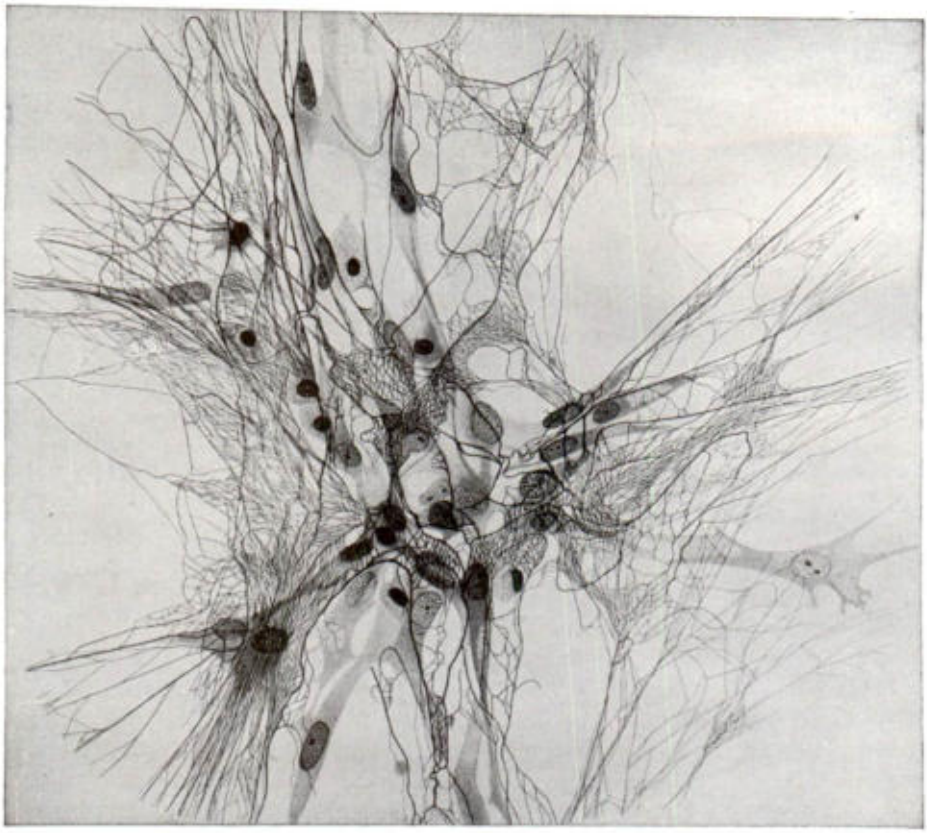


FIG. 35. Tissue culture of adult rabbit thymus. (Maximow.) Observe the syncytial appearance of the fibroblasts and the reticular network of fibers which are attached to cells in some regions and free in others.

directions in a plane or layer. With normal tensions they come to have parallel arrangements forming fiber bundles.

Elastic fibers probably develop in the same manner as the collagenous fibers but they develop later.

Ground Substance. The *ground substance* of connective tissue is a nonfibrillar homogeneous substance which varies from a fluid-like to a gel-like state. It is one of the two components of nonfibrillar intercellular substances of connective tissue. The other is tissue fluid which is related to plasma, and contains proteins, crystalloids, metabolites and gases. Both the ground substance and tissue fluid surround the fibrillar elements and cells of connective tissue.

In fresh spreads of connective tissue, ground substance has the same refractive index as water and isotonic saline solutions and so is invisible when the spreads are mounted in these media. It is visible when the spreads are mounted in a medium of a different refractive index such as serum or sugar solution (S. H. Bensley). In tissue which has been fixed it stains very little or not at all with the ordinary dyes but can be stained

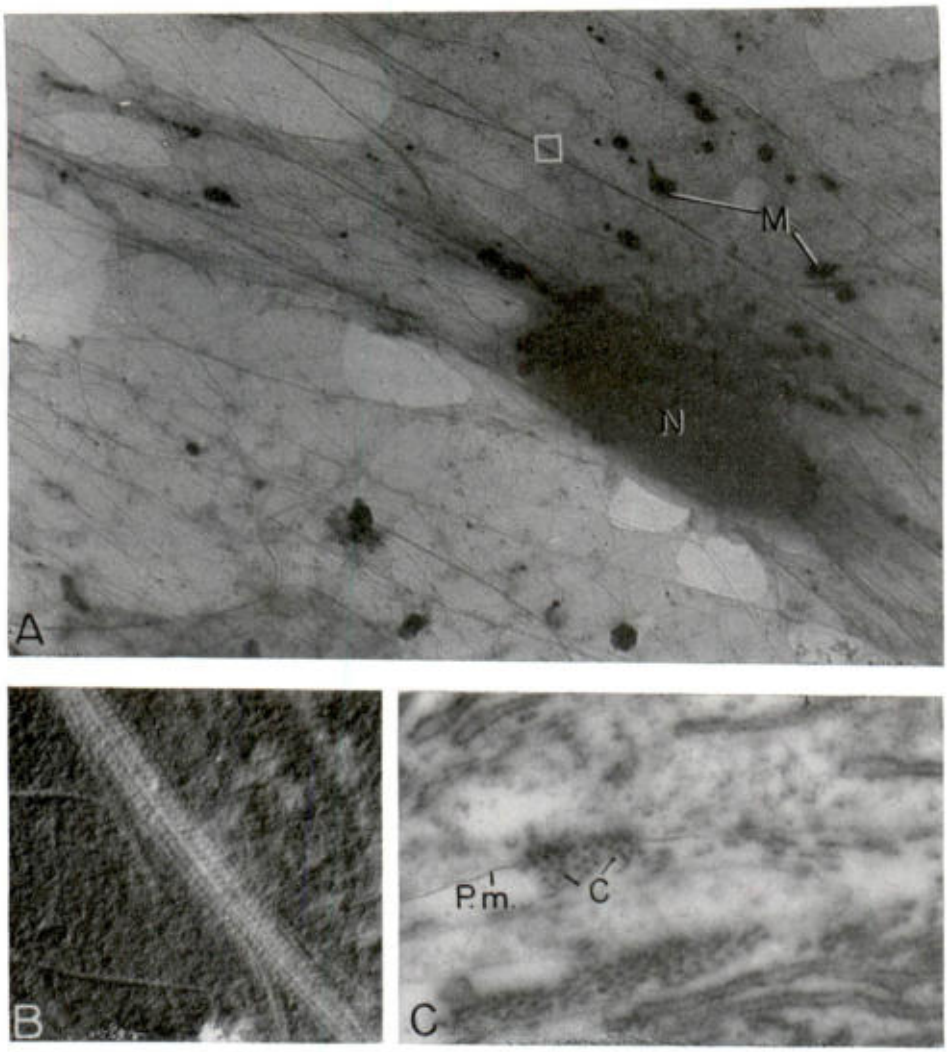


FIG. 36. Electron micrographs illustrating the relationship of fibroblasts to collagen fiber formation. *A*, Surface view of a fibroblast grown in tissue culture from an explant from the dermis of a 9-day-old chick embryo. The nucleus (*n*) and mitochondria (*m*) are indicated. Collagen fibers formed in association with the cell course from lower right to upper left. × 3,000. *B*, Higher magnification of the fiber shown within the small square of *A*. Note that the fiber is composed of many fibrils. × 20,000. *C*, Section through cells of the dermis of a 14-day-old chick embryo. A fiber similar to that illustrated in *B* is shown in a section vertical to the cell surface. No collagen fibrils (*c*) are evident within the cytoplasm; they appear first on the cell surface (*P.m.*). × 20,000. (Courtesy of Drs. Keith Porter and G. D. Pappas.)

selectively with the periodic acid-leucofuchsin technique. When properly fixed the ground substance shows a metachromasia when stained with toluidine blue. Ground substance is a mixture of mucopolysaccharides.

Ground substance becomes condensed beneath epithelium, capillaries and small veins, and with reticular fibrils forms basement membranes.

The ground substance appears to be of considerable physiological importance. Although much work remains to be done in order to clarify the problem, nevertheless, it appears that the substance is of importance in preventing the spread of noxious agents in localized infections. By chemical procedures it has been found that connective tissue, synovial fluid and some other fluids and tissues contain a viscous mucopolysaccharide—*hyaluronic acid*—isolated by Meyer and Palmer in 1934. Because of its capacity to bind water, this substance may be largely responsible for changes in the viscosity of ground substance. An enzyme, hyaluronidase, hydrolyzes it, reducing its viscosity, with a consequent increase in the permeability of the tissue. For instance, injections of India ink to which hyaluronidase has been added will spread much more rapidly than will injections of ink alone (*spreading reaction*). This enzyme (or enzymes) has been shown to be present in snake venom, in spermatozoa and in certain tumors, and to be formed by a few microorganisms. Its presence may increase their invasiveness.

The subject is complex. At least six different mucopolysaccharides have been isolated from adult connective tissues. The types and proportions of the different mucopolysaccharides (hyaluronic acid, chondroitin sulfate A, B and C, chondroitin and keratohyalin) vary in different locations and in different types of connective tissue. The reader is referred to papers by Meyer.

Functions of Loose Connective Tissue. Loose connective tissue loosely binds structures together and holds them in position. It acts as a padding and serves as a pathway for nerves and blood vessels.

Nutrient substances in their passage from the blood vessels to the cells of the body must traverse connective tissue as must also those products of metabolism which reach the blood and lymph capillaries (see Fig. 182.) There is a continual movement of intercellular fluid through the connective tissue.

The loose connective tissue plays an extremely important rôle in limiting the spread of localized infections and in the healing process. The localization invokes all elements of the connective tissue. The ground substance, though permeable, tends to inhibit the passage of the noxious agent. In addition to the phagocytic cells (neutrophilic leucocytes and monocytes) which migrate to the area from the blood, the tissue phagocytes (macrophages) multiply and mobilize. The fibroblasts also become active and, after a considerable time, will deposit a surrounding barrier of fibers. An area of infection is shown in Figure 33. In repair of wounds the fibroblasts increase in number and form fibers. Sprouts from the blood vessels grow into the delicate regenerating tissue which is known as granulation tissue.

Dense Connective Tissue

Dense connective tissue is chiefly characterized by the close packing of its fibers. It occurs in the form of sheets, bands and cord-like structures. Examples are the dermis, capsules of certain organs, aponeuroses, ligaments and tendons. Some of the deep fascia is intermediate between dense and loose connective

tissue. In most locations the main component is collagenous fibers but in a few of the ligaments, elastic fibers predominate.

Dense irregularly arranged connective tissue occurs in the form of sheets. The main component is coarse collagenous fibers but elastic and reticular fibers are also present. The fibers interlace and form a coarse tough feltwork, and some of them continue into adjacent tissue. Fibroblasts and some macrophages are present but show no special modifications. Examples of dense irregularly arranged connective tissue are: dermis (Figs. 37, 204), periosteum and perichondrium, and the capsules of some organs. The capsule of the testis (tunica albuginea, Fig. 354) is extremely dense.

Dense regularly arranged connective tissue occurs as cord-like structures, and as bands some of which, as in aponeuroses, may be very broad. The fibers are densely packed and lie parallel to each other forming structures of great tensile strength. This type of tissue comprises the tendons, ligaments and aponeuroses.

Tendons are composed almost entirely of white fibrous tissue (Fig. 38). The fibers are parallel and are closely packed in bundles which are so dense that they appear almost homogeneous. Fibroblasts are the only cell type present and they are few in number as compared to areolar tissue. In longitudinal sections of tendon, the fibroblasts or tendon cells are elongated and aligned in rows between the bundles of collagenous fibers (Fig. 38). In cross sections, the cells appear stellate in shape, with plate-like extensions between the collagenous bundles (Fig. 39).

Around each bundle of fibers is a small amount of areolar tissue and the whole tendon is surrounded by interlacing fibers.

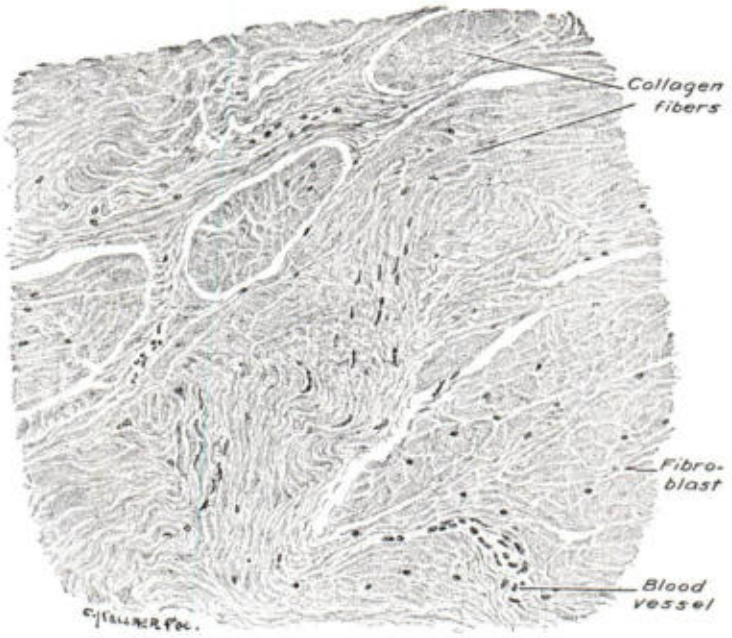
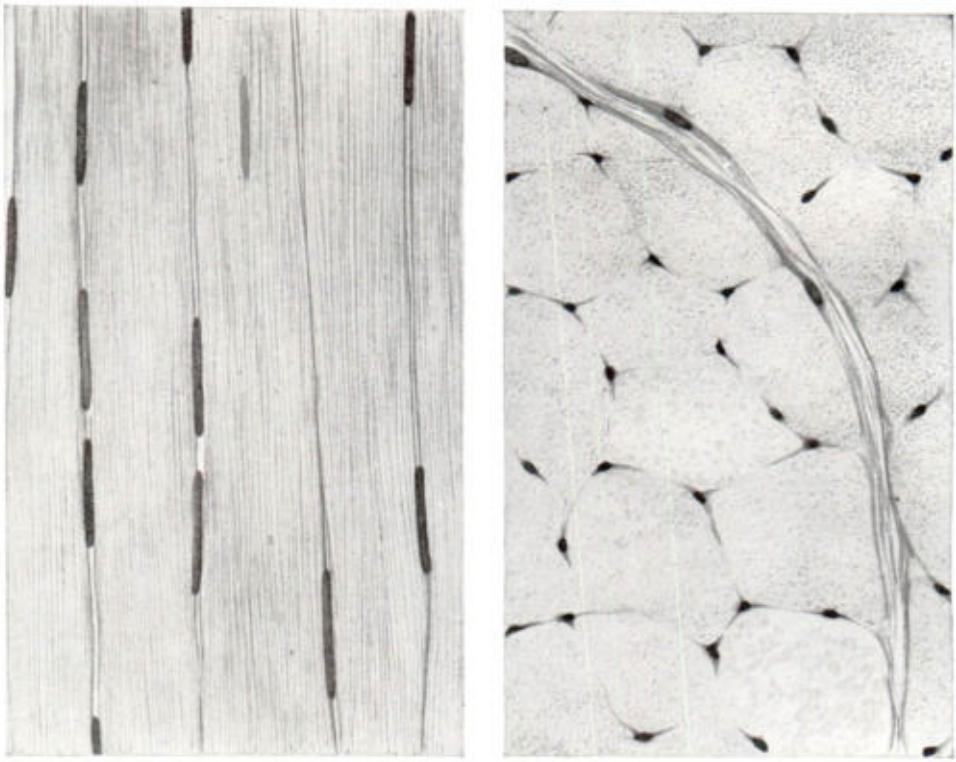


FIG. 37. Corium of human finger tip showing irregularly arranged compact bundles of collagenous fibers. ×300.



FIGS. 38 and 39. Sections of human achilles tendon. Fig. 38, (*left*) is from a longitudinal section and shows rows of dense-staining nuclei of fibroblasts (tendon cells) between bundles of regularly-arranged fibrils. Fig. 39, (*right*) is from a cross section. Note that the nuclei and the pale-staining cytoplasm of the tendon cells appear stellate in shape. A band of irregularly-arranged connective tissue separating tendon bundles is seen in the right side of the drawing. Both figures × 575.

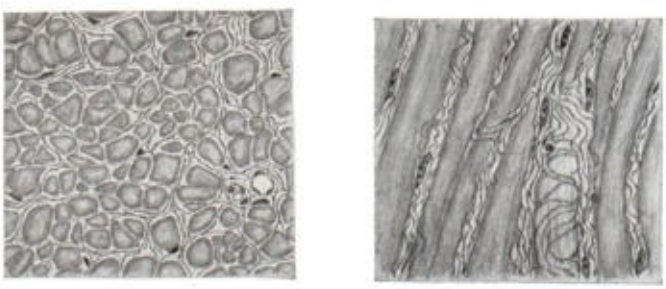


FIG. 40. Ligamentum nuchae of an ox. *A*, Cross section; *B*, longitudinal section. Slender and irregularly-arranged collagenous fibers are seen between the unusually large elastic fibers. ×435

Aponeuroses have the same composition as tendons but are broad and relatively thin. The fibers may be arranged in several superimposed layers, those of one layer running at an angle to those of adjacent layers. The layers may interweave.

Ligaments in most cases are structurally similar to tendons, being formed predominantly of collagenous fibers, but a few are composed almost entirely of elastic fibers.

The *yellow elastic ligaments* are formed of parallel coursing yellow elastic fibers which are bound together by a small amount of areolar tissue. The elastic fibers may be very large as in the ligamentum nuchae (Fig. 40). The series of ligaments (ligamenta flava) coursing between the arches of the vertebrae are also of the elastic type.

Reticular Connective Tissue

Reticular connective tissue is characterized by the presence of a cellular reticulum (reticular cells) and a fibrillar network. The fibrils are argyrophilic, and they branch and communicate, forming an open interlacing meshwork (page 64). The cells have a stellate shape with processes which extend in all directions and join the processes of neighboring cells (Fig. 41). Most of the protoplasmic processes are wrapped about or extend along the reticular fibers, but many of them lack this association, thus making part of the protoplasmic reticulum independent of the fibrillar one (Fig. 41). The relation of the fibers to the cells has been a much disputed topic. According to one view the fibers are extracellular as in other connective tissue, in close contact with the cells but not a part of them. Others maintain that the delicate fibers lie in the peripheral cytoplasm (ectoplasm) of the reticular cells and are always invested by a delicate protoplasmic membrane.

Morphologically, *reticular cells* probably have a closer resemblance to embryonic connective tissue or mesenchymal cells than do any other cells in the adult body. They have a large pale nucleus, rather abundant lightly staining cytoplasm but are without cytoplasmic granules or vacuoles under normal conditions.

Several different functional and de-

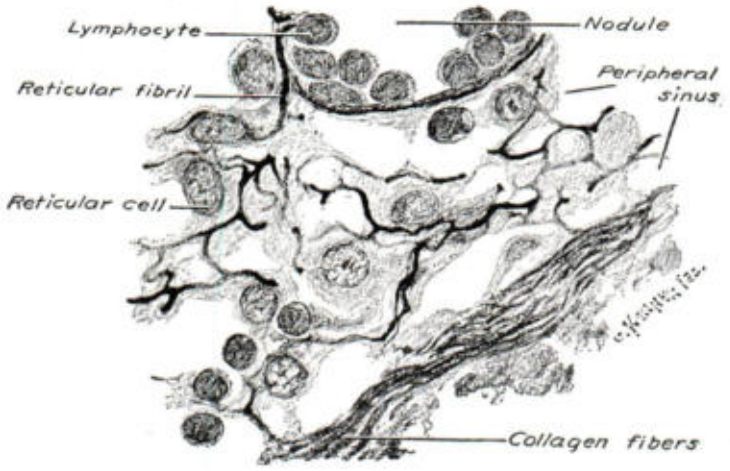


Fig. 41. Reticular tissue from lymph node of monkey. Bielschowsky-Foot and eosin-azure. ×1200.

velopmental potencies have been attributed to reticular cells. It seems likely either that the name includes several distinct types of cells which cannot be distinguished by morphological means alone or that they are not all in the same physiological state. Some of them may have retained their embryonic or developmental potencies while others have differentiated into either phagocytic or fibroblastic types of cells. The fibroblastic tendencies are shown by the cells associated with the fibrous elements of the reticular tissue. Other cells are so placed that they seem to make up part of the wall of a lymphatic sinus or blood sinusoid and in this condition may rightly be called reticulo-endothelial cells. When an animal is stained vitally with trypan blue (p. 75), many of the reticular cells segregate the dye into vacuoles and are therefore part of the "reticulo-endothelial system." In fact it is those phagocytic reticular cells which are placed in the walls of sinuses or sinusoids which have given the "system" its name. However, it should be borne in mind that many of the phagocytic cells are deep in the reticular tissue and have no contact with sinuses or sinusoids and that none of these phagocytic cells correspond to true endothelium. Reticular cells, in addition to being potentially phagocytic, also may give rise to the early stages of developing erythrocytes and leucocytes in the hemopoietic tissues.

Although reticular fibers are widely distributed in the body in association with fibroblasts, the occurrence of reticular fibers in association with reticular cells, i.e., *reticular tissue*, has a much more limited distribution. Reticular tissue forms the framework of lymphoid tissue (lymph nodes and nodules, spleen, thymus) and bone marrow (Fig. 41). In lymphoid tissue, the interstices of the reticular tissue are filled with lymphocytes; in bone marrow, blood-forming elements are present in the reticular tissue network.

Adipose Tissue (Fat)

Fat is considered by some as a special tissue arising from specific cells (steatoblasts), by others as a modified type of fibrillar connective tissue in which some of the cells have lost their fiber-forming capacity and have assumed the function of fat storage. Fat cells are found isolated or in groups in all loose connective tissue, but in certain places they are present in such large numbers and have an organization which justifies the designation of adipose tissue. The largest deposits of fat are found in the subcutaneous connective tissue (panniculus adiposus), in the kidney region, in the mesenteries and mediastinum, and in the cervical, axillary and inguinal regions.

Fat is different from the other connective tissues in that the cells, and not the intercellular substance, make up the bulk and determine the nature of the tissue. The cells are large and have an ovoid or spherical shape. The cytoplasm is displaced to the peripheral region of the cell by the presence of a single large fat droplet (Fig. 42). The nucleus, flattened and surrounded by a small amount of cytoplasm, is usually found pressed against the cell wall. In sections of fixed preparations in which the fat has been dissolved out, the cells appear as empty rings or ovals, or have a "signet ring" shape if the plane of section passes through the nucleus. When occurring singly or in small groups the cells retain their spherical or ovoid form; in denser masses they become polyhedral, due to the pressure of adjacent cells. Fat cells are usually arranged in groups or lobules, each lobule being separated from its neighbor by areolar connective tissue. Delicate connective tissue penetrates

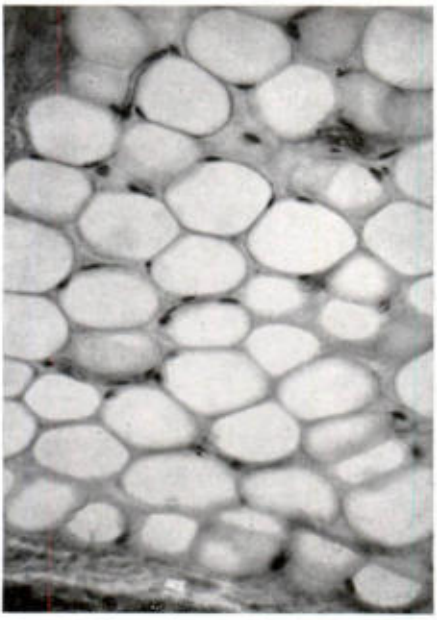


FIG. 42. Photomicrograph of fat from human subcutaneous tissue. × 190.

between the fat cells and serves as a bed for the numerous capillaries.

The appearance of the adult fat cell can be best understood by a reference to its histogenesis. In places where fat is to be formed certain cells of the embryonal connective tissue arrange themselves in groups in the meshes of a rich capillary network which marks the end of a small artery (Fig. 43). Such groups, each of which is destined to become an adult fat lobule, can be distinguished in man in the fourth month of embryonic life. The cells enlarge, lose their stellate form, become rich in protoplasm and have the general appearance of secretory cells (steatoblasts). Fine fat droplets are formed in the cytoplasm, which increase in number and finally coalesce forming a larger droplet. By further formation and fusion of fat droplets, the nucleus is pushed to one side and the cytoplasm gradually reduced to the thin membrane of the adult cell (Fig. 43).

The blood supply of fat is rich and the adult lobule retains its embryonal vascular relations, the vascular supply of each lobule being complete and independent. One artery runs to each lobule where it breaks up into an intralobular capillary network which in turn gives rise to the intralobular veins, usually two in number.

Chemically fat consists of the esters of glycerol and certain fatty acids (palmitic, stearic and oleic). It is not soluble in water or cold alcohol, but dissolves readily in ether, chloroform, benzol and xylol. Since the latter reagents are commonly used in histological technique, the fat is usually dissolved leaving an empty space or vacuole. When properly fixed, fat and fat-like substances stain black with osmic acid (osmium tetroxide). Other specific stains for fat are certain coal tar dyes, as Sudan III and Scharlach R.

The experiments of Schoenheimer have shown that the so-called fat deposits of the body are not inactive storehouses. He labelled or "tagged" molecules of carbohydrates and fats with atoms of heavy water or deuterium and followed their metabolism in the animal body. He found that mice synthesize fats from carbohydrate on a normal diet without overfeeding or fattening. The fats are rapidly replaced; the turnover in mice required only about six days.

The fat deposits of the body represent important food reserves which can be tapped in case the normal food supply becomes inadequate. When fat is given up by the cells, at least in the case of isolated fat cells or smaller scattered groups, the cells may revert to the type of a connective tissue cell, and are capable of again storing fat under favorable conditions. In the large fat masses, especially in older individuals, the cells when emptied of their fat sometimes become filled with a serous fluid which

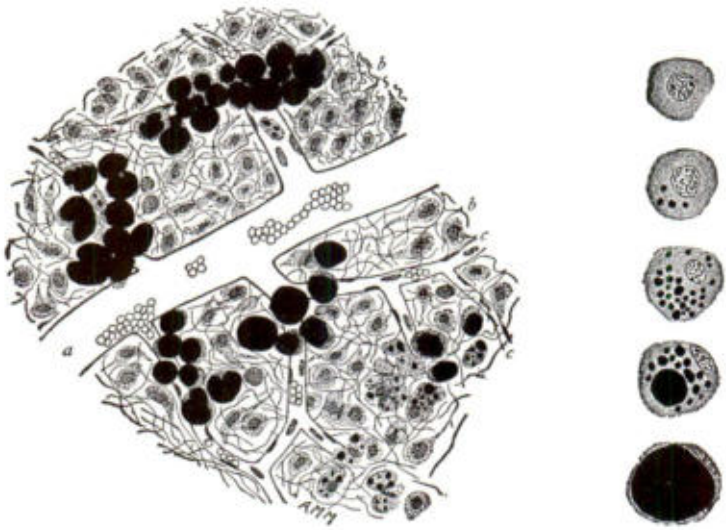


FIG. 43. Developing fat tissue from subcutaneous tissue of five-inch fetal pig. *a*, Arteriole breaking up into capillary network; *b*, embryonal connective tissue, embryonal cells from which fat cells are developing; *c*, capillaries. Fat droplets stained black. At the right are five individual cells showing stages of development from an embryonal cell to an adult fat cell.

also fills the spaces between the cells. This condition is known as serous atrophy of fatty tissue.

Besides its nutritive value fat has important mechanical functions. It forms plastic, shock-absorbing pads in the subcutaneous tissue of parts exposed to pressure, such as the gluteal region and the soles of the feet. It packs the orbital cavity and the angles of joints, acting as a guard against exaggerated movements. Such fatty pads are especially prominent in the hip, knee, shoulder and elbow joints, where they may be retained even in states of extreme emaciation.

As a nonconductor fat is also an important agent in the conservation of the body heat.

Rodents and a number of other animals have masses of *brown fat tissue* in addition to the common or white type. Brown fat is found in the interscapular region, in the inguinal area, and elsewhere. It was once considered as a *hibernating gland* but it is present in a number of animals which do not hibernate.

Each cell of brown fat contains a number of small lipid droplets whereas each cell of adult white fat contains only one large droplet (or vacuole). Therefore, brown fat is described as *multilocular* and white fat as *unilocular*.

Brown fat does not respond to nutritional changes as readily as ordinary fat. On the other hand, hypophysectomy brings about a more rapid lipid depletion in brown than in white fat.

Pigmented Connective Tissue

Pigmented connective tissue cells occur in the choroid and iris of the eye and in the corium of the dark skinned races (Fig. 44). The cytoplasm is filled to a varying degree with brown or black pigment which is usually melanin. Experiments with tissue cultures indicate that they are a specialized type of cell. The cells form true pigment in the cultures only if they are explanted from a tissue which would have grown pigment in the body.

Blood and Nerve Supply of Connective Tissue

Blood vessels and lymphatics are very numerous in loose connective tissue.

FIG. 44. Pigmented connective tissue cells from choroid coat of human eye. ×350.

The rich capillary plexuses are not, however, in the main destined for the tissue itself, but for the more active cells of epithelium and muscle. The connective tissue forms the supporting bed for all blood vessels and incidentally receives its own nutrition from them. In the dense connective tissues and tendons the blood supply is less abundant. In tendons, the blood vessels follow a straight course between the large fascicles and communicate with each other by short branches which run across the fascicles, forming a scanty capillary network with oblong meshes. The fibrous membranes of the periosteum and dura mater are more vascular, but here too the blood vessels are mainly destined for bone.

Lymphatics are numerous and form extensive networks, especially in the submucous, subserous and subcutaneous connective tissue. They are also present in fibrous membranes and tendons where they form both superficial and deep lymphatic plexuses.

Connective tissue is richly supplied with nerves which end in the tissue itself or go to epithelium and muscle. Special nerve endings are found in the tendons, fibrous membranes and in the periarticular connective tissue.

THE RETICULO-ENDOTHELIAL SYSTEM

The anatomical connotation of this name is rather unfortunate because the "system" to which it refers is based on physiological and pathological considerations. The earlier name suggested by Metchnikoff, "macrophages," might better have been retained, but since the former name has become firmly entrenched in a voluminous literature, it will be used in this discussion. The cells in this system do not form true endothelium, and the most widely distributed type of reticulo-endothelial cell, the connective tissue histiocyte, is not indicated by either the term "reticular" or "endothelial."

All highly phagocytic cells of the body, except leucocytes, belong to this system. They have no morphological characteristic which distinguishes them with certainty from other cells. The only certain method of identification is to inject into the living animal certain nontoxic dyes or some inert particulate matter such as the carbon particles of China ink. The macrophages take up the dye or other particles and can easily be identified in microscopic preparations. The injection of nontoxic dyes such as trypan blue or lithium carmine into a living animal is known as *vital* or *intravital staining*. Since the power to take up the dye and segregate it into vacuoles parallels the power to engulf particulate matter such as India ink or colloidal metals, the absorption of the dye has been called ultramicroscopic phagocytosis by Evans. After vital staining, the cells

containing dye are found in the loose connective tissue, in the reticular tissue or in the dilated blood sinuses of certain organs. In the connective tissue, they correspond to the *histiocytes* described above (p. 61). They are probably identical with the clasmatocytes of Ranvier, the leucocytoid and adventitial cells of Marchand, the rhagiocrine cells of Renaut, the resting wandering cells of Maximow, the macrophages of Lewis and Evans, the splenocytes and endothelial leucocytes of Mallory.

In the liver, the cells containing the dye are found in the sinusoids, either flattened against the wall so as to resemble endothelium or as stellate forms with their processes extending across the lumen after the manner of reticular cells. They are the *Kupffer* cells (see p. 382). In the lymphatic system, the phagocytosis is shown particularly by the large *reticular cells* of the sinuses of lymph nodes, but the dye is taken up also by the cells of the reticular tissue of the less organized lymphoid aggregations in many parts of the body. The spleen has a large number of cells which are marked by the dye, both in the reticulum of the pulp and in the walls of the blood sinuses. Some of the reticular cells of the bone marrow and cells in the walls of the sinuses of the suprarenal gland and hypophysis also show the phagocytic activity. The *microglia* of the nervous system take up the dye to some extent and since they have been shown recently to be derived from the mesoderm, they should be included in the system. Many of the phagocytic cells in the lymph nodes do actually line the lymph sinuses and at the same time send branching reticular processes into the lumen and into the denser tissue of the nodule. These are properly called reticulo-endothelium, but numerically they form only a small portion of the "system."

In the blood stream, a certain number of large mononuclear cells with phagocytic powers have been found. Mallory believed that they were endothelial in origin. Kiyono showed that they were histiocytes which had reached the blood stream. After vital staining, he found a number of the stained cells in the venous channels of the omentum, spleen and liver and traced them to the lungs where the greater number of these cells were filtered out of the blood by the lung capillaries. Some passed through the pulmonary circulation and reached the general circulation. He was able to distinguish these circulating histiocytes from the monocytes and lymphocytes. Recent experiments indicate that any of the phagocytic cells may detach themselves from their fixed positions and find their way into the blood stream, either indirectly through the lymph channels or directly as in the spleen and liver.

Although cells containing the dye are found in widely separated organs, the *reaction* is quite *specific*. Epithelium, nerve cells, and muscle take up the dye very slightly if at all. The true endothelial cells, lining the blood vessels and lymphatic capillaries, either fail to react to the dye or react so slightly that if a macrophage is near an endothelial cell, a quantitative difference is readily seen. The fibroblasts take up the dye more slowly and in smaller amounts than the histiocytes (Fig. 32). In the reticular tissue of the spleen, lymph nodes and other lymphoid tissue, there are undifferentiated cells which do not take up the dye although they are morphologically similar to the active cells.

The origin of these cells has not been determined. In the development of the embryo, the mesenchyme gives rise to two types of cells in the connective tissue: fibroblasts and wandering cells (Maximow). After the fibroblasts have once formed connective tissue fibers, they probably remain fibroblasts and do not change into other or more primitive types. The wandering cells, on the other hand, retain some of their primitive properties and may take one of several lines of development according to the

stimulus of body needs. Many of them become macrophages; others preserve embryological potencies which can be called forth under abnormal circumstances. The latter are called *undifferentiated mesenchymal cells.*

Some investigators believe that the cells of the reticulo-endothelial system retain *potentialities* for producing other types of cells, particularly blood cells. There is no evidence that they have such function in a normal individual, but under circumstances of abnormal stress they may reassume a primitive hemopoietic function. Maximow has shown in tissue cultures that monocytes and lymphocytes change into macrophages and eventually even into fibroblasts. In other experiments he has shown that the lymphocytes may become hemocytoblasts and form any or all of the cells of the blood. This experimental evidence would indicate that the macrophage or histiocyte is a more differentiated cell than the lymphocyte and that, if there is any universally potent cell in the adult, it is more likely to be the lymphocyte than the reticulo-endothelial cell. Certain of the cells of reticular tissue have been found to exhibit hemopoietic power, but it has not been shown that they are the phagocytic type of reticular cell.

The *functional importance* of the macrophages of the reticulo-endothelial system is very great. In a normal individual they are probably responsible for taking the broken down blood cells from the circulation and they may even store the iron which is to be used in hematopoiesis. In pathological conditions they are active agents in the removal of foreign bodies, bacteria, and broken down tissues or cells.

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VI

THE CONNECTIVE TISSUES: CARTILAGE AND BONE

CARTILAGE

Cartilage, like other connective tissues, consists of cells, fibers and ground substance. The last named, however, has physical properties which give to the tissue a compactness and elastic firmness, rendering it capable of withstanding a considerable degree of pressure and tension. In some of the lower vertebrates (elasmobranchs) the whole adult skeleton consists of cartilage, and in the mammals the greater part of the skeleton is first laid down in cartilage. In the adult body, cartilage covers the articular surfaces of bones, and it forms the sole skeletal support of the larynx, trachea, bronchi and certain other structures.

According to the nature and visibility of the fibrillar elements, cartilage is subdivided into three varieties: (1) hyaline, (2) elastic, (3) fibrous. Of these hyaline cartilage is the most widely distributed type.

Hyaline Cartilage

Hyaline cartilage (Figs. 45, 46, 68) appears as a bluish-white, translucent mass in the fresh condition. It forms the articular cartilage of joints, the costal cartilages, and the cartilages of the nose, larynx, trachea and bronchi. In the fetus, nearly all of the skeleton is first laid down as hyaline cartilage and is replaced later by osseous tissue in the formation of the bones.

The tissue is usually organized into plate-like masses which, with the exception of the articular cartilages, are invested by a fibrous membrane, the perichondrium (Fig. 45). Each cartilaginous plate forms a solid mass, consisting of cells and an apparently homogeneous intercellular matrix, the connective tissue fibrils being masked by the ground substance. The tissue is not supplied with blood vessels or nerves. Although blood vessels are occasionally found within some of the cartilages they are probably not supplying the cartilage but are merely passing through it on their way to other tissues.

The Cells. The cartilage cells (*chondrocytes*) are situated in smooth-walled spaces or *lacunae* (Fig. 45). Each cell has a large, centrally-placed spherical nucleus with intranuclear reticulum and one or more nucleoli. The cytoplasm is finely granular and contains fat droplets, glycogen granules and occasionally pigment.

In the living condition the cells completely fill their lacunae, but their large content of fat and glycogen leads to marked structural distortions in fixed preparations. These are dissolved out leaving the cells greatly vacuolated or shrunken into small masses separated by spaces from the walls of the lacunae. Often protoplasmic processes extend to the capsule giving the cell a branched appearance. In the living condition the

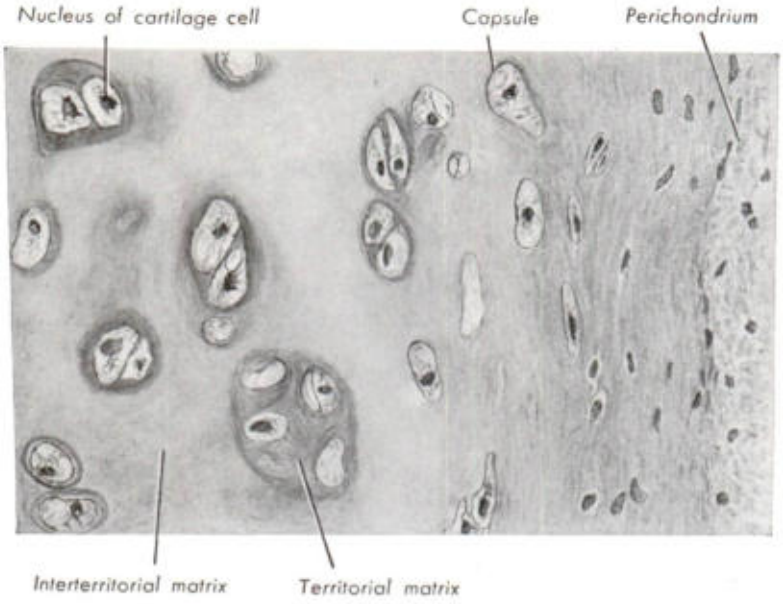

FIG. 45. Hyaline cartilage from the trachea of a boy seventeen years of age. Delafield's hematoxylin and eosin. ×435.

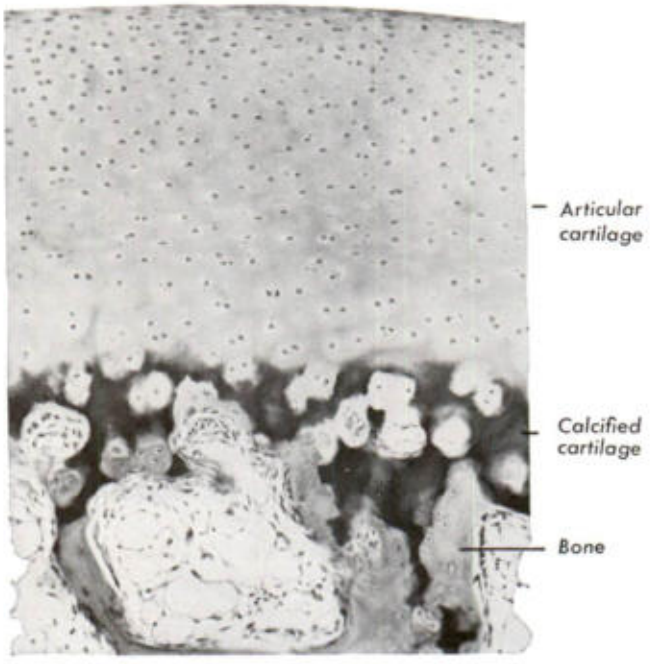

FIG. 46. Articular cartilage from a metatarsal bone of a rhesus monkey, two months old. Photomicrograph. ×165.

cells of adult cartilage are, however, unbranched.

In the center of the mass the cells are arranged in groups, each group representing the offspring of a parent cartilage cell (Fig. 45). The cells are spherical or ovoid, flattened on adjacent sides, their long axes directed radially to the surface of the plate. Toward the periphery the cells become progressively flattened and lose their definite grouping, until in the subperichondrial layers they appear as rows of narrow elongated cells whose long axes are parallel with the surface. The cartilage grades insensibly into the fibrous connective tissue of the perichondrium.

In embryonal cartilage (Fig. 47), before interstitial mitosis of cartilage cells has begun, the cells are not grouped but scattered singly in the cartilaginous mass. The branching shape which some of the cells show at this stage is lost as the cartilage matures.

The Intercellular Substance. The intercellular substance or *matrix* appears homogeneous in the fresh condition or in ordinary preparations. Only the walls of the lacunae stand out as more highly refractive rings which stain intensely with basic dyes. These so-called capsules are not of the nature of cell membranes, but represent a modification of the matrix surrounding the cell spaces.

The apparently homogeneous intercellular substance contains in reality numerous fine collagenous fibers which are masked by a ground substance of a similar index of refraction. By means of

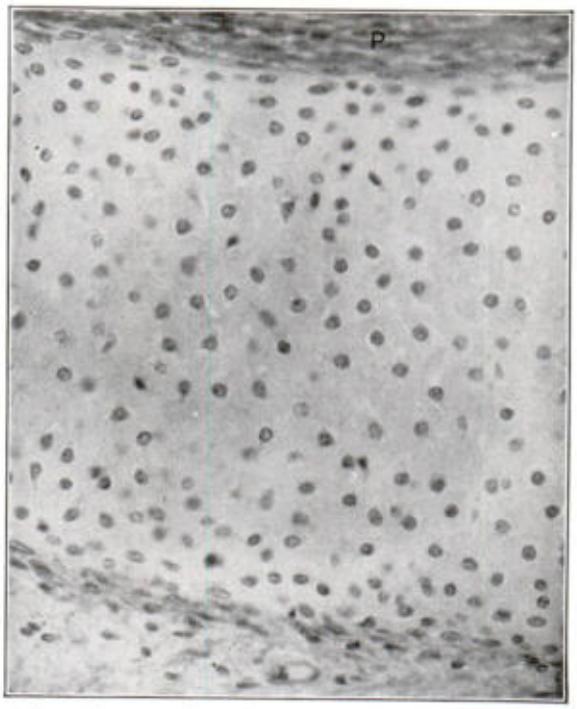

FIG. 47. Hyaline cartilage from trachea of seven-month human fetus. The cells are scattered singly in the homogeneous, strongly basophilic matrix. Some of the cells still have a branched appearance. *P*, Perichondrium. Photograph.

trypsin digestion or treatment with dilute alkalis, these fibrils may be demonstrated and their distribution studied. They are not arranged in bundles but form a fine felt-work. In the superficial portion of the plate they run parallel with the perichondrium, and become continuous without sharp transition with the connective tissue of the latter. In the deeper portions they run radially between the cell groups, while each group is itself surrounded by rows of concentrically arranged fibrils.

The *ground substance* consists of chondromucoid, chondroitin sulfuric acid and albumoid. Chondromucoid is a mucinogenous substance staining with basic dyes. It is soluble in alkalis and may be reprecipitated by acids. It is soluble in water. It is most abundant in embryonal cartilage, but in the mature tissue it is concentrated in the territories immediately surrounding the cell groups (*territorial matrix*) (Fig. 45). With age the chondromucoid content becomes greatly diminished.

The chondroitin sulfuric acid is mainly found in association with chondromucoid in the territorial matrix, and with the latter is responsible for the basophilic properties of cartilage. It is soluble in water. By treatment with alkalis, or by long immersion in water, the basophilic character of cartilage is lost.

Albumoid is an albuminoid substance rich in sulfur and related chemically to elastin and keratin. It is practically absent in fetal cartilage and in mature tissue is found mainly in the matrix between the cell territories (*interterritorial matrix*). It increases with age, and together with chondroitin sulfuric acid forms the main constituent of old cartilage matrix.

On boiling, cartilage yields a slowly gelatinizing heterogeneous substance (chondrin), which consists of gelatin, chondromucoid, albumoid and probably other substances. By previous removal of the chondromucoid, a purer and more quickly forming gelatin is obtained. Cartilage has a high water content (about 70 per cent) and is rich in sodium, potassium and chloride.

While the matrix of embryonal cartilage is uniformly basophilic (Fig. 47), adult cartilage shows interesting color reactions to histological dyes. When stained in a proper solution of a basic and acid dye, definite acidophilic and basophilic areas may be distinguished (Fig. 45). The capsule is the most basophilic structure and stains a deep blue. The territories immediately surrounding the cells likewise appear blue. On the other hand, the subperichondrial layers are stained red, and pink or reddish strands extend into the matrix between the cell territories. According to Hansen, the acidophilic collagen is completely masked by the large amount of chondromucoid and chondroitin sulfuric acid in the territorial matrix. That collagenous fibers are present in these areas can be demonstrated by proper technique.

Development and Growth. The blastema of cartilage is first recognized as an area of mesenchymal condensation. The cells become closely packed, the intercellular spaces disappear and the cell boundaries are difficult to make out, the anlage presenting the picture of a richly nucleated syncytium. In the interior of this *precartilage* the ground substance makes its first appearance as delicate, dark-staining lines or partitions which separate the individual cells and extend like a honey-comb throughout the entire mass. By the continued formation of the intercellular substance, the cells become widely separated from one another and the cartilage loses its richly cellular character. Collagenous fibers are formed simultaneously with the ground substance but soon become

masked, the whole intercellular substance appearing homogeneous and reacting strongly to basic dyes. The embryonal cartilage now appears as a plate consisting of cells scattered in a homogeneous matrix, and surrounded by a perichondrium of embryonal connective tissue (Fig. 47). Further growth takes place in two ways: (a) by the building up of masses through new formation at the surface, called appositional growth and (b) by the expansion of the whole internal mass, called interstitial growth.

At first growth is by peripheral accretions of cartilage formed in the perichondrial connective tissue. The innermost cells of the latter become transformed into cartilage cells by the formation around them of chondromucoid and chondroitin sulfuric acid. The collagenous fibers become masked, the intercellular substance assuming the homogeneous appearance of cartilage matrix. In this manner new cartilage cells and matrix are added to the cartilage of the original blastema. Such *subperichondrial* or *appositional* growth probably continues until the mature state is reached.

The first evidence of *interstitial* growth is seen in the mitoses of the cartilage cells. The cartilage cells divide into two, and the daughter cells may divide again, each cell group of adult cartilage representing the progeny of a single parent cell. The cells become separated from each other, each surrounded by its own capsule and intercapsular matrix. The old capsule sometimes remains, but usually it becomes less basophilic and merges into the territorial ground substance. This process of cell division and matrix formation takes place simultaneously through the cartilage mass and leads to a uniform interstitial expansion of the whole plate. Interstitial growth occurs mainly in young cartilage and gradually ceases, further growth being entirely appositional (subperichondrial).

Nutrition of Cartilage. Cartilage is devoid of vascular and lymphatic channels, hence nutrition is entirely by diffusion and imbibition. That the matrix is permeable even to coarse particles has been definitely shown. Arnold has demonstrated that injection of indigo carmine into the circulation leads to a deposition of the colored particles within the cell capsule as well as intercapsular matrix.

Age Changes. The poor nutrition is probably responsible for certain degenerative changes found in old cartilage, especially in plates of considerable thickness. The deeper portions of the plate show areas extending through many cell territories where the homogeneous matrix is replaced by closely packed coarse fibers (*asbestfaserung*, amiantine degeneration). Cavity formation due to the softening and liquefaction of these areas may ultimately result.

With old age, cartilage loses its translucency and bluish-white color and appears yellowish and cloudy. This change is due to a loss of chondromucoid and an increase in the amount of albumoid.

Calcification is likewise of common occurrence in old cartilage, and is usually associated with degenerative changes of the cartilage cells. The calcareous particles are at first deposited in the vicinity of the cells and then slowly spread into the matrix. Calcification of cartilage is a normal process during bone formation and will be described with the latter.

Regeneration. Regeneration of cartilage is a slow process and possibly does not occur at all, in many instances. When cartilage is broken, or injured, the wound is invaded by the perichondrial connective tissue which is gradually changed into cartilage. Such growth is in the nature of apposition and depends on

the presence of a perichondrium. Regeneration, in part at least, by interstitial growth has been observed, but is doubtless comparatively rare. In many instances of cartilage fracture, the pieces become united by dense fibrous tissue which may partly be replaced by a bony clasp.

Elastic Cartilage

Elastic cartilage appears more yellow and opaque than hyaline in the fresh condition, due to the large number of elastic fibers in its matrix (Fig. 48). These branch and run in all directions and form a dense network of anastomosing and interlacing fibers. In the peripheral layers the fibers are thin and the network wide-meshed; in the deeper portions they are thicker and more closely packed. As in hyaline cartilage the ground substance contains collagenous fibrils, which may be demonstrated by trypsin digestion.

Elastic cartilage develops from a hyaline blastema. Elastic fibrils are formed just peripheral to the cells and traverse the matrix as an elastic network. Growth of the cartilage takes place interstitially and subperichondrially. Calcification of elastic cartilage occurs very rarely, if at all.

Elastic cartilage occurs in the external ear, the Eustachian tube, the

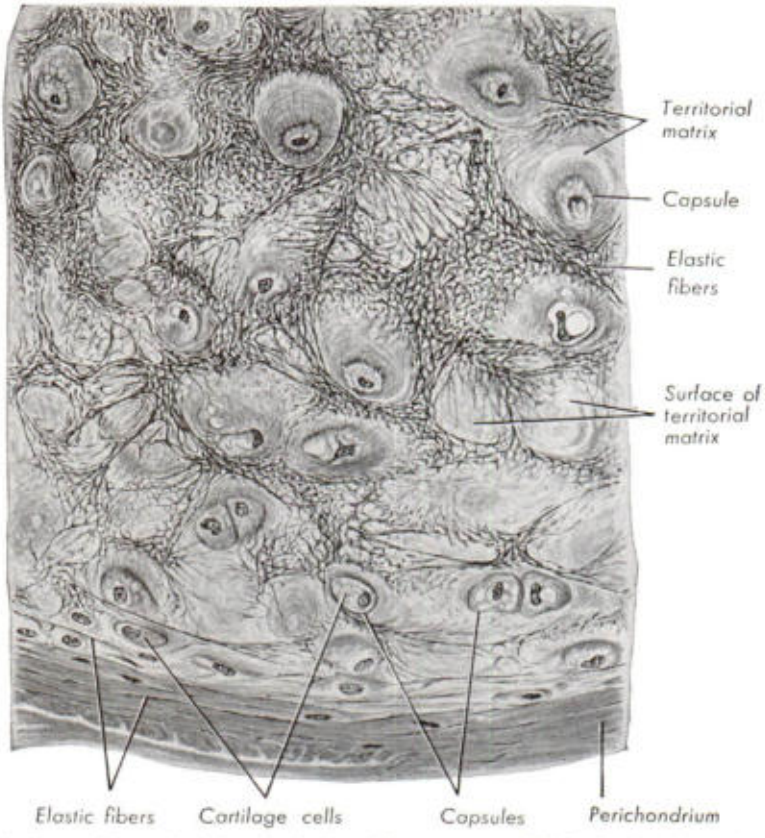

Fig. 48. Elastic cartilage of epiglottis of a boy thirteen years old. Stained with elastin H, Delafield's hematoxylin and eosin. ×435.

epiglottis and in some of the laryngeal cartilages.

Fibrous Cartilage

Fibrous cartilage (Fig. 49) is a combination of dense collagenous fibers and cartilage cells, the latter being enclosed in capsules which in turn may be surrounded by variable amounts of hyaline matrix. The relative proportions of collagenous fibers, cartilage cells and hyaline matrix vary greatly. The cartilage cells, enclosed in their capsules, frequently lie in rows between which are dense wavy bundles of collagenous fibers.

Fibrous cartilage occurs in association with some of the joints of the body. It is found where the attachment of a ligament or tendon to bone is adjacent to the hyaline cartilage of the articular surface or where hyaline cartilage intervenes between the attaching fibers and the bone. It occurs in considerable amounts in articular cartilages, in the glenoid and cotyloid ligaments, and in the ligamentum teres of the femur.

The *intervertebral discs* consist largely of fibrocartilage, which is continuous above and below with the articular cartilage of the adjacent vertebrae, and peripherally with the spinal ligaments. In the center of each disc is a gelatinous ellipsoid mass of variable extent known as the *nucleus pulposus*. Its center may contain fluid and cellular debris. Rupture of the disc and herniation of the nucleus pulposus into the spinal canal may be the cause of severe pain.

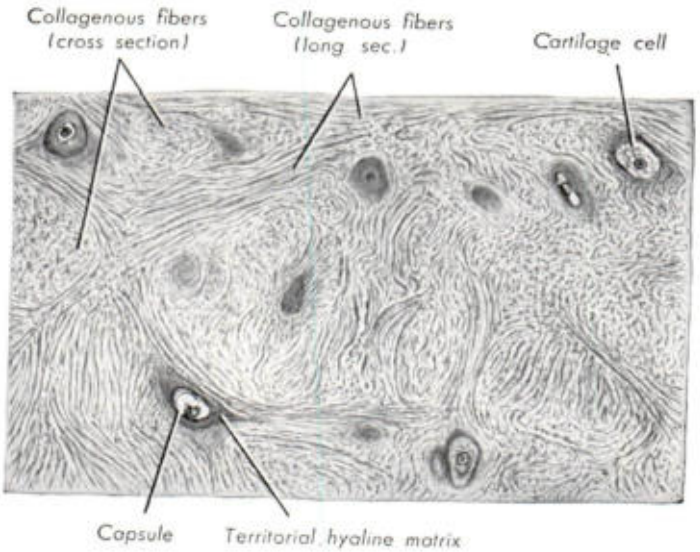

Fig. 49. Fibrocartilage from the intervertebral disc of a boy thirteen years old. The section was cut transversely to the broad (vertebral) surface of the disc. The part drawn is about 5 mm. from the ventral rim of the disc. Near the rim, the tissue is much like tendon. ×435.

BONE (OSSEOUS TISSUE)

Osseous tissue is a rigid form of connective tissue and is normally organized into definite structures, the bones. These form the skeleton, serve for the attachment and protection of the soft parts, and by their attachment to the muscles act as levers which bring about bodily motion.

Like cartilage or other forms of connective tissue, bone consists of cells, collagenous fibers and a viscid ground substance of a muco-albuminoid character, the various organic elements showing a definite structural organization characteristic of bone.

The rigidity of osseous tissue is due to the large amount of calcareous material deposited in its matrix. The inorganic matter constitutes about two-thirds of the bone weight and is composed of calcium phosphate (about 85 per cent), calcium carbonate (10 per cent), and small amounts of calcium fluoride and magnesium chloride. In spite of their large amount, the calcium salts are present in an extremely fine state of dispersion, and reflect the structural organization of the organic constituents with which they are intimately associated and combined. Thus when the organic constituents are completely destroyed by prolonged burning with free access of air (calcination), the inorganic material is left as a white brittle mass retaining the original form and size of the bone.

The shape and size of the bone are similarly retained when the lime salts are removed by decalcification, as with

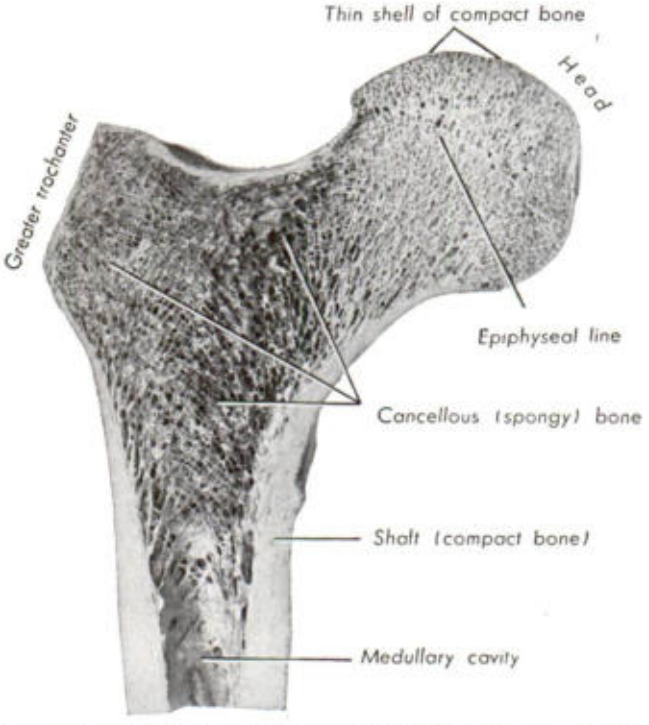

FIG. 50. A longitudinal section through the upper end of the femur of an adult male. The epiphyseal line of the great trochanter is not evident. Photograph.

prolonged immersion of the bone in a dilute mineral acid. The bone loses its rigidity, has a silky sheen, and is flexible but tough, and only slightly extensible. On boiling it yields a gelatinizing mass (ossein) consisting of gelatin and mucoalbuminoid substances from the matrix (osseomucoid, osseoalbumoid).

Gross Organization of Bone Tissue. Grossly, two types of bone may be distinguished: the *spongy* or *cancellous*, and the *dense* or *compact*. When a long bone is cut longitudinally (Fig. 50), it will be seen that the head or epiphysis has a spongy appearance and consists of slender irregular bone trabeculae or bars which anastomose to form a latticework in the meshes of which the marrow is contained. The thin outer shell, however, appears dense and white, and as the shaft is approached, the irregular marrow spaces of the epiphysis become continuous with the central medullary cavity of the shaft whose wall is formed by a thick plate of compact bone.

The spongy and compact varieties do not represent histologically distinct types of osseous tissue, but differ only in the degree of porosity. In spongy bone the spaces are large and the bony matter reduced to slender spicules and bars. Compact bone is likewise porous, but the channels are narrow, the bony tissue abundant and more densely packed. The histological character of the cells and intercellular substance is the same in both.

With very few exceptions, the compact and spongy forms are both present in every bone, but the amount and distribution of each type varies considerably. The diaphyses of the long bones consist mainly of compact tissue, only the innermost layer immediately surrounding the medullary cavity being spongy. The tabular bones of the head are composed of two plates of compact bone enclosing a marrow space bridged by irregular bars of spongy bone (diploë). The epiphyses of the long bones and most of the short bones consist of spongy bone covered by a thin outer shell of compact bone.

Each bone, except at its articular end is surrounded by a vascular fibroelastic coat, the *periosteum*. The so-called *endosteum* or inner periosteum of the marrow cavity and marrow spaces is not a histologically distinct membrane, but is a somewhat concentrated medullary connective tissue in immediate contact with the bone.

Finer Structure. The most characteristic feature of adult bone tissue is its lamellar structure, the fibers and calcified matrix being organized into thin layers or lamellae arranged in various ways. When the compact osseous tissue of a long bone is examined (Figs. 51, 52, 53), it is seen to be traversed by longitudinal channels, the *Haversian canals*, which anastomose with each other by oblique and transverse communications. From the periosteal and endosteal surfaces, somewhat narrower channels, *Volkmann's canals*, pierce the bone obliquely or at right angles to its long axis and communicate with the Haversian canals, thus establishing a continuous and elaborate canal system which lodges the blood vessels and nerves of the bone.

In a cross section of the bone, the Haversian canals are seen to be surrounded by a varying number (8–15) of concentric lamellae, these and the central canal constituting an *Haversian system* or *osteone* (Figs. 52, 53). The whole bone does not, however, consist of such concentric systems. In the periphery the lamellae run parallel with the surface and form a relatively thin outer layer of the bone. These are the outer *circumferential* or *general* lamellae (Fig.

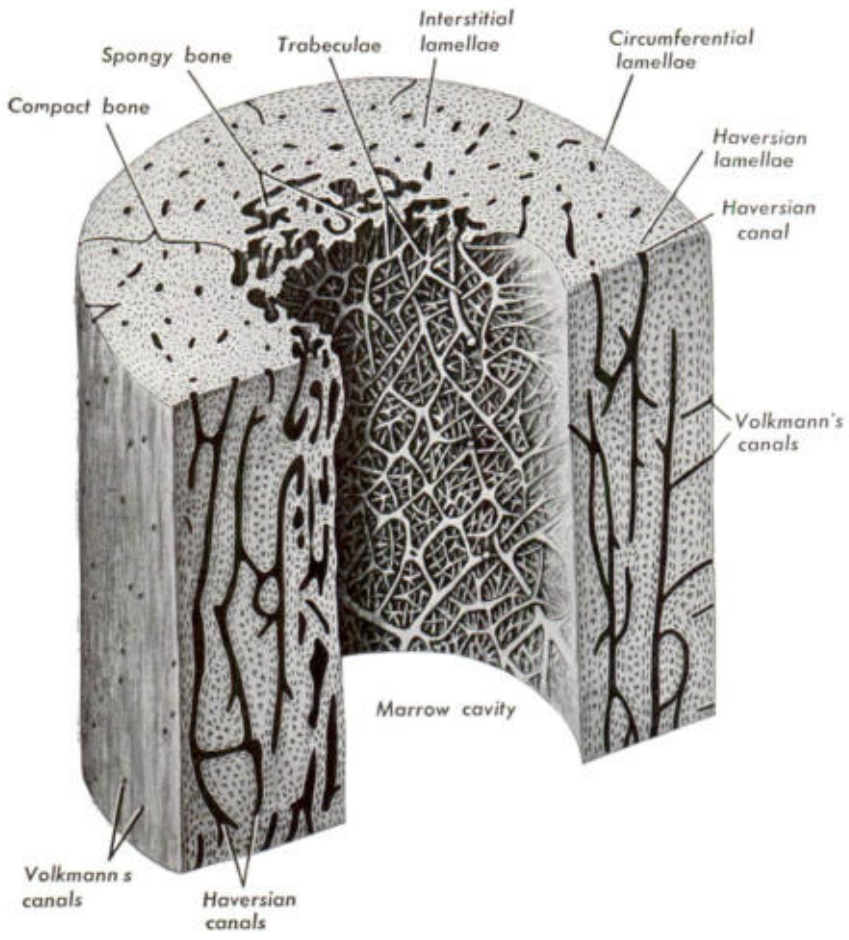

FIG. 51. Shaft of human humerus. Drawing made from macerated (undecalcified) bone. (From a chart by H. Poll.)

51). Similarly arranged inner circumferential lamellae separate the Haversian systems from the marrow cavity. Finally, the intervals between the Haversian systems are occupied by more irregular layers of bone which constitute the *interstitial* lamellae (Fig. 52). Adjacent lamellar systems are as a rule sharply delimited from each other by a dark-staining, thin layer of modified matrix (cement line, cement membrane).

Compact bone thus consists of branching and anastomosing tubular lamellae, whose intervals are filled in by interstitial lamellae and which are covered externally and internally by the more parallel running circumferential lamellae. The channels which pierce the bone from its outer and inner surface and become continuous with the Haversian canals, are not lined by concentric lamellae and are known as the canals of Volkmann.

The bone cells (*osteocytes*) are placed in flattened almond-shaped cell spaces or lacunae situated between or within the lamellae (Figs. 54, 66). Numerous delicate

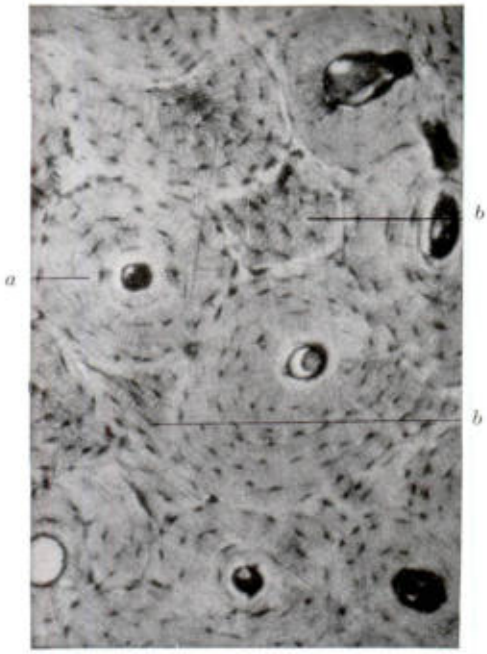

FIG. 52. Transverse section from the shaft of an undecalcified dried long bone. A slab of the dried bone was ground very thin and then mounted in thick balsam. In the spaces within the bone (lacunae, canaliculi and some of the Haversian canals) air was imprisoned so that they appear dark. *a*, Haversian system; *b*, interstitial lamellae. Photomicrograph.

channels, the *canaliculi*, extend at right angles from the lacunae, and many of these anastomose freely with the canaliculi of adjacent lacunae (Fig. 54). Many canaliculi of the outermost and innermost bony lamellae open respectively on the periosteal and endosteal surface, and in a similar manner the canaliculi communicate with the Haversian and Volkmann's canals. Thus besides the network of larger channels which carry the blood vessels, there is present a system of more minute canals, consisting of lacunae and canaliculi, which serves for the passage of nutrient fluids.

The wall of the lacuna immediately surrounding the cell is free from fibrils and shows a striking resistance to chemical reagents. When the rest of the matrix is dissolved by prolonged immersion in concentrated mineral acids, the wall or capsule remains intact and may be isolated with the enclosed bone cell. A similar resistance is shown by the modified matrix forming the cement membranes between lamellar systems.

The cells in the lacunae, *bone cells* or *osteocytes* (Figs. 54, 66), are flattened cells with a number of processes which extend into the canaliculi. They were bone forming cells (osteoblasts) but became imprisoned in the bone as it was deposited. Cells of similar origin form an incomplete lining of the Haversian canals and Volkmann's canals, and are present in the endosteum and inner layer of the periosteum. In the resting condition they are flattened branching cells similar to fibroblasts. With a stimulus such as is supplied by a fracture or physiological stress they again may become active bone forming cells.

The distribution of the collagenous fibrils can only be studied in decalcified preparations where proper precautions have been taken to prevent collagenous swelling. Otherwise the collagen appears as a fused homogeneous mass. The fibrils are arranged in delicate fascicles, separated by interfascicular matrix, which run a parallel course within a single lamella. The direction of the fibers may differ, however, in the various lamellae, the fibrils of one often passing at right angles to those of another. This is especially striking in transverse sections of the Haversian systems, where the concentric lamellae appear alternately striated and punctated. In the former the fibrils run somewhat circularly and hence are cut longitudinally. In the punctated lamellae the fibrils run more parallel to the long axis of the canal and appear in section as fine dots. The fibrils themselves

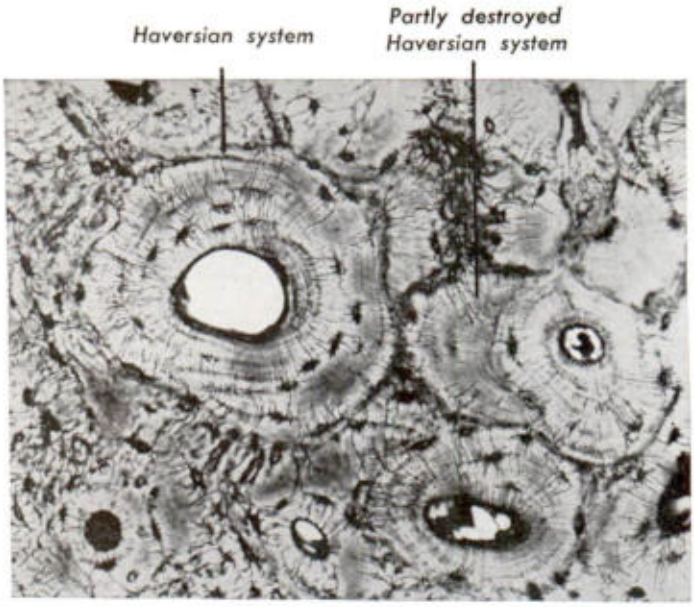

FIG. 53. Transverse section through the shaft of the femur of a young adult rhesus monkey. Decalcified, 6 μ section. The walls of the lacunae, canaliculi and Haversian canals are stained with a dye. Schmorl's thionin phosphomolybdic acid method. Photomicrograph. $\times 220$.

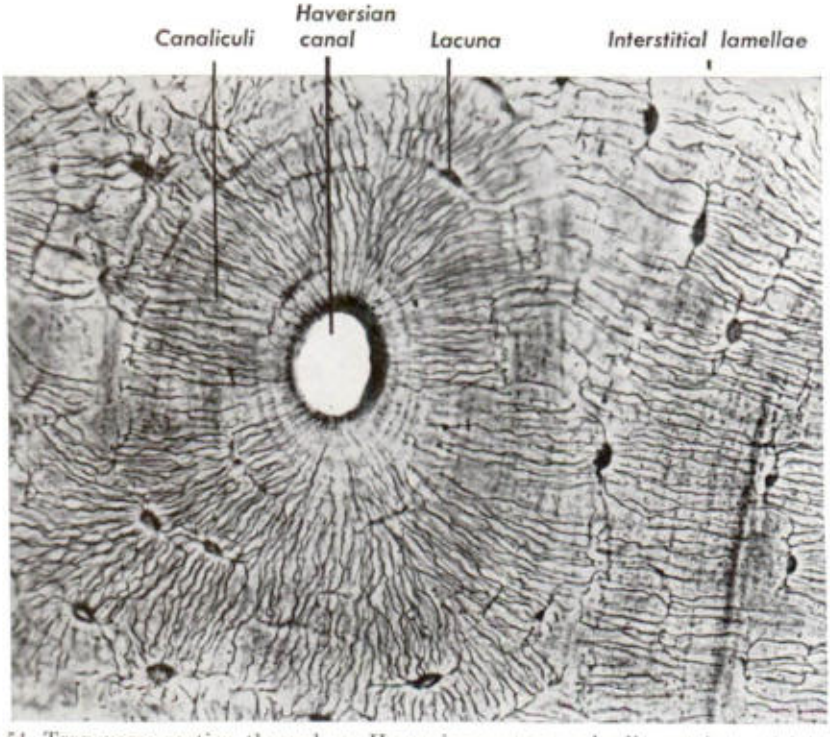

FIG. 54. Transverse section through an Haversian system and adjacent interstitial lamellae. Decalcified bone from the shaft of the femur of a young adult rhesus monkey. 6 μ section. Schmorl's thionin phosphomolybdic acid method. Photomicrograph. $\times 400$.

are not calcified and when removed by calcination the spaces occupied by them appear as delicate channels permeating the calcareous substance.

Besides the lamellar fibers, there are found in the outer layers of the bone the coarser perforating fibers of Sharpey (Fig. 55). They are continuations of the periosteal fibers and pierce the bone obliquely or at right angles to its long axis. They consist of collagenous or fibro-elastic bundles with uncalcified or only partly calcified matrix. Purely elastic perforating fibers are likewise found. They extend into the outer circumferential and interstitial lamellae, but never penetrate the Haversian systems. They are especially numerous in places where ligaments and tendons are inserted, and serve for firmer anchorage of these structures.

FIG. 55. Fibers of Sharpey in cross-section of decalcified phalanx from child of six years. The fibers of Sharpey are direct continuations of periosteal fibers. (After Petersen.)

Spongy bone shows the same lamellar structure but differs from compact bone in the more irregular arrangement of its lamellae (in trabeculae or spicules) and the relatively few Haversian systems.

Non-lamellar (Reticulated) Bone. The embryonal skeleton has at first no lamellated structure. The lacunae are large and irregularly disposed. The collagenous fibers are coarser and form an interlacing network similar to that of ordinary connective tissue (Fig. 56). It appears in fact as calcified connective tissue. Such reticulated bone forms a considerable part of the adult skeleton of lower vertebrates. In man it is replaced after birth by the lamellar variety, and

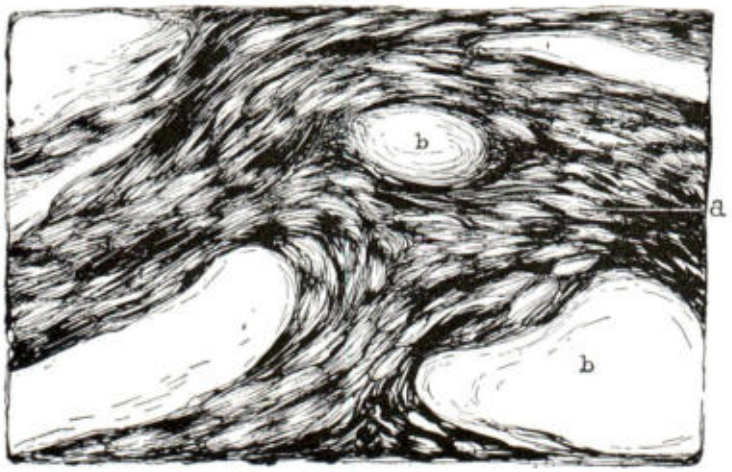

FIG. 56. Non-lamellar (reticulated) bone from the skull cap of a seven months fetus. *a*, Lacunae; *b*, marrow spaces. (After Petersen.)

only traces persist in the adult condition. It is found in the bony sutures, and often in places where tendons are inserted. Some interstitial lamellae occasionally show a reticulated structure.

DEVELOPMENT AND GROWTH OF BONE

According to the embryological origin there are two types of bone development, *intramembranous* and *intracartilaginous* or *endochondral*. In intramembranous bone formation the bone develops under or within a connective tissue membrane. It does not involve the removal of cartilage. In endochondral bone formation, cartilage is removed and replaced by bone. The development of the flat bones of the skull involves only the intramembranous type of bone formation. The development of the bones of the base of the skull, of the face and of the axial skeleton involves both types of bone formation, the bone around a cartilage (subperiosteal bone) being formed by the intramembranous type and the bone which replaces the cartilage being formed by the endochondral method. It should be kept in mind, however, that the fundamental process of bone deposition is the same in both types. In intracartilaginous bone formation there is the additional feature of the removal of the cartilage preparatory to the deposition of the bone.

Intramembranous Bone Formation. Since the development of the flat bones of the skull involves only intramembranous bone formation, it is an excellent place to study the structural features of the deposition of osseous tissue, uncomplicated by cartilage removal. In the locations where bone is to be laid down the mesenchyme becomes richly vascularized and active proliferation of the mesenchymal cells takes place. Within this primitive connective tissue membrane, some of the cells show structural changes which enable one to identify them as bone-forming cells or

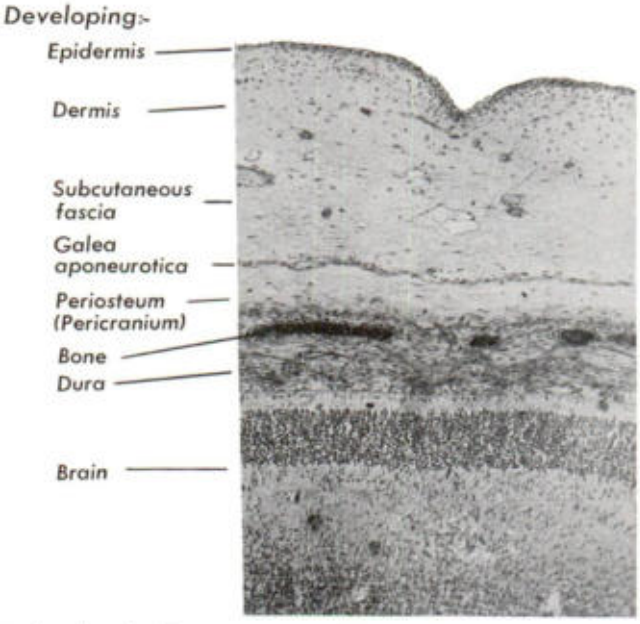

FIG. 57. Section showing the first appearance of bone in the parietal region of the developing skull. Human embryo of about two and one-half months. Photomicrograph. ×60.

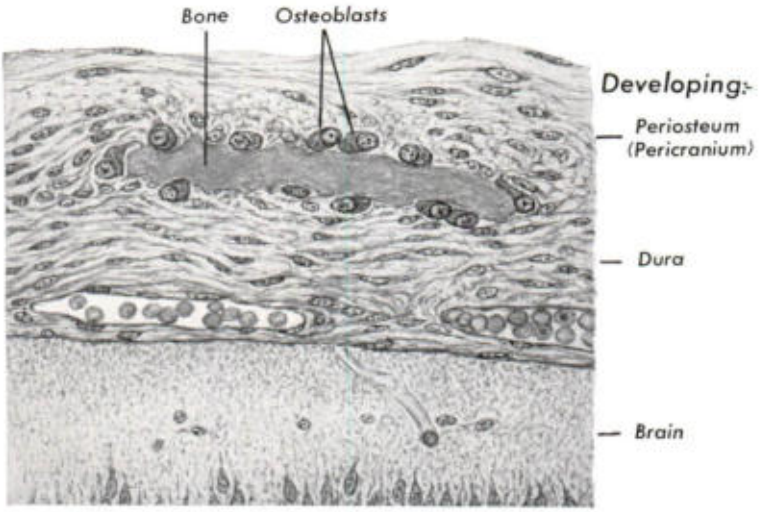

FIG. 58. One of the spicules of developing bone. Parietal region of the skull of a human embryo of about two and one-half months. Camera lucida drawing. ×420.

osteoblasts. They enlarge and their cytoplasm becomes more basophilic. Although the processes of the cells are retained, they are difficult to see and the cells thus frequently appear to have a smooth contour. In shape, the body of the cells is irregularly polyhedral or cuboidal.

Between the enlarged cells or osteoblasts an acidophilic hyaline ground substance appears. It masks the fibrils which were already present in the embryonal connective tissue as well as those that are subsequently formed by the osteoblasts. The intercellular substance or matrix, which thus is composed of a hyaline ground substance and collagenous fibers, is usually not calcified at first and is soft and easily cut. It is given the name of *osteoid* (resembling bone) and is the organic part of bone matrix without the inorganic constituent. As the deposition of the matrix progresses, certain of the osteoblasts, with their processes, are imprisoned by matrix being deposited around them, lacunae and canaliculi thus being formed. Since processes of adjoining cells connect with each other, the canaliculi necessarily connect adjoining lacunae. Only part of the osteoblasts are imprisoned, however, so that a layer of these cells is retained on the surface of the matrix. Their number is maintained by mitosis and probably also by the formation of osteoblasts from undifferentiated cells of the connective tissue.

CALCIFICATION. The intercellular substance of developing bone becomes calcifiable through some transformation which is supposedly brought about by the osteoblasts. In calcification the union of matrix and salts is very intimate due to the finely divided state and uniform distribution of the minerals. The minerals are deposited in the form of minute crystals which appear first in intimate association with the collagenous fibers. The crystals are too small to be visible under the light microscope, but they can be studied in electron micrographs where they are seen on the surface of the fibrils and in the interfibrillar areas. They have also been seen within the fibrils in the early stages of fetal bone development. The crystals are larger in adult than in

fetal bone. They are rod-shaped and tend to be oriented in the same directions as the fibers in adult bone.

There is often a lag in the deposition of mineral salts in the organic matrix. This accounts for the thin pale-staining layer of uncalcified osteoid frequently seen at the edges of growing bone (Fig. 59).

Although the formation of osteoid normally is followed by the deposition of bone salts in it, this is not the case when the calcium-phosphorus ratio in the blood is disturbed. This disturbance produces rickets, a condition in which there is an excessive formation of osteoid, particularly at the juncture of the epiphyseal plate (cartilage) and the shaft in endochondral ossification. Even though the bone is thickened at this zone due to abnormal amounts of osteoid, nevertheless it lacks rigidity and with pressure (and tension) becomes misshapen.

FURTHER GROWTH AND RESORPTION OF BONE. As growth continues at the foci of bone formation, plates and trabeculae of bone are formed. These enclose spaces (primary marrow cavities) and under very low powers of the microscope give to the bones, as a whole, a spongy appearance. In these spaces are richly-vascularized connective tissue and primitive marrow cells (Fig. 60).

Very early in development, osseous tissue is deposited on both the inner and outer surfaces of the flat bones of the skull and on their peripheral edges or margins. The latter produces an increase in the surface area of the bones and causes, or at least accompanies, the enlargement of the cranial cavity. Absorption of the inner (brain) surface of the plates soon begins, which still further increases the

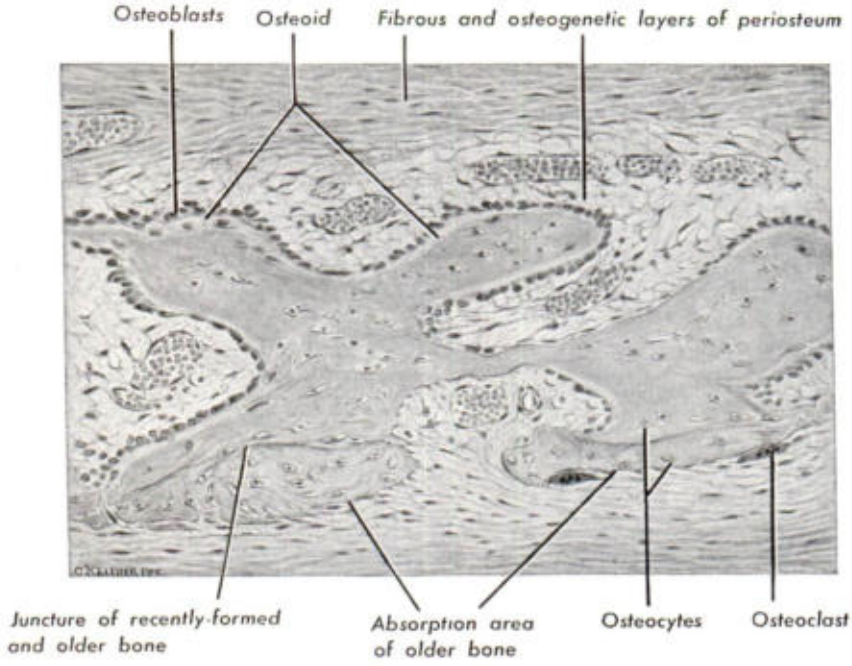

FIG. 59. Section through parietal bone of human fetus of three months. ×115.

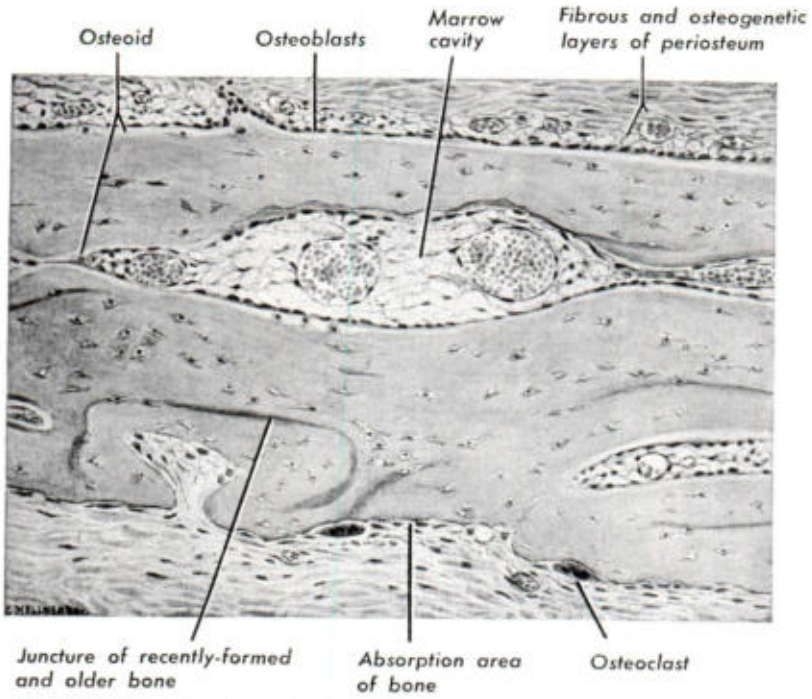

FIG. 60. Vertical section through parietal bone of human fetus of six months. ×115.

size of the cranial cavity. Bone deposition on the inner surface is not entirely absent, however, nor is bone resorption limited only to this surface.

OSTEOBLASTS. Osteoblasts are medium-sized cells which are usually arranged in a single row on the surface of growing bone. They are cuboidal or, more commonly, tall squamous with the long axis parallel to the surface of the bone. The cell bodies of the osteoblasts are separated from each other by intercellular spaces but cell processes, which can be distinguished by special methods, make contact between neighboring cells. The nucleus lies at one end of the cell, and close to it, in the cytoplasmic-rich part of the cell, one frequently sees a large and distinct clear zone which is the negative image of the Golgi apparatus (Fig. 65).

The cytoplasm of osteoblasts stains intensely with basic aniline dyes due to its high content of ribonucleic acid. As pointed out in Chapter I, RNA is abundant in cells which are active in protein synthesis. The cytoplasm also contains many small granules, about .3 μ in diameter, which stain with the periodic acid Schiff technique, indicating the presence of mucopolysaccharide. By a special technique, an abundance of alkaline phosphatase can be demonstrated in the cytoplasm of osteoblasts. It seems likely that the phosphatase, as well as the RNA and mucopolysaccharide granules, is related to the activity of the osteoblast in secreting material which forms the bone matrix (collagenous fibers and homogeneous ground substance).

The formation of bone in unusual regions of the body (metaplastic bone formation), as in the walls of sclerotic arteries, calcified foci in the lungs, in connective tissue adjoining a transplant of

transitional epithelium, etc., is somewhat difficult to correlate with osteoblastic specificity. The commonly accepted explanation is that fibroblasts with the proper stimulus and in the proper environmental condition, differentiate into osteogenetic cells as do mesenchymal cells in certain regions in the embryo.

OSTEOCLASTS. On the surface of bones (or calcified cartilage) where resorption is taking place, there are frequently, though not invariably, found large multinucleate giant cells which closely resemble the foreign-body giant cells seen at the sites of various pathological lesions (Fig. 65). They may occur in depressions in the bones (Howship's lacunae), on prominences, or on nearly flat surfaces. Osteoclasts appear to arise from mesenchymal cells, osteoblasts and, indirectly, from fibroblasts and reticular cells through the transformation of these cell types into osteoblasts.

The number of nuclei in the osteoclasts varies greatly. The cytoplasm is vacuolated and the zone in contact with calcified cartilage or bone usually has an irregular, comb-like appearance. Although this zone may appear to be a part of the cell, it is probably a fringe of collagenous fibers which are resorbed more slowly than the homogeneous ground substance of the matrix to which the osteoclast clings. They do not take up intravitally injected trypan blue but particles of degenerated cells have been seen in their cytoplasm. They are not actively phagocytic, however.

The fact that osteoclasts are usually present where bone and calcified cartilage are undergoing resorption led to the inference that they were the cells responsible for resorption. Although only a few can be found, in even a low power field of a bone or calcified cartilage undergoing resorption, nevertheless their total number is quite large. Dodds has estimated that there were 4000 of these cells on the calcified epiphyseal cartilage of the humerus in a growing kitten. When pronounced resorption of bone is produced by the administration of parathyroid extracts, osteoclasts in large numbers appear within 24 hours. Many of them will be located in depressions of the bone (Howship's lacunae). However, resorption may take place in the absence of osteoclasts, for instance in the so-called "creeping replacement" in bone transplants and in the removal of fragments of bone in fractures. It is attractive to attribute the resorption of bone to these

FIG. 61. Cartilaginous anlage of metacarpal bone of 4-cm. human fetus, showing changes in the central portion preparatory to ossification. The bone collar is beginning to form. Photograph.

cells but there is no direct evidence that they are the active agents in the resorption of either bone or cartilage.

Intracartilaginous Bone Formation. In this form of ossification, an embryonal type of hyaline cartilage precedes the formation of bone, and the shape of the bone will correspond more or less closely to that of the cartilage. During development this cartilage is replaced by bone except at the joint surfaces. This will not be completely accomplished, however, until the bone has achieved its full size, and growth has ceased.

During the replacement of the cartilage by bone, not only does the supporting function have to be maintained, but there is a continual increase in length and diameter, as well. The extensiveness of this growth can be realized by comparing the size of the fetal skeleton with that of an adult.

The first change which is indicative of beginning ossification takes place, in cartilages from which long bones will form, at about the center of the future shaft—the diaphyseal or *primary ossification center* (Fig. 61). The cartilage cells proliferate and hypertrophy and the lacunae correspondingly increase in number and size. The matrix between the lacunae becomes reduced in amount and forms but thin partitions. These, except for the cartilage capsules, then become calcified by the deposition of lime salts. This calcified portion stains more intensely with basic dyes than do the uncalcified capsules and the unchanged cartilage at either end of the ossification center.

At about the time that these intracartilaginous changes are clearly evident, the fibrocellular membrane of the cartilage (perichondrium) assumes an osteogenetic function. Some of its inner cells hypertrophy, become osteoblasts and a deposition of a perforated *bone ring* or *collar* around the center of ossification takes place. This bone collar is thin-walled and short at first but becomes progressively thicker-walled and longer as the ossification progresses. It is closely adherent to the cartilage and forms a splint that assists in maintaining the strength of the shaft which has been weakened by the dissolution of part of the cartilage. It is formed by an intramembranous type of bone development. The fibrocellular membrane which surrounded the cartilage has, in this region of ossification, now become a periosteum.

Vascular connective tissue sprouts—*periosteal buds*—from the periosteum now penetrate through apertures in the bone collar, into the periphery of the changed cartilage matrix (Fig. 62). They are highly vascular and although not containing definitive osteogenetic cells, they have undifferentiated cells which will form osteoblasts. These vascular buds erode the thin-walled partitions between the lacunae and thus form cavities, the *primary marrow spaces*.

The fate of the cartilage cells is not clear. Many of them show structural changes indicative of degeneration, and undoubtedly die. Some investigators maintain, however, that a part of the cartilage cells revert to a primitive cell type, thus forming elements of the primitive marrow.

The primitive marrow cavity increases in size through the continued confluence of lacunae, the walls of which are absorbed by the capillary tufts from the primitive marrow. Some of the calcified cartilaginous matrix temporarily escapes destruction, however, especially at the sides and towards the ends of the primitive marrow cavity. On the walls of these surviving trabeculae of calcified cartilage, cells appear which can be identified as osteoblasts, and osteoid is deposited,

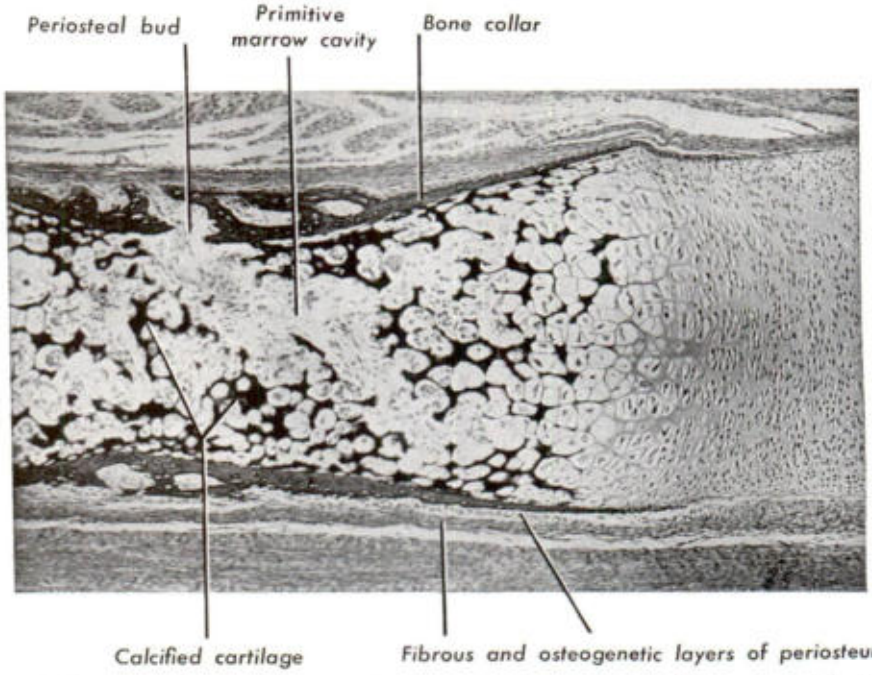

FIG. 62. Metacarpal bone of human fetus ten to twelve weeks old. The periosteal bud is only one of several as shown by an examination of serial sections. The primary marrow cavity is quite extensive. The zones of cell and lacunar enlargement and of cell multiplication are evident. Only a small amount of reserve cartilage has been included in the figure. (Photograph.)

which will later become calcified. These trabeculae will subsequently be resorbed as the marrow cavity continues to enlarge. They form a temporary supporting framework (Fig. 63).

The extension of the zone of ossification towards the ends of the cartilage is accompanied by an orderly sequence of changes in the cartilage similar to those which took place in the formation of the primary ossification center but the changes show a more distinct zonal arrangement (Fig. 64).

Several zones can be distinguished in the cartilage. Beginning at the ends of the cartilage and passing towards the ossification center, these zones which overlap each other somewhat are as follows:

1. A Zone of Reserve Cartilage. In the early phases of ossification, this zone is relatively long but later becomes shorter. It is composed of a primitive type of hyaline cartilage. Cell division and matrix formation occur in it and it grows in all directions although apparently at a slow rate.

2. A Zone of Cell Multiplication. This zone is formed of rows of cells, the cells of each row arising from a single cell in the reserve cartilage. The daughter cells do not form rounded or oval-shaped nests, but are more or less regularly arranged in rows which run parallel to the long axis of the cartilage. Each row consists of a number of cells (2–16). The lacunae containing the daughter cells are broad but flattened as are the cells, their long axes being transverse to the longitudinal axis of the cartilage. By these divisions of

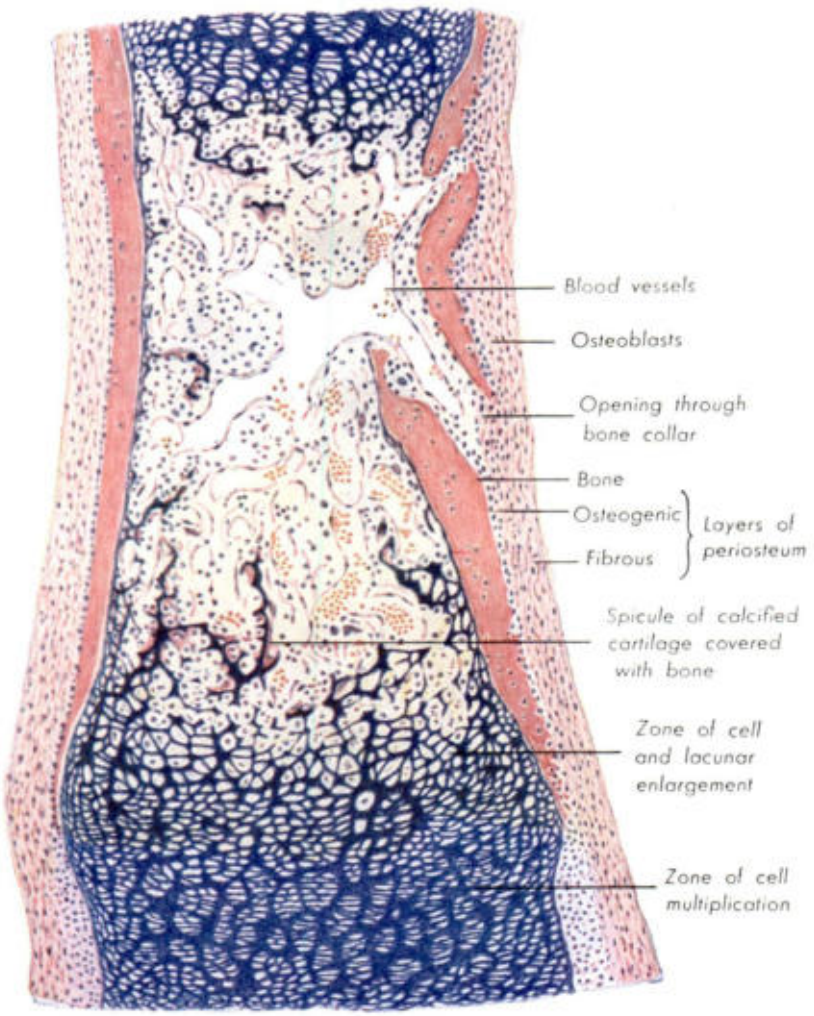

FIG. 63. Longitudinal section of middle phalanx of finger of a human embryo of about four months. Hematoxylin and eosin-azure II. ×80.

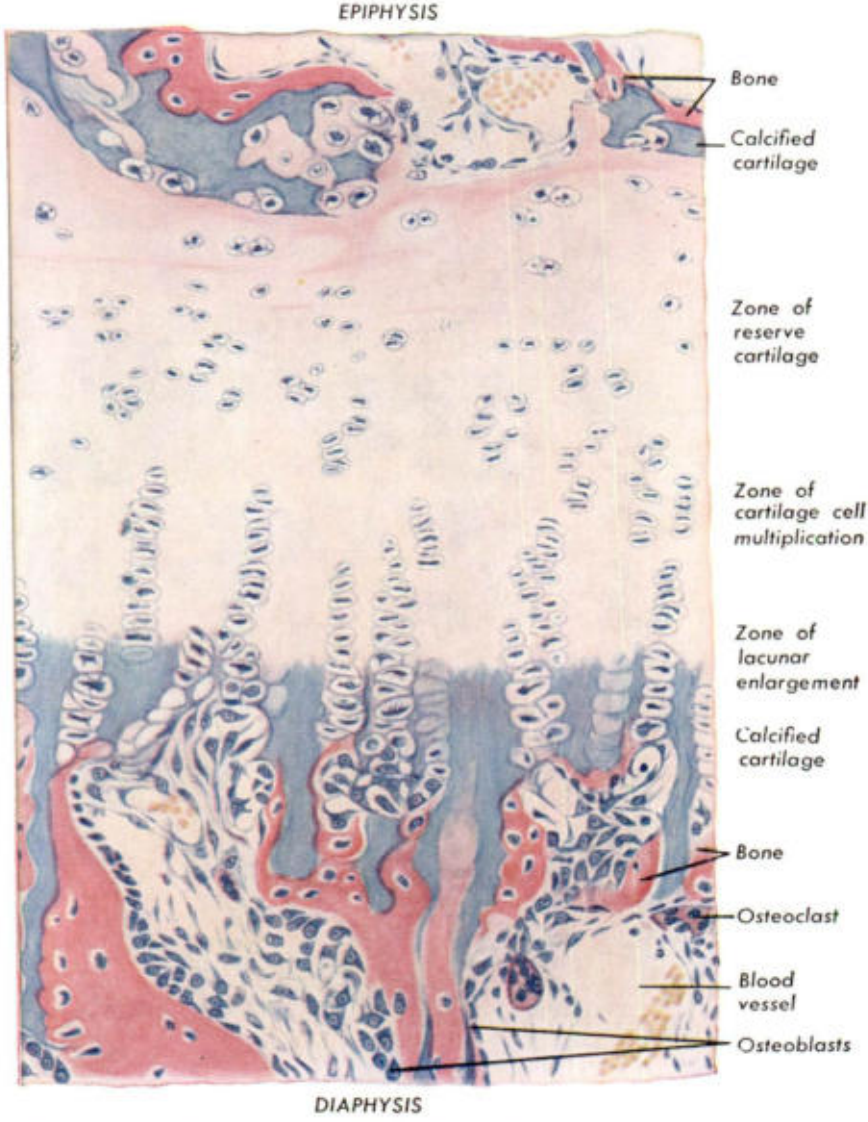

FIG. 64. Longitudinal section through the distal end of a metatarsal of a rhesus monkey two months of age. The epiphyseal (secondary) ossification center is well formed. Bone formation is taking place both at the epiphyseal and the diaphyseal surfaces of the epiphyseal plate. Delafield's hematoxylin and eosin. ×256.

cartilage cells and their distribution in rows, the length of the cartilage is increased more than is its diameter as new matrix is formed.

3. A Zone of Cell and Lacunar Enlargement. In this zone there is no further multiplication of the cells but they and their lacunae enlarge, becoming somewhat cuboidal in shape. This still further increases the length of the rows and of the cartilage columns (partitions) between the rows and correspondingly increases the length of the cartilage of this region. The cartilage separating the cells within a row, although originally small in amount, is somewhat reduced. It is composed only of capsular cartilage.

4. A Zone of Calcification. This zone is of variable width but is always narrow. It is limited to the distal part of the zone of enlarged lacunae.

5. A Zone of Cartilage Removal and Bone Deposition. In the outer part of this zone, the cartilage separating the cells within a row undergoes destruction, probably through the action of growing capillary tufts from the primitive marrow cavity. Longitudinal canals in the cartilage matrix are thus formed. Further towards the marrow cavity the calcified walls of these canals undergo a further destruction, though remnants of the longitudinal partitions remain. Some osteoclasts (chondroclasts?) are usually present. On the walls of remnants of the cartilage, osteoblasts appear and a coating of bone is deposited (Fig. 63).

As viewed in sections, cut parallel to the long axis of the developing bone, the cartilaginous walls between the longitudinal spaces look like isolated bars or trabeculae. They, however, are sections of continuous walls, as is shown by cross sections. They form an excellent framework to maintain the strength of the cartilage and their rigidity is increased by their calcification. Still further towards the center of the shaft, fragments of these walls, surrounded by bone, are present. Many of them, however, are undergoing

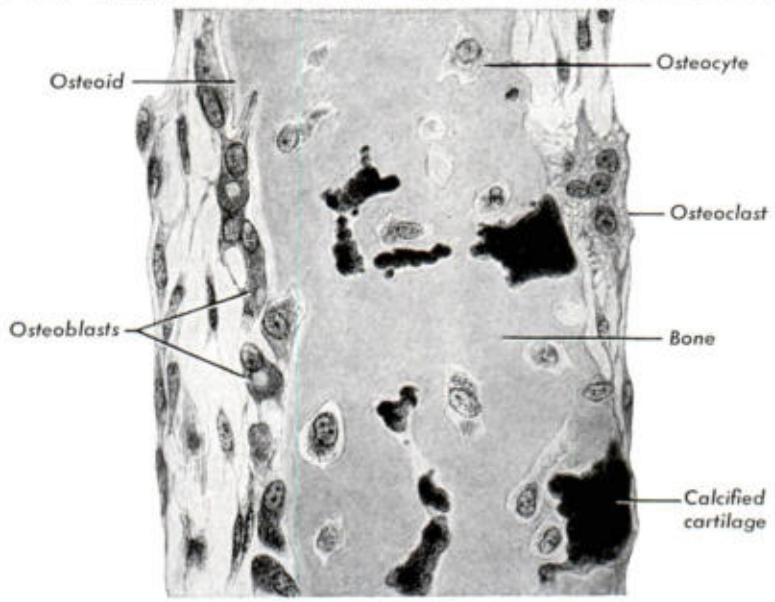

FIG. 65. Section of a trabecula in developing endochondral bone. Tibia of human fetus about three and one-half months old. Azure II-eosin stain. ×650.

resorption and all of them, as well as their covering of bone, will subsequently be resorbed as development proceeds and the marrow cavity enlarges (Fig. 65).

While these changes within the cartilage are taking place, the bone collar is increasing in length and in diameter by the deposition of new bone. The marrow cavity also increases in size not only by its longitudinal extension due to cartilage removal but also in diameter, due to resorption of the inner part of the bone collar or splint. These processes continue as development proceeds. The reserve cartilage, although undergoing cell division and growth, becomes relatively reduced in length, that part of it nearest the ossification center passing in turn through the stages of cell proliferation and row formation, and lacunar enlargement during which the cartilage is actually increased in length. The cartilage then calcifies and later the transverse partitions are dissolved through the action of penetrating vascular sprouts. The calcified longitudinal cartilage partitions subsequently are resorbed to a greater or lesser degree and on the remnants of their walls bone is deposited (Figs. 63, 64). These are subsequently resorbed as the marrow cavity increases in length. This process continues in both directions in the shaft.

At about the time of birth, ossification centers (epiphyseal or *secondary ossification centers*) appear in each end of the long bones. The cartilage in these centers passes through the same changes as in the diaphysis. The proliferation of the cartilage cells leads to nearly equal growth in all directions, however. The cartilage cells, each of which is enclosed in a lacuna, are arranged in irregularly-shaped nests and not in rows and the partitions between the nests run in various directions. As in the diaphysis the center is invaded by osteogenetic buds, and cartilage removal and bone deposition

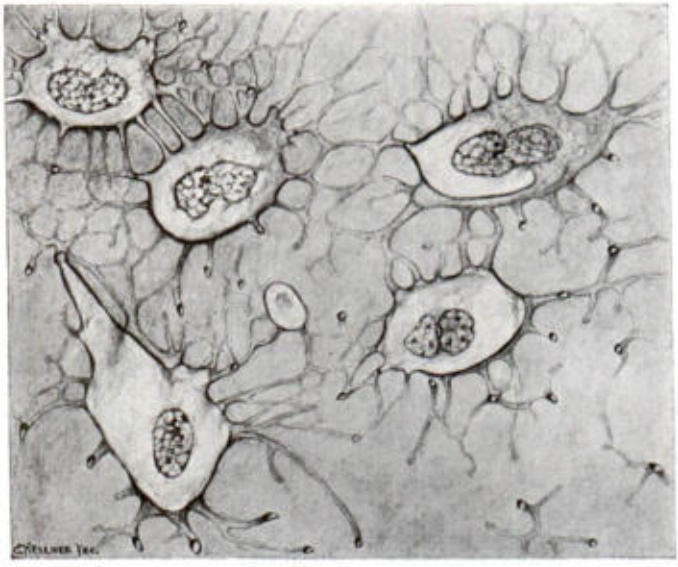

FIG. 66. Tangential section of bone collar (intramembranous bone) of developing ulna. Human fetus ten to twelve weeks old. It was possible to follow throughout a 10 μ section the extensive network of canaliculi due to the dark staining of their walls with the Azure II. Azure II-eosin stain. $\times 1350$.

take place. Bone trabeculae, however, remain in the epiphyseal cavity, giving it grossly a spongy appearance and the cartilage forming the articular surface persists, anchored to and supported by the underlying bone (Fig. 68).

The diaphysis continues its growth in length long after the epiphyseal centers appear. The cartilage plate which separates it from the epiphysis, called the *epiphyseal plate* (Fig. 64), continues to form new cartilage which is replaced by bone, a process which, as stated earlier, causes an increase principally in the length of the growing bone. When the cartilage of the epiphyseal plate is absorbed, growth of a bone ceases and the diaphysis is bound to the epiphysis by a bony union. The zone of this union is visible in the adult. It is called the *epiphyseal line*.

Haversian Systems. During the growth of the skeleton some of the spongy bone becomes transformed into compact bone. In this process lamellae of bone are deposited progressively inward on the surface of the cavities in the spongy bone until they are reduced to narrow canals. Each canal is traversed by blood vessels which originally were in the large cavity. The system of concentric lamellae with its canal and blood vessels forms an Haversian system.

Haversian systems are characteristically formed, however, in compact bones, i.e., in the shaft. A more complicated process from that stated above is involved in their formation for at the start of the process, there are no cavities of the size of Haversian systems present. The first step in the formation of Haversian systems in compact bone, is the erosion of tunnels in it by vascular sprouts from the medullary and periosteal surfaces.

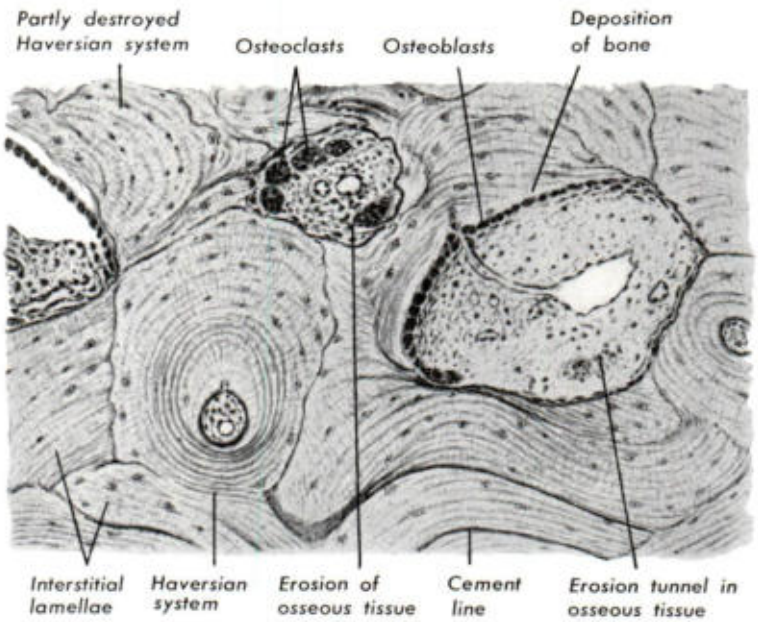

FIG. 67. Figure showing the absorption of bone and the formation of an Haversian system in compact osseous tissue. Proximal phalanx of the thumb of a child six years old. ×150. (After Petersen.)

The tunnels thus formed have irregular roughened surfaces but are of a fairly uniform diameter. When the absorption phase leading to the formation of the tunnel has been completed, osteoblasts differentiate from the cellular elements in the vascular sprouts. They come to lie next to the walls of the tunnel and deposit progressively inward concentric lamellae characteristic of an Haversian system. A narrow canal containing blood vessels is left—the Haversian canal. Parts of the original circumferential lamellae (ground lamellae) remain between the Haversian systems. However, new generations of Haversian systems keep forming throughout life although at a reduced rate in later years. In this process, parts of earlier formed Haversian systems as well as additional parts of the original circumferential lamellae are destroyed. Stages in the destruction of bone and its replacement by Haversian systems are illustrated in Figure 67.

The formation of Haversian systems appears to be due to mechanical stresses for it apparently leads to the formation of a stronger structure. It also serves in the maintenance of the calcium and phosphate levels of the blood, for bone serves as a reservoir or storehouse of these essential elements. When these elements are supplied from the bones they are not made available by their withdrawal leaving the organic elements of the bones intact (halisteresis) but both the salts and organic elements are removed. Destruction of bone can be produced by the experimental administration of parathyroid extract or by a tumor of the parathyroid gland which usually causes pronounced resorption of bone and its replacement by connective tissue (von Recklinghausen's disease).

It has been pointed out that perforating fibers of Sharpey do not enter the Haversian systems. An understanding of the developmental process will make the reason for this clear. Perforating fibers of Sharpey do not grow into bone. Rather they are radially or obliquely directed fibers which have been imprisoned by the advancing deposition of bone in much the same way that a branch of a tree becomes more and more enclosed in the growing trunk. When a vascular sprout forms a tunnel preceding the deposition of an Haversian system, the perforating fibers of Sharpey as well as the bone substance are resorbed and because of their method of formation the perforating fibers are not replaced.

Development of Short Bones. The development of the short bones is similar to that of the epiphyses of long bones. Ossification begins in the center of the cartilage and extends in all directions in the wake of the growing cartilage. Near the end of growth, the endochondral bone is invested with a thin compact layer of subperiosteal bone, except at the articular surfaces which remain cartilaginous.

Remodeling of Bone. It is obvious that bone, although dense, hard and rigid, is capable of changing somewhat in shape and also increasing or decreasing somewhat in amount, in response to environmental conditions. As already pointed out, these changes are not brought about by a remodeling such as might be done with a plastic wax, but involve the formation of new bone and the removal of bone already formed or the combination of both processes. This capacity to change in shape is well illustrated by the moving of improperly aligned teeth by the orthodontist and by the spontaneous movement of neighboring teeth after a tooth is extracted. The bony socket is resorbed ahead of the tooth undergoing movement and the shape and size of the socket is maintained by the formation of new bone back of the tooth. In other situations, as, for instance,

in increased muscular development, bone may be remodeled to meet the additional stresses imposed upon it. In old age resorption of the surfaces of some bones occurs. The changes in bone are much slower and less pronounced in the adult than during the period of growth. They probably occur to some degree throughout life and may be greatly accentuated, as at a fracture where a rebuilding and remodeling occurs in the injured bone.

Healing of Fractures. A fracture, as any traumatic injury, causes hemorrhage and tissue destruction. The first reparative changes thus are characteristic of those occurring in any injury of soft tissue. Proliferating fibroblasts and capillary sprouts grow into the blood clot and injured area thus forming granulation tissue. The area also is invaded by polymorphonuclear leucocytes and later by macrophages which phagocytize the tissue debris. The granulation tissue gradually becomes denser and in parts of it, cartilage is formed. This newly-formed connective tissue and cartilage is designated as a *callus*. It serves temporarily in stabilizing and binding together the fractured bone.

As the above described process is taking place, the dormant osteogenic cells of the periosteum enlarge and become active osteoblasts. On the outside of the fractured bone, at first at some distance from the fracture, osseous tissue is deposited. This formation of new bone continues towards the fractured ends of the bone and finally forms a sheath-like layer of bone over the fibrocartilaginous callus. As the bone increases in amount it invades the fibrous and cartilaginous callus and replaces it with a bony one. In the replacement of the fibrocartilaginous callus, the cartilage undergoes calcification and absorption as has already been studied in intracartilaginous bone development. Typical intramembranous bone formation also takes place. The newly formed bone is at first a spongy and not a compact type. It becomes transformed into a compact type and the callus becomes reduced in diameter. At the time when this subperiosteal bone formation is taking place, bone also forms in the marrow cavity. The medullary bone growing centripetally from each side of the fracture unites, thus aiding the bony union. The process of repair is, in general, an orderly process but varies greatly with the displacement of the fractured ends of the bone and the degree of trauma inflicted. Uneven or protruding surfaces are gradually removed and the healed bone, especially in young individuals, assumes its original contour.

The Periosteum and Endosteum

The *periosteum* is a fibrous membrane investing the bones, except at their articular surfaces. Its adherence to the bone varies in different places and at different ages. In the young bone it is easily stripped off. In the adult bone it is more firmly adherent and especially so at the insertion of tendons and ligaments, where more periosteal fibers penetrate into the bone as the perforating fibers of Sharpey.

The periosteum consists of two layers, the outer of which is composed of coarse fibrous connective tissue containing few cells but numerous blood vessels and nerves. The inner layer is less vascular but more cellular and contains many elastic fibers. During growth an osteogenetic layer of primitive connective tissue forms the inner layer of the periosteum. In the adult this is represented only by a row of scattered, flattened cells closely applied to the bone.

The periosteum serves as a supporting bed for the blood vessels and nerves going to the bone, and for the anchorage of tendons and ligaments. Its importance for bone regeneration has been a much

disputed topic. If the osteogenetic tissue is considered a part of the periosteum, the latter undoubtedly furnishes osteoblasts for growth and repair. However, the fibrous periosteum of the adult is itself a differentiated end product of the osteogenetic layer and probably plays no direct part in bone repair, but acts as an important limiting membrane controlling and restricting the extent of bone formation.

The *endosteum* lines the surface of cavities within a bone (marrow cavity, Haversian canals) and also the surfaces of trabeculae in the marrow cavity. In growing bone, it is formed of a delicate stratum of connective tissue beneath which is a layer of osteoblasts. In the adult, the osteogenetic cells become flattened, as in the periosteum, and are indistinguishable as a separate layer. They are capable of enlarging and becoming active, however, when there is a stimulus to bone formation, as after a fracture.

Marrow

Marrow is a soft tissue which occupies the medullary cavity of the long bones, the larger Haversian canals and all the spaces between the trabeculae of spongy bone. It consists of a delicate reticular connective tissue in the meshes of which lie various kinds of cells.

Two varieties of marrow are recognized: *red* and *yellow*.

Red Marrow. Red marrow (Fig. 78) is

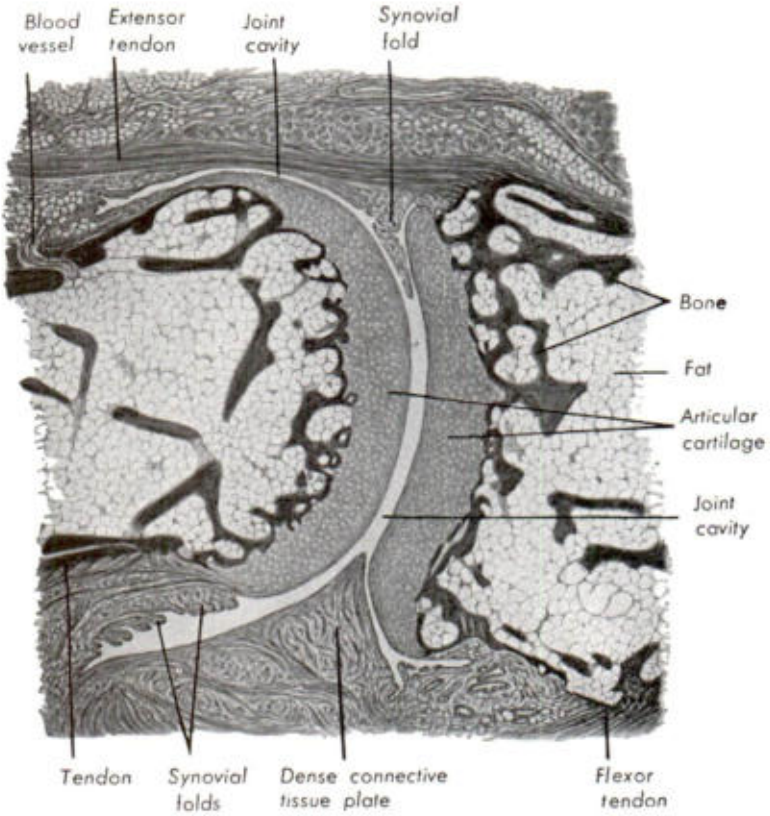

Fig. 68. Median sagittal section through the joint between the middle and terminal phalanges of middle toe. Human, ten years of age. The terminal phalanx is at the right. ×14.

the only type found in fetal and young bones, but in the adult it is restricted to the vertebrae, sternum, ribs, cranial bones and the epiphyses of long bones. It is the chief blood forming organ of the adult body, being the sole normal source of the red blood cells and granular leucocytes. During the fetal and growth period it forms part of the osteogenetic tissue which furnishes the osteoblasts for bone development. The cells of red marrow are discussed more fully in connection with blood formation (see p. 125).

Yellow Marrow. Yellow marrow consists in the main of fat cells (Fig. 68) which have gradually replaced the other marrow elements. Under certain conditions the yellow marrow of old or emaciated persons loses most of its fat and assumes a reddish color and gelatinous consistency. It is then known as "*gelatinous marrow*." With an adequate stimulus, such as fracture of the bone, yellow marrow may reassume the character of red marrow and play an active part in the process of bone repair.

Blood Vessels and Nerves

Bone is richly supplied with blood vessels which pass into it from the periosteum. Near the center of the shaft of a long bone a canal passes obliquely through the compact bone. This is known as the *medullary* or *nutrient* canal and its external opening as the *nutrient foramen*. A medullary artery runs through this canal to the marrow cavity. In its passage through the compact bone it communicates by branches with the blood vessels of the Haversian canals. On reaching the marrow cavity the artery divides into ascending and descending branches which supply all portions of the marrow, the terminal arteries opening into a network of *sinusoidal* capillaries characterized by their delicate walls and quite wide lumina.

The capillaries are drained by narrow and thin-walled veins which have no valves. The larger medullary veins pass through the medullary canal accompanying the medullary artery, and like the latter communicate by branches with the veins of the Haversian canals.

Besides the medullary canals the bone is everywhere pierced by the Volkmann's canals which serve for transmission of the numerous smaller vessels. In compact bone these vessels give rise to a network of branches which run in the Haversian canals. In spongy bone the network lies in the marrow spaces. Branches from these vessels pass to the marrow cavity, and there break up into a capillary network which anastomoses freely with the capillaries of the branches of the medullary artery.

Lymphatics. Lymphatics with distinct walls are present in the outer layer of the periosteum. Cleft-like lymph capillaries lined with endothelium accompany the blood vessels in Volkmann's and in the Haversian canals. The *lacunae* and *canaliculi* constitute a complete system of lymph channels which communicate with the lymphatics of the periosteum, of Volkmann's and the Haversian canals, and of the bone marrow.

Nerves. Both medullated and nonmedullated nerves accompany the vessels from the periosteum through Volkmann's canals into the Haversian canals and marrow cavities. Pacinian corpuscles occur in the periosteum. Periosteum is highly sensitive to painful stimuli, whereas osseous tissue is insensitive.

Joints (*Articulations*)

Two main types of connections between bones are distinguished: (1) Union without an articular cleft (*synarthrosis*), the joint being immovable or only slightly movable; (2) connection of bones with an articular cleft (*diarthrosis*, movable joint, Fig. 68).

1. In **synarthrosis** union may be by ligaments or dense fibrous tissue

(*syndesmosis*) or by means of cartilage (*synchondrosis*).

(a) In *syndesmosis* the connecting ligaments may be fibrous or elastic. Of the latter type are the ligamenta subflava which unite the vertebral arches. In the immovable articulations of the head bones (*sutures*), short fibers which are in the main continuations of the fibers of Sharpey, unite the serrated edges of adjacent bones.

(b) *Synchondrosis.* The cartilage is usually of the fibrous form, except nearest the bone where it is hyaline (see cartilage). The intervertebral discs consist of a fibrocartilaginous ring surrounding a central gelatinoid mass, the nucleus pulposus.

2. In a **diarthrosis** the bones are separated by an articular cleft (synovial cavity) and are more or less freely movable. The following structures must be considered: (a) the articular cartilage; (b) the interarticular cartilages or *menisci* and glenoid ligaments; (c) the joint capsule.

(a) The *articular cartilage* (Figs. 46, 68) covers the ends of the bones, and is usually of the hyaline variety, being the remains of the original cartilage in which the bones were formed. No perichondrium is present. The most superficial cartilage cells are flattened and arranged in rows parallel with the surface. In the deeper portion the cells are rounded and arranged in typical groups. The deepest layer, firmly apposed to the bone, is calcified.

In the acromio-clavicular, sterno-clavicular and costo-vertebral articulations the cartilage is of the fibrous form. The same is true of the cartilage covering the head of the ulna, while the surface of the radius which enters into the wrist joints is covered not by cartilage but by dense fibrous tissue.

(b) The *interarticular cartilages* and labra glenoidea serve to deepen the sockets for the articular ends and usually consist of fibrous cartilage.

(c) The *joint capsule* consists of two layers, an outer layer of dense fibrous tissue intimately blended with the ligamentous structures of the joint and known as the *stratum fibrosum*, and an inner layer, the *stratum synoviale* or *synovial membrane*, which forms the lining of the joint cavity. The outer part of the stratum synoviale consists of areolar tissue with its loosely arranged white and elastic fibers interlacing in all directions, and scattered connective tissue cells and fat cells. Nearer the surface of the membrane the fibers run parallel to the surface and the cellular elements are more abundant (Fig. 69). The cells are scattered among the fibers and are stellate branching cells

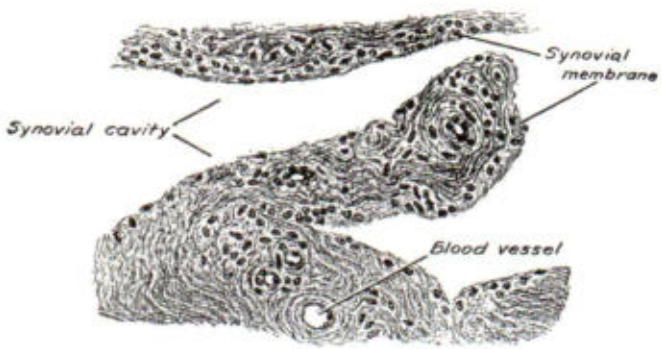

FIG. 69. Synovial membrane. ×320.

like those usually found in loose connective tissue. There is no definite cellular layer at the surface, as might be expected from the name synovial membrane. It is made up of mingled cells and fibers as if the synovial cavity were a cleft in the connective tissue, devoid of any specialized cellular lining.

From the free surfaces of synovial membranes, processes (*synovial villi, Haversian fringes*) project into the joint cavity (Figs. 68, 69). Some of these are nonvascular and consist mainly of stellate cells similar to those of the synovial membrane. Others have a distinct core of fibrous tissue containing blood vessels and covered with stellate connective tissue cells. From the primary villi small secondary nonvascular villi are frequently given off.

The *synovial fluid*, synovia, is probably in part secreted by the cells of the synovial membrane. It is a yellowish, viscid, muco-albuminous fluid, containing inclusions of cellular débris, fat droplets, etc., derived from the synovial membrane. The amount of synovia is greater during rest than during continued activity. It acts as a lubricating fluid facilitating the smooth gliding of the articular surfaces.

The larger blood vessels of the joint capsule lie in the outer layer of the stratum synoviale whence smaller vessels and capillaries pass to the inner layer and to some of the villi. The inner layer is richly, and the outer layer more poorly supplied with lymph capillaries. Nonmedullated nerve fibers are found in the connective tissue, some of them ending in end-bulbs or Pacinian corpuscles.

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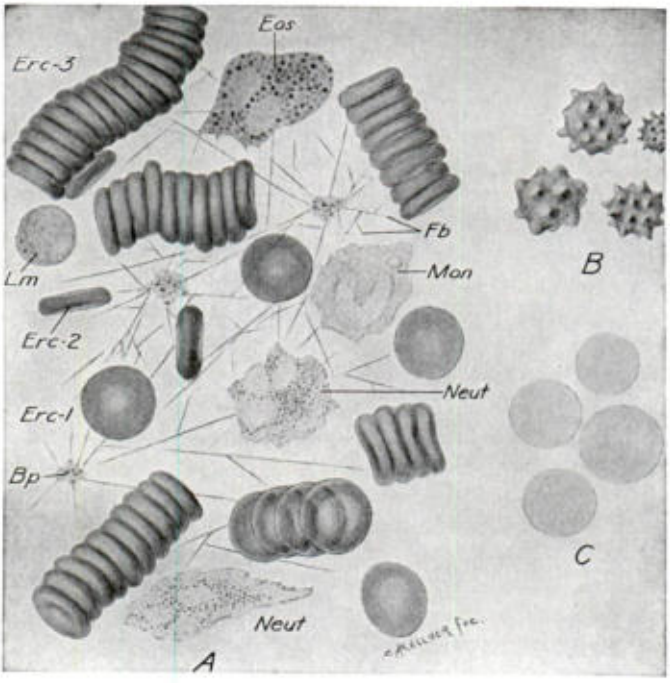

FIG. 70. *A*. Drawings of human blood cells from a thick film, fresh preparation. (Compare with living cells in thin film preparation, Figure 74.) *Bp*, Blood platelets; *Eos*, eosinophile leucocyte; *Erc-1*, *2*, and *3*, erythrocytes in surface view (1), in profile (2) and in rouleaux (3); *Fb*, strands of fibrin; *Lm*, small lymphocyte; *Mon*, monocyte; *Neut*, neutrophile leucocyte.

B. Crenation of erythrocytes after the addition of a few drops of 1.5 per cent NaCl to a fresh preparation.

C. Erythrocytes assuming spheroidal shapes and undergoing hemolysis after the addition of a drop of distilled water to a fresh preparation. All figures × 1160.

or megalocytes. Small forms are characteristically present in some other types of anemia (e.g., iron deficiency anemia) and are known as microcytes.

The corpuscles are very elastic and easily change their shape as may be seen when they squeeze through the narrowest capillaries or pass around the bend of a branching vessel. Experimentally this has been shown by corpuscles embedded in gelatin. When the latter is stretched, the erythrocytes likewise elongate, only to resume their normal shape when the tension is released. Another interesting physical characteristic is the tendency of the corpuscles to adhere to each other along their concave surfaces, thus forming rows or *rouleaux* like piled up coins (Fig. 70). Although the cause of this phenomenon is not entirely clear, it is usually explained as due to surface tension.

Chemically the erythrocyte consists of a protein and lipoid colloidal complex, of which the most important element is hemoglobin. Hemoglobin has the remarkable property of binding oxygen in a very loose combination (oxy-hemoglobin). The hemoglobin becomes saturated with oxygen in the capillaries of

the lung, and the circulating blood distributes this oxygen to the cells of the body in exchange for carbonic acid which constantly accumulates in the tissues. The corpuscle does not yield the oxygen directly to the cells, but the oxygen is first dissolved in the plasma at a level held constant by the erythrocytes.

The contents of the corpuscle are normally in osmotic equilibrium with the plasma, hence the plasma is said to be isosmotic or isotonic. Isotonic solutions may be prepared for study of the corpuscles outside the body; an 0.85 per cent solution of sodium chloride is approximately isotonic for mammalian blood. When *hypertonic* solutions are added to blood, the erythrocytes become shrunken and *crenated* (Fig. 70, *B*). This is explained as follows: the membranous coverings of the corpuscles are permeable to water and impermeable to sodium and potassium ions and therefore water passes from the corpuscles in an attempt to restore the osmotic equilibrium between the corpuscles and the surrounding medium whenever the latter is hypertonic. A few crenated corpuscles are usually found in fresh preparations of blood studied without the addition of hypertonic solutions; this results from evaporation which produces a slightly hypertonic solution and an altered pH. It should be added that crenation has also been produced experimentally in isotonic media and may not be entirely dependent on osmotic phenomena.

When blood is placed in distilled water or any *hypotonic* solution, water enters the corpuscles and they assume a spheroidal shape. The corpuscles lose their color by the escape of hemoglobin which becomes dissolved in the diluted plasma, and the colorless part which remains is known as the *stroma*, "*blood shadow*" or "*ghost*" (Fig. 70, *C*). Eventually, the shadows may also undergo solution. The process of extraction of hemoglobin is called *hemolysis* and the substances which effect it are known as hemolysins or hemolytic agents. Hypotonic solutions are not the only substances which produce hemolysis. Of particular interest is the fact that the plasma of one species may hemolyze the erythrocytes of another and that, in man, the serum of certain individuals may produce hemolysis in others. Hemolysis is of interest in clinical work since one of the types of anemia, *hemolytic anemia*, occurs when the erythrocytes within the body are hemolyzed at a rate which exceeds that of their formation.

Certain substances also bring about an *agglutination* or clumping of corpuscles. Agglutination may occur within the blood stream during certain pathological and experimental conditions and may thus produce a multiple thrombosis of the smaller vessels. Agglutinins present in the serum of some individuals may bring about an agglutination of erythrocytes in others; on this basis, individuals have been divided into several "blood groups." In giving blood transfusions, it is important to select donors from a blood group which is compatible with that of the recipient in order to avoid accidents which would result if the recipient's serum agglutinated the transfused, donor cells.

Histologically the erythrocytes appear entirely homogeneous in the fresh condition, when subjected to ordinary techniques, and in electron micrographs. The covering is a delicate plasma membrane formed by a condensation of the lipoprotein complex of the corpuscular substance.

It is thought by some that the contents of the corpuscle are of a jelly-like nature and consist of a morphological stroma with the hemoglobin and associated substances contained

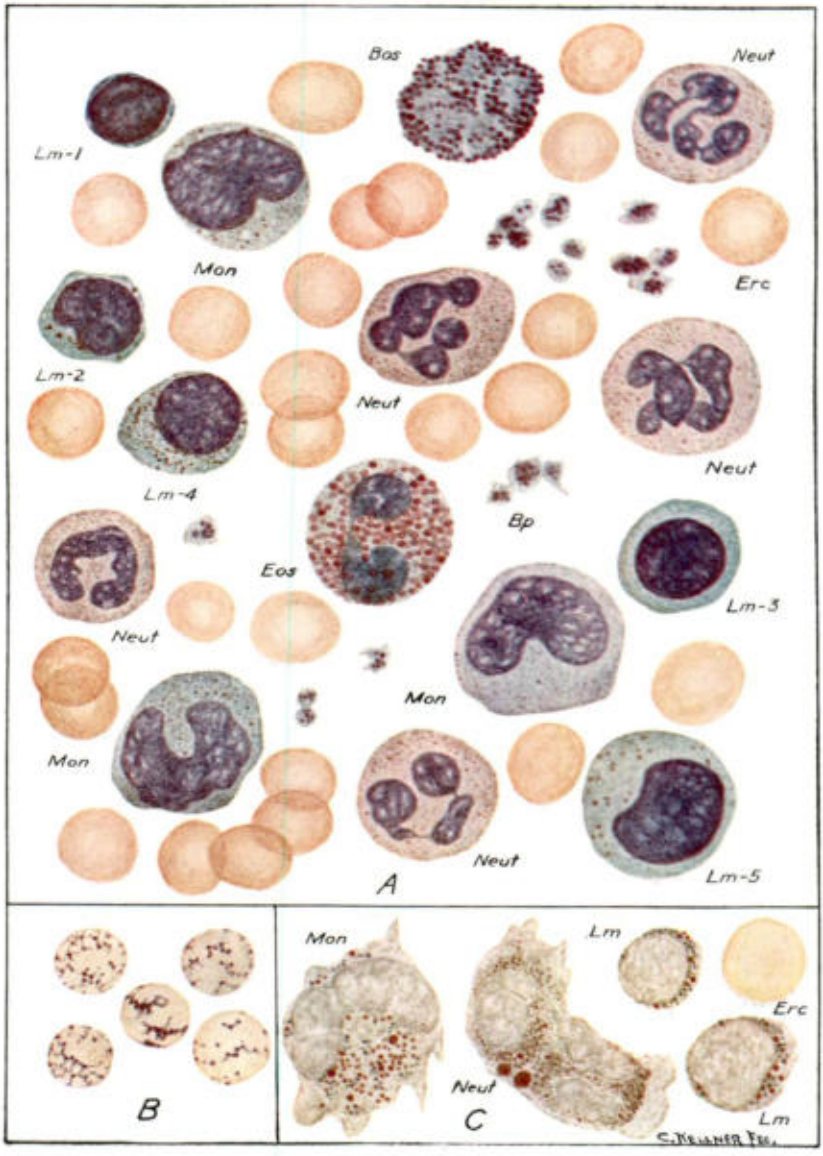

FIG. 71. *A*. Cells from normal human blood. Wright's stain. *Bas*, Basophile leucocyte; *Bp*, aggregations of blood platelets; *Eos*, eosinophile leucocyte; *Erc*, erythrocytes; *Lm-1-5*, lymphocytes, 1–3 are small and medium sizes and 4–5 are the less numerous larger forms; *Mon*, monocytes; *Neut*, neutrophile leucocytes.

B. Reticulocytes from normal human blood stained with dilute cresyl blue.

C. Blood cells as seen after about 20 minutes of staining with neutral red and Janus green in a supravital preparation. *Erc*, Erythrocyte; *Lm*, small lymphocytes with bluish green mitochondria and a few neutral red granules; *Mon*, monocyte with mitochondria and numerous neutral red granules and vacuoles of varying size; *Neut*, neutrophile leucocytes with staining of the neutrophile granules and the formation of a few large vacuoles which frequently appear after 15–20 minutes of staining. All figures × 1550.

in the meshwork. As evidence for this view it is pointed out that a network can be demonstrated by special methods after hemoglobin extraction and that corpuscles may be sectioned in an isotonic medium without an outpouring of hemoglobin. However, the histological pictures of a stroma are probably artifacts produced by the fixatives and the hemoglobin bearing fragments may be explained either by assuming that a new membrane is formed over the separated surfaces or that there is a coagulation of the contained substances. The more commonly accepted view holds that the contents are present as a relatively fluid colloidal mixture. Malarial parasites move freely within the erythrocytes; this would hardly be possible in a morphological structure having the consistency of a gel. Furthermore, microdissection studies have shown that the contents of the corpuscle flow out when the membrane is punctured by a needle.

A few of the erythrocytes show a reticular substance when supravitally stained with neutral red or cresyl blue (Fig. 71, B). These cells, *reticulocytes*, are immature erythrocytes and their reticulated appearance is thought to represent a stained precipitate of a residual protein rather than an actual morphological structure.

The erythrocytes are much more numerous than any of the other formed elements. The average is usually given as 5,000,000 per cubic millimeter of blood in a normal adult man (4,500,000 in woman). Actually, the average for healthy adult men is somewhat higher (5,742,000) but normal variations ranging from 4,000,000 to 6,000,000 are not uncommon (Mayers). There are also normal variations within the same individual which are associated with physiological changes, e.g., the increase after exercise. Many of these variations apparently represent a redistribution to the peripheral vessels rather than an actual change in total numbers. Life in high altitudes is accompanied by an increase to about 8,000,000. While the initial change in this case may be a redistribution through an outpouring of red cells from the spleen (Chapter XIII) there is also a real increase in total numbers in response to the lower oxygen tension. More pronounced variations occur under pathological conditions.

The surface area of a red corpuscle has been given as 128 square microns. From this one may calculate that the total surface area of five million corpuscles in a cubic millimeter of blood is 640 square millimeters and that in six liters of blood the total area available for respiratory function is 3840 square meters. This enormous area suggests the importance and the rapidity of the exchange phenomena between the corpuscles on the one hand, and the plasma and air on the other.

Under pathological conditions, not only the number, but the size, shape and hemoglobin content of the corpuscles may vary strikingly. The normal number may be present but the amount of hemoglobin reduced, as in some of the *secondary* (chlorotic) anemias. In the *macrocytic anemias*, (e.g., pernicious anemia which results from a deficiency of an erythrocyte maturation factor, vitamin B_{12}, which is normally stored in the liver), the cells are reduced in number but are abnormally large (macrocytes) and some of the cells have an increased content of hemoglobin. In *microcytic anemia*, (e.g., iron deficiency anemia), there is a decrease both in the number and in the size of the cells. Under most of these conditions, the cells may show a multiplicity of distortions in shape (poikilocytosis).

WHITE BLOOD CORPUSCLES (LEUCOCYTES)

The white blood cells contain no hemoglobin and differ from the red corpuscles in many other important respects. They possess a nucleus, hence are true cells, and they have the power of active ame-

boid movement by means of which they are able to pass through the walls of blood vessels. They are much less numerous than the red cells, the proportion being about one white cell to 600 red cells, or about 8000 to the cubic millimeter of blood, with a normal variation from 6000–10,000. Under pathological conditions the number may be greatly increased (leucocytosis); more rarely there is a reduction in number (leucopenia). At birth the leucocytes are more numerous (15,000–18,000 per cubic millimeter).

The erythrocytes spend their life span in the blood stream where their function is performed. Only under abnormal conditions, or when about to disintegrate are they found outside the blood vessels. It is quite different with the leucocytes. They function in the connective tissue where they have already been described as wandering elements. They arise, function and die outside of the blood stream which is to them merely a place of temporary sojourn, a means of transportation from their place of origin to their destination in the connective tissues.

The leucocytes may be subdivided into

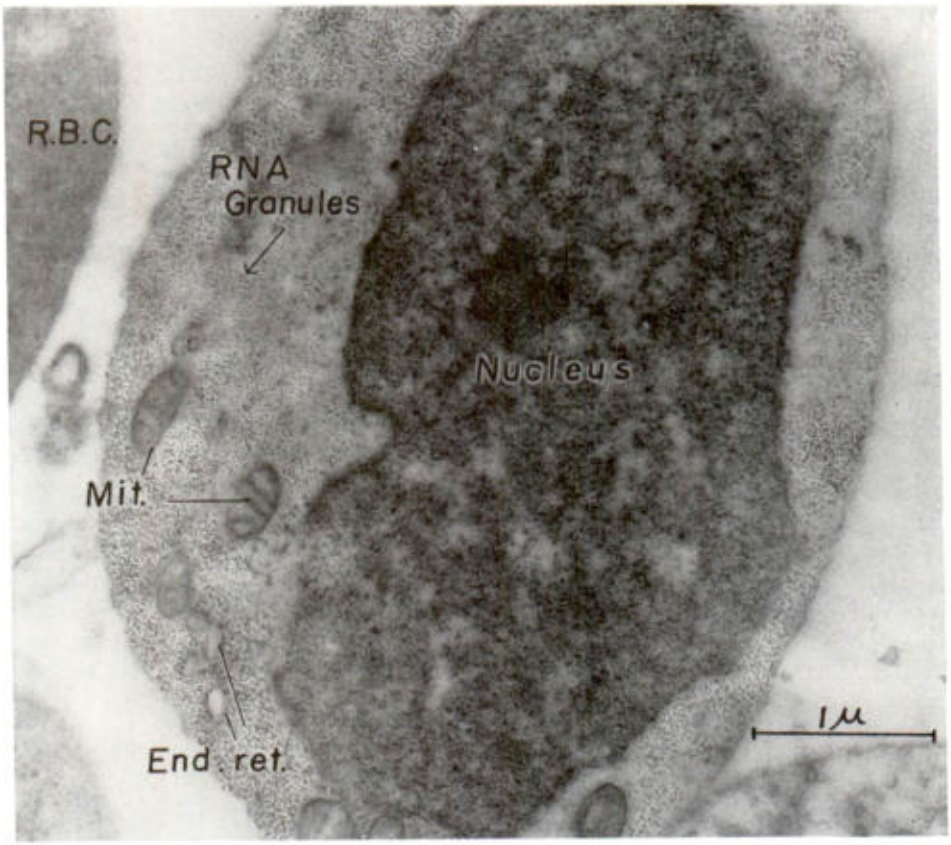

FIG. 72. Electron micrograph of a section of a human lymphocyte. Note sparse endoplasmic reticulum and numerous RNA granules. × 23,500. (Courtesy of Dr. J. A. Freeman; refer to Electron microscopic atlas of blood by Drs. F. N. Low and J. A. Freeman, Sr.)

nongranular forms (*agranulocytes*) and granular leucocytes (*granulocytes*). The cytoplasm of the granular leucocytes is characterized by the presence of numerous granules which may be seen in living cells as well as in fixed and stained preparations. In many of the cells these granules are fine and only slightly refractive but in others the cytoplasm is filled with coarse and highly refractive granules. Azurophilic granules may be found in the agranulocytes in Wright's stained dry smears but these granules are not constant in occurrence and are not of a specific type.

Types of Nongranular Leucocytes: Agranulocytes. The nongranular leucocytes include the *lymphocytes* which are small cells, about the size of erythrocytes, and a group of larger cells, *monocytes*, which have more cytoplasm and a more indented nucleus. The nongranular leucocytes are comparatively undifferentiated and can reproduce by mitosis. Such division does not take place as a rule in the blood stream itself but may occur in the connective tissues and in blood-forming organs.

Lymphocytes. The lymphocytes of the normal circulation vary from 6–10 μ with the majority being about 7–8 μ. They normally constitute about 20–25 per cent of the white blood cells. There is a considerable range for normal individuals and it is not uncommon to find lymphocyte counts as high as 35 per cent, or even 45 per cent. They have a relatively large spherical nucleus which may have a slight indentation on one side. The densely packed chromatin stains intensely; the nuclei are purplish-blue with Wright's stain. The basophilic cytoplasm forms a narrow rim around the nucleus and is a robin's egg blue after Wright's stain. It is usually homogeneous but may be slightly more basophilic at the border of the cell and paler adjacent to the nucleus. The basophilia of the cytoplasm is apparently related to the abundance of RNA which can be seen in the form of minute granules in electron micrographs (Fig. 72). Purplish, azurophilic granules occasionally appear in Wright's stained dry smears but, as already described, they are not specific since they are also found in monocytes and in certain immature cell types during blood development. The significance of these granules is not clear; the number of lymphocytes containing them varies at different times and in different individuals.

It has been noted above that a few of the lymphocytes of the normal circulation may be as large as 10 or 12 μ. The majority of these larger cells have a dense chromatic nucleus and they differ little from the small cells, except for size. Some of the larger cells may also appear to be intermediate between lymphocytes and monocytes. None of these larger cells should be confused with the immature type of large lymphocyte found in blood-forming organs. The latter type has a vesicular, undifferentiated nucleus with prominent nucleoli. Although the immature lymphocyte is usually confined to the blood-forming organs, it may become abundant in the blood stream during certain pathological states, as in lymphatic leukemia.

When supravital preparations are studied, the lymphocytes are stationary for a short time but after fifteen or twenty minutes they occasionally move in a manner which differs from that of the other leucocytes. They elongate by sending out a blunt pseudopod and advance by a sort of worm-like motion with the nucleus usually in front (Fig. 74). It should be added that the lymphocytes exhibit their characteristic motility only when the supravital preparations are kept at body temperature; they are usu-

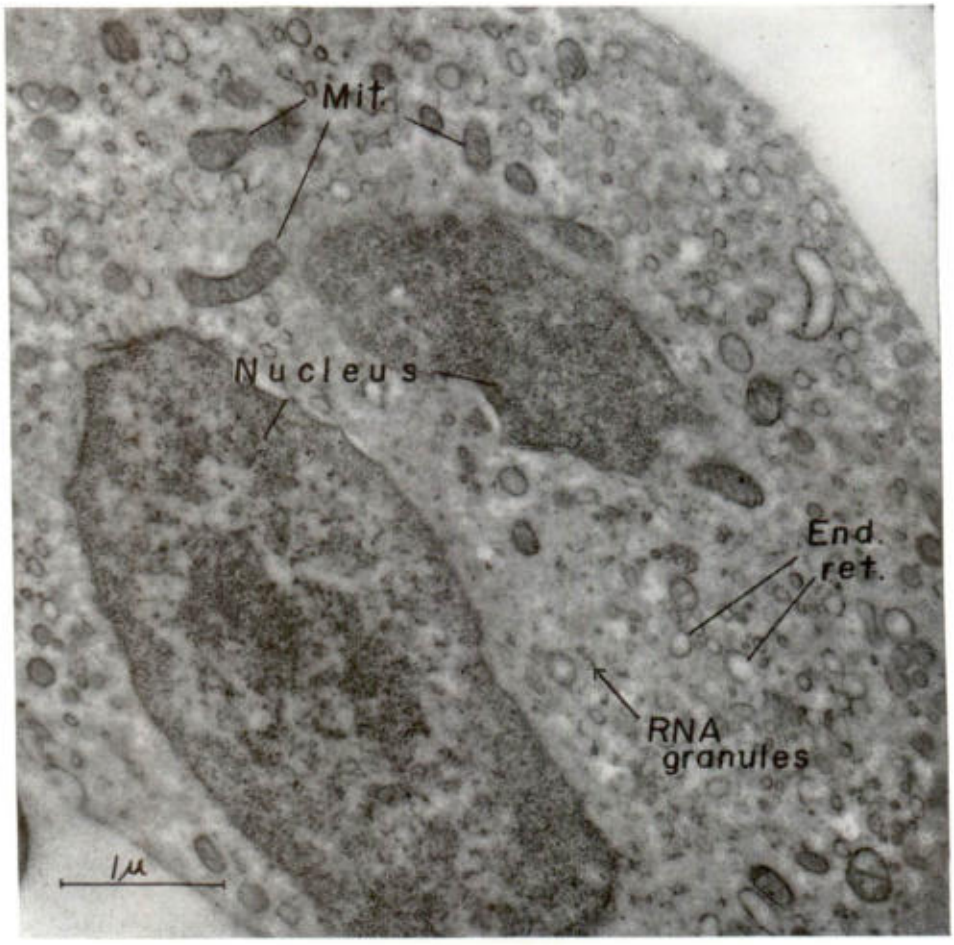

FIG. 73. Electron micrograph of a section of a human monocyte. Note well developed endoplasmic reticulum and many small mitochondria. The irregularly-shaped nucleus appears in two parts because of the plane of the section. × 23,000. (Courtesy of Dr. J. A. Freeman.)

ally nonmotile in preparations studied at room temperature. In supravital preparations stained with dilute solutions of neutral red and Janus green B, the mitochondria (stained bluish-green by the Janus green B) are in the form of granules and rods and frequently are found clumped at one side of the nucleus. Most of the lymphocytes also show a few neutral red granules and vacuoles (Fig. 71, *C*). Intermediate or medium-sized lymphocytes are more motile and form more neutral red vacuoles than the small lymphocytes (Sabin, 1923). In fact, it is frequently very difficult to differentiate some of the medium-sized lymphocytes from the small monocytes in supravital preparations. This is easier to understand, if one accepts the view that lymphocytes can differentiate into monocytes.

The lymphocytes migrate through the walls of the capillaries and display marked motility in the connective tis-

sues. Their function is not entirely clear but in the connective tissues they can apparently develop into plasma cells and monocytes and from the latter into macrophages. It has been noted, also, that a definite lymphocyte reaction accompanies diseases which are followed by a lasting immunity (Bunting, 1938). Experimental studies on the relationship of lymphocytes to antibody formation have given conflicting results; according to some investigators, the lymphocytes contain antibodies in their cytoplasm, but the best evidence indicates that antibodies are present within plasma cells which arise from a lymphocyte-like stem cell.

Monocytes or Large Mononuclear Leucocytes. Monocytes are large cells which constitute from 3–8 per cent of the leucocytes. In dry smears, they usually vary from 12–15 μ in diameter but when extremely flattened and stretched they may reach 20 μ. In supravital preparations, their diameter varies with the activity of the cell. The active monocytes send out numerous pseudopodia and naturally appear larger than the more rounded, inactive forms. The active cells are especially large when flattened in thin film preparations (Fig. 74).

The monocyte nucleus is ovoid, kidney or horseshoe-shaped, very rarely spherical, and usually eccentrically

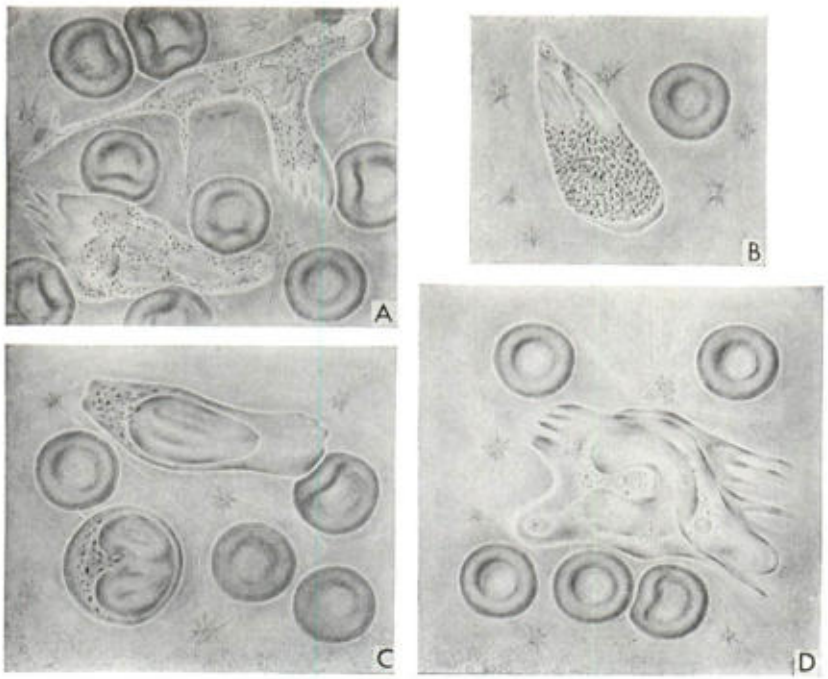

FIG. 74. Drawings of living human blood cells from a thin film preparation. *A*, Polymorphonuclear neutrophiles pushing their way between the red blood cells and the delicate fibrin network extending out from the small platelet masses; *B*, polymorphonuclear eosinophile; *C*, lymphocytes, the round resting stage and the elongated motile stage; *D*, monocyte with pseudopodia in the form of delicate undulating membranes. (Courtesy of Dr. C. M. Goss.)

cells, they have been found to range from 50–75 per cent in normal individuals. Under pathological conditions, the range is much greater. The polymorphous nucleus shows a variety of forms, usually consisting of 3 to 5 sausage-shaped masses of chromatin connected by fine threads and arranged in the form of an S or a horseshoe. The protoplasm is filled with fine granules which are neutrophilic, i.e., they have an affinity for the neutral stain of the Romanowsky compound rather than for the acid or basic portions of the dye. In some animals, rabbit and guinea pig, the granules take the acid stain and may be called *pseudoeosinophiles*. Since these cells vary in their staining reactions in different species, they are sometimes called *heterophilic* rather than neutrophilic leucocytes.

In differential counts, the neutrophiles are sometimes divided into sub-groups on the basis of the number of lobes to the nuclei. In the Arneth count, the neutrophiles are divided into five classes according to whether the nuclei are composed of 1, 2, 3, 4, or 5 (or more) lobes. Since there is some increase in nuclear segmentation as myelocytes differentiate into mature neutrophiles, it has been claimed that the cells with the most segmented nuclei are the oldest. As originally outlined by Arneth, the count was very complex and there was no definition of what constituted a division into lobes. The count was clarified by Cooke (1914) and by Cooke and Ponder (1927) who gave diagrams and definitions of the five classes. According to them, two parts of a nucleus are counted as separate lobes when they are connected merely by a thin chromatin filament (approximately no more than a thin nuclear membrane). When two nuclear segments are connected by any band of nuclear material broader than a chromatin filament, they are not counted as separate lobes. There have been a number of modifications of the count. One of the simplest is that of dividing the neutrophiles into two classes: *filament* types, or cells which have two or more nuclear lobes connected by thin chromatin filaments, and *non-filament* types, or cells which have only broad strands and no filaments connecting nuclear lobes. Normally, the non-filament types constitute 8–16 per cent of all of the neutrophilic leucocytes in the blood stream (Pepper and Farley). An increase in relative numbers of non-filament forms is designated as a "shift to the left"; an increase in filament types is a "shift to the right" and is considered a good sign because it is supposed to show that there is no longer any unusual demand on the bone marrow for younger cells.

In supravital preparations, the neutrophilic leucocyte is more active than any other blood cell. It advances by an ameboid movement, usually with the nucleus in the rear. At times, it is difficult to see the strands connecting the nuclear lobes and the cells may appear to be polynuclear instead of polymorphonuclear. The neutrophilic granules stain with neutral red, probably by an adsorption of the dye (Fig. 71, *C*). After fifteen or twenty minutes, some of the neutrophiles phagocytose the dye and form one or two neutral red vacuoles which occasionally become as large as one of the nuclear lobes. A few, small mitochondria stain with Janus green B.

After migration from the blood stream, the neutrophiles phagocytose bacteria and other small particles. They have been called the *microphages*, in contrast to the macrophages, which are larger cells that characteristically engulf larger particles. The neutrophiles elaborate powerful proteolytic enzymes which may act within the cell, as in the digestion of phagocytosed bodies, or are liberated and act outside the cell body. These leucocytes may in fact be considered as motile unicellular glands. Chemotactically attracted by devitalized tissue, bacteria or other foreign bodies, the cells reach these places by ameboid movement and there liberate the proteolytic enzymes, either by secretion or, more commonly, by disintegration and rupture of the leucocytes themselves which become the pus corpuscles. Although the neutrophiles are in the first line of defense

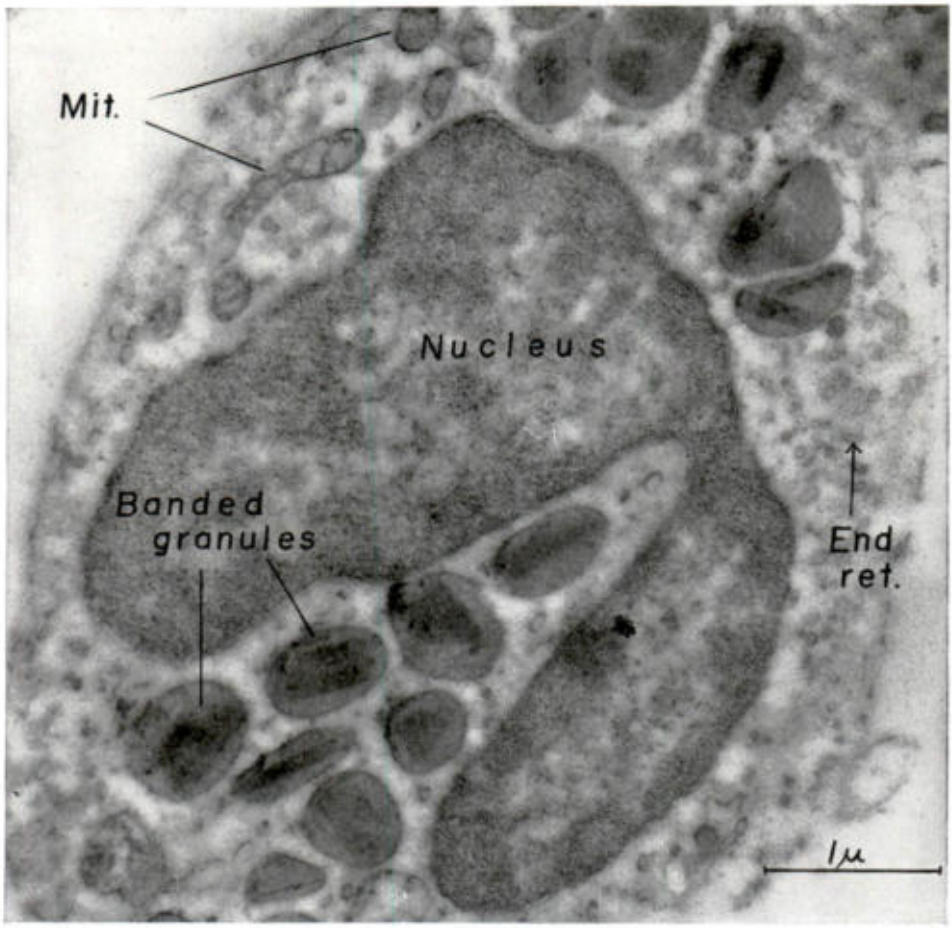

FIG. 76. Electron micrograph of a section of a human eosinophilic leucocyte. Note large "banded" granules. × 23,000. (Courtesy of Dr. J. A. Freeman.)

against invading organisms, they are not equally effective against all types. For example, they cannot successfully combat tubercle bacilli; in this case, the macrophages are the efficient agents.

Eosinophiles. The eosinophilic leucocytes (Fig. 71) normally constitute from 2–4 per cent of the white blood cells. They are somewhat larger than the neutrophiles (about 10–14 μ) and are characterized by an abundance of coarse, refractile granules of a uniform size which stain intensely with eosin or other acid dyes. In electron micrographs, the granules of the eosinophilic leucocytes are characteristically banded (Fig. 76). The nucleus is polymorphous, but the average number of lobes is less than in the neutrophiles, most commonly two.

In supravital preparations, the eosinophiles occasionally travel as rapidly as the neutrophiles but not for as long a period. Their granules stain intensely and uniformly with neutral red and a few mitochondria can be demonstrated with Janus green B.

The function of the eosinophiles is obscure. They show ameboid movement, but phagocytosis is observed only occasionally. They are more common in the connective tissue of certain places (e.g., intestinal mucosa) than in the blood stream. In certain diseases, such as bronchial asthma, skin diseases, parasitic infestations, etc., their number is greatly increased. The number of circulating eosinophiles is decreased after the administration of adrenocorticotropic hormone (ACTH) or after cortisone. Therefore, total eosinophile counts can give useful information during the clinical administration of these hormones although this information has not clarified the function of the eosinophile.

Basophiles. The basophilic leucocytes (Fig. 71) are present in blood in an almost negligible quantity, forming 0.5 to 1 per cent or even less of the total number of leucocytes. In size they vary from 8–10 μ. The nucleus is relatively large and irregularly polymorphous. The lobed nature is not as clearly defined as in other granulocytes and the chromatin network, which is less compact, takes a lighter stain. The cytoplasm contains a variable number of coarse granules which stain intensely with basic dyes. These granules are variable in size; a few may be as large or larger than the eosinophile granules but the majority are intermediate between the neutrophile and eosinophile types. Since the granules are soluble in water they are usually not found in sections prepared by the ordinary routine.

In supravital preparations, the basophiles are relatively inactive. Any motion which is observed is of the ameboid type. The basophilic granules are not as refractile as eosinophilic granules, are more variable in size, and do not stain as uniformly. Most of the granules give a deeper red reaction with neutral red.

The function of the basophilic leucocytes is unknown. They are increased in certain pathological conditions, e.g., myelocytic leukemia, Hodgkin's disease, smallpox, chickenpox, and chronic sinus inflammations. In some of the lower vertebrates (hellbender, mudpuppy, and certain turtles) they are normally more numerous than the other types of leucocytes.

BLOOD PLATELETS

Blood platelets (thromboplastids or thrombocytes, Fig. 71) are minute colorless bodies from 2 to 4 μ in diameter. In fresh blood their shape is round or ovoid; in fixed preparations they often appear stellate. Their number has been variously estimated, the average being about 200,000 to 300,000 per cubic millimeter. In the blood stream they are separate but show a marked tendency to agglutination when the blood is drawn. Structurally they consist of a central granular mass (chromomere) which stains deeply with basic dyes, and a peripheral hyaline zone (hyalomere).

There has been a tendency on the part of some observers to consider the platelets as true cells. Thus they have been described as ameboid, as having the same chemical composition as cells, and as containing either granules of chromatin or distinct nuclear structures. The so-called thrombocytes of lower vertebrates are larger than those of man, and are unquestionably nucleated cells. Their appearance is wholly unlike that of the human platelets and the relation of the two forms is doubtful.

The platelets presumably liberate an enzyme, *thromboplastin*, which affects the coagulation of the blood. Thromboplastin transforms *prothrombin* into *thrombin* and the latter, in turn, transforms fibrinogen into fibrin. Thromboplastin is also present in the plasma, as

well as in the platelets. Blood freed from platelets coagulates, though much more slowly, and lymph which has no platelets, likewise coagulates.

Pathologically the platelets may by agglutination give rise to colorless intravascular clots or thrombi. Deficiencies of circulating platelets are encountered clinically in conditions known as thrombocytopenia.

CHYLOMICRONS AND HEMOCONIA

Blood plasma contains very minute globules of fat (chylomicrons, about one micron in size) which are best studied by dark field illumination. They are particularly numerous in the plasma after digestion of a meal containing quantities of fat. In addition to the fat globules, blood plasma contains a variable number of small particles which may be observed in dry smears as well as in fresh preparations. These particles are known as hemoconia (blood dust). They are probably produced by disintegration of red corpuscles and leucocytes.

LYMPH

Lymph, like blood, consists of a fluid plasma in which are suspended various corpuscular elements. Red blood corpuscles and platelets are entirely missing and granulocytes are few in number, the chief cellular elements being lymphocytes.

The plasma of lymph is similar to that of blood but of less fixed constitution. It carries carbonic acid but very little oxygen.

During digestion the lymphatics of the intestine become filled with a large amount of fat globules. The lymph assumes a white color and is known as *chyle*. Many of the fat globules are removed and stored temporarily by the lymphoid organs before the lymph reaches the blood stream.

Lymph coagulates, though much more slowly than blood, the fibrin forming a colorless clot in which the cells are entangled.

DISPOSAL OF WORN-OUT CORPUSCLES

In contrast with many other cells of the body, the red and white corpuscles of the blood survive for only a relatively short period of time. The life span of the human erythrocyte as determined by tracer doses of radioactive isotopes is about 127 days (Shemin and Rittenberg, '46). From this data, it is obvious that billions of corpuscles are destroyed daily. The destruction is balanced so well by new blood formation that the characteristic number of corpuscles is constantly maintained under normal conditions.

Destruction of erythrocytes frequently begins within the blood stream itself by a disintegration of corpuscles into small hemoglobin retaining fragments, and is completed by the reticulo-endothelial cells of the blood destroying organs, chiefly by the spleen (see Chapter XIII). These cells remove the fragmented forms and also engulf many senile, worn-out erythrocytes in toto. The phagocytic cells break up the hemoglobin into an iron free portion (*globin*) and an iron retaining part (*hematin*). Hematin is further separated into *bilirubin* and *iron*. The bilirubin is transported to the liver to be excreted in the bile, and the iron is conserved by the macrophages to be used again in new erythrocytes developing in the bone marrow. The exact details of the transfer of iron and its utilization in hemoglobin formation have not been described.

The life span of the various types of leucocytes is quite variable. The length of life within the blood stream is probably about three days for the neutrophiles and less than 24 hours for the

lymphocytes. Fragile and nonmotile forms are frequently found in fresh preparations of blood but some investigators believe that many of these are produced in the technique of supravital preparations. Senile and dead cells are removed by phagocytosis in the spleen and liver. The migration of neutrophiles, especially pronounced during infection, and their disintegration in the connective tissues has already been pointed out (p. 120). They also escape by penetration through the lining epithelia of mucous membranes as illustrated by their presence in saliva. Eosinophiles show a particular tendency for migration into the connective tissues of the respiratory and gastrointestinal tracts where they eventually disintegrate. Some of the lymphocytes may undergo dissolution in the circulatory system, some may be destroyed while the blood courses through organs where cells of the reticulo-endothelial system are particularly numerous, and large numbers migrate into the connective tissues where they apparently disintegrate. Many are lost by migration into the lumen of the intestinal tract. There is also the probability that some lymphocytes develop into other cell types, especially monocytes and macrophages.

DEVELOPMENT OF BLOOD CORPUSCLES —HEMOPOIESIS

Throughout life there is active proliferation of the immature forms of blood cells to replace the mature corpuscles which are constantly being destroyed. The formed elements of the blood may be subdivided into two groups according to the location of their development in the normal adult. Lymphocytes and monocytes proliferate chiefly in the lymphoid tissues and only to a limited extent in bone marrow; they are classified *lymphoid elements*. Erythrocytes and granulocytes normally develop in red bone marrow (myeloid tissue); they are the *myeloid elements*. In certain pathological conditions, the myeloid elements may form in other locations such as spleen, liver, and lymph nodes; this is called extramedullary myelopoiesis.

The details of the early stages in blood development are not entirely clear. Similar stages of development have been given different names by different schools which emphasize the theoretical potencies of the developing cells rather than their structural features. For this reason, any description of the early cell types becomes involved with the theoretical considerations. According to one theory all blood cells, red and white, arise from a common stem cell of "lymphoid nature," the *hemocytoblast*; this is the monophyletic or unitarian theory and contrasts with the polyphyletic view that the several types of formed elements arise from different stem cells. According to the "dualist" theory, there are two stem cells: a *myeloblast* for all of the myeloid elements, and a *lymphoblast* for the lymphoid elements. The term "dualist" is also occasionally applied to those who believe that there is one stem cell for the red corpuscles and another one for the white corpuscles. Since investigators supporting the different theories frequently use different terminologies for similar cells, misunderstandings easily arise when the theories are compared. Fortunately, there is uniformity of agreement regarding structural features of the majority of the cells and the points of controversy are confined to the relationship and characteristics of the earliest stages which are of infrequent occurrence in normal adult marrow. In the account which follows, primary emphasis has been placed on the structural characteristics of the cells and some of the controversial

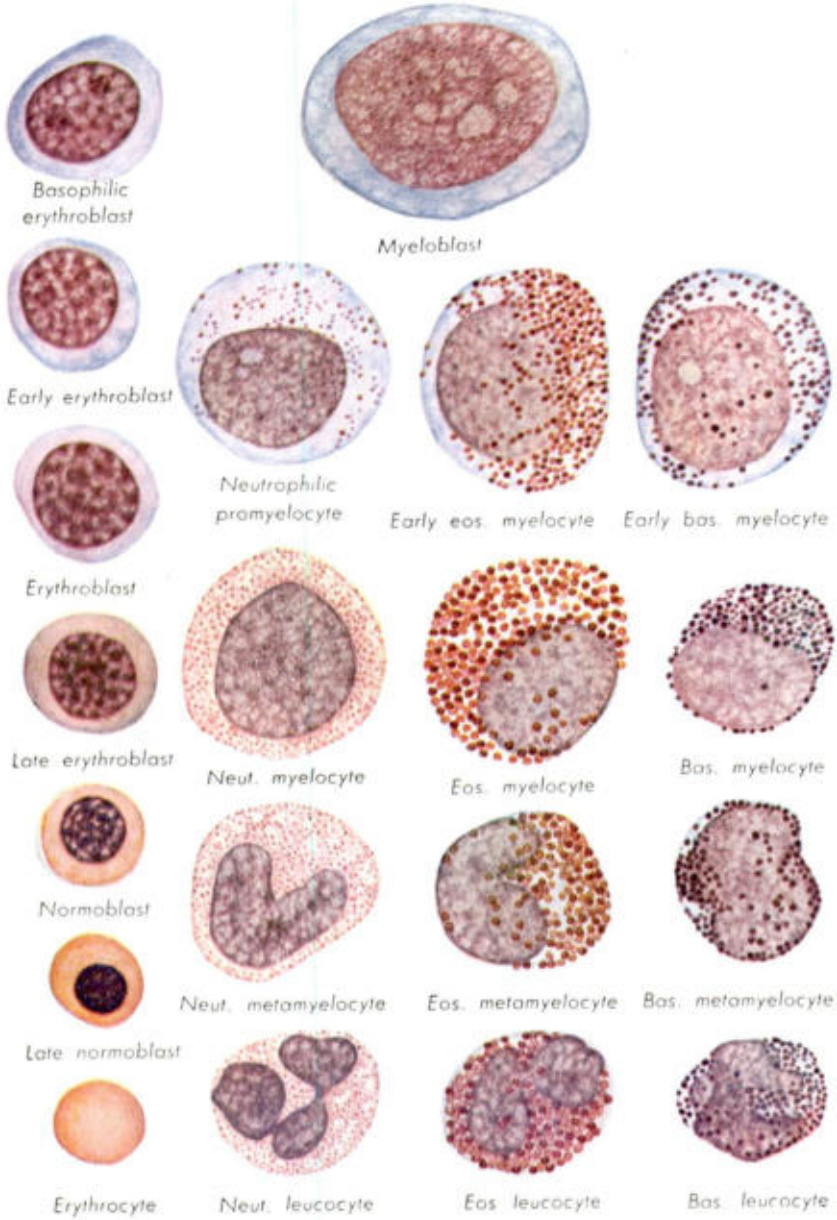

FIG. 77. Various stages in blood development as seen in dried *smears* of marrow from a human rib. *Wright's stain*. × 1560.

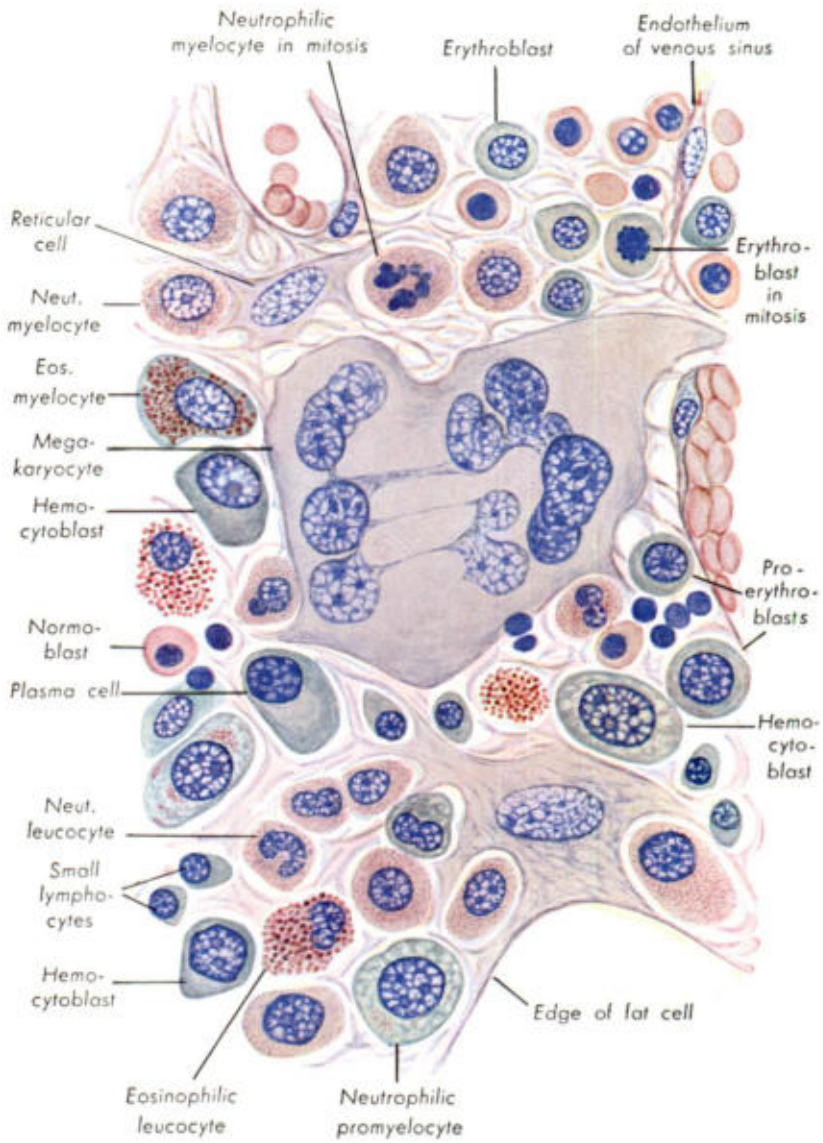

FIG. 78. Various stages in blood development as seen in a *section* of marrow from a human rib. Hematoxylin-eosin-azure. × 1365.

material has been confined to small print. It is suggested that the developmental stages may be followed more readily by the omission of all fine print in the first reading; reference should be made to these sections subsequently for an understanding of various views.

Development of Myeloid Elements

Red bone marrow, like lymphoid tissue, is composed of a framework or stroma of reticular cells and fibers with free cells located within the meshwork. The stroma of marrow is also characterized by the presence of a varying number of fat cells. The majority of the free cells in red marrow are the immature, developmental stages of the granular leucocytes and erythrocytes. Myeloid tissue may also contain giant cells (megakaryocytes), mature erythrocytes, granular leucocytes and a few lymphocytes, monocytes and plasma cells. The structure and vascular supply of marrow with its characteristic network of sinusoids have already been described (p. 104).

Granulocytes. The stages of granulocyte development in order of differentiation are: *reticular cells*, *hemocytoblasts* (*myeloblasts*), *promyelocytes*, *myelocytes*, *metamyelocytes*, and *granular leucocytes*.

Hemocytoblasts (Myeloblasts). The most immature, primitive free cells of marrow are characterized by a deeply basophilic cytoplasm and a relatively undifferentiated nucleus; they are the hemocytoblasts of the unitarian and the myeloblasts of the dualistic theories. Although the primitive cells vary in size, the majority of them are large, up to 15 μ or more. (Fig. 77 shows one of the larger cells of the type). The nuclei of these cells are rounded, relatively large and contain two or more coarse nucleoli. In dried smears treated with Wright's stain, the nucleoli are very pale and the arrangement of the granular chromatin frequently gives a characteristic sieve-like appearance (Fig. 77). The nucleus of this same cell type has a very different appearance in sections stained with hematoxylin-eosin-azure (see hemocytoblasts in Fig. 78). Here the nucleoli are stained intensely and the chromatin shows as a delicate reticulum enclosing vesicular-like pale staining areas. The cytoplasm is very basophilic both in sections and in dried smears and is a definite blue of the lymphocyte type after Wright's stain. Azurophilic granules are occasionally present in the cytoplasm but there are no granules of the specific leucocyte type.

The myeloblasts of normal adult marrow arise chiefly by mitotic divisions of their own type but new ones can differentiate from the reticular connective tissue cells which can round up, separate from the reticulum and become free (unattached) cells. Myeloblasts normally constitute only 0.3–5.0 per cent of the cells of marrow. In pathological conditions the myeloblasts may become more numerous and may appear in considerable numbers in the circulating blood, as in myelogenous leukemia. Here the increase is primarily the result of accelerated mitotic activity but new cells may also arise from the reticulum.

If all of the primitive cells of marrow are identical with the large lymphocytes of lymphoid organs, they obviously constitute a common stem type and may be called hemocytoblasts. According to this theory (unitarian), none of the primitive marrow cells characterized by basophilic, nongranular cytoplasm are definitely restricted to the development of myeloid elements.

On the basis of slight structural differences in dried smears, such as the more delicate nuclear membrane in the primitive marrow cells, the dualists separate these cells from lymphocytes and use the term myeloblast (Naegeli) instead of hemocytoblast. By the use of supravital stains Sabin has separated the early cells of marrow (the hemocytoblasts of the

unitarians) into primitive free cells and myeloblasts. The primitive free cells are said to develop into myeloblasts in the bone marrow and into lymphoblasts in the lymphoid organs. This view is in agreement with the unitarian theory that the first free cells arising from the reticulum of marrow and lymph nodes are similar but it differs from the unitarian theory in regard to the time when the earliest specificity appears.

There is considerable evidence in favor of the unitarian view. It is indeed true that the large cells in the germinal centers of the lymph nodes can normally be distinguished from the myeloblasts of marrow. However, these different types probably represent only minor modifications of the cells due to extrinsic factors. There is a good deal of evidence that under abnormal conditions, as in myeloid metaplasia, the large lymphocytes (lymphoblasts) of the lymphoid organs may give rise to granular leucocytes. Furthermore, Downey has shown that in acute lymphatic and in mixed leukemias, lymphocytes are derived from myeloblasts as indicated by the presence of intermediate stages between the latter and the small lymphocytes.

Myelocytes. The earliest myelocytes differentiate directly from the myeloblast and are known as *promyelocytes*. The first specific granules appear in the less basophilic areas of cytoplasm, especially in the region of the cytocentrum adjacent to the nucleus (Figs. 77 and 78). Azurophilic granules, characteristic of some of the myeloblasts, are frequently carried over into the promyelocyte stages but later disappear. A few azurophilic granules are shown in association with the neutrophilic granules in the neutrophilic promyelocyte illustrated in Figure 77. There is no evidence for the view that azurophilic granules develop into the specific granules; the differentiation from myeloblast to promyelocyte can occur in the absence of azure granules.

Although the promyelocyte granules show colors sufficiently specific for identification of the three types there may be some variations from the mature form. This is particularly true for the eosinophile where many of the early granules are less acidophilic.

In normal marrow, the promyelocytes constitute only about 4 per cent of the cells, (range, 1–8 per cent). They proliferate and differentiate into the *myelocytes* proper, which make up about 12 per cent of the marrow cells, (range, 5–20 per cent).

In differentiating from a promyelocyte to a myelocyte proper, the cell shows an increase in the number of its specific cytoplasmic granules, a decrease in the basophilia of its cytoplasm, and an increase in the density of the chromatin of the nucleus. After a number of generations, the myelocytes eventually reach a stage where they no longer divide. This stage is known as the *metamyelocyte*. It can usually be identified by its indented or kidney-shaped nucleus. The cells of this stage constitute about 20 per cent of the cells of normal marrow. They differentiate directly into the mature leucocytes, usually within the bone marrow. When they enter the circulation before completing their maturation they are known as the "juvenile" leucocytes of the Schilling differential count. Although they are usually scarce in the normal circulation, they become abundant in certain pathological states.

In each of the above myelocyte stages, the neutrophiles far outnumber the eosinophiles and basophiles. All three types of cells are well preserved in Wright's stained dried smears but the basophiles are generally indistinguishable in sections of material fixed in aqueous solutions since the basophilic granules are water soluble.

General Considerations of Granulocytopoiesis. When the percentages of the different myelocyte stages are added to the number of mature granulocytes present in marrow (20 per cent or more), it is found that the granular leucocyte line comprises approximately

60 per cent of the cells in normal marrow. Lymphocytes, monocytes, reticular cells, plasma cells and megakaryocytes may constitute another 10–20 per cent, leaving only about 20–30 per cent of the marrow cells in the erythrocyte line. Although the literature on differential counts for normal marrow gives an even greater range than that listed above, there is general agreement that the progenitors of the leucocytes outnumber those of the erythrocytes. The numerical preponderance of leucocytogenic over erythrocytogenic cells in marrow in contrast to the opposite relationship in blood can be explained partly by the fact that the leucocytes survive for a shorter time in the circulation than do the erythrocytes and also require a longer period for development and maturation in marrow.

Studies of bone marrow cells by the ultraviolet absorption method have shown that ribonucleic acid (RNA) is more abundant in the myeloblast than in any of the other developmental stages of the granulocyte line (Thorell, 1947). The high concentration of RNA is responsible for the marked basophilia of the cytoplasm at the myeloblast stage. It is thought that the high concentration of cytoplasmic RNA and the prominence of nucleoli are correlated with the rapid formation of cytoplasmic proteins during the early stages. It has been estimated that the total volume of all the promyelocytes is about eight times the total volume of the myeloblasts. This follows from the fact that the promyelocytes are more numerous and also from the fact that they are often larger than the myeloblasts (it has already been noted that the myeloblast illustrated in Figure 77 is one of the largest of its type). In the lineage from promyelocyte to myelocyte to metamyelocyte, the cells increase in number with a decrease in size so that the total volume of all the metamyelocytes is only slightly greater than the total volume of the promyelocytes. Development during these later stages is predominated by differentiation rather than by cell growth. The rapid decrease in cytoplasmic basophilia during these later stages appears to be correlated with the decrease in ribonucleic acid.

Erythrocytes. The stages of erythrocyte development in order of differentiation are: *reticular cells*, *hemocytoblasts* (*myeloblasts*), *basophilic erythroblasts*, *polychromatophilic erythroblasts*, *normoblasts*, and *erythrocytes*. The reticular cells and hemocytoblasts have been described in the preceding sections.

Basophilic Erythroblasts. The earliest cells which exhibit differentiation in the direction of erythrocytes are smaller than myeloblasts and their nuclei have a slightly coarser chromatin structure than that seen in myeloblasts (Fig. 77). Nucleoli are present but less prominent than in the myeloblast. The presence of hemoglobin is a differential characteristic in identifying the first step in the erythrocyte line but this can be seen better in living cells than in stained smears where the hemoglobin is obscured by the basophilia of the cytoplasm. However, it usually produces a slightly different shade from that which characterizes the myeloblast.

The terminology of the stages of the erythrocyte line is not as well defined as that for the leucocyte line. Some authors use the term *proerythroblast* for an intermediate stage between the myeloblast and basophilic erythroblast; others include the proerythroblasts within the general category of basophilic erythroblasts. It is unfortunate that the term *megaloblast* has also been used as a synonym for the earliest stage. The latter usage has led to confusion since the term megaloblast is regularly employed in clinical work to designate the primitive nucleated red cells of pernicious anemia

—cells which differ from those of normal development.

Polychromatophilic Erythroblasts. The basophilic erythroblasts undergo mitotic divisions and give rise to cells in which the hemoglobin is of sufficient quantity to be distinctly observed in stained preparations. There are many generations of erythroblasts and with each mitotic division there is a decrease in basophilia of cytoplasm and an increase in quantity of hemoglobin which is acidophilic.

The cytoplasm of the different generations of cells takes varying amounts of the acid and basic components of Wright's stain and therefore these cells show a "mixed color varying from purplish-blue to lilac or gray" and are called polychromatophilic. A few of these stages are illustrated in Figure 77.

The nucleus of the polychromatophilic erythroblast has a denser chromatin network than that of the basophilic erythroblast and the coarse chromatin bodies give a checker-board appearance which is characteristic of developing erythrocytes as contrasted with the more irregular arrangement of chromatin in the developing granulocytes. The polychromatophilic erythroblasts are smaller than the basophilic erythroblasts and larger than the normoblasts.

Normoblasts. After an unknown number of divisions the polychromatophil erythroblasts reach a stage (normoblast) in which the basophilia of the cytoplasm has decreased and the acidophilic hemoglobin increased to such an extent that the cytoplasm stains approximately as in the erythrocytes. The normoblasts are only slightly larger than the erythrocytes. Their nuclei are definitely smaller and denser in chromatin than in the erythroblasts. The normoblasts proliferate actively and eventually reach a stage in which the nucleus becomes pycnotic and no further division occurs. The chromatin becomes very compact, stains intensely, and some of the pycnotic nuclei assume bizarre shapes indicating a certain amount of fragmentation. Finally the nuclei (fragmented and unfragmented) are extruded from the cells. Some investigators believe that the nuclei normally undergo karyolysis and disappear intracellularly, but the fact that extruded nuclei are commonly found in bone marrow does not favor this view. Erythrocytes are occasionally found with small deep staining bodies (bodies of Howell-Jolly) which apparently are derivatives of nuclear fragmentation. The Cabot ring bodies sometimes found in erythrocytes of anemia were once described as remnants of nuclei but this has been shown to be incorrect. The configurations observed are laboratory creations and are the expression of cellular degeneration (Schleicher, 1942).

The youngest erythrocytes (*reticulocytes*) contain a delicate reticulum which can be demonstrated by supravital staining with dyes such as cresyl blue and neutral red (Fig. 71, *B*). The majority of the reticulocytes lose the reticular structure before leaving the marrow; normally the reticulocyte count is less than 1 per cent of the erythrocytes. Increased numbers may be called into circulation by repeated hemorrhages. Reticulocyte counts are used as an index of the effectiveness of therapy in pernicious anemia, an increase of reticulocytes after treatment being evidence of an increased production of young erythrocytes.

Extensive cell counts made on normal marrow of the rabbit (Sabin) show that less than 1 per cent of the nucleated reds are of the earliest basophilic erythroblast type. Under pathological conditions the proerythroblasts may become more numerous and new ones may develop from a more primitive cell type. The exact details of the development from the

primitive cell are controversial. According to the unitarian theory the primitive cell of origin is the hemocytoblast which in turn comes from the undifferentiated reticulum. On the other hand, Sabin believes that the earliest stage with hemoglobin differentiates directly as a daughter cell from the endothelium of the capillaries.

Normal marrow, in which normal erythrocytes develop through the stages of normal erythroblasts and normoblasts as described above is known as *normoblastic marrow*. This contrasts with *megaloblastic marrow* which occurs in pernicious anemia and other macrocytic anemias.

In addition to the different theories concerning the nature of the primitive cell of origin, there are differences of opinion as to whether the development of erythrocytes is extravascular or intravascular. According to the intravascular view, the red corpuscles develop in marrow capillaries which are temporarily closed off from circulation (Sabin, Doan, Cunningham and associates). Through experimental means (underfeeding, etc.) the red marrow of pigeons and rabbits was brought to a hypoplastic state resembling yellow marrow and the development of the early stages studied during the return to normal activity.

In ordinary sections, it is surprisingly difficult to determine whether the red cells develop outside or inside the vessels; one can usually find evidence for both views. The walls of the sinusoidal vessels are thin and frequently difficult to follow, but it is said that the details of the circulation can be seen more clearly when the vascular system of hypoplastic marrow is injected with India ink. The intravascular formation of red cells is an interesting idea and it may be added that the unitarians also agree to an intravascular differentiation of red cells (from hemocytoblasts) during embryonic development. They hold, however, that the evidence is not sufficiently conclusive in adult mammals.

It has been noted above that there is a general lack of agreement on terminology for the developmental stages of the erythrocyte. A Committee for Clarification of the Nomenclature of Blood Cells has attempted to clarify the problem by proposing a new set of terms as follows: rubriblast (for proerythroblast), prorubricyte (for basophilic erythroblast), rubricyte (for polychromatophilic erythroblasts), and metarubricyte (for normoblasts). It seems unnecessary for students to learn this additional set of terms until the new terminology has come into more general clinical usage.

General Considerations of Erythropoiesis. It has been found that ribonucleic acid (RNA) is more abundant in the basophilic erythroblast than in the later stages of the erythrocyte line. The concentration of RNA is responsible for the marked basophilia of the cytoplasm in the early stages and it is apparently correlated with the active synthesis of cytoplasmic proteins during the stages which show the greatest increase in total cell volume.

The normal development of erythrocytes is dependent upon many different factors. Obviously, all of the parent substances which enter into the formation of hemoglobin must be present. The absence of one of these, iron, is responsible for a microcytic type of anemia. Certain additional substances are necessary for the normal maturation of the erythrocytogenic cells. The absence of one of these, vitamin B_{12}, produces pernicious anemia. Vitamin B_{12} has been identified as the anti-pernicious anemia or liver factor. Actually, the real defect in pernicious anemia is the lack of some substance which is normally present in gastric juice (the intrinsic factor). The intrinsic factor facilitates the intestinal absorption of an extrinsic factor present in food (probably vitamin B_{12} itself). After absorption, B_{12} is stored chiefly in the liver until it is utilized later in bone marrow.

Megakaryocytes and Platelet Formation. The megakaryocytes are giant cells (30–100 μ) derived from the hemocytoblast and they apparently give rise to blood platelets. In the normal adult

they are usually said to occur only in marrow. During embryonic development, however, they are also present in other hemopoietic organs (liver, spleen). The nucleus is lobulated in a variable and complicated manner. In some cells there are only slight constrictions to indicate the presence of lobes but it is not uncommon to find forms with the nuclear lobes connected merely by delicate strands (Fig. 78). The cytoplasm is finely granular. Pseudopodia may extend through the walls of the sinuses and are said to form platelets by constriction and segmentation.

Megakaryocytes are limited in their life span and stages of degeneration are not uncommon. The nucleus becomes smaller, stains more intensely, and the protoplasm degenerates. The naked, fragmented nuclei may find their way into the blood vessels to be carried through the right side of the heart into the lung capillaries where they degenerate. In pathological conditions (Minot) entire megakaryocytes may be carried into the lungs and form an embolism of the vessels.

Development of Lymphoid Elements

Lymphocytes. In the germinal centers of lymphoid organs, some of the undifferentiated reticular cells develop into large and medium-sized lymphocytes characterized by basophilic cytoplasm and pale staining nuclei containing one or more large nucleoli. (See pages 253–254 and Fig. 203.) The most immature lymphocytes resemble the primitive cells of bone marrow and according to the unitarian theory of blood development they are merely the same cells in different locations and constitute a common stem type (hemocytoblast). On the other hand, the dualistic theory claims that the large lymphocytes are slightly different from the primitive marrow cells and that they can develop only into lymphocytes; therefore, they are known as *lymphoblasts*. These cells have an undifferentiated type of nucleus with coarse nucleoli and are not identical with the large lymphocytes found in the normal circulation (Figure 71, *A*, Lm-4). In sections stained with hematoxylin-eosin-azure, the appearance of the most immature lymphocytes is practically identical to that of the hemocytoblasts illustrated in Figure 78.

The large and medium-sized lymphocytes proliferate actively and develop into the small lymphocytes commonly found in the circulation. The small lymphocytes enter the circulation by way of the lymphatics and, to some extent, by penetration through the walls of the blood capillaries of lymphoid organs.

The differentiation of reticular cells into large lymphocytes normally accounts for only a small proportion of the total lymphocytes; their numbers are maintained chiefly by mitotic divisions of the large and medium-sized forms. This proliferation occurs in all parts of the lymphoid tissue and is not confined to the germinal centers which are intermittent in their activity. Although the small lymphocytes retain the ability to proliferate, their divisions are infrequent under normal conditions. According to the dualistic theory the small lymphocyte represents the end stage of a line of differentiation, but in the unitarian view, it is merely a temporarily inactive form of the hemocytoblast. It is claimed that the small lymphocytes can hypertrophy in the blood forming organs, becoming large-sized lymphocytes (hemocytoblasts) with unrestricted potencies for development into any of the formed elements of the blood.

Monocytes. There are numerous theories for the origin of monocytes. They have been derived from histiocytes, monoblasts, lymphocytes, and hemocytoblasts.

As evidence for the development of monocytes from histiocytes, it may be pointed out that, in tissue culture experiments, macrophages have not only been developed from monocytes but have also been made to reassume the appearance of monocytes by varying the culture medium. This, however, is not convincing evidence that the monocytes of the circulating blood normally arise from the connective tissue histiocytes.

According to another theory (Sabin and others), undifferentiated reticular cells form primitive free cells which develop into myeloblasts, lymphoblasts, and monoblasts. Monoblasts are said to form chiefly in the spleen. The main criticism of this view is that it is extremely difficult to differentiate the monoblast from the lymphoblast. The unitarian theory makes no distinction between these cells and holds that monocytes develop from hemocytoblasts. It has also been shown by Bloom, that the small lymphocytes can change into monocytes in tissue culture. Since the small lymphocyte is considered as merely a form of the hemocytoblast, the experiments by Bloom are not in disagreement with the unitarian theory. It is said the change from small lymphocytes to monocytes may occur in blood vessels anywhere but that it is most likely to take place where the circulation is sluggish as in the sinuses of the spleen. This theory has a number of points in its favor and also furnishes an interesting explanation for the fact that the circulating blood usually contains a few intermediate cells which are very difficult to definitely diagnose as either typical lymphocytes or monocytes.

EMBRYONIC DEVELOPMENT OF BLOOD CELLS

Blood development begins shortly after the formation of the germ layers. It is initiated in the third week of development in the extra-embryonic mesoderm of the yolk sac by the formation of *blood islands*. These consist of mesenchymal cells which have become less irregular in shape and whose cytoplasm has assumed a definitely basophilic character. The cells proliferate actively and become more closely packed, forming solid cellular clusters, but soon show a differentiation in two directions. The peripheral cells of the cluster become flattened and form the primary endothelium of the first blood vessels. The central cells become detached and float freely in the plasma which is gradually accumulating (Fig. 79). These isolated mesenchymal cells with their vesicular pale-staining nuclei and basophilic homogeneous cytoplasm represent the first primitive blood cells or *hemocytoblasts*. In this manner both endothelium and primitive blood cells are formed simultaneously from the mesenchyme.

Some of the hemocytoblasts remain as primitive blood cells characterized by a basophilic cytoplasm but the majority of them become transformed into *primitive erythroblasts* and primitive nucleated erythrocytes by the elaboration or accumulation of hemoglobin in their cytoplasm. The primitive erythroblasts are characterized by their large size and they form a special race of their own which has but a transient existence. Under normal conditions they develop only in the embryonic yolk sac but cells of a similar type may develop in adult bone marrow in pernicious anemia. In some mammals (rat, mouse), the primitive strain is the only type of red cell formed in the yolk sac but in human embryos a few *definitive erythroblasts*—cells identical with the normal erythroblasts of marrow—develop in the yolk sac after six or seven weeks (Bloom and Bartelmez).

The hemocytoblasts are continuously being detached throughout the embryonic period. New ones also can differentiate from the reticular connective tissue cells throughout life. For a limited embryonic period, hemocytoblasts also arise from the primitive endothelium. Among the free cells which are formed, there are some which differ in structure from the hemocytoblasts. They have smaller darker staining nuclei and a vacuolated cytoplasm which is only slightly basophilic. They are

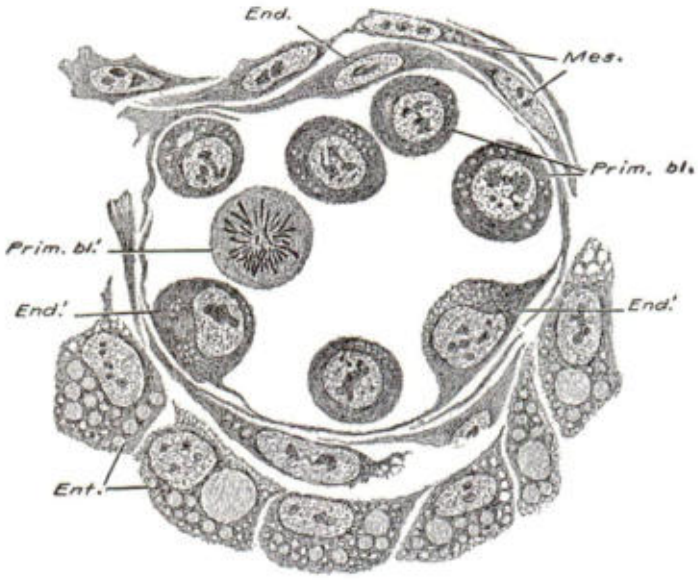

FIG. 79. Section through a vessel of the area vasculosa of an eight and one-half day rabbit embryo. *End*, Primitive endothelium; *End'*, primitive endothelial cells rounding off and beginning to differentiate into primitive blood cells; *Ent*, entoderm; *Mes*, mesenchyme; *Prim. bl*, primitive blood cells; *Prim. bl'*, primitive blood cell in mitosis and with cytoplasm showing differentiation toward the primitive erythroblast. (Redrawn from Maximow, Arch. mikr. Anat., 1909, vol. 73.)

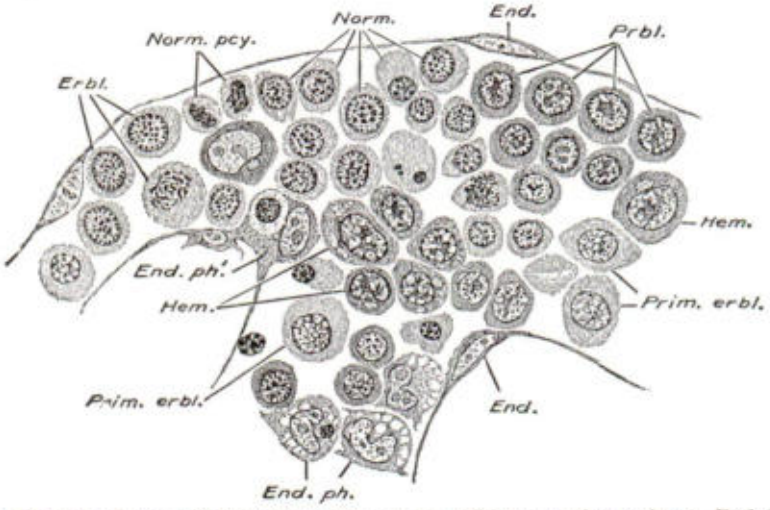

FIG. 80. Portion of a vessel from the yolk sac of a 13½ day rabbit embryo. *End*, Endothelial cells; *End. ph*, phagocytic cells which have developed from the primitive endothelium and *End. ph'*, a phagocytic cell which is still attached to the endothelium; *Erbl*, erythroblasts; *Hem*, hemocytoblasts; *Norm*, normoblasts; *Norm. pcy*, normoblasts with pycnotic nuclei; *Prbl*, proerythroblasts; *Prim. erbl*, primitive erythroblasts. Magnification is slightly less than that of Figure 79. (Redrawn from Maximow, Arch. mikr. Anat. 1909, vol. 73.)

actively phagocytic and readily store vital dyes. They are the first histioid wandering cells and correspond to the histiocytes of later life.

The liver forms the second important hemopoietic organ of the fetus. Blood formation begins at about six weeks, before the spleen and marrow have appeared. Here too the mother stem cell (hemocytoblast) is a large cell with a pale-staining vesicular nucleus and basophilic cytoplasm. The hemocytoblasts are probably in part brought in by the circulation. In part they are formed from the scanty mesenchyme between the liver cords and from the endothelium of the sinusoids. The hemocytoblasts divide actively and differentiate into nucleated and non-nucleated red cells, granular leucocytes and megakaryocytes. The nucleated red cells (normoblasts) are predominant in the fetal circulation during the second month. Blood destruction also occurs in the fetal liver as evidenced by pigment formation, nuclear disintegration, and phagocytosis by histiocytes. The liver remains the most active place of blood formation until the middle of fetal life. Then its activity slowly decreases, and at birth or soon after, hemopoiesis ceases under normal conditions.

The spleen appears as a blood-forming organ in the latter part of the second month and remains active until the eighth month. After this period erythropoiesis gradually diminishes and ceases soon after birth, but lymphocytes and monocytes are formed throughout life. In the lymph nodes the embryonal formation of erythrocytes is of very little extent and does not occur in the parenchyma. Clusters of developing erythrocytes have, however, been observed in the septal connective tissue in the vicinity of blood vessels. The lymph nodes function throughout life as the chief organs of lymphocyte formation. Here again the mother stem cell (lymphoblast) resembles the hemocytoblast, having the large, pale-staining nucleus and nongranular basophilic cytoplasm. These cells are formed by proliferation of their own kind and by new detachment from the reticulum of lymph nodes and spleen.

The most important and permanent hemopoietic organ is the red marrow of bones which makes its appearance in the third fetal month. The perichondral mesenchyme invades the cartilaginous primordia of the bones and constitutes the primitive marrow. Again mesenchymal cells detach themselves to become hemocytoblasts (myeloblasts) and histiocytes, marrow being apparently able when needed to furnish such free elements throughout adult life. From the hemocytoblasts are formed the megakaryocytes, and the erythroblasts and myelocytes which proliferate and differentiate into the erythrocytes and granulocytes.

With the advent of bone marrow as a hemopoietic organ in the third month, the number of circulating normoblasts is decreased and the nonnucleated erythrocytes become predominant in the circulation.

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VIII

MUSCLE

Three types of muscle are distinguishable histologically: *smooth*, *skeletal* and *cardiac*. Smooth or *non-striated* muscle lacks the cross striations seen in other muscle and it is *involuntary*, being especially associated with viscera which are not under voluntary control. Skeletal muscle is *striated* and *voluntary*, making up the bulky muscles of the body. Cardiac or heart muscle is *striated* but *involuntary*.

SMOOTH MUSCLE

Smooth muscle consists of fusiform or spindle-shaped cells with abundant cytoplasm in whose central thickest portion the nucleus lies. As a rule, the cells are gathered into dense sheets or bands, but they may occur as isolated units scattered among the connective tissue fibers in a tissue like the dartos tunic of the scrotum. The fresh smooth muscle tissue is pale pink in color, tough and resistant to stretching. In this condition the outlines of the cells are difficult to distinguish, the nuclei are occasionally visible as oval granular objects and the entire mass has a faint longitudinal striation.

Within the bands, the cells are roughly parallel to each other but are irregularly and densely packed together so that the narrower portion of one cell lies against the wider portions of its neighbors. The shape varies according to the organ containing the muscle: for example, very long and slender in the walls of the intestine (Fig. 81), short and thick in the walls of small arteries, or thrown into irregular folds and twistings by elastic fibers in the walls of large arteries. The greatest diameter varies from 3 to 8 microns, the length from 15 to 200 microns, except in the pregnant uterus where it may exceed half a millimeter (500 microns). The complete outlines of the cells cannot be made out with certainty in a longitudinal aspect of either fresh material or prepared sections, due to the overlapping. The outlines are easily seen in cross sections, however, where they are round, oval or flattened and vary greatly in size because the individual cells, due to their fusiform shape, may be cut through their thick central portions or at the narrow ends (Fig. 82). The individual cells can be separated from one another by teasing after treatment with nitric acid (Fig. 81) so that the shape of the cells, the central nuclei and faint longitudinal marking or striation can be seen.

The *nucleus* conforms to the outline of the cell as a whole and may be, therefore, oval, a flat disc or a long rod (Fig. 81). The cytoplasm near the nucleus is relatively undifferentiated. It contains mitochondria, a Golgi apparatus, and occasional fat droplets. The principal mass of the cytoplasm has been specialized

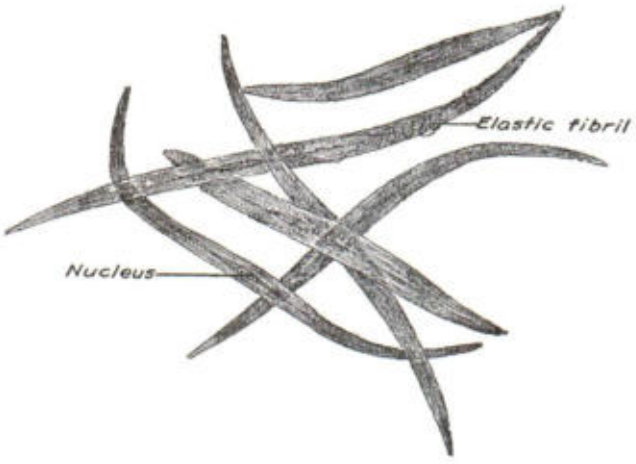


FIG. 81. Smooth muscle cells teased from intestine treated with nitric acid. Unstained. × 800.

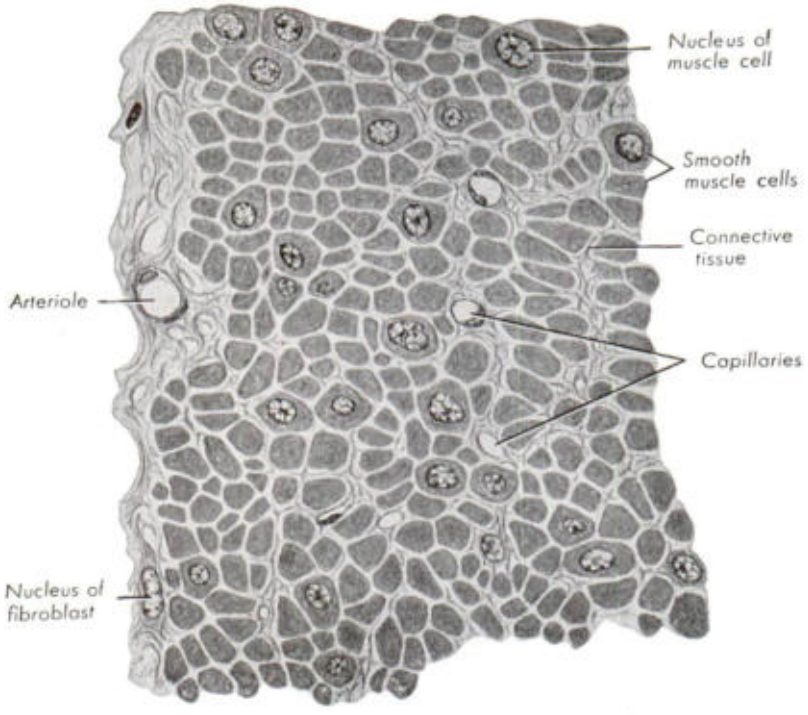


FIG. 82. Smooth muscle cut transversely, from the muscularis externa of human stomach. Hematoxylin and eosin. × 1665.

for contraction. In ordinary slide preparations, it appears quite homogeneous and is acidophilic. Differences in the intensity of staining may be associated with the state of contraction or relaxation as will be pointed out a little later.

Although the greater part of the *cytoplasm* appears homogeneous in the living cell it is possible to bring out definite longitudinal fibrillae by subjecting the cells to the appropriate treatment. The entire outer cytoplasm may be filled with very fine fibrillae or there may be differences in the outermost layers, in which case, the more peripheral fibrillae are coarser and stain more heavily. In slides prepared according to the usual methods, the cytoplasm has a vague longitudinal marking, such as that shown in Figure 83. The external surface of the cytoplasm functions as a cell membrane, but it does not form a well-defined sarcolemma as in skeletal muscle.

Within the bands of muscle, the individual cells are held together by an investment of connective tissue consisting of reticular fibers and fine collagenous fibers. By special techniques, elastic fibers can be demonstrated also. The latter are occasionally seen in macerated preparations as tiny coils around the cells (Fig. 81). Between the larger bundles of fibers there are coarser collagenous and elastic fibers (Fig. 82). Since the smooth muscle of mammals either ends in soft parts or forms more or less continuous circular or spiral bands, there is no specialized connective tissue attachment such as the tendon of skeletal muscle.

The *contraction* of smooth muscle is slow and sustained. This is quite the opposite of skeletal muscle which contracts rapidly and is soon fatigued. Two types of contraction have been observed. Each individual cell may contract in its entirety, or the contraction may pass over the cell in a wave, only part of each cell being in a state of contraction at a given instant. When the cells have been caught in a completely contracted state by the fixative, they appear shortened and stain more intensely. If a wave has been caught passing over the cell by the fixing reagent, the contracted portion is bulged and shortened and is more heavily stained than the rest of the cell, so that it looks like a swollen dark segment of the cell. The *contraction bands* have a tendency to extend in lines across the

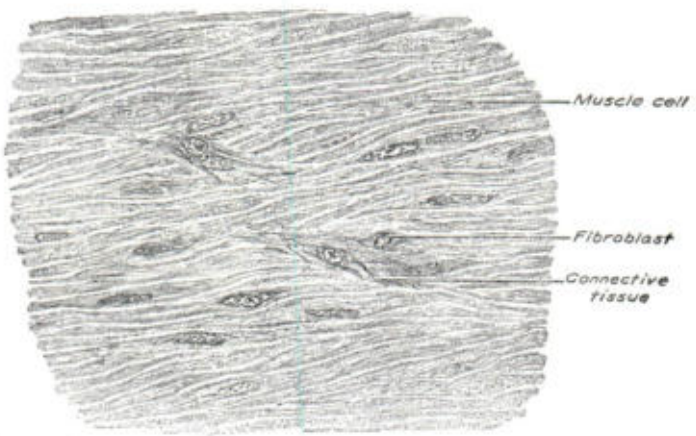


FIG. 83. Smooth muscle cut longitudinally. Human intestine. × 500

whole sheet of muscle so that the contracted swelling involves the center of one cell and the narrower extremities of its neighbors at the same time (Fig. 84).

There are *nerve endings* about smooth muscle cells, but motor terminations for every cell have not been demonstrated. It seems altogether probable, therefore, that a type of transmission of impulses other than nervous is utilized. One possibility is that the mechanical pull exerted by one cell in contraction serves as the stimulus for its neighbors; another is that a chemical agent comes into action.

Smooth muscle is not as richly supplied with blood vessels as skeletal or cardiac muscle. The arteries and veins are carried in the coarser septa of connective tissue. The capillaries lie in connective tissue between thin layers or small groups of cells rather than about individual cells. Connective tissue cells are rare among the reticular fibrils between the muscle cells unless associated with the blood vessels or larger connective tissue laminae.

Smooth muscle is found in the wall of the alimentary canal from the middle of the esophagus to the anus; gall bladder and hepatic ducts; dorsal wall of trachea, and whole bronchial tree; ureter, bladder, urethra, corpora cavernosa, testes and ducts, prostate and Cowper's glands, broad ligament, oviduct, uterus and vagina; blood vessels, larger lymphatics and spleen. Smooth muscle occurs in the skin in connection with hairs (arrectores pilorum) and occasionally in other places (e.g., corrugator cutis ani). In the eye, it is found in the iris and ciliary body.

Development of Smooth Muscle. Smooth muscle is derived from the loose mesodermal network of the embryo known as mesenchyme. Exceptions are found in the iridic muscle of the eye and in the modified muscle cells in the walls of sweat glands which are derived from the ectoderm. The forerunners of the muscle cells cannot be distinguished at first from the connective tissue elements with which they are mingled. The cells increase in number by mitotic division. Some of them become recognizable as *myoblasts* by their elongated nuclei and

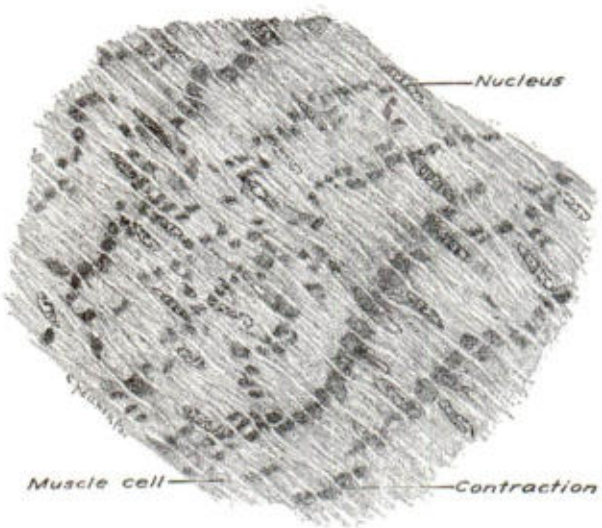


FIG. 84. Smooth muscle from human intestine showing contraction nodes. × 500.

spindle shape. The new myoblasts continue to arise by differentiation from the network during the earlier stages of development. Later the division of existing myoblasts gradually takes the place of this differentiation in the production of new muscular elements.

Longitudinal markings may be seen at the surface of some of the elongated cells. This is probably the beginning of differentiation into muscle cells. Later the longitudinal striation can be resolved into homogeneous fibrillae in the peripheral cytoplasm. At first the myofibrillae are delicate threads; later the peripheral fibrillae become thicker and fine fibrillae appear in the deeper layers of the cytoplasm.

Scattered oval nuclei around and among the groups of elongated myoblasts mark the presence of connective tissue fibroblasts. As the muscle cells develop into sheets or bundles, the fibroblasts lay down collagenous, elastic and reticular fibers.

Proliferation of smooth muscle cells by mitotic division has been found in the uteri of virgin rabbits treated with female sex hormone. The formation of new muscle cells from undifferentiated cells during pregnancy has been described. The regenerative powers of the muscle coats of the alimentary tract are limited, however, and the healing of wounds takes place principally by scar formation. The muscle cells of the walls of new blood vessels associated with healing processes have been described as coming from primitive types of perivascular cells.

SKELETAL MUSCLE

The smallest independent units of skeletal muscle are called fibers. Although they are frequently referred to as cells, the more specific term, fiber, is preferable. They are much more complicated than the cells of smooth muscle; they have many nuclei and are larger than most cells. The fibers are grouped together into bundles called fasciculi. In some muscles (gluteus maximus, deltoideus), the bundles are larger than in others giving the muscle a coarse-grained appearance when seen with the naked eye. The larger muscles are composed of many fasciculi.

Fibers. The *fibers* are cylindrical or prismatic structures whose greatest length in man has been found to be 4 cm. Excised muscle, more particularly that removed after rigor mortis has set in, can be teased into its constituent fibers. The separation is made easier by maceration or treatment with nitric acid, but the ends of the fibers appear torn and broken (Fig. 87). Huber teased all the fibers of individual fasciculi from rabbit muscles, preserving their entire length intact and at the same time keeping them in their original relationship to the length of the fasciculus. He found three types of fibers: (1) those extending from one end of the fasciculus to the other; (2) those beginning at one or the other end of the fasciculus and terminating within the substance of the bundle; and (3) those having both ends within the muscular substance (Fig. 85). The fibers are the same length as the fasciculi if the latter measure less than 1.5 cm. and may be shorter than 1 mm. in the muscles of small animals and in the very small muscles of man such as the M. stapedius. A common average length for a fiber is 3 cm., the greatest length 4 cm. It should be mentioned, in this connection, that fasciculi are often much shorter than the whole muscle, particularly in penniform muscles. The fibers which are attached at the tendinous ends of a fasciculus terminate abruptly in flat or bluntly rounded extremities, whereas the terminations within the

substance are long tapering points. Muscles which end in the skin or mucous membranes, such as those of the face and tongue, may have branched ends; this condition is well illustrated in the frog's tongue.

The *diameter* of the fibers varies from 10 to 100 microns, so that, in many cases, they are visible to the naked eye. Although fibers of different thickness are intermingled in the same muscle, there is a more or less typical size for each muscle, and some correlation has been found between the heaviness of the work a muscle performs and the thickness of its fibers. Fibers in the delicate ocular muscles are much smaller than those of a bulky muscle like the gastrocnemius (Fig. 86). The fibers of a well nourished individual are thicker than those of one who is emaciated. Moreover, the increase in size of a muscle which takes place during the growth of an individual or which is brought about by exercise, is due to an increase in the size of its fibers rather than an increase in their number. The fibers of amphibians and fishes are generally thicker, while those of birds are thinner than those of mammals.

Completely surrounding the muscle fiber is a sheath, the *sarcolemma*. An elastic, transparent, homogeneous membrane, it is difficult to see in fresh muscle

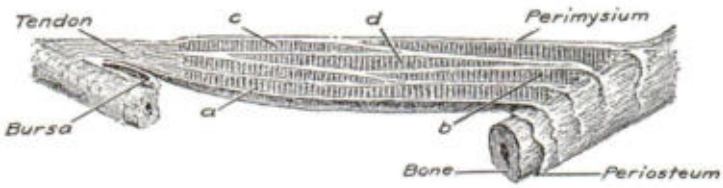
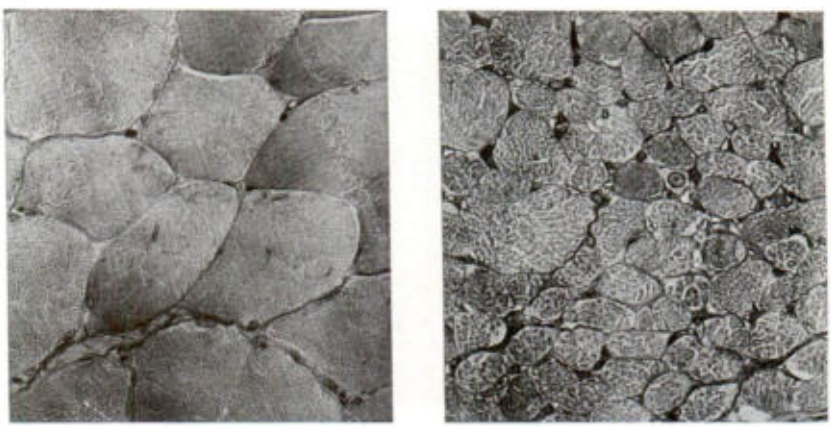


FIG. 85. Diagram of attachment of muscle to skeleton and relation of fibers to each other within a fasciculus. *a*, Fiber extends the length of fasciculus; *b*, fiber begins at periosteum but ends in muscle; *c*, begins at tendon but ends in muscle; *d*, both ends of fiber within the muscle. (Redrawn from Braus.)



A *B*

FIG. 86. Relative size of muscle fibers. *A*, Gastrocnemius. *B*, Ocular muscle. Human. The muscles were taken from the same subject, a middle-aged adult, and were photographed at the same magnification. × 335.

unless the fibers have been teased and torn. In the latter case it may be seen projecting beyond the interior or extending between the fractured and retracted ends of the fibrillar mass (Fig. 87). In fixed preparations, it does not stain readily, but can be seen clearly in thin longitudinal sections. Microdissection studies have shown that it is a part of the fiber, a cell membrane elaborated by the protoplasm during development. Electron micrographs show that it is actually composed of a double membrane. Inside the sarcolemma are the nuclei and a cross striated substance composed principally of the *myofibrillae*. Surrounding the fibrillae and accumulated near the nuclei is the more fluid portion of the fiber which is called the *sarcoplasm*. It corresponds in many respects to the cytoplasm of other cells.

The skeletal muscle fiber has many *nuclei*, several hundred appearing within a fiber of average size. The characteristic

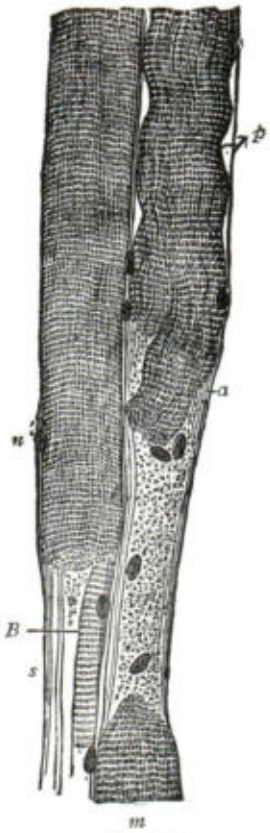


FIG. 87

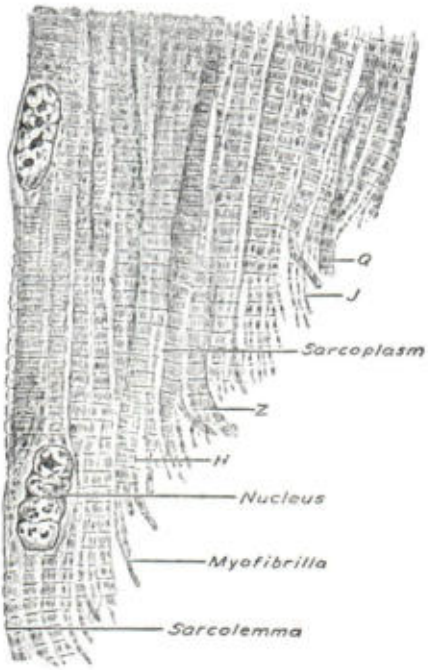


FIG. 88

FIG. 87. Semidiagrammatic drawing of parts of two muscle fibers which have been broken, showing the relations between muscle substance proper and sarcolemma. (Ranvier.) *m*, *a*, Retracted ends of muscle substance, between which is seen the sarcolemma with several adherent muscle nuclei; *B*, thin layer of muscle substance which has adhered to the sarcolemma; *n*, muscle nucleus; *s*, sarcolemma; *p*, space between sarcolemma and muscle substance.

FIG. 88. Human muscle fiber showing individual fibrillae. × 1500.

position for these nuclei in mammals is directly under the sarcolemma (Fig. 91), but occasionally, a fiber may be found which has nuclei in the interior. They are flattened oval bodies, frequently elongated and of approximately the same size as the nuclei of neighboring connective tissue cells. They are scattered along the fiber in irregular spirals or longitudinal rows. In fresh muscle they are difficult to see, faintly outlined against the background of striated substance. In fixed preparations they show a loose network of chromatin threads and granules. The interior position is more frequently encountered in less differentiated muscles, particularly in the so-called dark fibers (see p. 146) or those rich in sarcoplasm. Interior nuclei are of common occurrence in the lower vertebrates, and in the skeletal muscles of insects it is usual for the nuclei to form an axial column in the center of the fiber.

Myofibrils. Skeletal muscle examined with a comparatively low magnification has a striking and characteristic appearance due to the regular alternation of light and dark stripes or striations across each fiber. These striations are visible whether the muscle is living, freshly removed from the body, or fixed and stained, and in either ordinary transmitted light or polarized light. Examination with higher magnification, however, reveals that the bands are not homogeneous. They are produced by alternating light and dark segments of longitudinally arranged elements, the myofibrillae.

The *myofibrillae* impart to the muscle fiber as a whole an appearance of longitudinal striation. They range from 1 to 2 microns in diameter for the most part, but may be as small as 0.2 of a micron. They are visible within fibers of teased fresh muscle as well as in fixed slides and often protrude from the frayed

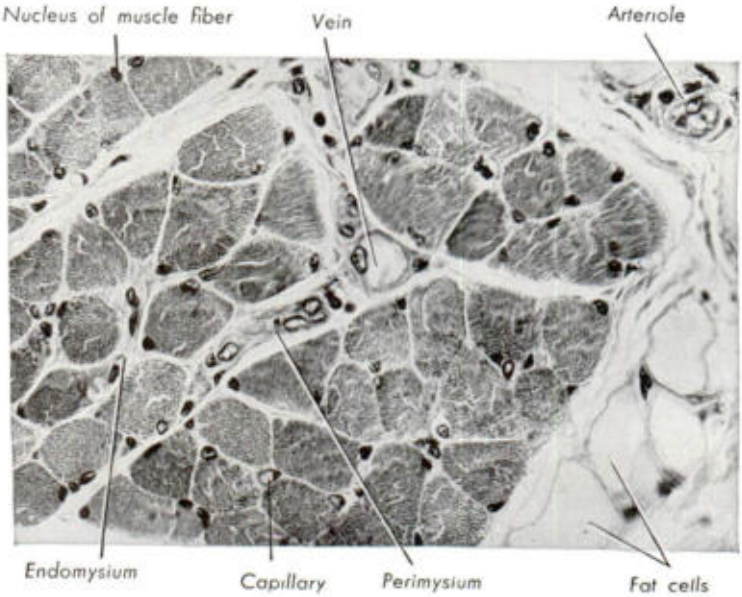


FIG. 89. Cross section of skeletal muscle. Human tongue. Hematoxylin and eosin. Photomicrograph. × 425.

FIG. 90. Cross section of human skeletal muscle fiber, drawn with camera lucida. × 1500.

ends of torn fibers (Figs. 87 and 88). Similar segments of adjacent fibrillae are placed side by side in an intact fiber to form the characteristic cross striations seen in the fiber as a whole.

In a cross section of a fiber, the cut ends of the myofibrillae are usually visible as definite areas with individual size and shape. They are separated from each other by narrow spaces which represent the *sarcoplasm*. When the sarcoplasm is distributed irregularly, the fibrils appear in groups known as Cohnheim's fields (Fig. 90).

The *cross striations* are confined to the myofibrillae and they usually stand out clearly in longitudinal sections of fixed muscle. They color readily with a variety of dyes but the iron hematoxylin of Heidenhain is a particularly good stain for the minute structure of the striations (Fig. 92). The more refractive segments are deeply colored, while the less refractive ones are pale. The dark band is known as the A or Q stripe—A from its anisotropic refractive property and Q from the German word Querscheibe meaning cross-disc (Figs. 91 and 92). The light band is designated I or J—I from its isotropic refractive property and J from the German terminology. Each of these bands is bisected by a narrow line: that in the I band stains deeply and is designated Z from the German Zwischenscheibe, between-disc; the line bisecting the A (Q) band is pale and is designated H, both from the German word hell, meaning light, and from the name of the discoverer, Hensen. Within the H line or disc there is a narrow stripe designated by the letter M. The Z line is also called Krause's membrane because this author first described its structure as a membrane extending across the entire fiber, bridging the spaces between the fibrillae and being pierced by them. However, electron

micrographs show that the Z line is confined to the myofilaments.

The portion of a fibrilla or column between two successive Z lines is called a *sarcomere*. Its height in relaxed mammalian muscle is 2 to 3 microns. It may be stretched to a greater length and in greatly contracted fibers it may be less than one micron. Insect muscles with sarcomeres 14 microns high have been described.

It is known that muscular tissue in general is doubly refractive in polarized light. As early as 1858, however, Brücke discovered that all parts of the striated fiber did not possess this property to the same extent. When a fiber is viewed with crossed Nicol prisms, the A band is visible as a bright band while the I is dark and invisible. In order to avoid confusion in terminology, it is important for the reader to notice that this light A band corresponds to the dark, heavily stained A of fixed sections.

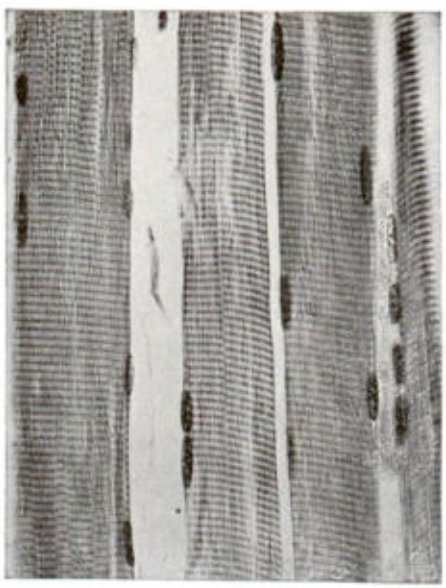


FIG. 91. A longitudinal section of human pectoralis minor muscle. Hematoxylin and eosin. Photomicrograph × 425.

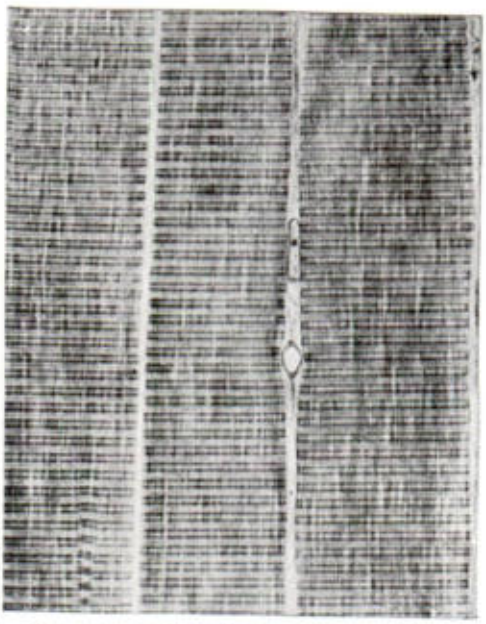


FIG. 92. Longitudinal section of human skeletal muscle fibers. Each dark disc (*A*) is bisected by a very distinct light zone or band (H). Bisecting each light disc (I) is a very narrow but distinct dark line or Krause's membrane (Z). A capillary and also the nucleus of a fibroblast are evident between two of the fibers. The nuclei of the muscle fibers are not visible. Phosphotungstic acid hematoxylin. Photomicrograph × 905.

As already noted, the A band is anisotropic whereas the I band is relatively monorefringent or isotropic.

Studies with the electron microscope have revealed all of the cross bands observed with the ordinary light microscope plus a few additional bands. These studies have also established the fact that each of the myofibrils is composed of a number of thinner, thread-like elements known as *myofilaments*. According to one view there are two types of myofilaments: *myosin* myofilaments which are confined to the A band, and *actin* myofilaments which extend from the Z line through the I band and into the A band to the neighborhood of the H

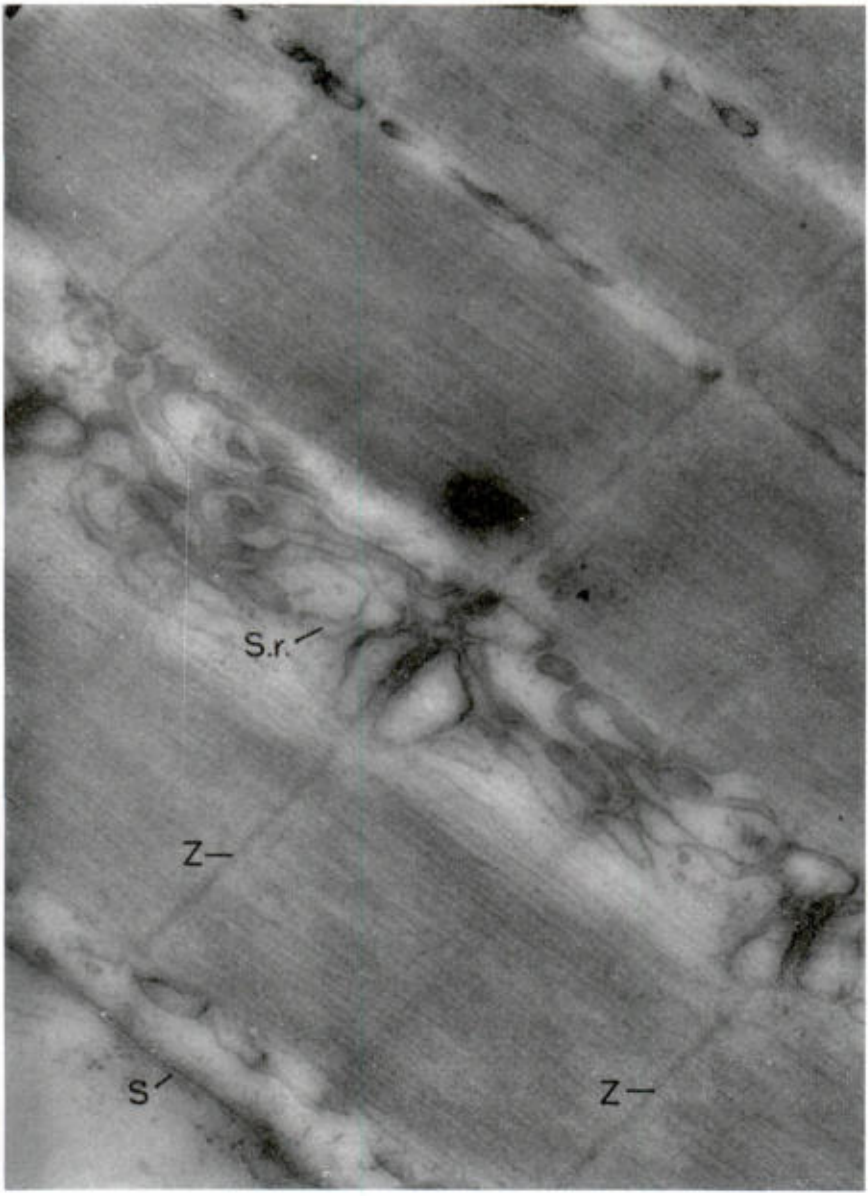


FIG. 93. Electron micrograph of the marginal portion of a skeletal muscle fiber from myotome of Amblystoma punctatum. Three myofibrils, each composed of numerous myofilaments, run across the image from upper left to lower right. The cross bands or striations are not as distinctly shown as in some cases but the sarcoplasmic reticulum (*S.r.*) is particularly clear. It courses predominantly in the long axis of the fiber except in the area adjacent to the Z disk. The sarcolemma (s) is seen at the lower left. × 40,000. (Courtesy of Dr. Keith Porter.)

line where they unite by means of an unidentified protein with the actin filaments in the other half of the sarcomere. According to another view, the myofilaments are all alike but are thicker in the region of the A band where they contain both myosin and actin. They are thinner in the I bands where they are composed of actin only. It should be noted that the two views agree that myosin is confined to the A band.

The Sarcoplasm. The sarcoplasm fills all the interstices between the myofibrils. It is less refractile than the fibrillae and appears as a clear homogeneous substance containing scattered mitochondria, lipid droplets and glycogen.

Fibers which are rich in sarcoplasm have a dark appearance in the fresh state and are called "dark fibers" whereas those that are poor in sarcoplasm are called "light fibers." These two types of fibers are intermingled in human and most other mammalian muscles.

The *sarcoplasmic reticulum* (corresponding to the endoplasmic reticulum of other cells) can be seen in electron micrographs (Fig. 93). The reticulum and its associated RNA granules are found in the regions which appear as homogeneous sarcoplasm under the light microscope. It is composed of a network of cisternae or membranous tubules which course between the myofibrils and join the sarcolemma opposite the Z

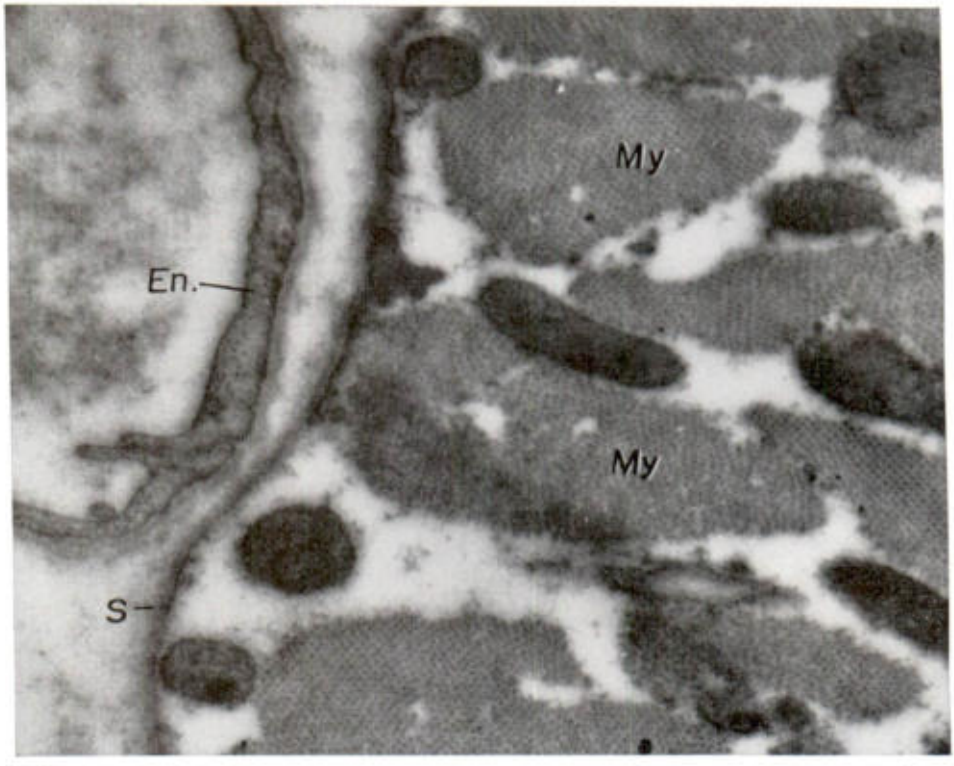


FIG. 94. Electron micrograph, showing myofibrils (*My*) cut transversely. Note that each myofibril contains many myofilaments. *S*, sarcolemma; *En.*, endothelial cell of capillary blood vessel. From section of cardiac muscle. × 30,750. (Courtesy of Dr. Dan Moore.)

lines. The transverse arrangement and density of the network at the level of the Z lines probably accounts for the earlier erroneous descriptions of fusion of Z lines with the sarcolemma.

Sarcosomes were observed as granular elements in fresh muscle many years ago. They may appear less abundant or may even be lacking in fixed material because of the chemical treatment to which the fibers have been subjected. They tend to lie in clumps where there are accumulations of sarcoplasm just under the sarcolemma, near the nuclei, and between the myofibrils. Studies with the electron microscope indicate that most of the structures originally described as sarcosomes are mitochondria. They are oval or rod-shaped and usually aligned with the long axis of the muscle fiber.

Changes During Contraction. Morphological changes associated with contraction have been studied both in living and in fixed muscle. In both cases, it is seen that the fiber as a whole becomes shorter and broader when it contracts. Each sarcomere also becomes shorter and broader. The isotropic or I band becomes particularly thin during contraction; in fact, it seems to disappear when the fibers are stimulated to contract by 50 per cent. At this time, the A substance of each half of a sarcomere appears to fill the regions formerly occupied by the I bands and the H line becomes more obvious. This produces the so-called reversal of striations. When the portions of the A substance from adjacent sarcomeres fuse along the Z line, they give a dense, darkly-stained structure known as the *contraction band*.

Reversal of striations in contracted muscle was observed first under the light microscope. Later studies with the electron microscope suggest several possible explanations for the appearance of reversal. According to the view which holds that there are two types of myofilaments (thick myosin filaments in the A band and thin actin filaments in the I band and in a part of A), it is thought that contraction is accompanied by a sliding of the thin (actin) filaments along the thick (myosin) filaments until the former are completely within the A band. According to the view that there is only one type of myofilament (a thin actin filament throughout the sarcomere accompanied by myosin in the A band), contraction is accomplished by an altered molecular arrangement which draws the I band material into the A band.

The contractile protein, actomyosin, is a complex of actin and myosin in which cyclic changes occur with the cooperation of adenosinetriphosphate (ATP). The length (or contraction) of each protein thread is dependent upon the state of combination of its components, actin and myosin. As noted above, the morphological configuration of these components during contraction is controversial.

When the nerve to a muscle is stimulated, there is a change in electrolyte balance along the sarcolemma and it has been suggested that the impulse for contraction may be conveyed to the interior of the fiber by the sarcoplasmic reticulum which unites with the sarcolemma opposite the Z lines. As a consequence of nerve stimulation, the actomyosin complex absorbs adenosinetriphosphate (ATP) and certain cations (K^+ and Mg^{++}). In the process of contraction and relaxation, adenosinetriphosphate (ATP) is changed to adenosinediphosphate (ADP) with the release of free phosphate ions which supply the source of energy. The ADP is later rephosphorylated into ATP and the muscle is ready for contraction again. Mitochondria, carrying the enzymes of the

citric acid cycle, are important in muscle function because they play an important role in the formation and maintenance of ATP.

Connective Tissue. Surrounding each muscle fiber is a sheath irregularly woven out of very delicate reticular fibrils (Fig. 95). It is called the *endomysium*. At many points the delicate fibrils combine into stronger strands which merge with collagenous connective tissue fibers. The collagenous fibers, mingled with elastic fibers, form a more or less complete connective tissue sheath about groups of a dozen or more muscle fibers to make up the fasciculi. The fasciculi are in their turn bound into larger and larger orders of bundles and the entire muscle has its outer investment, the deep fascia seen in gross anatomy. The outermost sheath of connective tissue is called the *epimysium*, while the tissue surrounding the fasciculi and dividing the muscle by septa is called the *perimysium*.

Muscle-tendon Junction. At the ends of a muscle, its fibers are attached securely either to tendon, to periosteum or to some fibrous connective tissue structure. The terminations of the muscle fibers are rounded, abruptly conical or long and tapering and in certain organs, such as the tongue, may be split into digitations. Studies on the muscle-tendon junction have dealt chiefly with the question whether the fibrillae of muscle and tendon are continuous with each other or are discontinuous and separated by the sarcolemma.

In sections stained with the ordinary dyes, the fibrillae of the muscle fiber often appear to be continuous with those of the tendon, no definite boundary between them being discernable. The striations of the muscle fiber decrease in distinctness so that the exact point where they disappear and the tendon fibers begin is uncertain. In other preparations the muscle fiber seems to fit into a cup-shaped depression in the tendon and the two are quite sharply delimited. There is usually an increased number of nuclei at the junction but it is difficult to determine whether they are muscle or connective tissue nuclei.

From a study of sections stained for

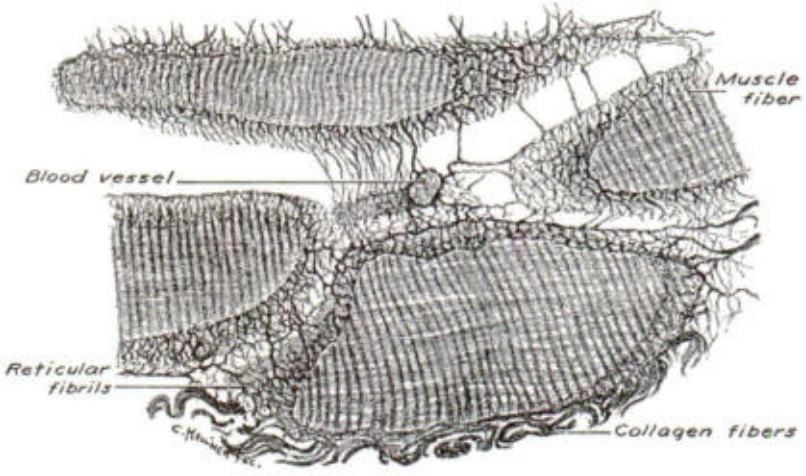


FIG. 95. Reticular fibrils of endomysium about skeletal muscle fibers. Foot silver method. × 900.

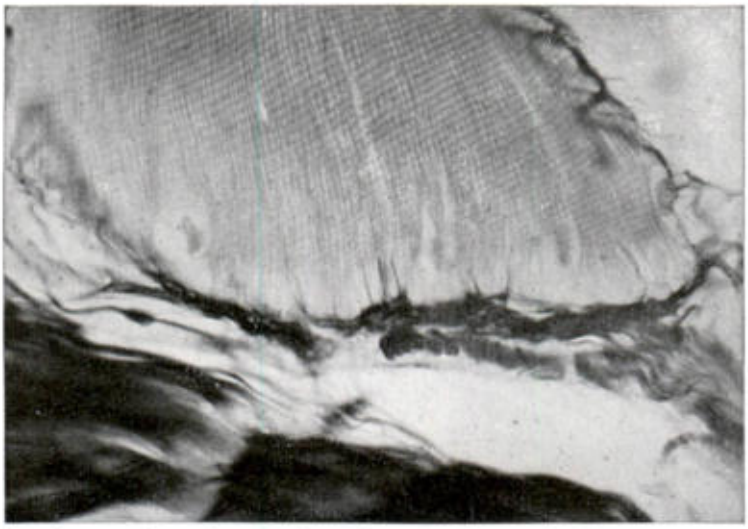


FIG. 96. Attachment of muscle to tendon. The end of the muscle fiber is clearly outlined and is enclosed by the argyrophil net which sends straight, needle-like projections into invaginations of the terminal surface of the fiber. A capillary containing red blood corpuscles lies between the tendon (below) and the termination of the muscle fiber. Monkey. Bielschowsky-Masson. × 600. (After Goss.)

reticular fibers by the method of Bielschowsky, Goss reports that the argyrophil fibers have an arrangement peculiar to the attachment of muscle to tendon. Near the end of the muscle fiber the argyrophil fibrillae become thickened, run parallel to the axis of the fiber and converge over its termination to form a strand which is continuous with the fiber bundles of the tendon. The more centrally-placed fibrillae of this strand form a cup-like network over the end of the muscle fiber. The ends of the muscle fibers may be smooth and regular, but more frequently they are deeply indented. The indentations are occupied by argyrophil fibers which project into them from the cup-shaped network. These projections are either straight spikes, spiral threads or irregular septa. In these preparations it seems clear that muscle fibrillae are not continuous with those of the connective tissue.

Electron micrographs have confirmed the above findings that connective tissue fibrils insert into indentations in the sarcolemma.

Blood Vessels. The larger branches of the arteries penetrate the muscle by following the laminae of the perimysium. The arterioles which penetrate the fasciculi give off capillaries at abrupt angles. The capillary supply is very rich (Fig. 98), several capillaries having contact with each muscular fiber. The veins follow the arteries; even their smallest branches have valves.

Lymphatic capillaries are not found between the individual muscle fibers. They are present, however, in the connective tissue septa and along the blood vessels.

Nerves. Every skeletal muscle fiber receives at least one motor nerve ending from the efferent cerebrospinal system. The minute anatomy of the motor end plates is described in Chapter X. The sensory or afferent fibers are principally

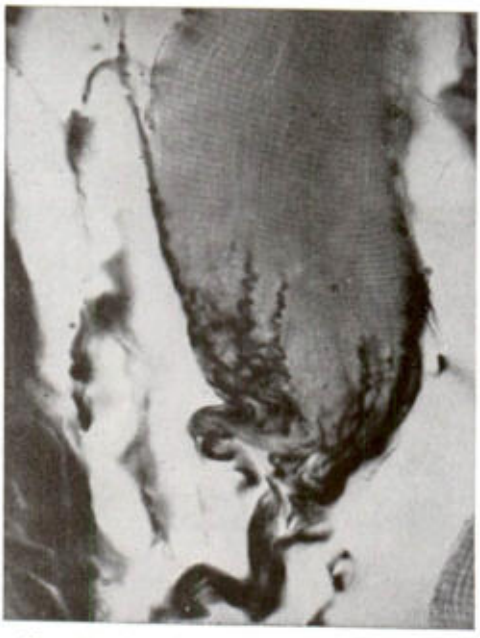


FIG. 97. Attachment of muscle to tendon. The thickened argyrophil fibrillae enclosing the end of the fiber form a narrow strand which courses toward the tendon in the lower left corner. Spiral argyrophil projections invaginate the end of the fiber. Monkey. Bielschowsky-Masson. × 900. (After Goss.)

associated with specialized end-organs known as neuromuscular spindles.

Development of Skeletal Muscle. The skeletal muscles of the trunk are derived from the myotomes of the embryonic segments or somites. The limb muscles are formed from the mesenchyme of the limb buds and the muscles of the tongue from head mesenchyme.

As in the case of smooth muscle, the first evidence of differentiation is the elongation of the nuclei and cell bodies to form myoblasts. At first the cytoplasm is without specialized cytological structure. Soon homogeneous fibrillae may be seen, particularly in the peripheral portions. After a distinctly elongated form has been established, fibrillae containing the alternating light and dark segments of cross striations begin to appear.

Two methods of formation of multinucleate fibers from the mononucleate myoblasts have been described. According to one the fibers are formed by the fusion of many myoblasts. According to the other they are elaborated from single myoblasts. Studies by de Rényi and Hogue on skeletal fibers of the chick embryo in tissue cultures favor the development from single myoblasts. They were able to show by microdissection that the apparently fused fibers were closely adherent, but retained their individuality. These investigators demonstrated a sarcolemma completely surrounding all fibers, over the ends as well as along the sides. It was described as a structureless membrane of unusual thickness to which the fibroblasts were firmly adherent.

The later development of the large adult multinucleate fibers is less disputed. The nuclei divide but the cytoplasm fails to undergo cleavage. It is possible that most of the nuclear division is by mitosis in the primitive fibers, but in the later stages this type of division is seen so rarely that amitosis has been assumed to take its place.

The primitive fibers have scattered striated myofibrillae at the surface and a central core of nuclei and sarcoplasm. As the fiber grows, it increases in length and breadth. The number of nuclei keeps pace with the growth in bulk. Greater breadth is brought about largely by the formation of more fibrillae. The new fibrillae are formed by longitudinal splitting of those already present, according to Heidenhain. In later fetal life and after birth the fibers are well filled by the masses of fibrillae and most of the nuclei change from a central to a peripheral position.

The number of muscle fibers apparently

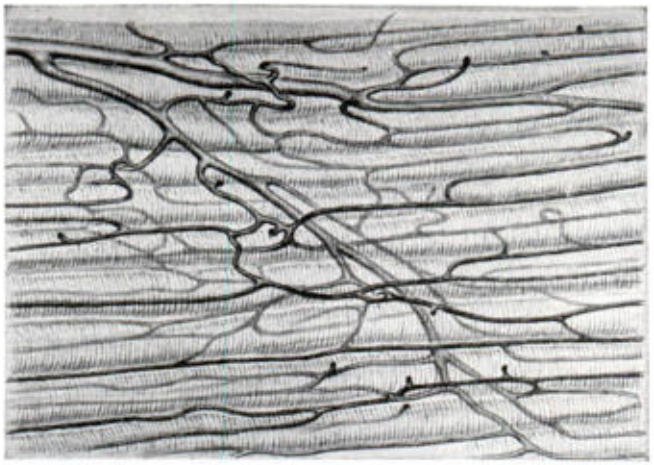


FIG. 98. Arterioles and capillaries in skeletal muscle. The vessels were filled with gelatin colored with carmine.

does not increase during the last month of fetal life or after birth. Increase in the over-all size of a muscle is then brought about by the increase in diameter of the fibers through the formation of more myofibrillae.

Not all of the primitive muscle fibers survive. Many of them fail to establish themselves as necessary units of the muscle and degenerate.

Regeneration is restricted in adult mammalian muscle by the lack of capacity to produce new fibers. Partial regeneration from remnants of injured or degenerated fibers occurs. The cut end of a fiber may send out a branched pseudopodium containing groups of nuclei, in a manner similar to the behavior of muscle fibers in tissue cultures. The cytoplasm of the pseudopodium is at first without specialized structure but later develops fibrillae and cross striations. Although there is some inherent capacity for growth and regeneration of adult skeletal muscle fibers, union between cut ends of fibers is more commonly effected by the connective tissue elements.

CARDIAC MUSCLE

The myocardium of vertebrate hearts is composed of an interlacing network of muscle fibers which have no apparent terminations except at the atrio-ventricular junction. In the mammalian heart, the net is irregularly and incompletely subdivided by connective tissue into bundles and laminae which wind about the heart in long spirals, particularly in the ventricles. The fibers within a bundle are roughly parallel, but the bundles themselves course in different directions in the deeper and more superficial layers so that any section through the myocardium will present groups of fibers cut longitudinally, transversely and with varying degrees of obliquity.

Fibers. Individual units such as the cells of smooth muscle or the giant multinucleate fibers of skeletal muscle are not obvious in the adult heart. The muscular substance is composed of strands of fibers, 9 to 20 microns in diameter, connected with each other by anastomosing branches which appear to be fused into a continuous network or syncytium when observed under the light

microscope (Fig. 99). Studies with the electron microscope show that the network is composed of separate cells (fibers) which are in intimate contact rather than in a continuous syncytium (Fig. 102). When treated with strong alkali, the strands of fibers break into mono- or bi-nucleate portions of fibers, more or less cylindrical or prismatic in shape but with a variable number of branches

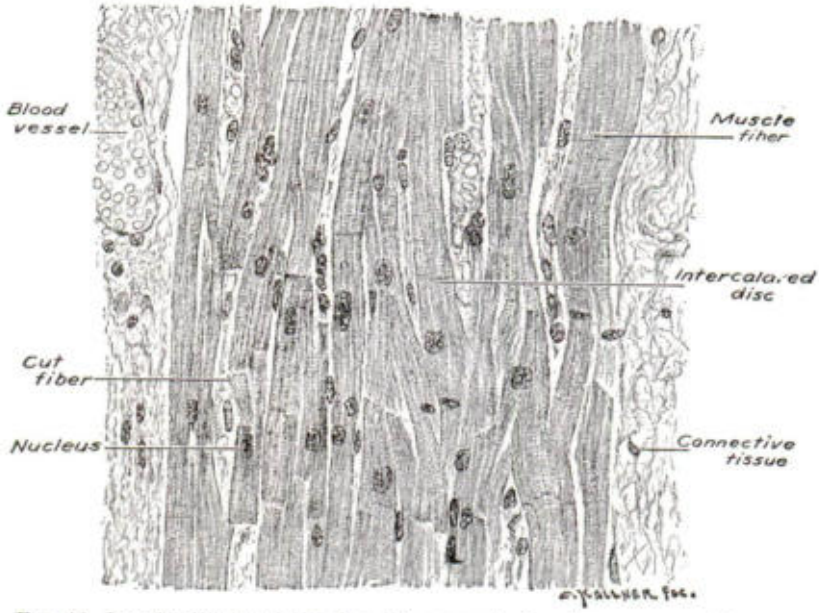


FIG. 99. Longitudinal section of cardiac muscle from human ventricle. × 300.

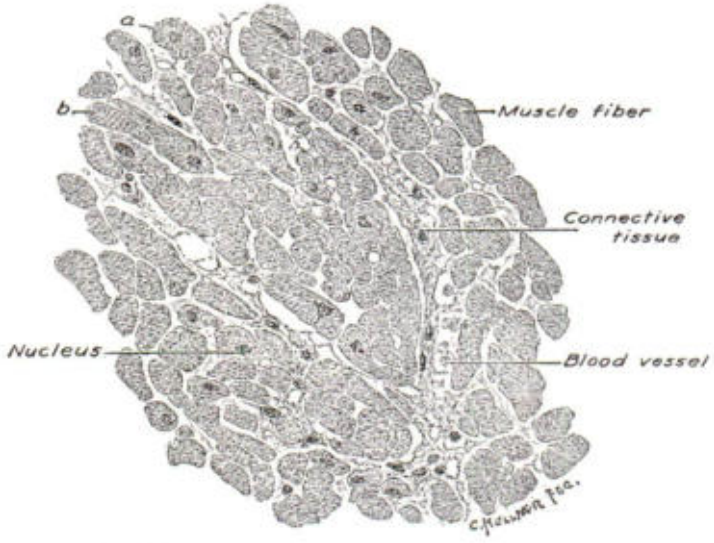


FIG. 100. Cross section of cardiac muscle from human ventricle. *a*, Shows spoke-like arrangement of cut ends of myofibrillae; *b*, shows platelike arrangement. × 300.

extending out from the sides at acute angles.

The *minute structure* of the myofibrils of cardiac muscle is essentially the same as that of skeletal muscle. The cross striations however, are closer together and not as clearly visible. The myofibrillae are thicker and more closely packed together at the periphery of the fiber but may be so fine and sparse in the center that there appears to be an axial core of sarcoplasm (Fig. 101). In cross section, the arrangement of the cut ends of the myofibrils frequently suggests short parallel bands or the spokes of a wheel (Fig. 100).

The *nuclei* are in the interior of the fibers, most frequently near the central axis. They are oval in shape, quite large, sometimes half the diameter of the fibers (Figs. 99, 101). There is an accumulation of sarcoplasm at their poles which contains mitochondria, fat droplets and granules.

A *sarcolemma* which can be dissected off as a structural entity like that of a skeletal fiber has not been demonstrated. There is, however, a protoplasmic surface layer which has greater mechanical strength than one would expect from a naked plasma membrane.

The *sarcosomes* or *mitochondria* are even more abundant in cardiac muscle than in the "dark" skeletal fibers. Neutral fat droplets are common, especially in a well nourished individual. Human

FIG. 101. Longitudinal section of muscle from left ventricle of monkey. The wider dark cross lines are intercalated discs. Sarcosomes may be seen in the clearer areas adjacent to the nuclei. Photomicrograph. × 805.

hearts, in later adult life occasionally have small brownish pigment granules in the sarcoplasm at the poles of the nuclei.

Intercalated Discs. Intercalated discs are peculiar to cardiac muscle. They are cross bands 0.5 to 1 micron thick, i.e., less than a cross striation or sarcomere, which are strongly refractive in fresh muscle and deeply stained in fixed material (Fig. 101). They often follow an irregular course, giving the appearance of "step-formations." Electron micrographs show that the intercalated discs are located at the junctions of the cells (Fig. 102). Those which are observable under the light microscope apparently represent a condensation of material along the inner surfaces of the intercellular membranes.

Connective Tissue of Cardiac Muscle. In the mammalian heart, a net of reticular fibers and fine collagenous fibers surrounds each muscle fiber (Fig. 103). It corresponds to the endomysium of skeletal muscle but it is more irregular in its arrangement because the cardiac muscle cells are connected in a branching network. Between bundles of muscle fibers, there are coarser collagenous fibers and elastic fibers. These regions correspond to the perimysium of skeletal muscle. The connective tissue is particularly dense at the atrio-ventricular

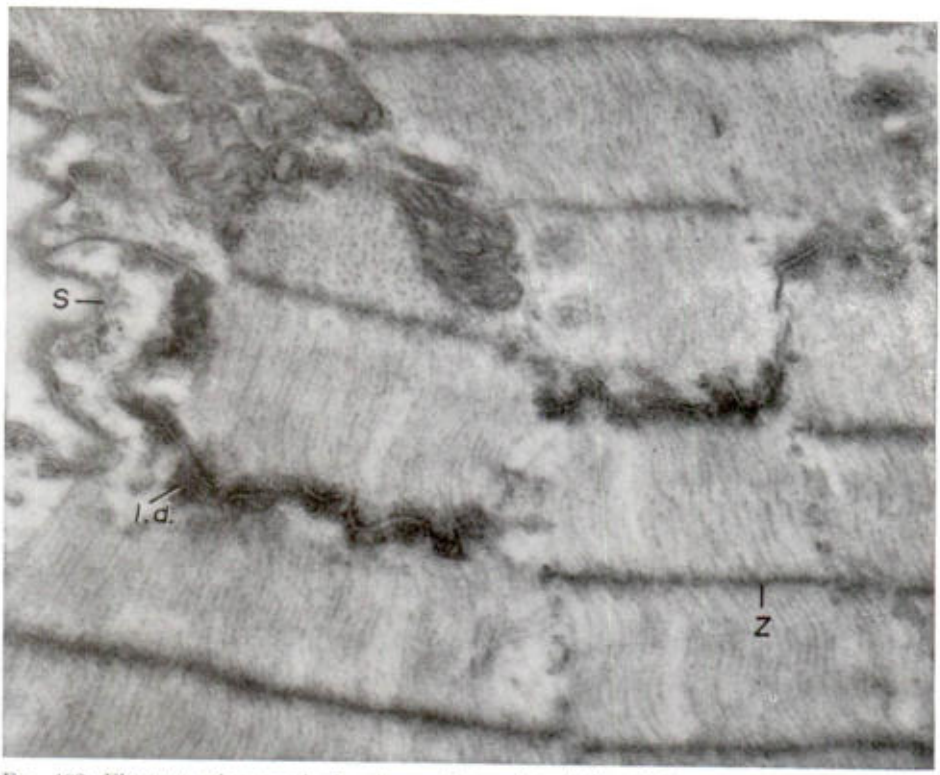


FIG. 102. Electron micrograph showing an intercalated disc (I.d.) of human cardiac muscle. Note the paired membranes within the disc which apparently represent the cell membranes of adjoining muscle fibers. The membranes of the disc become continuous peripherally with the sarcolemma (*S*). (Courtesy of Dr. D. W. Fawcett.)

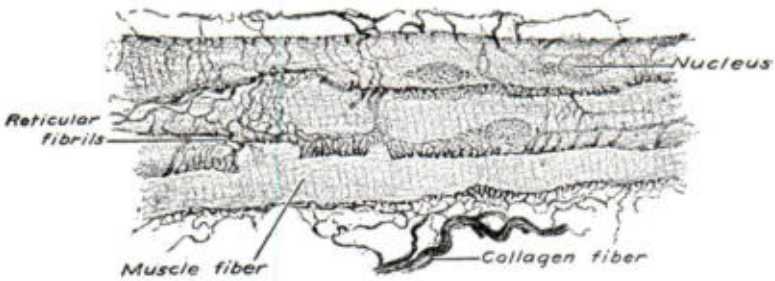


FIG. 103. Reticular fibrils about cardiac muscle fibers. Foot silver method. × 900.

junction. The topographical arrangement of the connective tissues in the heart is described in Chapter XII.

Blood Vessels and Nerves of Cardiac Muscle. Branches of the coronary arteries and veins penetrate the myocardium by coursing among the collagenous and elastic fibers of the larger bundles of connective tissue. An extensive plexus of blood and lymph capillaries is found in the connective tissue network surrounding each muscle fiber. The blood supply of cardiac muscle surpasses that for skeletal muscle.

Branches of sympathetic and parasympathetic nerves follow the connective tissue pathways and terminate in fine endings on the muscle fibers. The rate of muscle contraction is accelerated by stimulation of the sympathetics and retarded by the parasympathetics.

Conduction System. Cardiac muscle fibers, because of their intimate contact in a network and their inherent capacity to conduct, are capable of transmitting a contractile impulse over the entire heart. However, some of the fibers are modified in structure and conduct at a rate surpassing that of the typical cardiac fibers. The special conduction elements are known as *Purkinje fibers*; a description of their structure and distribution is given in Chapter XII.

Development of Cardiac Muscle. The heart muscle or myocardium is derived from the splanchnic mesoderm. It is recognizable at a very early age in mammals. The cells may contract before they have specialized structures such as fibrillae and cross striations. At this stage the cell boundaries can be distinguished in both living and fixed material under the light microscope. The fibrillae occur first in the peripheral cytoplasm, but as they increase in mass with development, the nuclei do not leave their central position as in skeletal muscle.

The details of the development of the fibrillae and their cross striations have been studied in fixed material and the process seems to be essentially the same as in skeletal muscle. Additional information has been obtained from tissue cultures of cardiac muscle (for description see Chapter II). Fibrillae are not seen in the living cells but are found in fixed cultures, both before and after striations appear. The first evidence of striation in many of the living cells is an alignment of the enlarged mitochondria into regular rows, both longitudinally and transversely. The mitochondria are not concerned in the process, however, except as they are necessary components of growing cells. This is indicated by their passive motion during contraction of these cells.

Regeneration of Cardiac Muscle. There is little or no regenerative capacity of cardiac muscle fibers following injury or destruction. Healing is accomplished by scar formation. In this connection

it is interesting to note that Törő (1939) reported some improvement in the healing process following the use of an extract of embryonic hearts.

Hypertrophy of the heart following any condition which places an excessive functional demand on the organ is accomplished by an increase in the size of the fibers rather than by an increase in their numbers. Likewise, growth of the heart during childhood is accomplished by an increase in the size of the fibers.

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IX

ORGANIZATION OF NERVOUS TISSUE

Like other tissues of the body, nervous tissue is composed of discrete structural units. These are the nerve cells, or *neurons*, and every neuron of the body is an integral part of a single organized system, the *nervous system*. Nerve cells have the function of initiating and conducting that peculiar form of protoplasmic activity known as the nerve impulse. For the performance of this function, neurons are linked together in linear fashion to form the interconnected *conduction pathways* over which nerve impulses travel. By means of these pathways certain parts of the body, the *receptors*, which are sensitive to changes in the external or internal environment, are brought into communication with those parts which are capable of an adaptive response to the environmental changes. These parts, the *effectors*, are usually either muscles or gland cells.

The great variety of response exhibited by the animal body is made possible only by the great number and variety of interconnections of the conduction pathways. The neurons make up all the conduction pathways and together with certain supporting tissues constitute the nervous system. It is therefore evident that a preliminary consideration of the arrangement and mutual relationships of the elements of nervous tissue should lead to a better appreciation of the structure of those elements. It is the purpose of this chapter to survey briefly some of the chief features of the organization of the nervous system.

The Neuron. The structural unit of the nervous system is the *neuron*. Although exhibiting great variety in form, every neuron is characterized by having one or more cytoplasmic processes. Some of these processes, termed *axons*, may extend for long distances as *nerve fibers*. They are the parts which are most highly modified for the specific function of conduction. Other cytoplasmic processes, called *dendrites*, generally terminate near the cell body in a diffuse branching. *Multipolar* neurons possess a number of dendrites and one axon. *Bipolar* neurons have but two processes, both of which structurally are axons. Still another type, the *unipolar* neuron, has but one process, an axon, which divides not far from the cell body into two long branches.

Although many neurons are usually linked together to form a conduction pathway, the relation between any two neurons is, so far as is known, purely a contact relationship. The nerve impulse is transmitted from one neuron to another neuron with which it is in contact, the region of contact being known as the *synapse*. This characteristic feature of the nervous system of all vertebrates leads to its designation as a synaptic nervous system. All neurons normally conduct a nerve impulse in one direction only. This one-way conduction, known

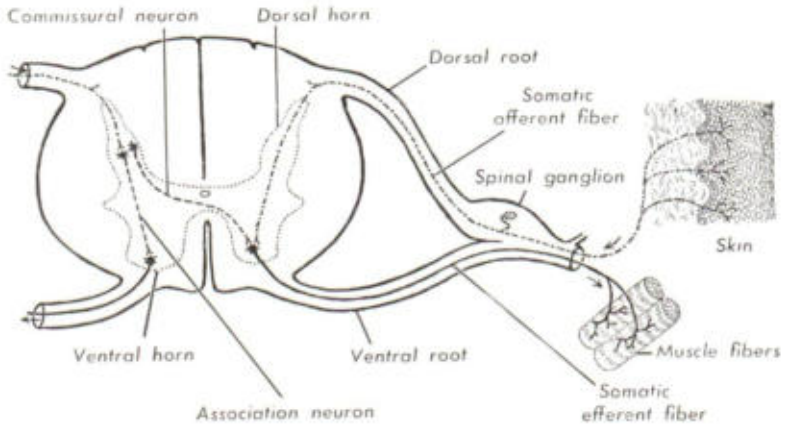

FIG. 104. Diagrammatic representation of a cross section of the spinal cord, showing simple reflex arcs. The direction of conduction is indicated by arrows.

as *dynamic polarization*, is due to the synapse, which has the capacity of transmitting an impulse in one direction only.

Organization of the Nervous System. The entire nervous system may be divided into the *central nervous system*, consisting of the *brain* and *spinal cord*, and the *peripheral nervous system*, which includes the *cranial* and *spinal nerves* with their ganglia and the peripheral parts of the *autonomic nervous system*. The divisions are interconnected by the nerve fiber pathways. For example, processes from some of the nerve cell bodies of the central nervous system emerge and course peripherally in the spinal and cranial nerves, and conversely, processes from cell bodies in spinal and cranial nerve ganglia enter the central nervous system.

Some of the fibers in the spinal and cranial nerves have terminations in peripheral structures where they may be affected by environmental changes. Nerve endings which are affected by (i.e., are receptive to) environmental stimuli are known as *receptors* and the nerve fibers so excited are *sensory* fibers. Since they conduct impulses toward the central nervous system, they are also termed *afferent* fibers. Other fibers in the spinal and cranial nerves carry impulses from the central nervous system to peripheral structures, such as muscles and glands, which may be stimulated to activity. The structures activated are referred to as *effector* organs and nerves that transmit impulses which stimulate effectors are known as *motor* fibers. Since the fibers conduct impulses away from the central nervous system they are also referred to as *efferent* fibers.

An arrangement whereby a motor and a sensory neuron are linked together synaptically as a receptor-effector mechanism constitutes the simplest type of *reflex arc*. An example of this type of combination is illustrated in Figure 104, which represents a cross section through the spinal cord and a spinal nerve. The cell body of the sensory neuron is located outside the spinal cord where together with other similar cell bodies it forms a spinal ganglion. The sensory neuron is unipolar, that is, it has only one process. A short distance from the cell body this single process, which is structurally an axon, divides into a peripheral process with a receptor ending in the skin and a

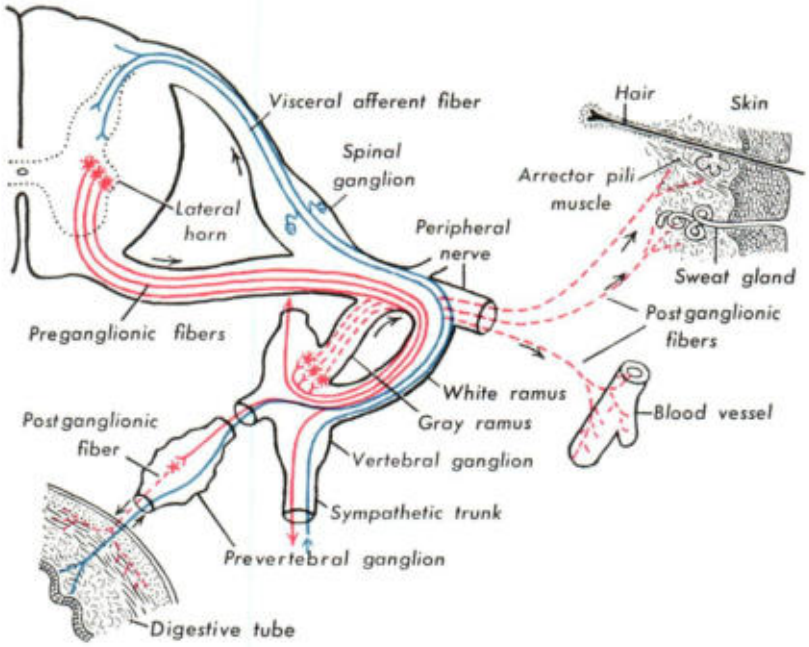

FIG. 105. Diagram showing the relation of visceral efferent neurons to thoracic spinal cord, sympathetic trunk and visceral structures. Preganglionic fibers are shown in solid red lines, postganglionic fibers in broken red lines and sensory fibers in solid blue lines. The direction of conduction is indicated by arrows.

central process which enters the spinal cord by way of the *dorsal root* of the spinal nerve. The central process may terminate in contact with either the dendrites or the cell body of a multipolar motor neuron located in the ventral part of the cord. The axon of this second neuron leaves the cord by way of the *ventral root*, joins the sensory fibers to form the *spinal nerve*, and courses peripherally to terminate on an effector (skeletal muscle, in the example illustrated in Figure 104).

The two-neuron reflex, described above, although theoretically possible, is a much more simple arrangement than that which is usually found in mammals. More commonly a third neuron (or even more neurons) lying entirely within the cord is interposed between the sensory and motor neurons in the reflex arc. This is an *association* neuron, or, if its axon crosses to the opposite side of the cord, a *commissural* neuron (Fig. 104). These axons may also run to some other level, thus bringing into functional connection different regions of the central nervous system. Inasmuch as both the central process of the sensory fiber and the axon of the association neuron may give off several branches, or *collaterals*, it is evident that there are possibilities for a great variety of conduction pathways. A nerve impulse may traverse several association neurons and take a number of different pathways before it reaches effector organs.

The arrangement of the nerve cells and fibers within the spinal cord makes it possible to differentiate two distinct areas of tissue: a thick peripheral layer of *white matter* and a central column of *gray matter*. The white matter is composed primarily of longitudinally directed nerve fibers, many of which are covered by sheaths of a white, fatty substance, *myelin*. The gray matter is composed principally of nerve cells and transversely directed fibers. Many of the fibers of the gray matter are unmyelinated, but a considerable number of myelinated ones are present also. In transverse section, the gray matter is shaped like the letter H. Its dorsal wings constitute the *dorsal horns* and its ventral ones the *ventral horns*, in which lie the cell bodies of the motor neurons. The two lateral gray areas are connected across the midline by a transverse bar, the *gray commissure*, in which lies the small central canal. It is, of course, evident that the dorsal and ventral horns seen in cross section actually are columns of gray matter extending the length of the cord.

Functional Classification of Nerve Fibers. It has already been stated that the dorsal roots are composed of afferent, or sensory, fibers and the ventral roots of efferent, or motor, fibers. These together comprise the spinal nerve. Those afferent fibers which are distributed to sensory endings in the body exclusive of the viscera, are termed *somatic afferent* fibers; those providing the sensory innervation of the viscera are the *visceral afferent* fibers. Both functional types have their cell bodies in the spinal ganglia (Figs. 104, 105).

A great many of the efferent fibers are distributed to the voluntary skeletal muscle of the body and are therefore termed *somatic efferent* fibers. Other efferent fibers terminate on smooth muscle and glandular epithelium of visceral structures; these are the *visceral efferent* fibers.

THE AUTONOMIC NERVOUS SYSTEM

The visceral efferent components of both the cranial and the spinal nerves pursue a different course than do the

somatic efferent fibers, for two neurons are always involved in the conduction of a visceral impulse from the central nervous system to the effector organ.[1] They also differ physiologically in that the essentially visceral reflexes which they mediate are not subject to direct voluntary control and are also more or less diffuse, rather than localized, in their effects. Because of these and certain other differences, it has been found convenient to consider the visceral efferent neurons of the body as a separate physiological system for which the name first given by Langley, the *autonomic nervous system*, is commonly used. According to this definition, the autonomic system includes only the visceral efferent (motor) innervation and does not include either the visceral afferent (sensory) fibers or those higher centers in the central nervous system which influence the visceral activities. There has been some tendency in recent years to broaden the usage of the term "autonomic" to include all of the neural apparatus concerned with visceral function. In this sense the term becomes more synonymous with "visceral" or "vegetative."

It must be emphasized that the autonomic nervous system is purely a functional grouping of efferent neurons and is in no sense an anatomical division. These neurons lie within the central nervous system, in outlying ganglia and in visceral nerve plexuses and spinal nerves. Their fibers are present in all spinal nerves and in most of the cranial nerves. The reflexes which it governs may be initiated by sensory impulses flowing over somatic afferent or visceral afferent fibers or coming from any receptor organ. The stimuli may be in the external environment or they may arise within the body. The autonomic nervous system brings about and coordinates the visceral responses and is thus purely a motor system.

The efferent fibers to visceral structures leave the central nervous system at three levels, making it possible to recognize three divisions of the autonomic nervous system (Fig. 106). In the *cranial division*, fibers leave by way of the third, seventh, ninth, tenth and eleventh cranial nerves. Other visceral efferent fibers emerge through the thoracic and the upper lumbar spinal nerves; these constitute the *thoracico-lumbar division*. The *sacral division* includes visceral efferent fibers in the second, third and fourth sacral nerves.

Whatever their level of origin, all visceral efferent pathways involve two successive neurons (Fig. 105). The first of these has its cell body within the central nervous system and its axon terminating in a peripheral autonomic ganglion; it is therefore termed a *preganglionic* neuron. In the ganglion it makes synaptic connection with a second multipolar neuron whose axon terminates in the effector organ (muscle or epithelium). This is the *postganglionic* neuron.

In the cranial and sacral divisions the preganglionic fibers generally end in *terminal* ganglia which lie near or within the walls of the structures which they innervate. In this and other respects, as well as in their response to certain drugs, the cranial and sacral divisions resemble each other and differ from the thoracico-lumbar components. They are therefore grouped together as the cranio-sacral or *parasympathetic* division of the autonomic nervous system.

The thoracico-lumbar visceral efferent outflow is the *sympathetic* division of the autonomic nervous system. Its pregan-

[1] The term viscera is used in its broad sense to include all organs and parts of the body concerned with its maintenance and reproduction, in contrast to the somatic organs which have to do more directly with adjustment to external conditions (Herrick).

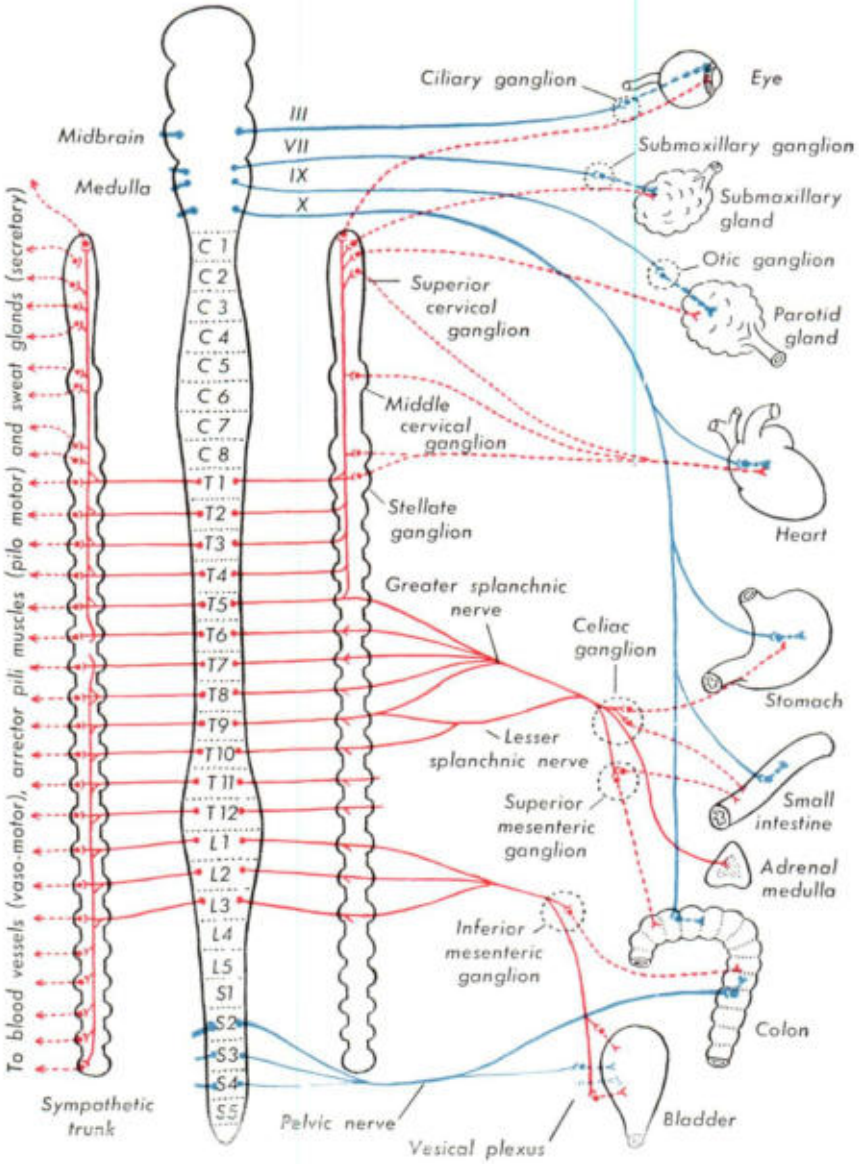

FIG. 106. Diagrammatic representation of some of the chief conduction pathways of the autonomic nervous system. For clearness, the nerves to blood vessels, arrector pili muscles and sweat glands are shown only on the left side of the figure and the pathways to other visceral structures only on the right side. The sympathetic division is shown in red, the parasympathetic division in blue. Solid lines represent preganglionic fibers; broken lines represent postganglionic fibers.

glionic fibers terminate either in *vertebral* or *prevertebral* ganglia. The vertebral ganglia are a series of ganglia, connected linearly by nerve fibers, which lie along the ventro-lateral aspects of the vertebral column and thus form two *sympathetic trunks* extending on either side the length of the vertebral column. The prevertebral, or *collateral*, ganglia are aggregations of postganglionic neurons associated with visceral nerve plexuses in the abdomen (Fig. 106).

It can be said in general that most visceral organs are innervated by both the parasympathetic and sympathetic divisions, the effects of which are usually antagonistic. For example, the parasympathetic fibers to the heart transmit impulses which tend to inhibit the cardiac rhythm; impulses from the sympathetic fibers, on the other hand, accelerate it. In the case of the stomach, parasympathetic impulses excite muscular contraction and sympathetic impulses inhibit it. The stimulation of the parasympathetic contributes to the conservation of bodily energy; the stimulation of the sympathetic assists the body in meeting emergencies.

Sympathetic Division. The sympathetic trunks are composed of a series of vertebral ganglia containing the cell bodies of postganglionic neurons and connected in linear order by ascending or descending nerve fibers (Fig. 106). In the cervical region there are three ganglia: the *superior cervical* ganglion, which is the largest; the *middle cervical*, sometimes absent; and the *inferior cervical*, which may be fused with the first thoracic to form the *stellate ganglion*. In the thoracic region, the ganglia, 10 or 11 in number, are segmentally arranged. Three or four ganglia are associated with the lumbar level and four or five with the sacral region.

Each sympathetic trunk is connected with the spinal nerves of its side by a series of communicating rami composed of nerve fibers. These are of two types. One type, the *gray communicating rami*, consists of fibers mostly devoid of myelin; these are found connecting the trunk to every spinal nerve. The other type, the *white communicating rami*, is limited to the thoracic and first three or four lumbar nerves and is not present at cervical or sacral levels (Fig. 106).

Preganglionic fibers of the sympathetic division have their cell bodies located in the *intermediolateral cell column (lateral horn)* of the thoracic and upper lumbar levels of the spinal cord. The myelinated fibers emerge through the ventral roots and reach the nearby sympathetic trunk by way of the white communicating rami. Within the sympathetic trunk the preganglionic fiber may take one of three courses:

(1) It may terminate in this level of the trunk in synaptic relation to a postganglionic neuron whose nonmyelinated axon joins the corresponding spinal nerve by way of the gray communicating ramus. With the various other fibers of this nerve the postganglionic fiber courses peripheralward to terminate in the smooth muscle of a blood vessel (vasomotor), the arrector pili muscle of a hair (pilomotor), or among the epithelial cells of a sweat gland (secretory) (Fig. 105).

(2) Many of the preganglionic fibers from the white rami pass directly through the sympathetic trunk without interruption and continue to the prevertebral (collateral) ganglia, from which postganglionic fibers course to the visceral organs. Preganglionic fibers such as these give the appearance of forming branches from the sympathetic trunks. Those from the fifth to ninth thoracic ganglia form the *greater splanchnic nerves* which terminate in the *celiac ganglia*, which are prevertebral. The

lesser splanchnic nerve is formed by fibers from the ninth and tenth thoracic ganglia. The postganglionic fibers contribute to the formation of the celiac plexus and pass directly to their terminations in the viscera.

(3) A great many of the preganglionic fibers, upon reaching the sympathetic trunk, course either caudally or cephalically in this trunk before they have a synaptic juncture with postganglionic neurons. These fibers form the pathways for the visceral efferent outflow to regions of the trunk which do not possess white rami. For example, preganglionic fibers originating at levels as low as the seventh thoracic terminate in the superior cervical ganglion. From this ganglion, nonmyelinated postganglionic nerve fibers run to various visceral structures. Some accompany the internal carotid artery as the internal carotid plexus and furnish the pathway by which impulses reach the dilatator pupillae muscle of the eye. Other fibers accompany the external carotid artery and reach the submaxillary gland, carrying impulses which stimulate secretion. Other fibers form the superior cervical cardiac nerve to the cardiac plexus and conduct impulses which accelerate the rhythm of the heart.

Other pathways of the sympathetic division are shown diagrammatically in Figure 106.

Parasympathetic Division. It has already been noted that most structures innervated by the autonomic system receive a double nerve supply in the form of fibers from its sympathetic and parasympathetic divisions, and that the action of these two divisions is generally antagonistic.

The cranial parasympathetic preganglionic neurons lie in the midbrain and medulla, sending their axons out over the oculomotor, facial, glossopharyngeal, vagus and accessory nerves. These preganglionic fibers are myelinated and course in their respective nerves to terminal ganglia located near or within visceral structures.

In the case of the oculomotor nerve, the preganglionic fibers enter the orbit with this nerve but diverge to reach the ciliary ganglion, forming its short motor root. The ciliary ganglion, which lies against the lateral surface of the optic nerve, contains the postganglionic neurons whose axons course in the short ciliary nerves to the eyeball. In the eyeball they are distributed to the ciliary muscle of accommodation and the sphincter muscle of the iris. The antagonistic action of parasympathetic and sympathetic nerves is here evident, for the parasympathetic fibers bring about contraction of the pupil. It has already been noted that sympathetic postganglionic fibers from the superior cervical ganglion cause dilation of the pupil.

The vagus nerve contains many preganglionic fibers. The cell bodies lie in the medulla and many of the fibers pass to the cardiac plexus and terminate in synaptic relation to ganglion cells located on the surface of the atria and roots of the great vessels or in the subepicardium of the atrial walls. The axons of these cardiac ganglion cells are short postganglionic fibers which end in the heart muscle. They are inhibitory in function.

The parasympathetic innervation of the gastro-intestinal tract and other abdominal viscera is through efferent fibers of the vagus nerve and also the pelvic nerve, which arises from nerve cells in the lateral horn of the second, third and fourth sacral segments of the spinal cord. These are preganglionic fibers. Those which innervate the gastro-intestinal tract course without interruption to terminate in its wall. Here they are synaptically related to postganglionic neurons which, with associated plexuses of nerve fibers, form two ex-

tensive ganglionated plexuses, the *enteric ganglionated plexuses*. One of these, the *myenteric plexus* or *plexus of Auerbach*, is situated between the longitudinal and circular layers of muscle. The other, the *submucous plexus* or *plexus of Meissner*, lies in the submucosa. These plexuses are composed of small ganglia connected to each other by strands of nerve fibers. Other strands connect the plexuses with each other.

Preganglionic fibers which pass to pelvic reproductive and urinary organs terminate in synaptic relation to the cell bodies of postganglionic neurons located in or near the walls of these viscera.

The nerve fibers of the enteric plexuses fall into the following classes:

(*1*) *Postganglionic sympathetic fibers*, derived from postganglionic neurons located chiefly in prevertebral ganglia. They end on the smooth muscle of the gut and among epithelial cells. Their impulses usually inhibit gastro-intestinal motility.

(*2*) *Preganglionic parasympathetic fibers*. These are fibers of the vagus or, in the descending colon and rectum, the visceral branches of sacral nerves. They end in synapses with the ganglion cells of the myenteric or submucous plexuses.

(*3*) *Postganglionic parasympathetic fibers*, which are axons of the above-mentioned ganglion cells. They innervate smooth muscle and epithelium of the gut and their impulses usually excite gastro-intestinal motility.

(*4*) *Visceral afferent fibers*. These fibers are sensory in function and are therefore, by definition, not components of the autonomic nervous system. In the enteric plexuses these afferent fibers intermingle with the visceral efferent fibers. They play an important part in gastro-intestinal reflexes. Some of the sensory fibers from the gut course in the vagus to the sensory ganglia of this nerve and from there to the medulla. Other sensory fibers course in the visceral nerves, then through the vertebral sympathetic ganglia and the white rami communicantes to their cell bodies in the spinal ganglia and then to the spinal cord (Fig. 105).

On the basis of physiological experiments, it has been suggested that there are afferent neurons lying entirely within the enteric plexuses and serving to mediate local reflexes which are independent of the central nervous system. Although the physiological findings are difficult to explain on any other basis, histological studies have failed to demonstrate satisfactorily the existence of such neurons.

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X

NERVOUS TISSUE

The preceding chapter has discussed the manner in which nervous tissue is organized to form a functioning nervous system. It has also been noted that the nervous system is composed of a vast number of units which are so arranged and interrelated that they can coordinate and integrate the manifold activities of the body. The function of the nervous system is thus not only to conduct nerve impulses which produce activity on the part of some muscle fiber or gland cell but also to regulate these activities so that together they are adapted to the well-being of the individual organism. Furthermore, since a major part of the adjustment of the organism is an adjustment to external influences, some parts of the nervous system must be exposed to, as well as responsive to, a variety of such external influences.

It is evident, therefore, that an understanding of the functional activity of the nervous system must rest on a knowledge of its structural organization. This knowledge, in turn, can be approached only through a study of the elements which comprise the nervous tissue itself. It is the purpose of this chapter to consider the histological features of those elements.

The nervous system, central and peripheral, is composed of the following elements:

(1) The nervous elements proper, the neurons. These have the specific nervous function of conduction and possess certain common structural characteristics, although exhibiting a great variety of form.

(2) The interstitial tissue of the nervous system. This includes elements which in part are supportive in character, although their association with the nervous elements is often so intimate as to suggest other important roles. The interstitial tissue includes the neuroglia of the central nervous system, the neurilemma cells of the peripheral nerve fibers, and the satellite cells of the cerebrospinal and sympathetic ganglia.

(3) Connective tissue, which is associated with the above elements in the structure of some parts of the nervous system. Connective tissue forms the membranous investments (the meninges) of the central nervous system and forms the sheaths which envelop the peripheral nerves. It also contributes to the capsules of the ganglia and is associated with the sensory nerve fiber endings in the formation of sense organs.

The neurons and the interstitial tissue, with one exception, are of ectodermal origin. The connective tissue is, of course, of mesodermal origin.

THE NEURON

The neuron may be defined as a nerve cell with all its processes. A striking feature of the neuron is the length of its protoplasmic processes which may reach for very considerable distances away

from the cell body. This is but another example of the close correlation which exists between structure and function, for a specific function of the nervous system is conduction and the apparatus for the performance of this function is provided by the extended processes of the neurons.

As already stated in the preceding chapter, the neurons may be classified as unipolar, bipolar, and multipolar, according to whether they have one, two or several processes.

Multipolar Neurons. Although a number of processes arise from the cell body of a multipolar neuron, they are of only two structural types. One type of process is a nerve fiber process and is known as the *axon* (axis-cylinder process, neuraxon or neurite). Each neuron usually

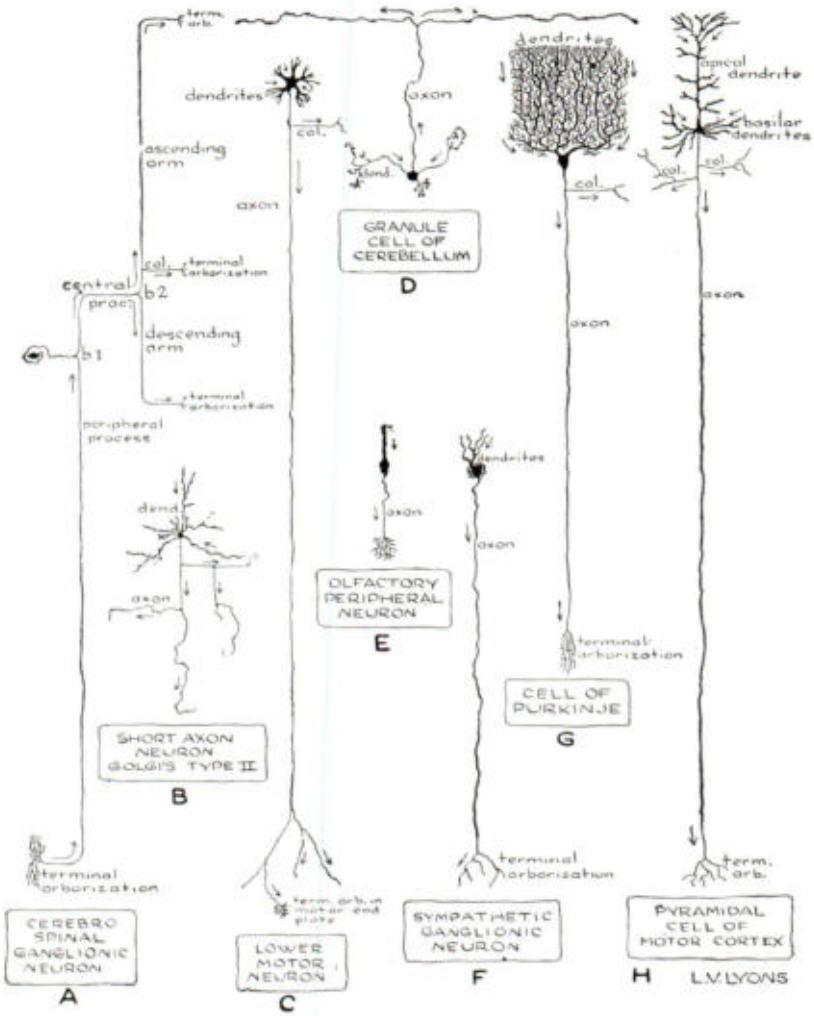


FIG. 107. Some of the principal forms of neurons. The sheaths are not shown. The axons, except in *B*, are shown much shorter in proportion to the size of body and dendrites than they actually are. The direction of conduction is shown by the arrows. *Col*, collateral branch; *proc*, process; *term. arb*, terminal arborization.

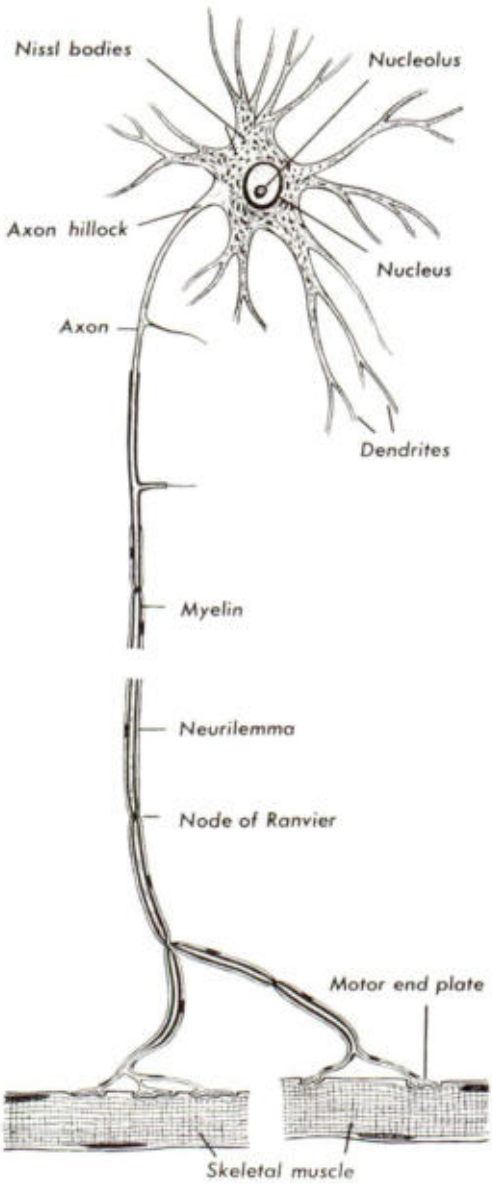


FIG. 108. Diagram of a motor neuron from the ventral horn of the spinal cord. The cell body and all its processes (dendrites and axon) constitute a single cell known as the neuron. The axon is shown in the drawing with an interruption; if shown completely and at the same scale as the cell body it would extend more than 200 feet for a neuron of this type.

has only one axon. The other type of process is known as the *dendrite* (protoplasmic process, dendron). Each multipolar neuron has a number of dendrites.

Dendrites bear more structural resemblance to the cell body than do axons and as the term *protoplasmic process* implies, they appear to be simple extensions of the cell body. They are of tapering shape and rough contour, fork repeatedly at acute angles and generally terminate not far from the cell body (Fig. 108).

The axon of a multipolar neuron is usually given off from a small conical elevation on the cell body, which is known as the *implantation cone* or *axon-hillock* (Figs. 108 and 113). In some instances, the axon may arise directly from the cell body in the form of a thin thread or it may be given off from one of the larger protoplasmic trunks. Whatever its origin, it may be distinguished from the dendrites by its smoother contour and more uniform diameter, although for a short distance from the axon-hillock it may be constricted. Not far from the cell body it generally gives off several branches, or collaterals. Both the axon and its collaterals usually end in more or less diffuse terminal arborizations called *telodendria*. In many instances the axon is an extremely long process and may extend a distance of several feet away from the cell body (Fig. 107, *C* and *H*).

Most of the neurons whose cell bodies lie in the central nervous system and in the autonomic ganglia belong to the multipolar type (Fig. 107, *B*, *C*, *D*, *F*, *G* and *H*).

Bipolar Neurons. Bipolar neurons give off two processes, one at either end of the cell body, but both processes have the structural characteristics of an axon. Examples of such neurons are found in the spiral and vestibular ganglia

of the acoustic (eighth cranial) nerve. Bipolar neurons of modified type are also present in the retina.

Unipolar Neurons. The majority of the neurons in the cerebrospinal ganglia are nerve cells possessing a single process which divides not far from the cell body into two branches, one proceeding to some peripheral structure and the other entering the central nervous system. These T-shaped neurons are unipolar cells (Figs. 107, *A*, 135). Both arms of the single process have the structural features characteristic of an axon and may give off collaterals.

In their early development, these cells are of typical bipolar form but as they develop, the two processes converge at one side of the cell body and fuse together for a short distance. The result is a single T or Y-shaped process (Fig. 109). On the basis of development, therefore, this type of neuron is sometimes classified as bipolar (or pseudo-unipolar).

The extreme length which the process of one of these neurons may attain will be appreciated if one considers that a single nerve cell located in a spinal ganglion may not only send a peripheral process to one of the toes but its central branch, after entering the spinal cord, may bifurcate into two branches, one of which ascends the whole length of the spinal cord. In this case, the total length of the neuron would equal approximately the distance from toe to nape of neck.

It will be noted that in the above discussion the terms "axon" and "dendrite" have been used with reference to two types of nerve cell processes which are structurally different. Thus the two processes of a bipolar neuron of the spiral ganglion of the ear, for example, would be considered as axons, since they are structurally identical and have the histological characters of an axon. So, too, would the single branched process

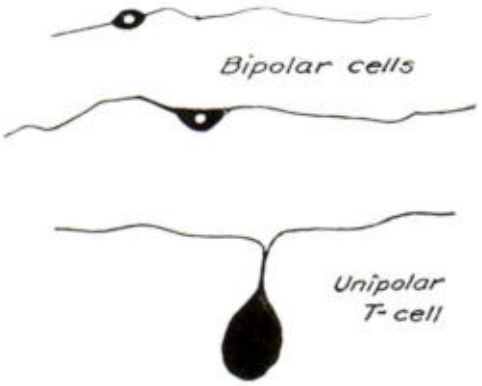


FIG. 109. Unipolar and bipolar neurons. (Redrawn from von Möllendorff.)

of the spinal ganglion cell be regarded as an axon, with the peripheral branch conducting impulses toward the cell body and the central branch conducting them away from the cell body to the central nervous system.

It should be noted, however, that the terms "axon" and "dendrite" are often used with reference not to the structure of a particular neuron process but to the direction in which it normally conducts the nerve impulse. According to this usage, all processes which normally conduct toward the cell body are termed dendrites and those which conduct away from the cell body are termed axons. Thus the peripheral process of a spinal ganglion cell (sensory neuron) would be called a dendrite, although it is identical in structure with the central process, or axon. Used in this manner, the terms denote physiological rather than structural characteristics.

The difficulty of applying them on this basis to a histological preparation need not be pointed out, for under the microscope the direction of conduction in the neuron processes is not revealed. Furthermore, in the case of the peripheral branch of the sensory neuron referred to above, it is known that it does conduct im-

pulses distally (in a direction away from the cell body) in the phenomenon of antidromic conduction.

Inasmuch as we are here particularly concerned with the histological features of nervous tissue, the terms "axon" and "dendrite" will be used to denote neuron processes possessing different structural characteristics. These will be described more fully in the following pages.

General Characteristics of the Nerve Cell Body. Although the neuron has been defined as a nerve cell with all of its processes, the term "nerve cell" is commonly used to designate the neuron body and its processes exclusive of the nerve fiber process. As in most other cells, this consists of a mass of cytoplasm surrounding a nucleus. There is no definite cell membrane other than the plasma membrane formed by the cytoplasm of the cell surface but this plasma membrane undoubtedly plays an important part in the physiology of impulse conduction.

Nerve cell bodies vary considerably both in form and in size. Variations in size range from the very small granule cells of the cerebellum, which are about 4 μ in diameter, to the large bodies of the motor cells of the ventral horns of the spinal cord, which may attain a diameter of 135 μ. Variations in forms of neurons are innumerable but there are certain well marked groups such as the pyramidal cells of the cerebral cortex (Fig. 110), the Purkinje cells of the cerebellum, with their fan-shaped dendritic arborizations (Fig. 111) and the large multipolar neurons of the ventral horns of the spinal cord (Fig. 112).

The neuron body, with its nucleus, is the genetic center of the neuron, the

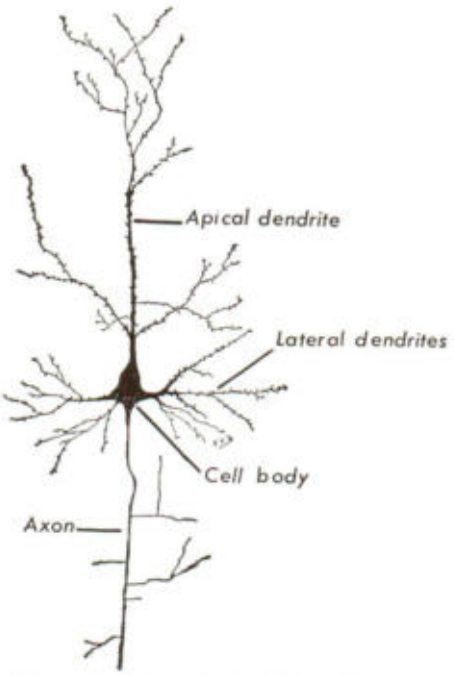


FIG. 110. Pyramidal cell from human cerebral cortex. Only a part of the axon is included in the drawing. Gold chloride method.

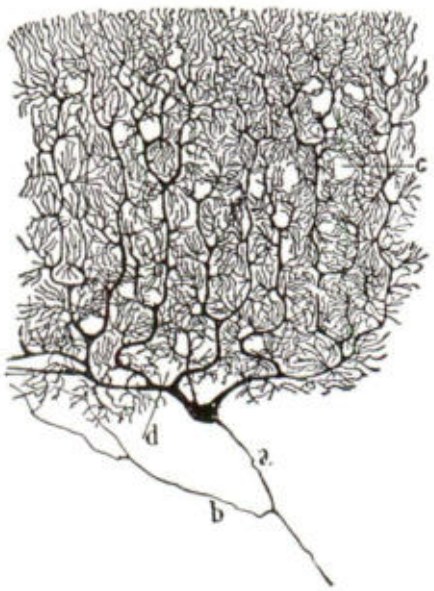


FIG. 111. Purkinje cell of adult human cerebellum. Golgi preparation. (Cajal.) *a*, Axon; *b*, recurrent collateral; *c*, spaces occupied by blood-vessels; *d*, spaces occupied by basket cells.

various processes being outgrowths from it. If these processes are cut off from the nucleated portion of the neuron, they degenerate and ultimately disappear, for, as in other cells, the nucleus is the trophic center of the neuron.

Nucleus of the Nerve Cell. The nucleus of the nerve cell is a spherical structure. Although considerable variation in the size of nerve cell nuclei exists there is nevertheless a fairly constant ratio between the size of the nucleus and the size of the nerve cell. Usually the nucleus is situated approximately in the center of the cell body in large nerve cells, the most striking exceptions being its eccentric position in the cells of Clarke's column in the spinal cord and in cells of sympathetic ganglia. Eccentricity of the nucleus is seen in various pathological conditions and when the axon of the cell is injured (see p. 190).

Although a single nucleus is the rule, binucleate cells occur in the sympathetic ganglia.

The nuclear membrane is always well defined. Internally there is a linin network with widely dispersed basichromatin. The nerve cell nucleus thus characteristically has a vesicular appearance, and stains lightly in comparison with the nuclei of most other cells.

The nucleus of a nerve cell usually contains a relatively large nucleolus. The latter contains both basophilic and acidophilic substances, namely ribonucleic acids and basic proteins. Consequently, the nucleolus may appear to be either basophilic or acidophilic in stained preparations, depending on the combination of dyes used. In toluidine blue-erythrosin preparations, it usually appears to be basophilic (Fig. 113, *A*). A small body, about one micron in diameter, is often seen adjacent to the nucleolus. It is known as the nucleolar satellite (Fig. 113, *C*). It apparently contains the desoxyribose type of nucleic acid rather than the ribose type found in the nucleolus. It has been claimed that the nucleolar satellite, as well as the nucleolus, may be concerned with nucleoprotein synthesis.

FIG. 112. Bodies and portions of dendrites of several somatic, efferent neurons (lower motor neurons) in the ventral gray horn of the lumbo-sacral spinal cord of an infant about one month old. The neurofibrillar structure is shown and also the nuclei (light areas) in some of which the more darkly staining nucleoli are faintly shown. Portions of dendrites and axons of other neurons fill the field. Photograph. Reduced silver method of Cajal.

Cytoplasm of the Nerve Cell Body. Before considering the various elements which can be identified in the cytoplasm of the nerve cell, it may be appropriate to point out that it is necessary to employ a variety of technical methods to demonstrate these constituents. No single

preparation can show them and it is necessary to build up a composite picture of nerve cell structure from a study of several preparations prepared by different techniques.

The cytoplasm of the cell body (that part surrounding the nucleus) is sometimes referred to as the *perikaryon*. In it there may be found, with various methods of fixation and staining, the following structures or substances: (1) neurofibrillae; (2) the chromophil substance (Nissl bodies, tigroid bodies); (3) the Golgi apparatus; (4) mitochondria; (5) cytoplasmic inclusions, such as fats, and pigment.

(1) The *neurofibrillae* are delicate fibrils, continuous throughout the cell body and its processes, which are present in all neurons. In the reduced silver preparations by which they are usually demonstrated they appear as dark, slender filaments which course parallel with one another in the axon and dendrites but cross and interlace in the cell body (Fig. 112). Studies with the electron microscope reveal that each neurofibril is in turn composed of a number of more slender elements which are somewhat irregular in their arrangement.

There is considerable evidence that neurofibrillae are real structures and not fixation artifacts. They have been studied in living nerves from invertebrates by microdissection methods (de Rényi, 1932) and they have been demonstrated in chick ganglion cells in tissue culture (Weiss and Wang, 1936).

The cell substance which surrounds and separates the neurofibrillae is often referred to collectively as the perifibrillar or interfibrillar substance or *neuroplasm*. It is obvious that the neuroplasm also contains the various other cytoplasmic elements enumerated above and demonstrated by other methods than those employed for the demonstration of neurofibrillae.

(2) The *chromophil substance* usually appears, in appropriately fixed and stained preparations, as very small granules which are clumped together, in a variety of shapes, to form small aggregations known as *Nissl bodies*. They are usually larger in motor than in sensory neurons. Because of the striped appearance which collectively they may impart to a cell, they are sometimes also called tigroid bodies (Fig. 113).

The Nissl bodies are found in the perikarya and dendrites of all large and many small nerve cells but they are absent from the axon and the axon-hillock. They stain sharply with basic aniline dyes, resembling basichromatin in this respect. They differ from chromatin however, in that they are composed chiefly of the ribose type of nucleic acid and nucleoprotein rather than the desoxyribose type. This is readily established by treating sections with an enzyme, ribonuclease, which selectively removes ribonucleic acid (compare Figs. 113, *A* and 113, *B*).

Electron micrographs show that the Nissl bodies are composed of a particularly dense network of cisternae (endoplasmic reticulum) and associated RNA granules. The three-dimensional reconstruction of endoplasmic reticulum shown in Figure 3, Chapter I, is based largely on studies of nerve cells (Palay and Palade, 1955). Ribonucleic acid (RNA) is usually most abundant in cells which are most active in synthesizing proteins. Offhand, it may seem surprising that RNA is such a prominent constituent of mature neurons which are neither increasing in size nor in number. However, it seems that protein is used and replenished almost continuously during the life of the nerve cell. The aggregate of RNA within the cell is apparently in a state of flux but is usually maintained at a fairly constant level while protein synthesis balances protein usage or loss.

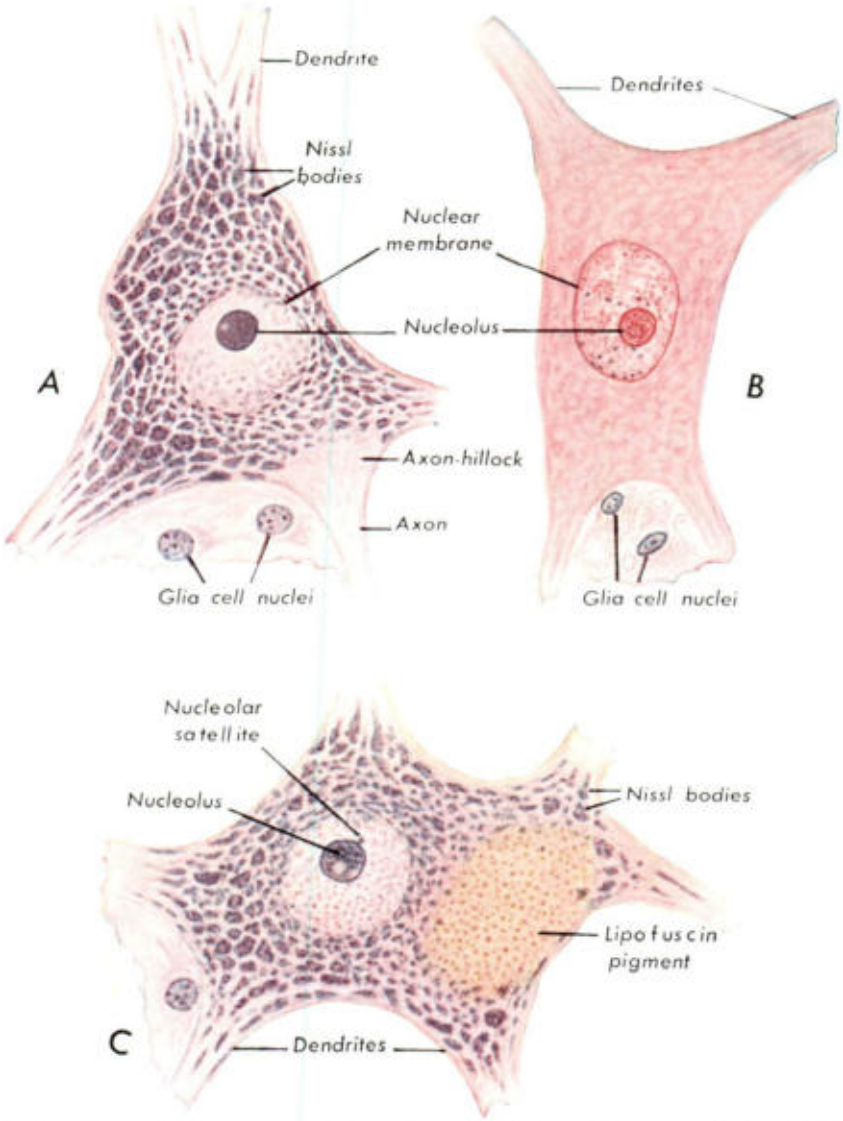


FIG. 113. Drawings of motor neurons from the ventral horn of human spinal cord. Stained for Nissl bodies by the toluidine blue-erythrosin method. *A* and *C*, stained without previous use of enzymes; *B*, the appearance of a cell with the same stains after previous treatment with an enzyme, ribonuclease, which specifically removes ribosenucleoproteins. × 1900.

During prolonged periods of stimulation, the rate of protein usage may exceed that of nucleoprotein synthesis. Then, there is a reduction in the amount of Nissl (RNA) material—a condition known as chromatolysis. Chromatolysis is particularly marked during the early stages of degeneration and regeneration following injury of the axon (see under Degeneration and Regeneration). In normal conditions, different cells of the same type in the same preparation may show some variations in the amount of Nissl material due to different physiological states. However, different cells of a particular type usually present an approximately similar Nissl picture (equivalent picture) which furnishes a norm for comparison with cells showing pathological changes.

The relation which the appearance of the Nissl-stained cell bears to the structure of the living protoplasm has long been a much discussed question. There is good evidence now that "Nissl bodies pre-exist in the living neuron and are essentially unchanged after good cytological fixation" (Deitch and Murray, 1956). When tissue culture preparations of ganglion cells are studied under the phase contrast microscope, dense areas are seen in the same locations as the Nissl bodies observed after fixation. Evidence for the existence of Nissl substance in the form of organized masses is demonstrated also by the discreteness of the bodies after high speed centrifugation and subsequent staining (Fig. 114).

(3) The *Golgi apparatus* is found in all nerve cells and it was in nerve cells that Golgi first discovered this structure. It responds to pathological conditions and in nerve cell injury it may disintegrate and disappear.

(4) *Mitochondria* are found as granular or rod-shaped bodies scattered throughout the neuroplasm, in the axon as well as in the cell body and dendrites. In fresh nerve cells, they can be supravitally stained with Janus green B.

(5) *Inclusions.* Some nerve cells contain granules of a dark brown pigment, melanin. This occurs in certain cells of the olfactory bulb, the locus caeruleus in the floor of the fourth ventricle, in the substantia nigra of the midbrain, and in certain cells scattered through the reticular formation. It is also present in some spinal and sympathetic ganglion cells. Nothing is known of its significance.

Another type of pigment inclusion in the cytoplasm of nerve cells is a fatty lipofuscin pigment of a yellowish or brownish color which is insoluble in the usual lipid solvents. It appears in the form of granules which may be dispersed throughout the cell but are usually, as they increase in number, aggregated in some part of the cell body in a dense rounded mass (Fig. 113, *C*). They are blackened by osmic acid and stain with Scharlach R. Lipofuscin is not present in the nerve cells of the newborn infant but appears in increasing amounts with increasing age.

The Dendrites or Protoplasmic Processes. The dendrites appear to be simple unmodified extensions of the cell body which serve to increase the surface area of the cell. They branch repeatedly,

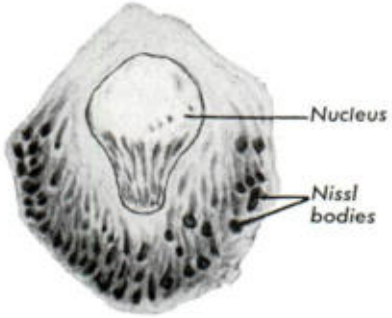


FIG. 114. Spinal ganglion cell of rat. The cell was centrifuged for a half hour at about 400,000 times the force of gravity. The Nissl bodies have moved toward the centrifugal pole. (Redrawn after Beams and King.)

are of tapering form and usually terminate at no great distance from the cell body in profuse terminal arborizations, the telodendria. They have a much rougher contour than do axons and their surfaces are often beset with fine spiny or knobbed excrescences (gemmules) (Fig. 110).

They are composed of the same cytoplasmic elements as make up the nerve cell body—principally neurofibrillae and neuroplasm. In the larger cells, they contain elongated Nissl bodies as well as mitochondria. The Golgi apparatus may also extend into the dendrites.

General Characteristics of the Axon and its Sheaths (The Nerve Fiber). The axon is that part of the neuron which is most highly modified for its particular function—conduction. As compared with the dendrites, its great length in many cases has already been noted. It has a smoother contour than the dendrites and is of more uniform diameter. It may branch or give off collaterals which are at right angles to the main axon in the central nervous system. As a rule, the axon ends in a terminal arborization.

The axon, or axis-cylinder, differs from the cell body in that it consists almost entirely of neurofibrillae embedded in the homogeneous perifibrillar substance, here called the *axioplasm*. This may contain mitochondria but the chro-

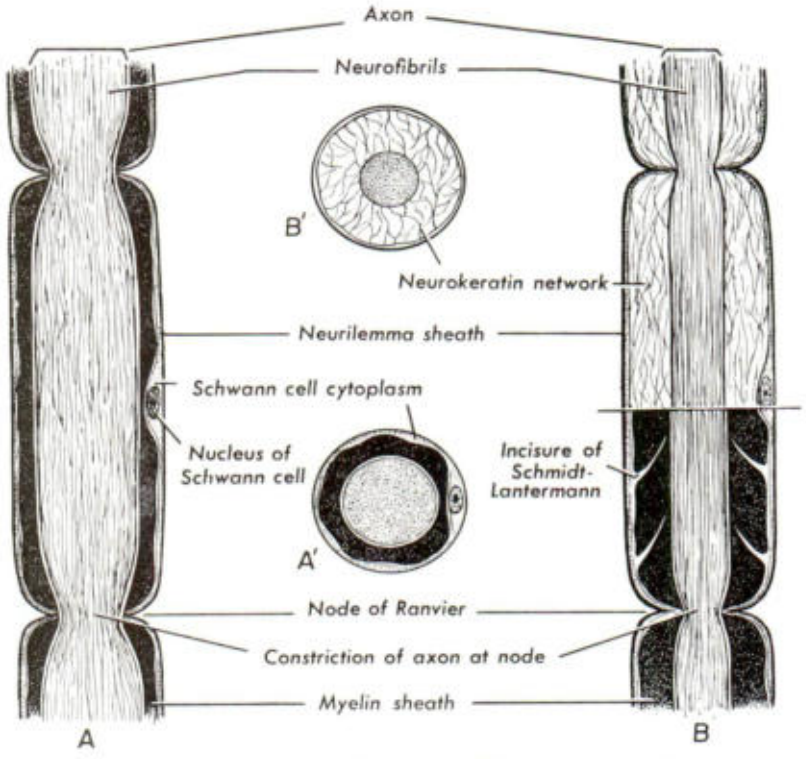


FIG. 115. Diagram of structure of medullated nerve fibers. *A*. Longitudinal section of a single internode, showing the axon and its sheaths at relative diameters based on studies of living nerve fibers. *A'*. Cross section at the level of the Schwann cell nucleus in *A*. *B*. Longitudinal section of an internode showing shrunken axon as seen in most fixed preparations. *B'*. Cross section of fiber shown in *B*.

mophil substance and Golgi apparatus are lacking.

Axons usually have one or more sheaths for at least a portion of their course. The axon and its sheaths, when they are present, constitute the *nerve fiber*. These sheaths are an outer delicate nucleated membranous sheath, known as the *sheath of Schwann* or *neurilemma*, and a thicker sheath lying within this, next to the axis-cylinder of the nerve fiber, and composed principally of a fatty material called *myelin*. This latter sheath is called the *myelin sheath* or *medullary sheath* (Fig. 115). Those nerve fibers possessing a myelin sheath are known as *myelinated* or *medullated* nerve fibers; those with little or no myelin are classified as *amyelinated* or *nonmedullated* nerve fibers.

Myelinated Nerve Fibers. The peripheral myelinated nerve fibers are somewhat different in structure from the central myelinated fibers, i.e., those lying within the central nervous system. The former possess a neurilemma sheath, which is absent in the central fibers. Accordingly, the peripheral myelinated fiber is composed of (1) an axon, (2) a myelin sheath and (3) a neurilemma sheath; the central myelinated fiber is composed of an axon and a myelin sheath.

(1) The *axon* is composed of neurofibrils continuous with those of the cell body and, like them, lying in a perifibrillar substance or neuroplasm which also contains the short rod-like mitochondria. In the fresh condition, the axon is broad and either appears homogeneous or shows faint longitudinal striations corresponding to the neurofibrillae. In a study of the living nerve fiber by microdissection, de Rényi found that the axis-cylinder is in a soft gelatinous condition, more solid at the nodes than in the internodes.

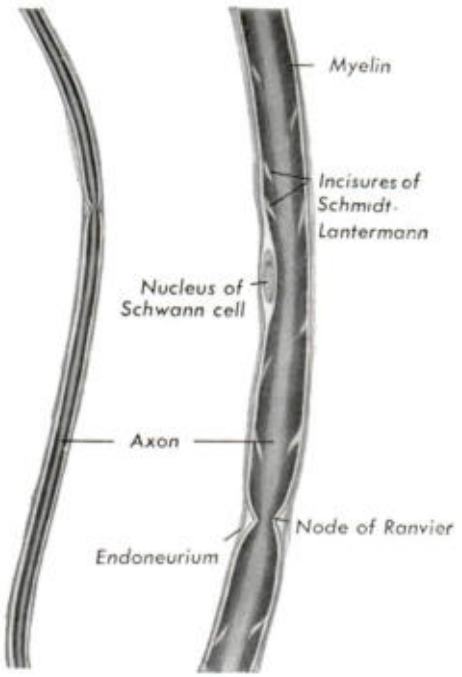


FIG. 116. Semischematic drawing of two myelinated nerve fibers teased from a sciatic nerve fixed with osmic acid. The unstained axons are completely invested, except at the nodes of Ranvier, by myelin which is stained black with osmic acid.

Fixatives usually cause the axon to shrink down to a thin axial thread, whence its older name of axis-cylinder (Fig. 119). In fixed preparations, therefore, the axon appears in cross section as a small dark spot in the center of a relatively large myelin space (Fig. 121). This gives a false impression of the ratio between the diameters of the axon and myelin sheath, which in the living condition is said by de Rényi to be as 6 to 1 or 7 to 1. In carefully prepared celloidin sections, this ratio is more nearly approached (Fig. 117). At the nodes of Ranvier, the axon shows a slight constriction and the neurofibrillae are more closely grouped together

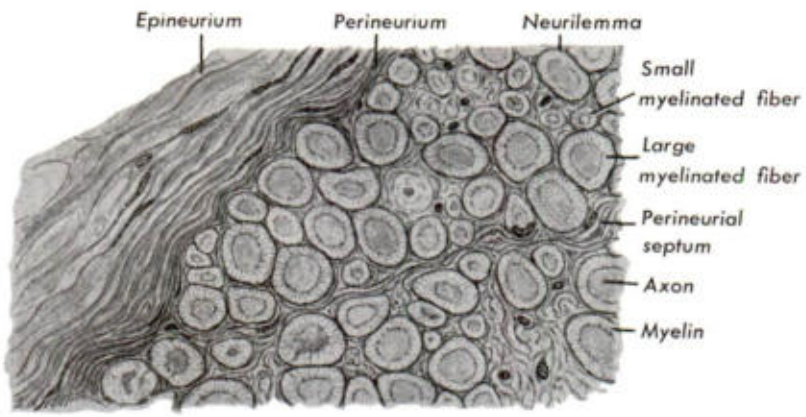


FIG. 117. Cross section of sciatic nerve of monkey. Hematoxylin and eosin stain after celloidin technique, showing only slight shrinkage of axons. × 500. Compare with Figure 118.

The neurofibrillae were held to be the specific conducting substance of the nerve fiber at one time, but experimental studies have failed to support this view. The interesting suggestion has been made that they may be concerned with metabolism or with mechanical support of nerve fibers.

(2) The *myelin sheath* (Figs. 117 and 120) is a thick sheath composed chiefly of a semi-fluid substance known as myelin. This is a complex substance containing, among other components, cholesterol, phospholipins and certain cerebrosides. In the fresh state, the myelin has a glistening homogeneous white appearance, which gives to those regions of the central nervous system where it predominates the name "white matter." When fixed in osmic acid, the myelin sheath becomes gray or black. In ordinary sections, prepared by methods which involve the use of lipid solvents, the myelin sheath is dissolved, leaving in its place a space, which is bridged by a network of fine trabeculae, the *neurokeratin network* (Figs. 115, 119). Under the electron microscope, myelin appears to consist of concentric layers or lamellae which are explained by its method of formation.

The myelin sheath is not continuous but is interrupted at intervals of from 80 to 600 μ by the *nodes of Ranvier* (Fig. 116). The myelin sheath is also absent where the fiber branches and at the beginning and at the termination of the fiber. The length of the internode is usually proportional to the diameter of the axon, the larger fibers having the longer internodes. The thickness of the myelin is also usually proportional to the diameter of the axon.

In osmic fixed specimens, the myelin sheath of the internode region is sometimes interrupted by oblique, funnel-shaped clefts which extend from the neurilemma to the axon. These are known as the *clefts* or *incisures of Schmidt-Lantermann* (Fig. 115). Although there is disagreement on the question of whether these clefts are fixation artifacts or whether they exist in living specimens under normal conditions, they have been described for living nerves viewed by polarized light (von Muralt, 1946).

(3) The *neurilemma* or *sheath of Schwann* (Fig. 115) is a delicate tube-like membrane which encloses the myelin. Although at the nodes of Ranvier the neurilemma dips inward to come in contact with the axon, it has been shown

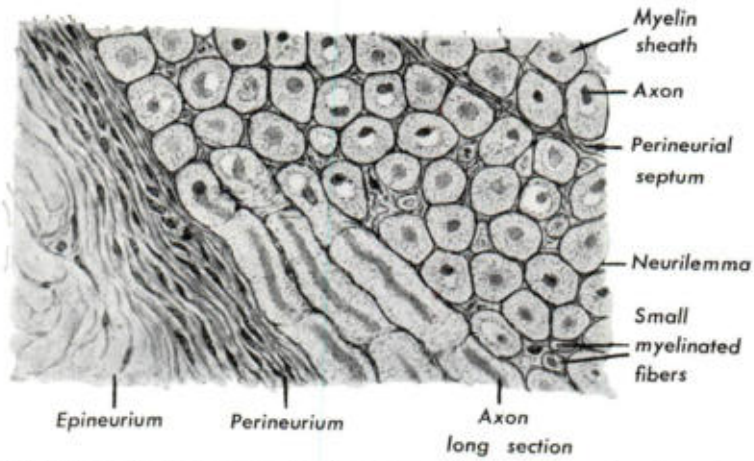


FIG. 118. Cross section of sciatic nerve of monkey. Hematoxylin and eosin stain, after paraffin technique, showing about an average amount of shrinkage of axons obtained by this method. × 500.

by microdissection studies that it is a continuous membrane, uninterrupted at the nodes. Against the inner surface of the sheath, usually about midway between each two nodes, is an oval nucleus surrounded by an area of cytoplasm, from which cytoplasmic strands radiate, branching and anastomosing to form a thin flat cytoplasmic network which lines the inner surface of the sheath. This is a cell of Schwann (Fig. 122).

It is known that the process of myelin formation in peripheral nerves is definitely dependent upon the presence of the neurilemma sheath cells. This process was observed by Speidel in the living nerve fibers of the transparent tail fins of amphibian larvae. He was able to watch the growth of nerve fibers for hours at a time and at frequent intervals during several months of observation. According to this author's account, the distal portions of growing nerve fibers are naked axis cylinders but subsequently sheath cells (cells of Schwann) migrate out and take up stations at intervals along the naked axon. In those fibers destined to become myelinated, the first myelin is

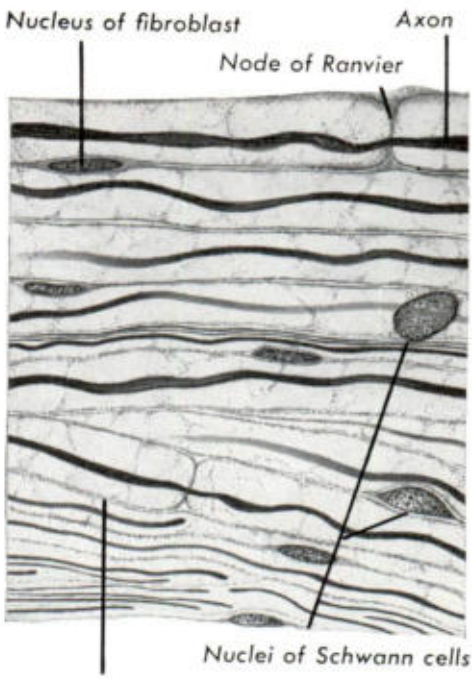


FIG. 119. Longitudinal section of brachial nerve of monkey. Cajal silver stain. Most of the fibers shown are myelinated but a few nonmyelinated (or thinly myelinated) fibers are present in the lower left portion of the field. × 700.

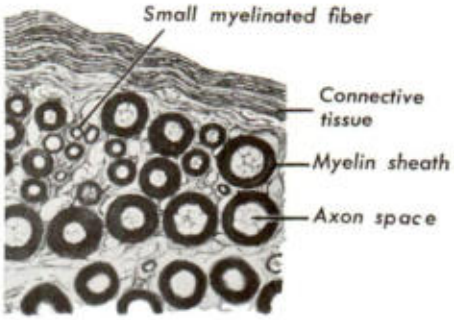


FIG. 120. Cross section of sciatic nerve of monkey. Osmic acid fixation (myelin stain). × 667.

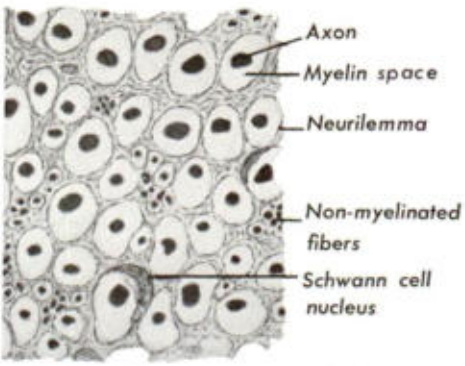


FIG. 121. Cross section of sciatic nerve of monkey. Cajal silver stain for axons. × 667.

formed in the vicinity of a sheath cell nucleus; additional myelin is formed in either direction until a complete myelin segment has been established between two nodes of Ranvier. In this manner new myelin segments are added successively in a proximo-distal direction, following the advance of the growing nerve tip.

Speidel also observed the end-to-end anastomosis of two myelin segments, with obliteration of the intervening node. In this case, the superfluous Schwann cell abandoned its position and migrated to another nerve.

Electron micrographs and tissue culture studies have clarified our knowledge of the process of myelin formation. Electron micrographs showing different stages of the process indicate that the Schwann cell cytoplasm encloses the axon and that the cell then rotates around the axon, leaving the latter surrounded by several concentric layers. In some manner, the cytoplasm is squeezed out of the inner layers and myelin is deposited in its place. This is known as the "jelly-roll" hypothesis of myelin formation (Figs. 123, 124). Tissue culture studies have demonstrated the actual rotation of the Schwann cell around the axon in living preparations.

In addition to the above-described sheaths, the fibers of peripheral nerves have, outside the neurilemma, a connective tissue sheath of mesodermal origin known as the *endoneurium* or *sheath of Henle*, which does not, however, form a part of the nerve fiber proper. The endoneurium consists of delicate connective

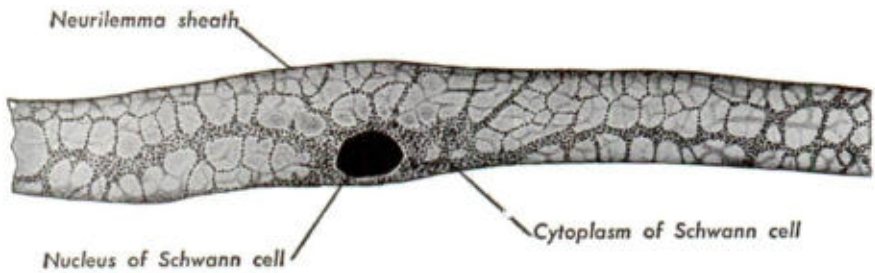
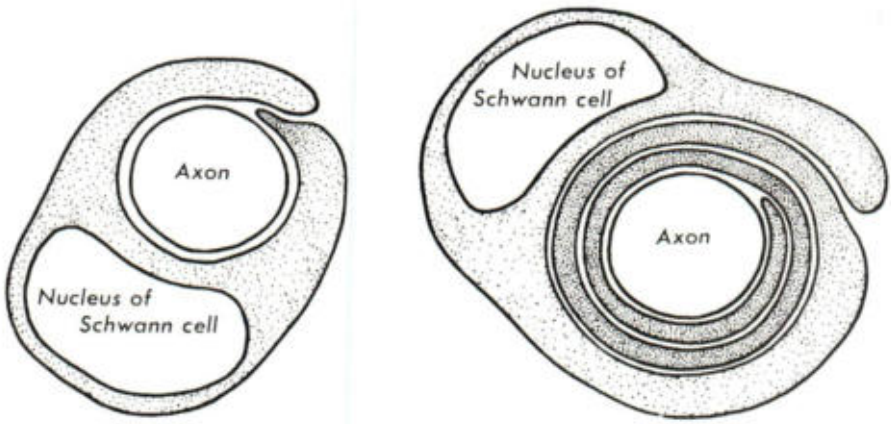


FIG. 122. Portion of a myelinated nerve fiber from the cat, showing the extent of the cytoplasm of the Schwann cell. (Redrawn from Nemiloff.)



FIGS. 123 and 124. Diagrams showing the "jelly-roll" hypothesis of myelination by rotation of the sheath cell around the axon. The condensed cytoplasm of the inner spirals of the cell is supposedly squeezed into the less compressed part of the cell, leaving concentric layers of myelin. According to another hypothesis the layers of myelin are "secreted" by the Schwann cell and rotation of the cell is not an essential part of the process. (Above figures are based on electron micrographs and on diagrams shown by Drs. B. B. Geren and F. O. Schmitt, See Symposium on Fine Structure of Cells, 1955).

tissue fibrils, connective tissue cells, and a homogeneous interfibrillar ground substance. The fibrils are disposed chiefly in a longitudinal direction and they are closely adherent to the neurilemma sheath.

Myelinated nerve fibers in the central nervous system. There is no neurilemma sheath in the central nervous system, the neurilemma cells being represented by the neuroglia cells investing the nerve fibers. Irregular and rather indefinite nodes of Ranvier have been described. The structure of the myelinated fibers of the central nervous system is otherwise similar to the above-described structure of a medullated nerve fiber with a neurilemma. It must be remembered that many nerve fibers lie partly within and partly outside the central nervous system.

Myelinated nerve fibers vary greatly in size. The fine fibers have a diameter of from 2 to 4 μ, those of medium size from 4 to 10 μ, the largest from 10 to 20 μ.

A thick myelin sheath appears to be related to the speed of conduction of the nerve impulse since the largest axons with the thickest sheaths generally have the highest rate of conduction. The myelin sheath is regarded by some as having an insulating function, preventing the nerve impulse in one fiber from diffusing to adjacent fibers. Others assign to it a nutritive role in relation to the enclosed axon. The phenomenon seen in secondary degeneration of nerve fibers indicates a close metabolic relation between the two.

Amyelinated or Nonmedullated Nerve Fibers. In the peripheral nervous system the majority of the axons of the autonomic ganglia and the processes of many of the smaller cerebrospinal ganglion cells have no obvious myelin sheaths. These amyelinated peripheral nerve fibers are sometimes called *fibers of Remak*. Special methods have indicated that there is a thin layer of fatty molecules even on these fibers which are ordinarily classified as nonmedullated. The axis cylinder is very slender and the thin neurilemma sheath contains scattered nuclei which represent the Schwann cells. The latter are not arranged segmentally

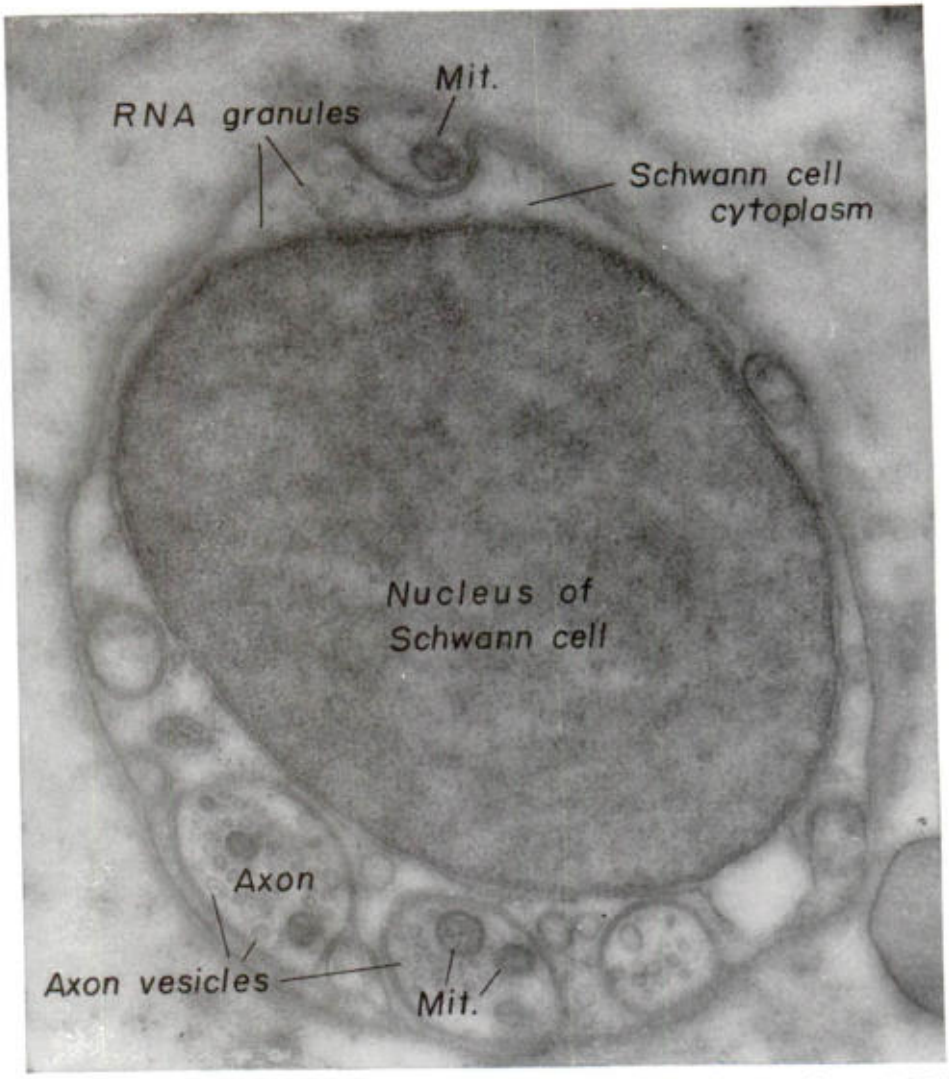


FIG. 125. Electron micrograph of cross section of non-medullated nerve fibers. × 29,120. (Courtesy of Dr. G. D. Pappas.)

and there are no nodes of Ranvier. Electron micrographs of such fibers show that the cytoplasmic extensions of one Schwann cell usually enclose several axons (Figs. 125, 126). In ordinary preparations, it is difficult to distinguish the nonmedullated nerve fibers from adjacent connective tissue fibers.

In the central nervous system there are also many amyelinated nerve fibers. Here the neurilemma is, of course, also lacking and the fibers appear to be naked axons embedded in the neuroglia.

RELATIONS BETWEEN NEURONS. THE SYNAPSE

While there remains much that needs to be learned concerning the histological

relations between neurons, it seems certain that the relations are of the nature of contact rather than complete structural continuity. This junction of two neurons is called a *synapse*. The synapse may be defined as the point of contact of the cell membrane (plasma membrane) of an axon terminal with the cell membrane of another neuron.

Although a nerve fiber is capable of conducting a nerve impulse in either direction from a point of stimulus, the synapse can transmit an impulse in only one direction. This leads to a one-way, end to end conduction along all neurons known as dynamic polarization.

The synapse may be between the axon termination of one neuron and the cell body of another (axo-somatic synapse) or it may be between the axon terminals and the dendrites of another neuron (axo-dendritic synapse). In the central nervous system, the interwoven telodendria of axon and dendrite arborizations forms a dense feltwork of fibers which is termed *neuropil*.

The axon terminals of a single axon may form synapses with both the body and the dendrites of another neuron. Various methods of contact occur, among which are: (1) by neuropodia or terminal boutons; (2) by gemmules; (3) by parallel apposition. In the first method the axon terminals end in small bulb-like expansions in contact with the body or dendrite of another neuron. Each expansion or neuropodium consists of neurofibrillar loops embedded in perifibrillar substance; sometimes there are simply small neurofibrillar rings (Fig. 127). Studies with the electron microscope show many minute *presynaptic vesicles* in the terminal portion of the axon (Figs. 128, 129). It has

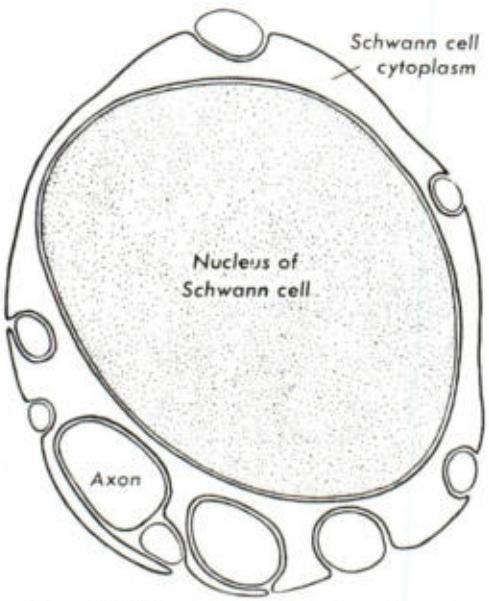


FIG. 126. Diagram showing how the cytoplasm of one sheath cell partially encloses several non-medullated axons. Based on electron micrograph of Fig. 125.

FIG. 127. Axo-somatic and axo-dendritic synapses. Terminal arborizations from axons of other nerve cells are shown as neuropodia or terminal boutons on the cell body and dendrites of a nerve cell from the olivary body of the medulla of a cat. (After Bielschowsky.)

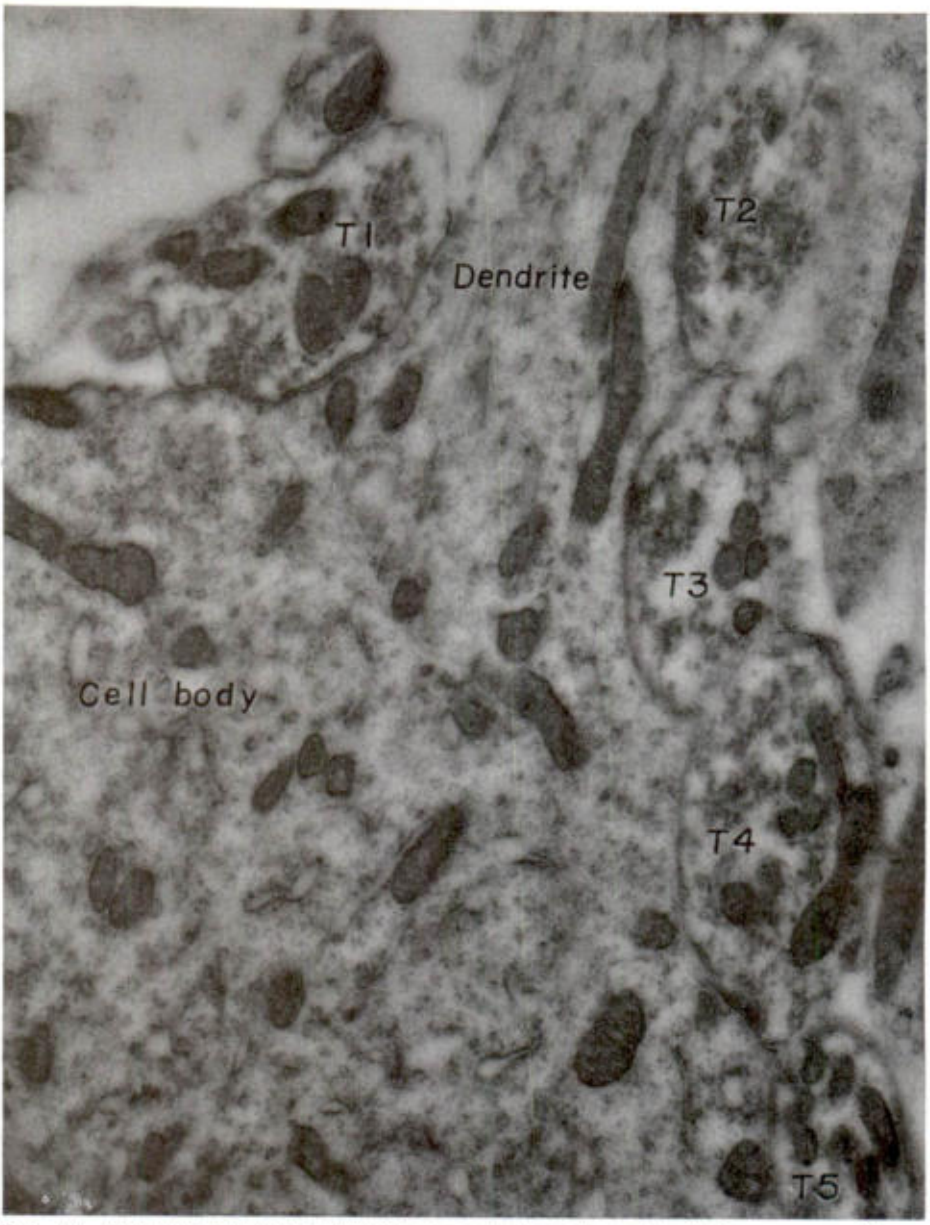


FIG. 128. Electron micrograph through axo-somatic and axo-dendritic synapses. The axon terminals (T-1 to T-5) are seen in close apposition with the body and with the dendrite of another neuron. × 34,000. (Courtesy of Dr. S. L. Palay.)

been suggested that they may contain acetylcholine which is released as a chemical mediator for transmission of the nerve impulse.

In the gemmule type of synapsis, axon processes running at right angles to the dendrites of another neuron appear to come in contact with their gemmules.

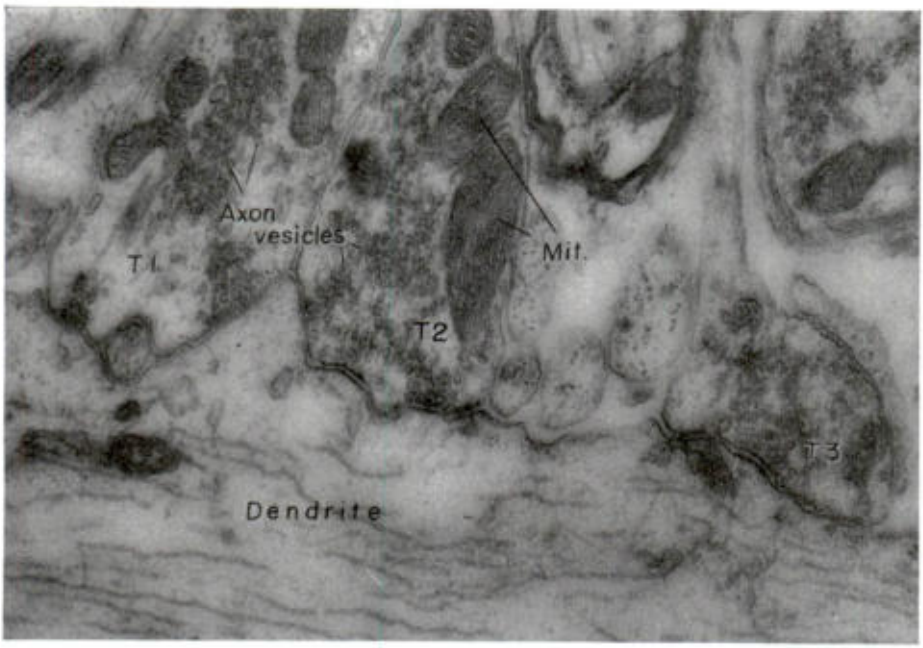


FIG. 129. Electron micrograph through axo-dendritic synapse. Three axon terminals (telodendria, T-1 to T-3) are seen in the upper part of the figure. Note that the axon terminals have many mitochondria and numerous small vesicles. The apposing synaptic membranes, consisting of the membrane of the axon and the membrane of the dendrite, are separated by a narrow space only about 200 A in width. × 43,000. (Courtesy of Dr. S. L. Palay.)

In the third type, the axon terminal comes into lengthwise apposition with the dendrite, cell body or, in some cases, the amyelinated portion of the axon of the receiving neuron (Fig. 130). It is obvious that one axon may carry impulses to a number of neurons and that each neuron may receive impulses from the axons of a number of other neurons.

TRANSMISSION OF THE NERVE IMPULSE

We have very little knowledge as to the physiological significance of the structural elements of the nerve fiber in relation to conduction of the nerve impulse. The neurofibrillae, the neuroplasm, the axon as a whole, the surface membrane of the axon—each has been proposed as the structural element most intimately concerned with transmission of the nerve impulse. The last named enjoys the greater popularity today.

No visible structural changes take place in a nerve fiber during conduction but we know that this functional activity is accompanied by an increased consumption of oxygen, the elimination of carbon dioxide and the production of heat. A temporary change in the electrical potential of the nerve fiber also attends the passage of the impulse; this is a sharp rise in the negative electrical potential, producing a small but measurable electric current which accompanies the impulse along the fiber. This is the "action current" of the nerve fiber.

Considerable evidence has accumulated in support of the view that a chemical substance is liberated at axon terminations—adrenine at sympathetic terminations on effectors, acetylcholine at

FIG. 130. Parallel axo-dendritic synapses formed by the terminal arborization of a climbing fiber (black) with the dendrites (stippled) of a cell of Purkinje; *a*, axis cylinder of a climbing fiber just before breaking up into its terminal arborization; *b*, body of a cell of Purkinje. (Cajal.) Chrome silver method of Golgi.

parasympathetic terminations, at terminations of axons in skeletal muscle fibers and at synapses in sympathetic ganglia.

THE PERIPHERAL NERVES

The nerve fibers, coursing from their cell bodies to their terminations in some peripheral structure, are grouped together in bundles to form the peripheral nerves. The fibers connected with the spinal cord form the spinal nerves and those connected with the brain comprise the cranial nerves.

Spinal nerves contain four functional types of nerve fibers: somatic efferent, visceral efferent, somatic afferent, and visceral afferent (Figs. 104, 105). The somatic efferent fibers are myelinated axons of multipolar cells situated in the ventral horn of the spinal cord. They leave the cord in several bundles which join to form the ventral or motor root. The amyelinated visceral efferent fibers arise from sympathetic ganglion cells and join the nerve trunk by way of the gray communicating ramus. The afferent fibers, somatic and visceral, are peripheral processes of unipolar neurons located in the spinal ganglion, on the dorsal root. These leave the ganglion and join with the fibers of the ventral root to form the mixed spinal nerve trunk. Some of these afferent fibers may be amyelinated. The connection of the ganglion with the spinal cord is by means of the central processes of the ganglion cells, which together enter the cord as the dorsal, or sensory, root.

Of the cranial nerves, some are purely efferent, others purely afferent, while still others contain both efferent and afferent fibers. The same fundamental relations hold for the cranial nerves as for the spinal nerves. The afferent fibers arise from cell bodies in ganglia outside the central nervous system and the efferent fibers either from neuron bodies lying within the brain or from cells in autonomic ganglia. The optic "nerve" and possibly part of the trigeminal and certain other cranial nerves form exceptions to this statement. The optic nerve actually is a fiber tract connecting the retina—an outlying evaginated part of the neural tube—with the brain, while the trigeminal and possibly certain other cranial nerves contain some afferent fibers originating from cell bodies lying within the brain. The olfactory nerve arises from cells in the olfactory mucous membrane, which thus retain their primitive position at the surface of the body.

In structure, the peripheral nerves consist of nerve fibers supported and enclosed by connective tissue (Fig. 131). Enclosing the entire nerve is a thick

sheath of connective tissue, the *epineurium*. It is composed of irregularly arranged collagenous and elastic fibers together with fibroblasts and histiocytes. When the nerve fibers are arranged in several distinct fasciculi, as is often the case, these bundles are separated by extensions of the epineurium. Each fasciculus is surrounded by a fairly dense sheath composed of several concentrically arranged layers of connective tissue, the *perineurium*. The collagenous fiber bundles of the perineurium are relatively fine in contrast to the coarse bundles characteristically present in the epineurium (Figs. 131, 118). Septa from the perineurium may extend into the nerve bundle.

Finally, the individual nerve fibers themselves are surrounded by a delicate connective tissue sheath, the *endoneurium*. This sheath is continuous with the perineurium and is composed of fine connective tissue fibers, together with fibroblasts and histiocytes.

Blood vessels run in the epineurium and perineurium. These sheaths become progressively thinner as the nerves branch and become smaller.

On emerging from the central nervous system, the root fibers receive an investment of connective tissue (endoneurium and perineurium) as they pass through the pia mater. This is reinforced by additional connective tissue (epineurium) as the nerve passes through the dura mater.

THE GANGLIA

Cranial and Spinal Ganglia. The cranial and the spinal ganglia consist of aggregations of afferent neurons situated on the sensory roots of their respective nerves. Each ganglion is surrounded by a connective tissue capsule which is continuous with the epineurium and perineurium of the peripheral nerve (Fig. 132). Connective tissue trabeculae extend from the capsule into the ganglion to form a framework. Within the ganglion, the nerve cells are separated into irregular groups by strands of connective tissue and by bundles of nerve fibers (Fig. 132).

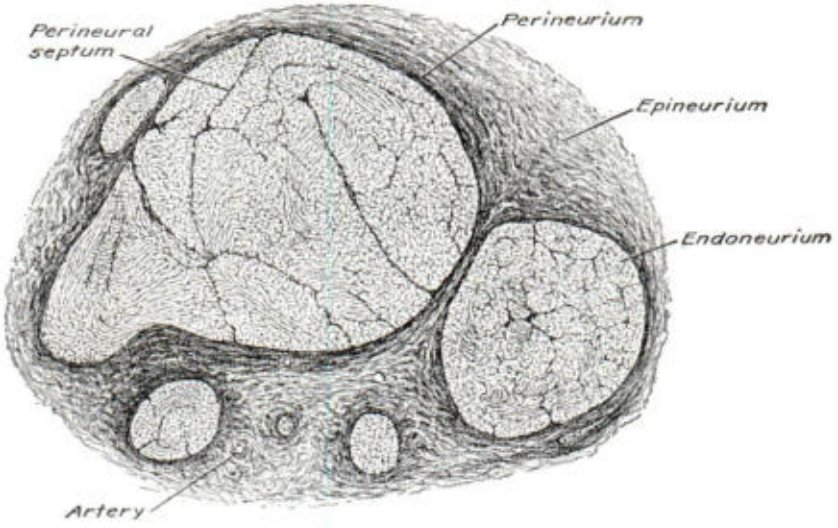


FIG. 131. Cross section of sciatic nerve of monkey.

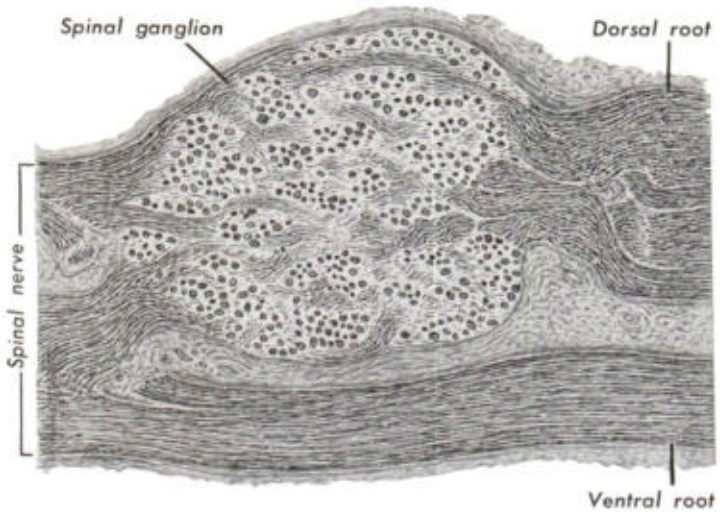


FIG. 132. Longitudinal section through a spinal ganglion of an infant, two months of age. Cajal silver. × 28.

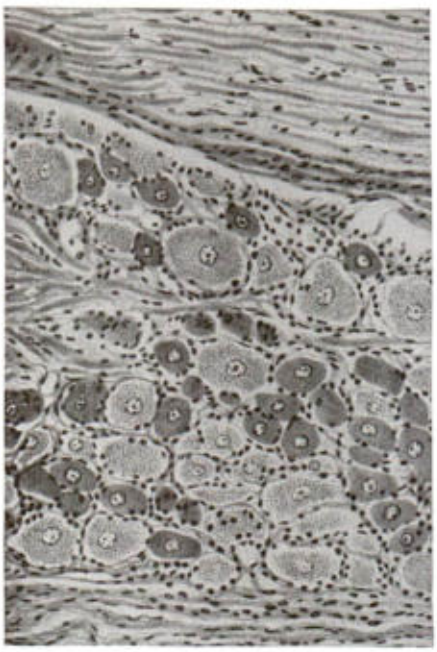


FIG. 133. Section of a human spinal ganglion. Hematoxylin-eosin stain. Two principal types of ganglion cells are shown, a large clear type with well marked Nissl bodies and smaller, more darkly staining type. The nuclei of the capsule cells are also evident. (Ph. Stöhr, Jr., From v. Möllendorff, Handbuch der mikroskopischen Anatomie des Menschen.)

Each ganglion cell is enveloped by a double layer. The outer part, or *capsule*, is composed of flat fibroblasts and connective tissue fibers which are continuous with the endoneurium. The inner part consists of a layer of fusiform or stellate cells closely applied to the surface of the ganglion cell (Fig. 134). These inner cells are the *satellite cells*, or *amphicytes*. They are not of connective tissue origin but are derived from the same neural ectoderm as are the ganglion cells themselves.

The cells which comprise the cranial and spinal ganglia are unipolar neurons. The cell bodies vary in size from 15 μ to 100 μ. Many of the smaller ones are reported to give rise to amyelinated fibers concerned with the conduction of pain impulses. Each cell contains a centrally placed rather vesicular nucleus with a prominent nucleolus. The chromophil substance may be scattered throughout the cytoplasm as diffuse granules or it may form rather small, finely granular Nissl bodies which are concentrically arranged around the nucleus (Fig. 134). With silver stains, the neurofibrillae often

give the appearance of forming large or small loops within the cell body.

Most of the ganglion cells have one principal myelinated process which, at some distance from the cell body, divides into a peripheral branch which courses in the peripheral nerve and a central branch which enters the central nervous system as a sensory root fiber. The manner in which this single process arises from the cell varies. The single process, or axon, usually comes off the cell body by a single root (Fig. 135) but occasionally it arises from the cell body by several smaller roots which fuse. The course of the axon in the neighborhood of the cell body varies for different neurons. In some cases, the axon is coiled and looped around the cell body to form an intracapsular "glomerulus" (Fig. 135); in other cases, it follows a relatively straight course from its cell body to the point where it divides into a central and a peripheral process. The majority of the large ganglion cells have axons of the coiled, "glomerular" type whereas most of the small, darkly staining "obscure" cells have the nonglomerular type. It should be noted that special methods of technique are necessary for demonstrating the intracapsular "glomerular" course of an axon.

The Autonomic (Sympathetic and Parasympathetic) Ganglia. The majority of the autonomic ganglia resemble the cranial and spinal ganglia in having a connective tissue capsule and framework. The neurons, however, are multipolar cells with numerous branched dendrites and a single axon which forms the amyelinated visceral efferent nerve fiber (Fig. 136). The cell bodies vary in size from 20 μ to 45 μ. The nucleus is relatively large and vesicular, is round or oval in shape and has one or more well-defined nucleoli. The nucleus is often eccentrically located. Binucleate cells are not uncommon and multinucleate neurons have been observed in sympathetic ganglia.

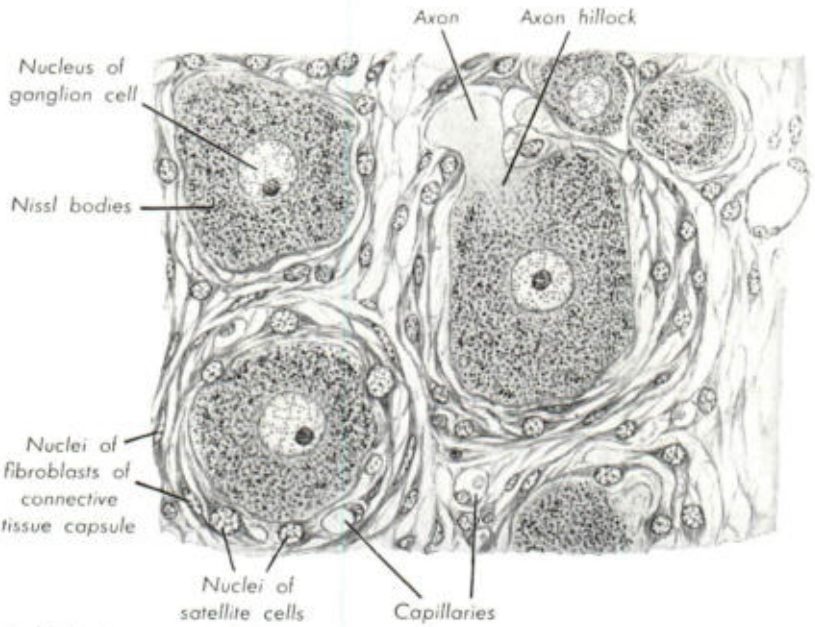


FIG. 134. Unipolar nerve cells from a Gasserian (cranial) ganglion stained by the Nissl method. Human, 19 years of age. × 640.

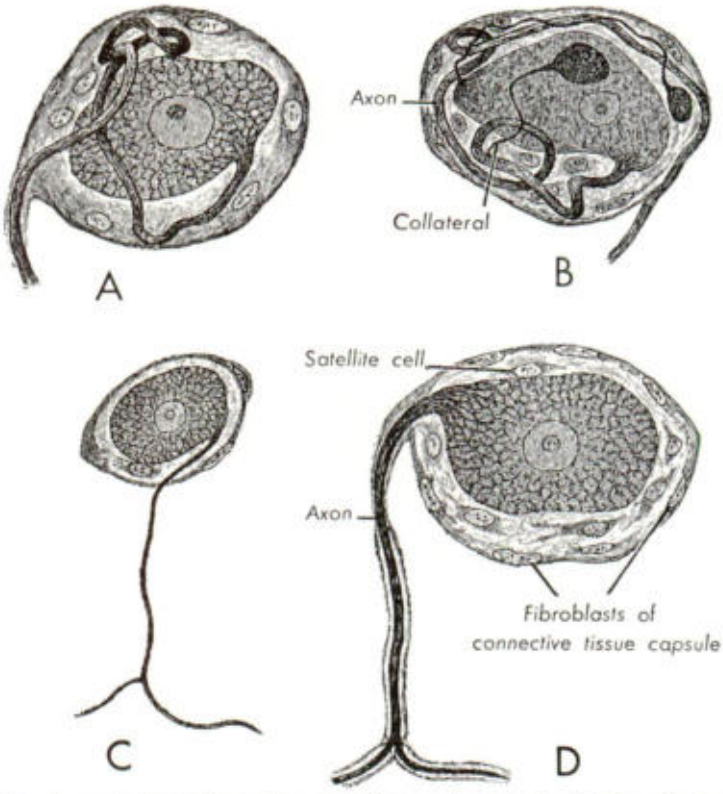


FIG. 135. Cerebrospinal ganglion cells and their capsules, Cajal's silver stain. *A*, large unipolar cell with single process forming an intracapsular coil or glomerulus; *B*, large cell with main process forming a glomerulus and short processes ending in intracapsular bulbs. The short processes may arise either from the cell body or as collaterals from the main process. *C*, small cell with nonglomerular, nonmyelinated process; *D*, large cell with nonglomerular, myelinated process. *A*, drawn from a 35 μ section of the Gasserian ganglion of the trigeminal nerve of a 17-year-old boy, × 920. *B*, *C*, and *D*, redrawn from Cajal.

The chromophil substance is in the form of Nissl bodies which may be distributed uniformly throughout the cytoplasm or may be confined either to the perinuclear zone or to the peripheral cytoplasm.

In the larger ganglia, each cell is surrounded by a layer of satellite cells, as in the spinal ganglia. The shorter dendrites of the ganglion cell terminate within the capsule but the longer dendrites pass through the capsule and form their telodendria in the intercellular plexus of fibers.

Often, two ganglion cells may share a single capsule. In the smaller terminal ganglia, such as those in the intestinal wall, many of the ganglion cells may lack capsules. Parasympathetic ganglion cells are found in almost all visceral structures.

DEGENERATION AND REGENERATION OF NERVE FIBERS

The individuality of the neuron and the interdependence of its parts are strikingly exemplified by its behavior when injured. When a nerve trunk is cut

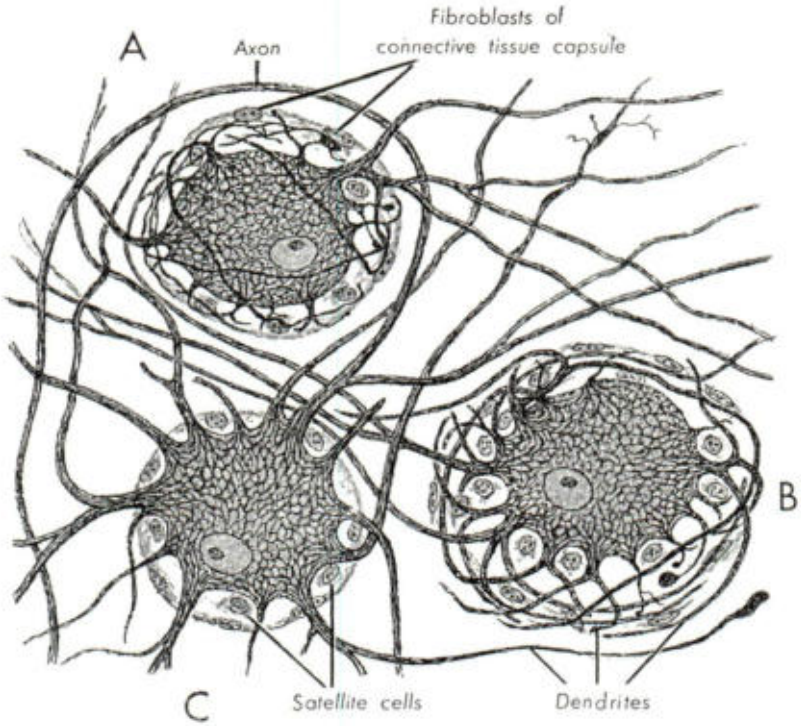


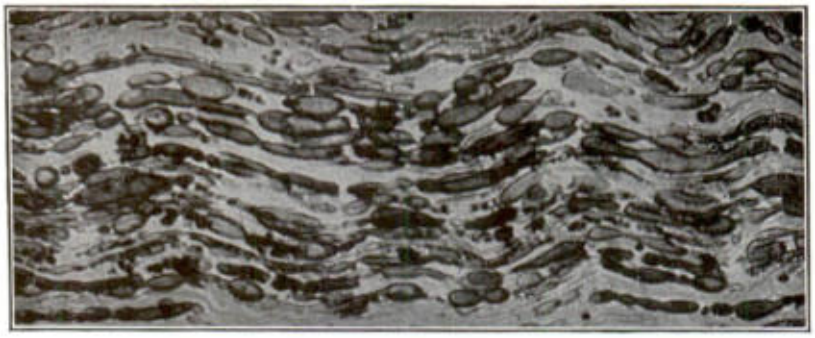
FIG. 136. Sympathetic nerve cells, human, 36 years of age. Cajal's silver stain. *A* and *B*, cells whose dendrites form a pericellular plexus. *C*, cell with long dendrites. Redrawn from Cajal.

across, certain changes take place in the cut ends for a short distance on either side of the cut. These are degenerative changes of a traumatic nature, involving necrosis of the injured parts. On the proximal side of the injury (toward the cell body), degenerative changes may extend the distance of a few internodes but very soon regenerative processes are initiated leading to a new growth from the end of this central stump.

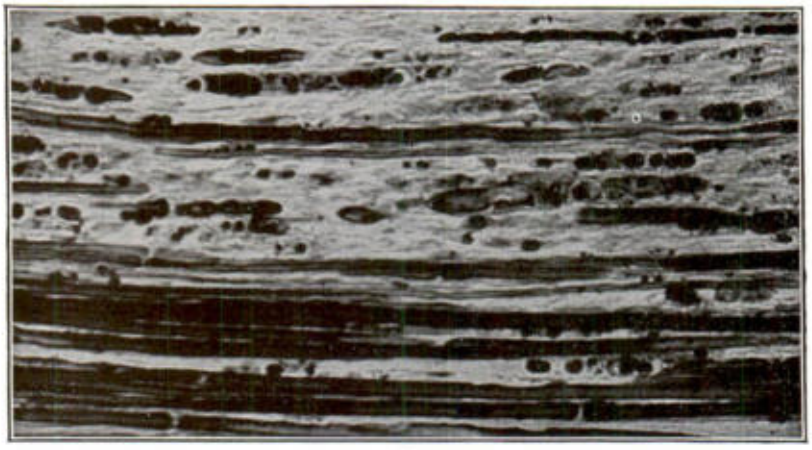
Distal to the site of injury, however, the degenerative changes are so extensive and continuing as to lead to the complete breakdown and disappearance of this portion of the nerve fibers, including their terminal arborizations. This process is known as *secondary* or *Wallerian degeneration*. In terms of the neuron concept, this means that an axon cut off from its cell of origin degenerates and disappears and this behavior of the axon accords with the fact that the cell body is the trophic center of the neuron.

The first changes seen in the distal portion of the cut nerve occur in the axons themselves. They lose their uniform contour and become swollen. The neurofibrillae become irregular and granular and change in their staining reactions. Within three to five days after section of the nerve, the axons break up into irregular twisted segments, which finally undergo complete disintegration.

Coincident with these changes in the axon, degenerative changes also take place in the myelin sheath. During the first few days, there is a fragmentation of the myelin so that it becomes broken



A



B

C

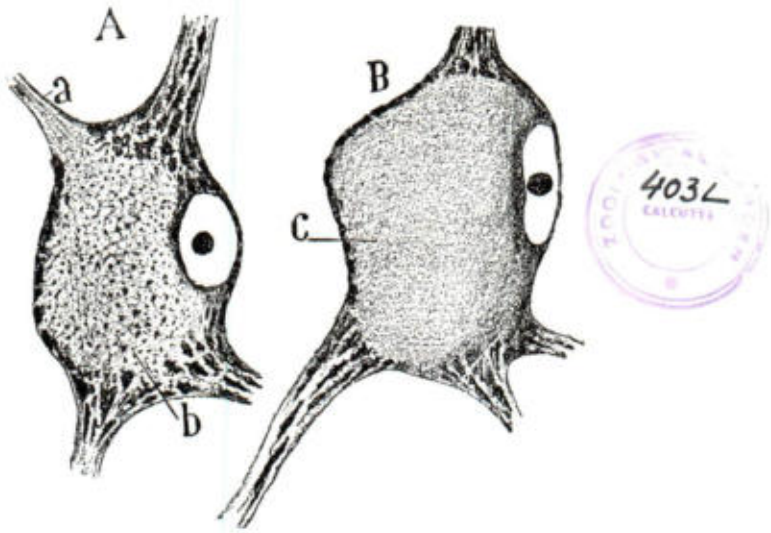
FIG. 137. *A*, Distal stump of a nerve cut 3 to 5 days previously. Osmic stain. *B*, Distal stump of a nerve partly cut 12 to 15 days previously. Osmic. Normal fibers and two nodes of Ranvier are shown at the bottom. *C*, Distal stump of a nerve cut 12 to 15 days previously. Osmic and Weigert's iron hematoxylin. In addition to the degenerating myelin, several band fibers and their nuclei (e.g., at *n*) are shown. *A*, *B* and *C*, photographs.

into spherical, oval or elongated segments which surround the fragments of the axon (Fig. 137, *A*). In the succeeding days, these myelin fragments undergo further breakdown into smaller droplets, until finally the myelin is completely disintegrated and disappears (Fig. 137, *B* and *C*). Coincident with these physical changes, chemical changes are also taking place in the myelin which result in its separation into its component substances and their breaking down into simpler fatty substances which, unlike normal myelin, give the reaction to the Marchi stain.

While the axon and myelin sheath are disintegrating, the Schwann cells of the neurilemma sheath enlarge and their nuclei divide mitotically without accompanying division of the cytoplasm. Thus there is formed a band or tube of syncytial cytoplasm containing many Schwann cell nuclei. These bands or tubes are known as "band fibers" or "protoplasmic fibers." They are very important in supplying pathways for the regenerating axons from the central stump of the severed nerve. Although the Schwann cells of the band fibers may play a part in the removal of the debris formed by the degeneration of the peripheral axon and myelin, this function is performed chiefly by macrophages which invade the Schwann tubes from the surrounding connective tissue.

Similar changes take place in degenerating amyelinated fibers, except that no myelin is involved.

The extensive degenerative processes in the peripheral stump are not the only changes which follow nerve section. The primary or traumatic degeneration occurring in the proximal stump at the site of the injury has already been mentioned. Degenerative changes also occur in the neuron body itself. *Chromatolysis*



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CALCUTTA

FIG. 138. Two motor cells from ventral horn of spinal cord of rabbit; fifteen days after cutting major sacro-sciatic nerve. *A*, Cell in which the Nissl bodies in the center of the cell appear disintegrated (*b*) and nucleus eccentric; *B*, cell showing more advanced chromatolysis, the chromophil substance being present as bodies only in the dendrites and in the form of a homogeneous mass around the nucleus; nucleus causes bulging of surface of cell. The cells also show turgescence.

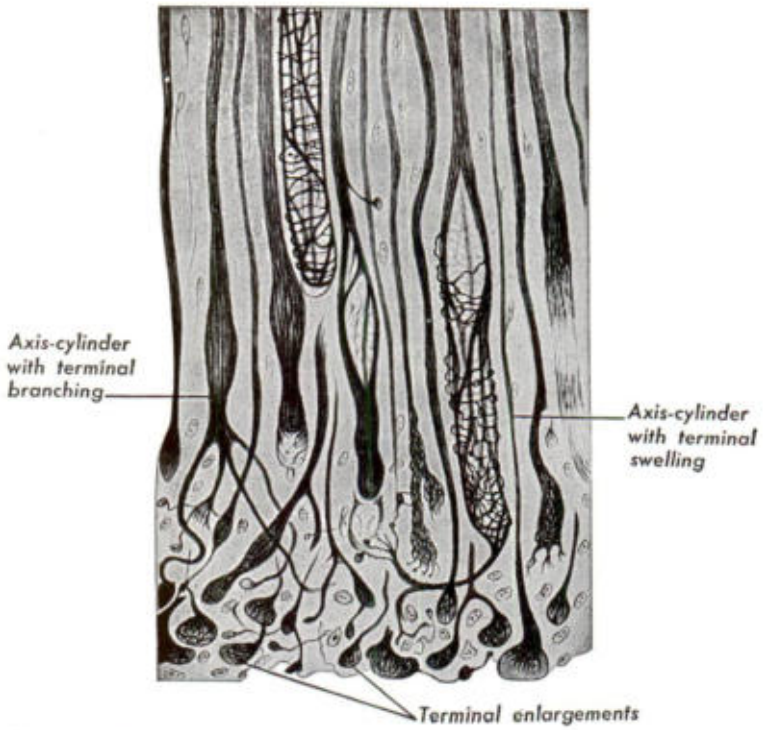

FIG. 139. Regenerating axons in the central stump of the sciatic nerve of a cat two and one-half days after section of the nerve. (Redrawn from Ramon y Cajal.)

(i.e., dissolution of the Nissl bodies) may begin as early as the first day after nerve section. It usually reaches its peak during the second and third weeks. Associated with chromatolysis, there is a change in the cytoplasmic osmotic pressure and an uptake of water with consequent swelling of the cell body and a displacement of the nucleus to an eccentric position (Fig. 138). The extent and rapidity of these changes depend upon the type of neuron and on the nature and location of the injury, an injury near the cell body causing more effect centrally than one at a distance. If the axon is injured very near the cell body, the latter may ultimately die.

If the neuron body survives, fiber regeneration takes place. The axons of the proximal stump of the severed nerve form bulbous enlargements and axonal sprouts within a few days (Fig. 139). Usually it takes about two weeks for the axonal sprouts to cross the scar area to enter the Schwann tubes of the distal stump of the severed nerve. The growing tip of the axonal sprout advances more rapidly after it has entered the Schwann tube. Axonal growth across the scar is facilitated by bridges which are formed chiefly by proliferation and migration of the Schwann cells and fibroblasts. The axonal sprouts are unable to cross the gap when the latter is too long or when it becomes filled with dense collagenous fibers. Transplants are made in order to facilitate the growth of axonal sprouts across gaps of any appreciable size (e.g., after gunshot wounds).

Each sprout from the proximal end of

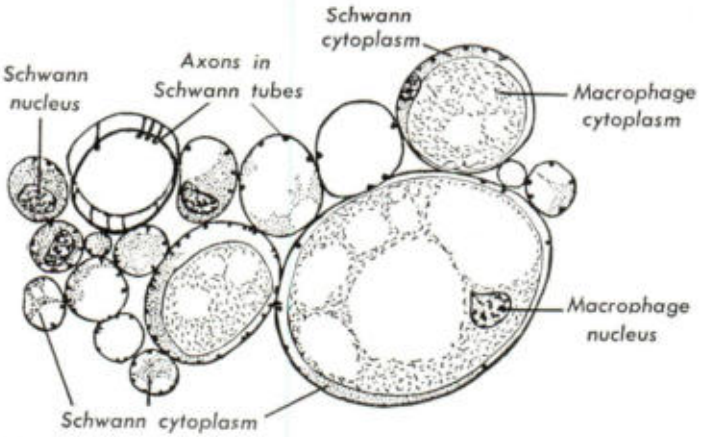

FIG. 140. Diagrammatic representation of a cross section of a portion of the peripheral stump of a rabbit sciatic nerve severed 25 days previously. The peripheral portions of the severed axons together with their myelin sheaths have degenerated and have been phagocytosed by macrophages. Most of the Schwann tubes contain several axonal sprouts which have grown from the central (proximal) portion of the severed nerve. As many as 50 sprouts may grow from the end of one severed axon but not all of these succeed in crossing the scar to enter the Schwann tubes in the peripheral stump. At the stage shown, the regenerating axons are of fine caliber and are unmyelinated. (After J. Z. Young.)

a severed nerve usually splits into a number of branches, sometimes as many as 50. This increases the chance of appropriate connections. A number of branches may enter a single Schwann tube (Fig. 140). Some of these branches enlarge, others degenerate (Fig. 141). Eventually, only one axon is left per tube.

During the course of regeneration, the rate of synthesis of ribonucleoprotein eventually overtakes the rate of demand. Then, Nissl material begins to reappear in the neuron cell body, cell turgescence gradually subsides, and the nucleus reassumes its normal position.

At first, the regenerating fibers possess only a neurilemma sheath, formed by the Schwann cells. Myelin segments are added subsequently, apparently through the agency of the Schwann cells. The regenerated axons are slender at first and they require a long period of time to attain the diameter, myelin thickness, and conduction rate of a normal adult fiber.

The rapidity of the above degenerative and regenerative processes varies according to the metabolic rate of the animal. They are more rapid in warm-blooded than in cold-blooded animals.

In the central nervous system of mammals, where Schwann cells are lacking and no band fibers are formed, regeneration does not occur as readily as in the peripheral nervous system. However, recent studies have shown that axons will bridge the gap between the cut ends of a transected spinal cord in mammals when special surgical procedures are used to prevent connective tissue from growing into the gap.

The importance of degenerative processes from the standpoint of neuroanatomy lies in the fact that by using the proper technical methods, it is possible to trace the connections between cell bodies and nerve fibers throughout

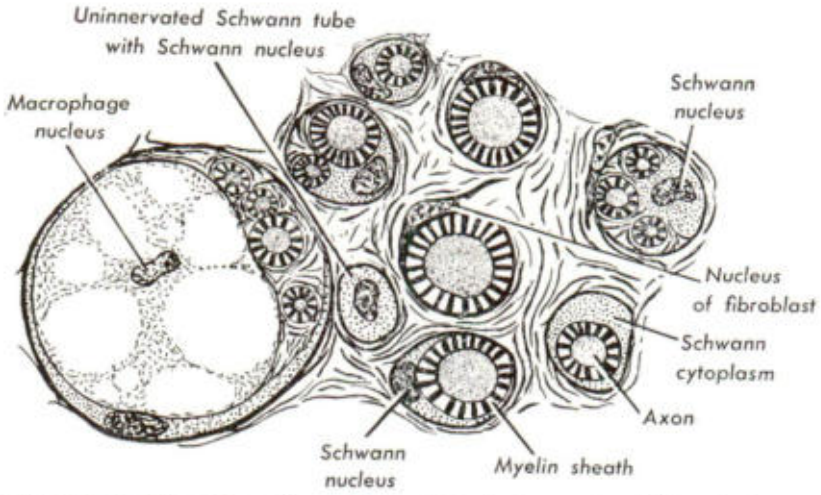

FIG. 141. Diagram of a cross section of the peripheral stump of a rabbit sciatic nerve severed 150 days previously. The regenerating axons are of larger caliber than at the earlier stage shown in Figure 140. They have also become myelinated, even in the tube which still contains a macrophage. The supernumerary axons eventually disappear and the remaining ones attain a normal diameter, with one axon per tube. (After J. Z. Young.)

the nervous system. The Marchi method, in which osmic acid is used after potassium bichromate fixation, restricts the staining to the fatty products of myelin degeneration, the normal myelin staining very lightly. In this way, even scattered nerve fibers, if stained in the proper stage of degeneration, can be traced throughout their whole length. To ascertain the cell bodies to which the cut fibers belong, the Nissl method of staining the chromophil substance is usually employed and the abnormal cells distinguished by the characteristic changes already described.

NERVE TERMINATIONS

The axons which form the peripheral nerve fibers terminate in peripheral structures, to which or from which they convey nerve impulses. The efferent fibers terminate in tissues which are excited into activity by the nerve impulse—the somatic efferent fibers on skeletal muscle and the visceral efferent on cardiac and smooth muscle and on secretory epithelium. These constitute *effector* organs. The afferent fibers end freely in the tissues or in specially organized structures, where in either case they receive stimuli which cause them to convey nerve impulses to the central nervous system. These nerve endings are the *receptors*.

Terminations of Somatic Efferent Fibers. The cell bodies of these fibers lie in the ventral horns of the spinal cord or in the motor nuclei of cranial nerves in the brain. The axons themselves form part of the ventral roots and efferent fibers of the peripheral nerves and terminate in the skeletal muscles of the body and head. The nerve fibers enter the perimysium externum and internum, in which they may bifurcate several times, thus permitting one neuron to innervate more than one muscle fiber. Finer muscles concerned with delicate gradations of precise movement generally receive a more abundant supply of nerve fibers. In one of the extrinsic eye muscles, the superior oblique muscle, the number

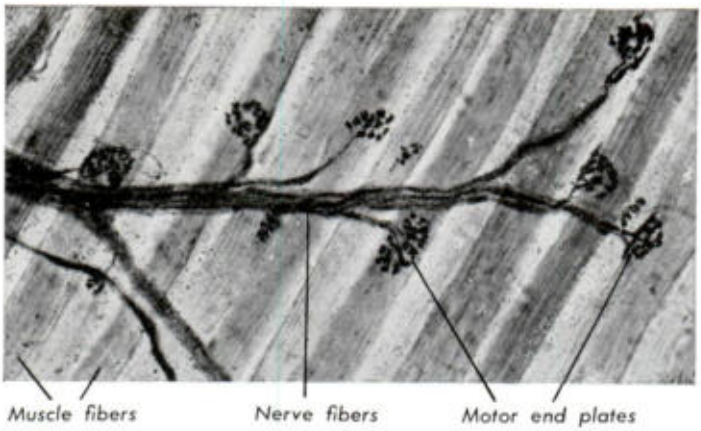

FIG. 142. Photomicrograph of motor nerve ending in intercostal muscle. Gold chloride method. × 215.

of nerve fibers to muscle fibers may approach a one to one ratio. In most muscles, however, the ratio is much lower and many muscle fibers are innervated by the terminal branches of one nerve fiber.

After repeated branchings in the connective tissue of the perimysium, the nerve fibers pass to the individual muscle fibers where they terminate in structures known as *motor end plates* (Fig. 142). At the end plate region, the myelin is lost and the endoneurium becomes continuous with a layer of reticular fibers over the sarcolemma. The Schwann cells continue as a partial covering around the branched terminations of the axon which extend into grooves or depressions of the sarcolemma (Fig. 143). Although the nerve ending on skeletal muscle was once described as hypolemmal, it appears that it actually does not penetrate into the sarcoplasm. It is separated from the sarcoplasm by a membrane, or subneural apparatus, which is continuous with the sarcolemma of other parts of the muscle fiber. Beneath the end-plate region there is an increase in the number of muscle nuclei in a region known as the *sole* of the motor plate. In ordinary sections, the muscle nuclei of this region appear to be adjacent to or even intermingled with some of the Schwann nuclei due to the manner in which the ending extends into grooves of the muscle fiber (Fig. 143).

Electron micrographs have confirmed the epilemmal rather than the hypolemmal location of the axon termination, as shown by the light microscope in Figure 143. However, they fail to show a continuity of Schwann cell cytoplasm around the termination as indicated in Figure 143.

Terminations of Visceral Efferent Fibers. The visceral efferent fibers from the autonomic ganglion cells terminate in the following effectors: heart muscle (cardiomotor); smooth muscle of viscera (visceromotor), of blood vessels (vasomotor), and of hairs (pilomotor); and glandular epithelia (secretory). These fibers are amyelinated.

In heart muscle and in smooth muscle, the fibers form plexuses around the muscle bundles. From these plexuses, fine nerve fibers pass to the individual muscle fibers, where they end in one or two nodular enlargements either on or within the muscle fibers.

In glandular epithelium, the visceral

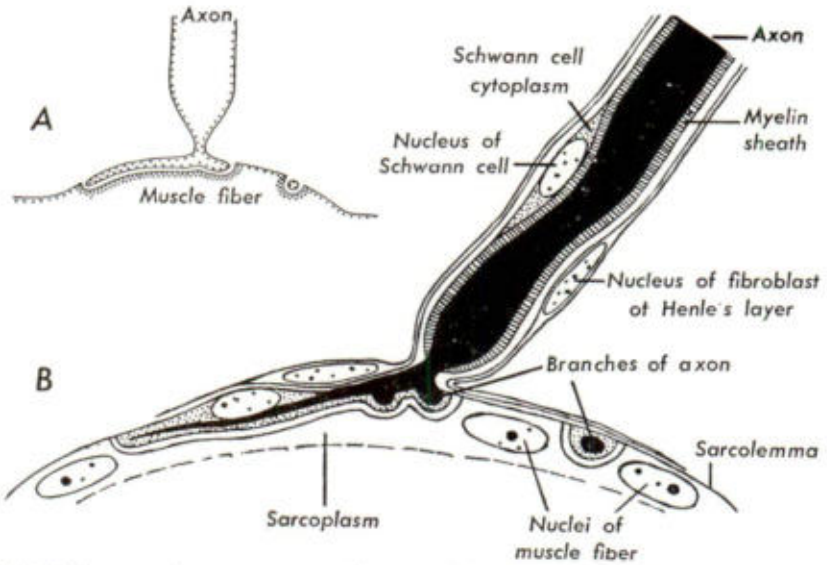

FIG. 143. Diagrams of motor nerve endings on skeletal muscle. *A*, General relationship of the nerve ending to the muscle fiber. A space is left between the nerve ending and the muscle fiber for clarity in illustration; actually, the two structures are in intimate contact. Stippled areas in *A* indicate the regions with the highest concentration of cholinesterase.

Figure *B* shows the detailed relationship of the nerve ending to the muscle fiber. The branches of the axon are covered by a thin sheath of cytoplasm homologous with that of the Schwann cells on the other parts of the fiber. Beneath the ending there is a specialized substance or subneural apparatus (double line in diagram) which is continuous with the sarcolemma of the rest of the muscle fiber. According to this view, the nerve ending merely indents the modified sarcolemma of the subneural region. (Slightly modified from Couteaux.)

efferent fibers form a plexus beneath the basement membrane, through which the fibers pass to terminate in free endings on the gland cells.

Classification of Terminations of Afferent Fibers. Those parts of the body which receive stimuli and contain the terminations of peripheral afferent fibers are known as receptors. The receptors have the function of responding to various physical and chemical stimuli and, furthermore, each kind of receptor has the specific function of reacting to one particular kind of stimulus. The nature of the reaction naturally also depends upon the central connections of the afferent nerve fibers and upon the effectors to which the nerve impulse is ultimately conducted, but the property of reacting differently to different kinds of stimuli rests primarily upon the peculiar discriminative capacity of the receptors.

The receptors may be classified in several more or less overlapping ways:

(1) Some receptors are found more or less widely distributed over the body. These may be termed collectively receptors of general bodily or *somaesthetic* sensibility (touch, pressure, pain, temperature, position, movement, visceral). Other receptors are found aggregated only in certain places in the head, where they constitute the *organs of special senses* (smell, sight, taste, hearing and head position and movement).

(2) Another distinction may be made between *exteroceptors*, the receptors af-

fected by external stimuli (touch, light pressure, cutaneous pain and temperature, smell, sight and hearing); the *proprioceptors*, which are affected by stimuli arising within the body wall, especially those of movement and posture; and the *enteroceptors*, which are affected by stimuli arising within the viscera.

The modes of termination of the peripheral processes of the cranial or spinal ganglion cells in receptors are so varied and complicated as to make impracticable any structural classification except in the broadest sense. However, the terminal arborizations of the afferent fibers follow one of two structural arrangements. Either they terminate freely among epithelial cells, muscle fibers or connective tissue elements or they are surrounded by special connective tissue capsules. The distinction can thus be made between *free* or *nonencapsulated* sensory endings and *encapsulated* ones.

Free or Nonencapsulated Afferent Endings. These endings are found in practically all epithelia of the body, in connective tissue, in muscle and in serous membranes.

In the skin, and in those mucous membranes which are covered by stratified squamous epithelium, the nerve fibers of a given branch separate as they approach the epithelium, lose their myelin sheaths and form a subepithelial plexus. From this plexus, axons or their branches enter the epithelial layer and split into minute fibrils, the terminal arborizations, which penetrate between the epithelial cells and terminate there, often in little knob-like swellings. In the skin, the nerve endings do not penetrate beyond the cells of the stratum granulosum.

Essentially similar free nerve endings are seen in other epithelial surfaces, such as the mucosa of the respiratory tract (Fig. 144).

Another form of free nerve ending is the *peritrichial* ending, in which sensory fibers encircle the hair follicle and terminate principally in the connective tissue sheath and vitreous membrane of the follicle. Fine nerve fibrils may also extend into the outer epithelial root sheath to end among epithelial cells.

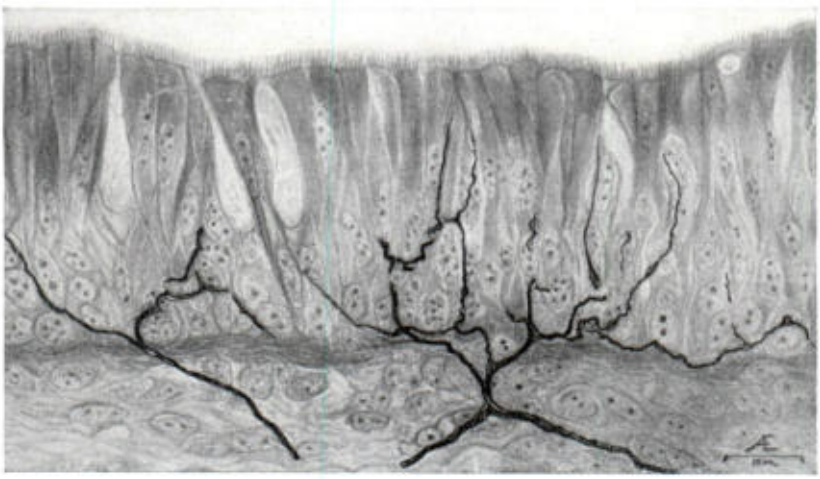

FIG. 144. Nerve endings in bronchial epithelium (pseudostratified ciliated columnar epithelium). Reduced silver method of Cajal. (After A. Elftman.)

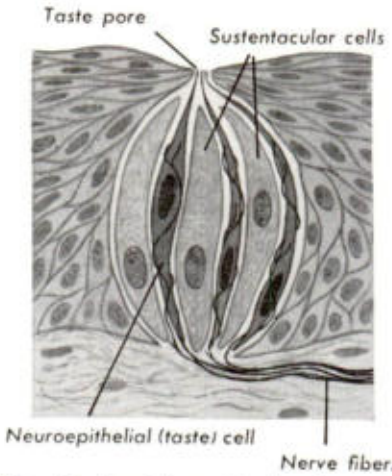

FIG. 145. Semischematic drawing of taste bud from circumvallate papilla of tongue. Special techniques are required to demonstrate the nerve fibers which end on the neuroepithelial cells.

In general, these intraepithelial endings terminate among epithelial cells which do not differ from the adjoining epithelial cells. In the deeper epithelial layers of the skin, however, there may be seen leaf-like expansions of a nerve terminal, each of which forms a *meniscus* in contact with an epithelial cell which stains differently from the other cells. This is a *tactile cell* and the whole apparatus is known as a *tactile corpuscle of Merkel*.

This tendency to modification of epithelial cells receiving special sensory nerve terminations is particularly well exemplified in certain *neuro-epithelial* cells, such as the taste cells of the taste buds (Fig. 145) and the hair cells in the sensory epithelia of the organ of Corti, cristae and maculae of the internal ear (Chapter XXII). In these membranes, the sensory nerve fibers terminate in profuse arborizations around the special epithelial cells. In the neuro-epithelium of the olfactory organ and in the retina of the eye, the sensitive cells are themselves nerve cells. (See Chapter XXII.)

Aside from the free nerve endings in epithelia, sensory fibers also terminate diffusely in connective tissue. Although these terminations may vary in form, they have in common the fact that they all end by a branching of the sensory fibers among the fibers and cells of the connective tissue. Such endings are found extensively in the dermis and subcutaneous tissue and in the connective tissue of mucous and serous membranes, the periosteum and blood vessels, to name a few instances.

The nerve ending may be in the form of a network, a myelinated fiber losing its myelin sheath and dividing repeatedly into a number of varicose fibrils which spread among the connective tissue fibers, anastomosing and interlacing with each other to form a network.

In other instances, the afferent fiber may end in a profuse arborization. Repeated branchings of the fiber produce within the connective tissue a tree-like pattern of innumerable fine amyelinated nerve threads.

In addition to arborized terminations in the interstitial connective tissue of muscle, nonencapsulated sensory nerve endings are also found around the individual muscle fibers themselves (Fig. 146).

Encapsulated Afferent Endings. These include such structures as the *end bulbs*, the *tactile corpuscles of Meissner*, the *Pacinian corpuscles* or *corpuscles of Vater-Pacini*, the *muscle spindles* or *neuromuscular bundles*, and the *muscle-tendon spindles* or *organs of Golgi*.

Of the encapsulated sensory endings, probably the simplest are the so-called end bulbs. These are spherical or oval in shape and consist of a thin lamellated capsule of flattened connective tissue cells and fibers surrounding a central cavity, the *inner bulb*. Within the inner bulb, the naked axons of one or more myelinated fibers end. In some inner

FIG. 146. Afferent nerve endings in muscle (smooth) of one of the larger bronchi. Seven-day-old puppy. Reduced silver method of Cajal. (After A. Elftman.)

bulbs, the axon may terminate in a number of branches which twist and interlace to form a spherical skein-like mass known as a glomerulus. An example of this type is seen in the *end bulbs of Krause* in the conjunctival connective tissue.

End bulbs are found in the lips, in the mucous membranes of the tongue, cheeks, soft palate, epiglottis, nasal cavities, lower end of rectum, peritoneum, serous membranes, tendons, ligaments, connective tissue of nerve trunks, synovial membranes of certain joints and the external genitals, especially the glans penis and clitoris.

The *tactile corpuscle of Meissner* (Fig. 147) offers an example of a more complex encapsulated tactile corpuscle. They occur especially in the hairless portions of the skin and are most numerous in the finger tips, the palms of the hand and the soles of the feet. Lying within the connective tissue of the dermal papillae, they are oval bodies which are composed of flattened, connective tissue cells in the form of horizontal lamellae surrounded by a connective tissue capsule. Two or more coarse myelinated nerve fibers are distributed to each corpuscle. As the fibers reach the corpuscle, the sheaths of Henle join the connective tissue capsule, the myelin sheaths disappear, and the naked axons pass into the corpuscle where they branch and pursue a spiral course among the connective tissue elements. They end in flattened expansions consisting of neurofibrils and perifibrillar substance. In addition to the terminations of coarse myelinated fibers, many tactile corpuscles and other encapsulated receptors may also contain the endings of fine amyelinated fibers, the significance of which is not known.

The corpuscles of Meissner are assumed to respond to tactile stimuli and are probably the ones most concerned with discriminative touch.

The *Pacinian corpuscles*, or corpuscles

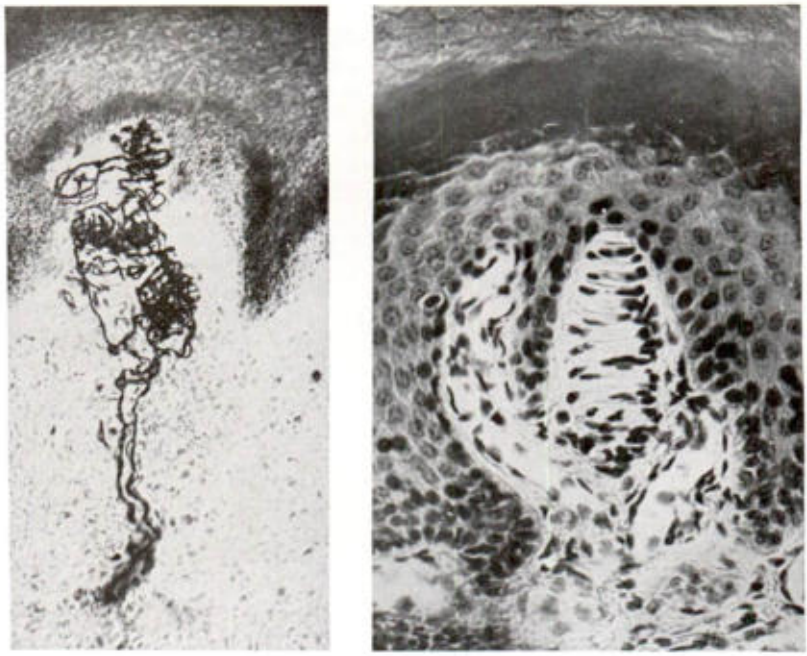

FIG. 147. Photomicrographs of Meissner's corpuscles in dermal papillae of human skin. At the *left*, stained with silver, showing the axon; at the *right*, stained with hematoxylin and eosin, showing the connective tissue elements of the nerve ending. Only a portion of the epidermis is included in the field. (Courtesy of Dr. A. Elwyn.)

of Vater-Pacini, are laminated elliptical structures which differ from the simpler end bulbs already described chiefly in the greater development of the connective tissue capsule. They are relatively large structures which are visible to the naked eye (Fig. 148). The capsule is formed by a large number of concentric lamellae, each lamella consisting of connective tissue fibers lined by a single layer of flat connective tissue cells. The lamellae are separated from one another by a clear fluid or semi-fluid substance. As in the simpler end bulbs, there is a central cavity within the capsule known as the inner bulb. Each Pacinian corpuscle is supplied by a single myelinated nerve fiber. As it enters the base of the corpuscle, its myelin sheath ends and the sheath of Henle merges with the connective tissue lamellae. The axon extends through the center of the inner bulb, often terminating in a knob-like expansion.

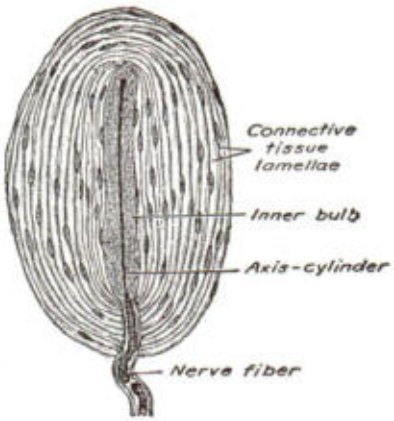

FIG. 148. Pacinian corpuscle. Human. (Redrawn from Cajal.)

Fine blood vessels enter the base of

the corpuscle along with the nerve fiber and break up into capillary networks among the lamellae. They do not enter the inner bulb.

The Pacinian corpuscles are found in the deeper subcutaneous connective tissue, especially of the hand and foot, in the parietal peritoneum, pancreas, mesentery, penis, clitoris, urethra, nipple, mammary gland, and in the connective tissue in the vicinity of tendons, ligaments and joints. Their form and to some extent their position indicate that they are stimulated by deep, or heavy, pressure. Their presence in viscera does not conflict with this view since they probably respond to internal pressure changes in this location.

In skeletal muscle, sensory nerves terminate in end bulbs and in complicated end organs called *muscle spindles* or *neuro-muscular bundles*. The muscle spindle (Fig. 149) is an elongated cylindrical structure within which are one or several muscle fibers, connective tissue, blood vessels, and myelinated nerve fibers. The whole is enclosed in a connective tissue capsule which is pierced at various points by one or more nerve fibers. The thinner nerve fibers are generally motor fibers and end on the muscle fibers in typical motor plates. The thicker myelinated sensory fibers lose their myelin as they branch repeatedly within the spindle. The axons then terminate around the enclosed muscle fibers in close apposition to the sarcolemma (epilemmal ending). Frequently the ending is in the form of a spiral; it may also form a series of rings or an arborization.

The muscle fibers of the spindle are thinner than ordinary fibers and are richer in sarcoplasm. They also contain more nuclei, particularly in the regions surrounded by nerve fibers.

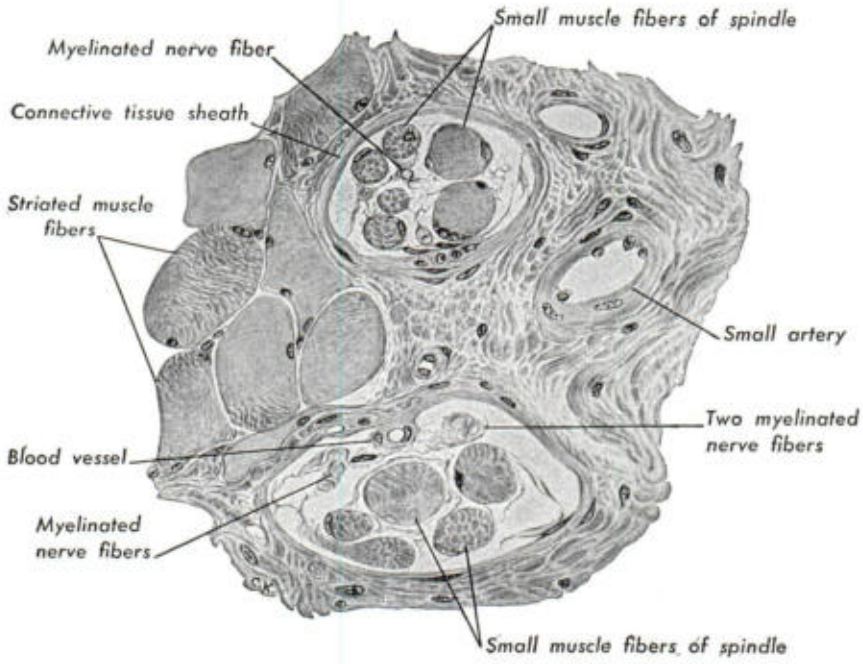

FIG. 149. Cross section of two muscle spindles in skeletal muscle of monkey.

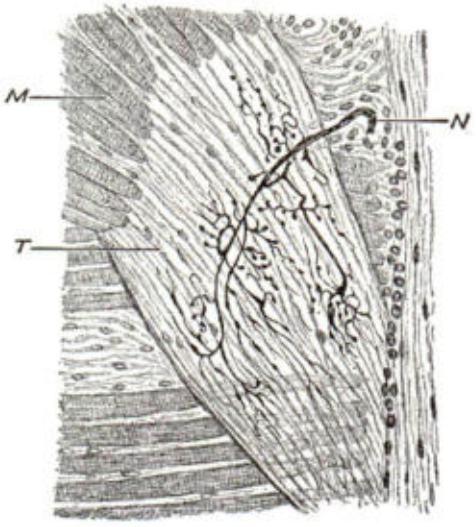

FIG. 150. Musculotendinous bundle from a six months human fetus. *M*, Muscle fibers; *N*, nerve fiber; *T*, tendon fibers. (Redrawn from Tello.)

At the junction of muscle and tendon are found the elaborate sensory structures known as the *muscle-tendon spindles* or *organs of Golgi* (Fig. 150). This is a spindle-shaped body composed of several tendon bundles covered by a thin capsule. Into this there enter one or several afferent nerve fibers which break up into complicated arborizations upon the tendon bundles.

The proprioceptive stimuli of position and movement due to the constant or varying tension of voluntary muscles and their attached tendons are undoubtedly received by the neuromuscular spindles and organs of Golgi.

NEUROGLIA, THE INTERSTITIAL TISSUE OF THE NERVOUS SYSTEM

Most organs of the body have a connective tissue framework which not only serves as a vascular bed but also constitutes a supporting skeleton from which finer connective tissue fibers form a structural interstitial groundwork for the particular cellular elements which comprise the organ. In the brain and spinal cord, connective tissue is limited to the enveloping membranes, the meninges, and a slight amount which accompanies the blood vessels. The supportive function is performed instead by a special group of cells and fibers which form the interstitial tissue of the nervous system. This is the *neuroglia* (Gr. *neuron*, nerve + *glia*, glue).

In ordinary microscopic preparations of the central nervous system, many nuclei are seen which belong neither to nerve cells nor vascular tissue. These nuclei, which do not possess nucleoli, belong to neuroglia cells. The neuroglia, frequently referred to simply as the *glia*, may be divided into the following classes: (1) *astroglia* or *astrocytes*, (2) *oligodendroglia*, (3) *microglia* or *mesoglia*, (4) *ependyma*. To these should probably be added for the peripheral nervous system, (5) the *neurilemma cells of Schwann* of the peripheral nerves and the *satellite cells* which surround the cell bodies of the spinal and cranial ganglia. These probably bear a greater resemblance to the oligodendroglia than to the other classes of neuroglia. All of the above except the microglia are of ectodermal origin.

It should be emphasized that special, and often difficult, selective staining methods must be employed to demonstrate the various structural features of the neuroglia. The student should bear in mind that most of the features which are described in the following paragraphs will not be visible in the ordinary preparations.

Astrocytes. As the name implies, the astrocytes are stellate shaped cells with many cytoplasmic processes. They may be divided into *protoplasmic astrocytes* and *fibrous astrocytes*.

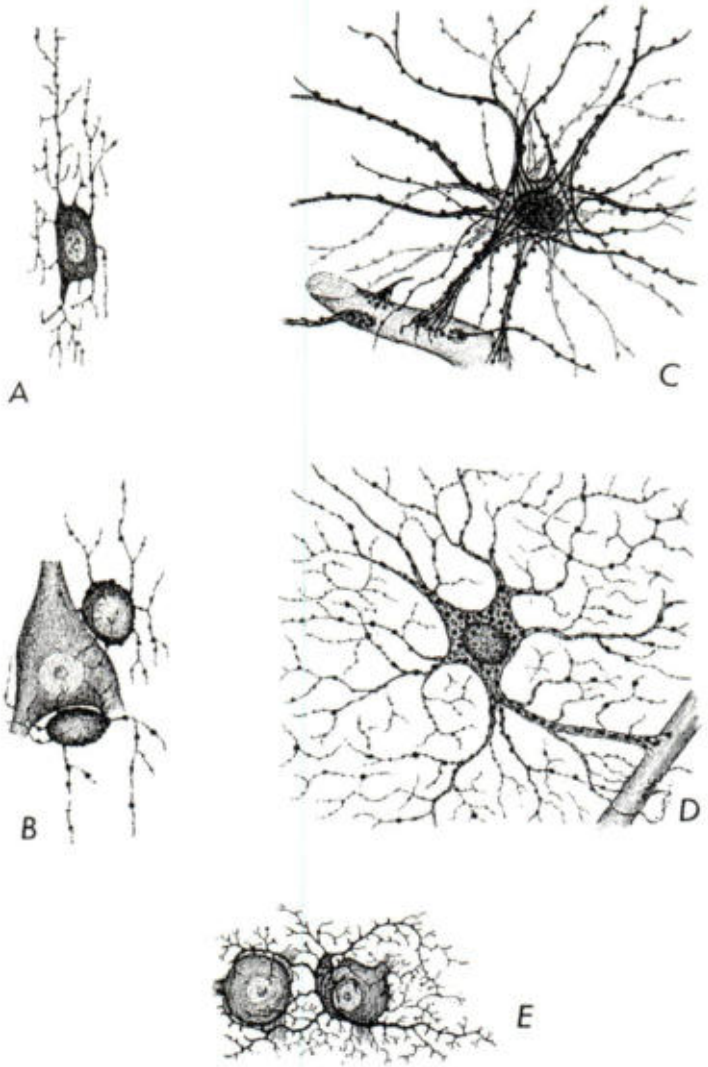

FIG. 151. *A*, Oligodendroglia cell in the white matter (interfascicular form); *B*, two oligodendroglia cells lying against a nerve cell (perineuronal satellites); *C*, fibrous type of astrocyte with one or two processes forming foot plates against a neighboring blood vessel. The foot plate process as well as others contains glia fibers. *D*, Protoplasmic (nonfibrous) astrocyte with foot plate. Gliosomes are shown in all the above as small enlargements on the processes and as dark granules in the cell body. *E*, Microglia cell. Being next to a nerve cell body it is also a perineuronal satellite. Its processes typically embrace another neuron also. The microglia cells have the same form in the white matter but are less numerous. Note the absence of gliosomes and vascular feet, the elongated nucleus, and the terminal spines on its processes. Stained by del Rio-Hortega's modified reduced silver stain. (Redrawn after Penfield.)

The protoplasmic astrocytes (Fig. 151, *D*) have numerous processes which extend out from the cell body in all directions and branch frequently. The nucleus is irregularly oval and has scant chromatin and no nucleolus. Small round or slightly elongated granules in the cytoplasm of both the cell body and processes are known as *gliosomes*.

The protoplasmic astrocytes are found principally in the gray matter of the brain and spinal cord. Often they partly envelop the neuron bodies and then constitute one of the varieties of *satellite cells*. One or more of the processes of an astrocyte have peculiar terminal expansions, the *foot plates* or *perivascular feet*, which are applied to the walls of capillaries or small blood vessels.

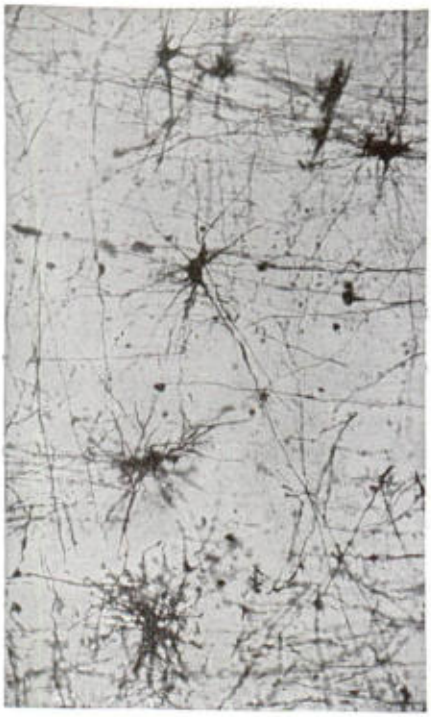

FIG. 152. Several astrocytes stained by Golgi's chrome-silver method. The cells are in the white matter of the cord and are of the fibrous type though the one at the bottom appears to be transitional to the protoplasmic form. A number of characteristic straight unbranched neuroglia fibers are seen in various parts of the field. Photograph.

The fibrous astrocytes differ from the protoplasmic type in having fewer processes but these are much straighter and longer (Fig. 151, *C*). They possess gliosomes and coursing through their cytoplasm are fine, straight, unbranched fibers—the *neuroglia fibers*. These can be demonstrated only by certain special stains, when it may be seen that they not only course from one process through the cell body and into another process but often appear to leave the cell body or process completely. For some time it was thought that the neuroglia fibers were separate elements, akin to the connective tissue fibers. The best technical methods, however, indicate that normally the neuroglia fiber always bears a thin sheath of cytoplasm.

The fibrous astrocytes are found chiefly in the white matter (Fig. 152). Like the protoplasmic ones, they bear perivascular feet.

Oligodendroglia (Gr. *oligos*, few + *dendron*, tree + *glia*, glue). These cells were given their name by del Rio-Hortega because of the fact that their branches were small and few as compared with the astrocytes (Fig. 151, *A* and *B*). They are smaller cells than the latter and their processes are more delicate. Their processes never form perivascular feet but the cell body may be applied to the surface of a capillary as a *perivascular satellite*.

The oligodendroglia are present in both gray and white matter. In the gray, they usually lie in close apposition to neuron bodies as *perineuronal satellites* (Fig. 151, *B*). In the white matter, they lie in rows between the myelinated nerve fibers with their processes forming an investment around the myelin. They

are here known as the *interfascicular glia*. There is some evidence that their relationship to the myelin sheaths in the central nervous system is similar to the relationship of the Schwann cells to the myelinated fibers of peripheral nerves.

Microglia. The microglia cells are small cells with small deeply staining nuclei, often of elongated or irregular shape and bearing some resemblance to fibroblast nuclei (Fig. 153). The cells have delicate tortuous processes which bear small spines but their cytoplasm contains neither fibers nor gliosomes (Fig. 151, *E*).

Microglia cells are found in both gray and white matter, but they are less numerous in the latter. In the gray matter they may be perineuronal satellites. Although they never have perivascular feet, the cells themselves are frequently found on blood vessels as perivascular satellites.

The microglia are of mesodermal origin and appear to enter the central nervous system not long before birth, perhaps arising from the pia mater.

In certain pathological conditions, the microglia cells show transitions to elongated cells known as *rod cells*. In trauma and other destructive lesions of the nervous system, the microglia cells may show great phagocytic activity, swelling and forming intraprotoplasmic granules chiefly of a fatty nature, thereby becoming *compound granular corpuscles*. These are phagocytic cells which have ameboid motion and should be included as a part of the reticulo-endothelial system (see Chapter V).

The various forms of nuclei seen in the astroglia, oligodendroglia, and microglia are shown in Figure 153.

Ependyma. The ependyma (Fig. 154) in ordinary preparations appears to consist of closely packed elongated nuclei, lying in the thick walled portions of the neural tube next to the cavities of the spinal cord and brain (central canal and ventricles). Their long axes are perpendicular to the cavity and they present the appearance of a columnar epithelium. The cells to which they belong are, in fact, columnar epithelial cells with, however, many of their inner processes ramifying more or less deeply in the wall of the neural tube. The ependyma cells contain neuroglia fibers and are to be regarded as neuroglia cells which have retained their embryonic shape and position. They may have, in certain forms and in certain places at least, cilia or flagella (Fig. 154) which protrude into the neural cavity.

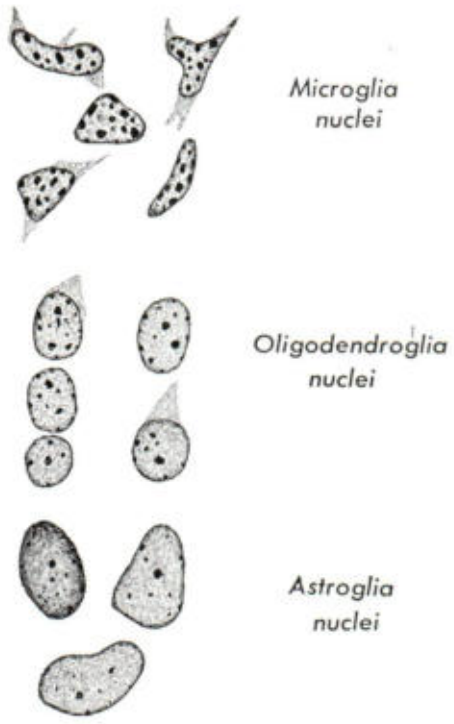

FIG. 153. Types of neuroglia nuclei. (Redrawn after Penfield.)

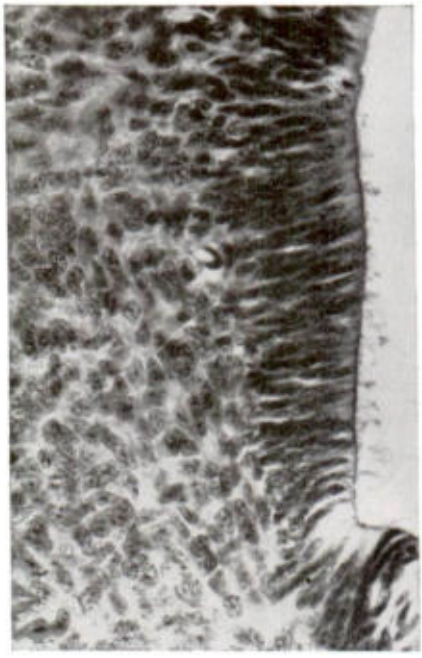

FIG. 154. Photomicrograph from a section of the spinal cord of a 25-mm. pig embryo, showing columnar-shaped ependymal cells with flagella at the right side of the figure and nuclei of spongioblasts and neuroblasts at the left. × 407.

HISTOGENESIS OF NERVE TISSUE

The first appearance of the nervous system is a thickening of the ectoderm, the *neural plate*. While the plate is closing to form the *neural tube*, its epithelium becomes thicker, changing from a cuboidal or low columnar to a high columnar type, the cell outlines at the same time become more indistinct and the nuclei assume positions in various depths of the epithelium, except an outer *marginal layer* which contains no nuclei. Near the central canal mitotic figures are present, the rounded dividing cells being called *germinal cells*. Later, another layer differentiates from the outer part of the columnar or *nuclear layer*, known as the *mantle* layer.

As shown by the chrome-silver and reduced silver methods, two lines of cells differentiate, *neural* and *glial*. The cells are neuroblasts and spongioblasts. In the rounded germinal cells, which at first have little cytoplasm, there appears in each, in the side away from the central canal, an increase of cytoplasm in which neurofibrillae and other cell constituents can be demonstrated. This is the apolar stage of the neuroblast. The cell body migrates from the central canal, to which it often remains attached by a process, and at the same time sends out a process toward the periphery, the axon, which ultimately either grows out of the neural tube as an efferent root fiber or up or down in the marginal layer as a longitudinal nerve fiber. As long as it retains its attachment to the central canal, the neuroblast is in its *bipolar* stage. It soon, however, loses this attachment and thereby becomes *unipolar*. Very soon dendrites appear and the neuroblast attains its *multipolar* form. It is the change, by migration and differentiation, into these final stages which produces the appearance of the mantle layer.

At the same time, other cells, which apparently in some cases never have lost their columnar character and are known as *spongioblasts*, undergo parallel changes. Originally columnar, with nuclei near the central canal, or bipolar by migration of their nuclei, they lose their connection with the central canal, becoming *unipolar spongioblasts*. Other processes appear and the cells become *astroblasts* which may acquire connections with blood vessels by means of "end feet." Further branching and loss of polarity leads to the formation of the definitive *protoplasmic astrocytes* which, by the formation of glia fibers, may become *fibrous astrocytes*. Other cells of a more undifferentiated type (*medulloblasts*) may, by proliferation and differentiation, form spongioblasts, oligodendroblasts or neuroblasts. Cells in the thick walled portions of the neural tube which remain attached to the central canal become *ependymal spongioblasts* and finally *ependyma cells*; those remaining in the thin walled portions (telae) form the inner lining of the choroid plexuses and telae and become the *choroidal epithelial cells*.

These changes are shown schematically in Figure 155 but are probably not as simple as they are shown, for it is not certain how many generations of undifferentiated cells there may be or how far the earlier stages sketched may retain their capacity for differentiating in more than one direction.

As already stated, it is believed that the microglia migrate inward from the pia and are therefore of mesodermal origin. The histogenesis of the neuroglia forms the basis of the classification of the gliomata and may throw

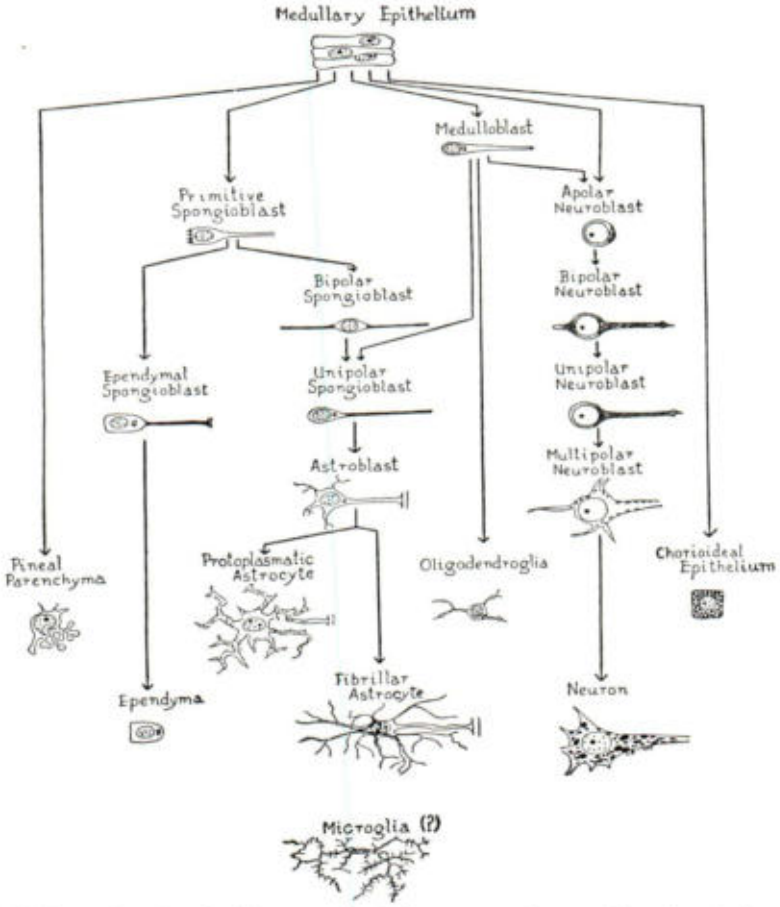

FIG. 155. Schema showing the histogenesis of the nerve and neuroglia cells. (Bailey and Cushing.)

light upon their nature and causes of formation.

In the cells which become detached from the dorsal part of the neural tube (*ganglionic crest*) a similar differentiation takes place. Some cells become larger (neural *ganglioblasts*) and send one process into the neural tube (afferent root fibers) and another to the periphery, thereby becoming *bipolar ganglion cells* which later may become unipolar by a recession of the body from the two processes. Other cells either form satellite cells around the bodies of ganglion cells or sheath cells around their processes, ultimately becoming the *neurilemma cells*. They play an active rôle in the formation of the myelin sheath as probably do the oligodendroglia cells in the neural tube. Cells which migrate still further from the neural tube differentiate into *sympathoblasts* and finally into the *sympathetic (autonomic) ganglion cells* on the one hand, into sheath cells on the other. Still others of these cells differentiate into the *chromaffine cells* of the medulla of the adrenal gland and certain other related structures.

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XI

THE SPINAL CORD, CEREBELLAR CORTEX AND CEREBRAL CORTEX

MEMBRANES OF THE BRAIN AND CORD

The brain and spinal cord are enclosed by two connective tissue membranes, the *dura mater* and the *pia-arachnoid*, the latter usually being described as two membranes, the *pia mater* and *arachnoid* (Fig. 156). These membranes are collectively known as the *meninges*, the dura mater being the *pachymeninx* and the pia-arachnoid the *leptomeninx*, or *leptomeninges*.

The *dura mater* is the outer of the two membranes and consists of dense fibrous tissue. The *cerebral dura* serves both as an investing membrane for the brain and as periosteum for the inner surfaces of the cranial bones. It consists of two layers. The inner layer is composed of dense connective tissue which is lined on its inner surface by a single layer of flat cells. The outer layer, which forms the periosteum and is similar in structure to the inner layer, is much richer in blood vessels and nerves. The *spinal dura* corresponds to the inner layer of the cerebral dura, the vertebrae having their own separate periosteum. It contains more elastic tissue than the inner layer of the cerebral dura but otherwise resembles it in structure. The outer surface of the spinal dura is covered with a single layer of flat cells, and is separated from the periosteum by the *epidural space*, which contains anastomosing thin-walled veins lying in an areolar tissue rich in fat. The inner surface of the spinal dura is also lined by a single layer of flat cells. Beneath the spinal dura, between it and the arachnoid, is the *subdural space*, a narrow cleft containing fluid. It has no direct communication with the subarachnoid space.

The *pia mater* closely invests the brain and cord, extending into the sulci, and protrudes into the ventricles at the thin walled portions of the brain, where it is lined on its inner surface by a layer of cells of neural origin, the choroid cells. The pia consists of white fibrous tissue and contains the blood vessels which send branches into the nerve tissue.

The *arachnoid* (outer portion of the pia-arachnoid) passes over the sulci without dipping into them and extends out a short distance along the roots of the cerebrospinal nerves, and along the optic nerve to the eye. The arachnoid is partly separated from the pia by numerous spaces across which trabeculae pass connecting pia and arachnoid. These are the *subarachnoid spaces* or *space* and can be looked upon as dilations of pial spaces separated from the subdural space by a membrane, the arachnoidal part of the pia-arachnoid. In

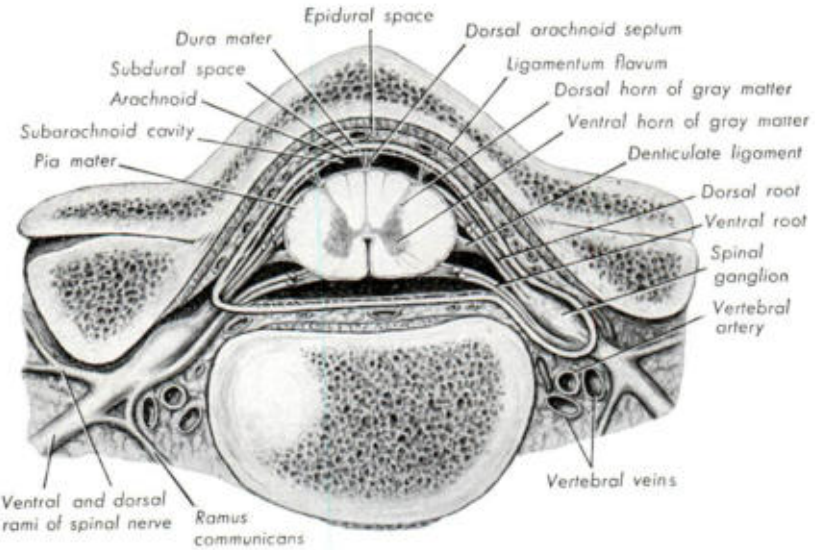

FIG. 156. Transverse section through the fourth cervical vertebra, showing the coverings of the cord. (Rauber-Kopsch, Anatomie.)

other words they are intraleptomeningeal spaces. The subarachnoid spaces dip into the sulci. In the cerebral pia-arachnoid the subarachnoid spaces are traversed by numerous connective tissue trabeculae and there is no clear distinction between pia and arachnoid (Fig. 157). In the spinal pia-arachnoid there are few trabeculae and the subarachnoid space is thus a more continuous cavity and the arachnoid a more distinct membrane. The trabeculae and arachnoid contain delicate strands of connective tissue covered with a single layer of flat or low cuboidal cells which also extends over the outer pial surface. This layer of cells (mesenchymal epithelium) lines the subarachnoid spaces. The cells ordinarily have large pale oval nuclei.

In certain places the arachnoid sends prolongations into the dura which protrude into a venous sinus or venous lacuna. The prolongations contain spaces, traversed by trabeculae, which may be regarded as continuations of the subarachnoid space. These arachnoidal outgrowths are covered with the usual layer of low cells and are known as *arachnoid villi* (Fig. 157). They are most numerous along the longitudinal fissure of the cerebral hemispheres where they are in relation with the superior longitudinal sinus and are also found along the transverse, cavernous and superior petrosal sinuses and elsewhere.

The subarachnoid space contains cerebrospinal fluid. There is direct communication in the roof of the fourth ventricle between the subarachnoid space and the brain cavity (ventricle) by means of an aperture in the caudal part of the thin roof of the fourth ventricle (the foramen of Magendie or metapore) and an aperture in each of the thin-walled lateral recesses of the fourth ventricle (the foramina of Luschka). The subarachnoid space communicates with the tissue spaces within the nerve sheaths, which in turn communicate with lymphatics.

It also communicates with the tissue spaces around the adventitia of the blood vessels which penetrate the central nerv-

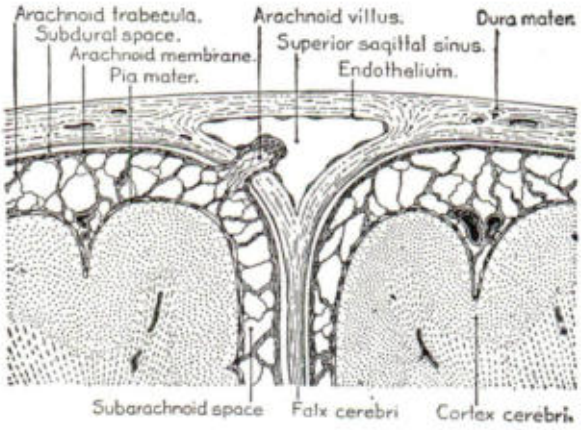

FIG. 157. Schematic diagram of coronal section of meninges and cerebral cortex, to show relation of arachnoid villus to dural venous sinus. The potential subdural space is necessarily shown of greater size than is normal; the subarachnoid space is also increased in width to illustrate the character of the subarachnoid mesh. The nuclei of the cells lining the subarachnoid space are faintly shown. (Weed.)

ous system from the pia mater. These *perivascular spaces* are lined by a thin membrane of mesenchymal epithelium derived from the pia mater (Fig. 158). This pial prolongation comes to an end before the capillaries are reached. It was once thought that the perivascular spaces continued along the capillaries and became continuous with perineuronal spaces around the bodies of the nerve cells. Later studies indicate that these spaces are probably artifacts.

Blood Vessels. The spinal dura and the inner layer of the cerebral dura are poor in blood vessels. The outer layer of the cerebral dura, forming as it does the periosteum of the cranial bones, is rich in blood vessels which pass into and supply the bones. The pia is very vascular, especially its inner layers, from which vessels pass into the brain and cord. The arachnoid is nonvascular.

As stated above, the capillaries of the brain and cord are apparently devoid of perivascular spaces comparable to those found in other tissues. This may be one of the factors responsible for the *blood-brain barrier*, (*hemato-encephalic barrier*), i.e., a barrier which normally prevents injected dyes, such as trypan blue, from passing from capillaries into the central nervous system. Differences in the permeability of the capillaries and their enclosure by processes (perivascular feet) from glial cells have been listed as other factors for the selective barrier between blood capillaries and the neurons of the central nervous system.

Cerebrospinal Fluid. The cerebrospinal fluid is a clear colorless fluid which occupies the ventricles of the brain, the central canal of the spinal cord, the subarachnoid and perivascular spaces and their continuations. It is very slightly viscous and of low specific gravity (1.004 to 1.006). It contains small quantities of inorganic salts, chiefly sodium chloride and potassium chloride, as well as small amounts of dextrose and traces of proteins. It normally contains very few cells. Its quantity in the nervous system of an adult man is probably between 100 and 150 c.c. The bulk of the cerebrospinal fluid is probably formed

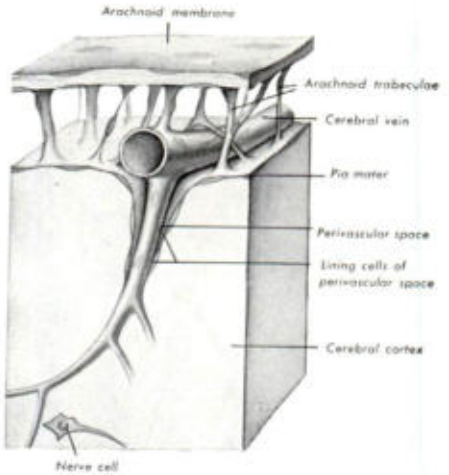

FIG. 158. Schematic diagram of leptomeninges and nervous tissue, showing the relationship of the subarachnoid space, blood vessels, and nerve cells. Numerous nerve cells and glia cells would be present in the area shown; only one neuron is shown for purposes of clarity. (Modified from Weed.)

by the activity of the epithelial cells lining the choroid plexus and telae, from which it passes into the ventricles and thence by the foramina of Magendie and Luschka into the subarachnoid spaces. The drainage of the fluid appears to be chiefly by filtration through the walls of the arachnoid villi into the cerebral venous sinuses.

TELAE CHOROIDEAE AND CHOROID PLEXUSES

Certain parts of the wall of the brain are composed solely of a thin non-nervous epithelial membrane, a lamina epithelialis, which is covered externally by a highly vascularized pia mater. These two layers together constitute the telae choroideae which form the roof of the fourth ventricle, the roof of the third ventricle, and parts of the walls of the lateral ventricles. Projecting into the ventricles from the telae choroideae there are complex folds and invaginations containing tortuous networks of small vessels and capillaries, the choroid plexuses, derived from the vessels of the pia mater, and covered completely by an epithelial choroid lamina, which is a continuation of the epithelial lamina of the tela choroidea. (The term "choroid plexus" is often used in referring to the entire mass of infolded membranes, rather than to the network of blood vessels alone.)

The modified ependymal epithelium covering the choroid plexuses is a simple cuboidal to low columnar type with a rather granular cytoplasm. In electron micrographs, the free surface of the cells appears to be thrown into fine cytoplasmic projections, resembling a striated or brush border. These cells are thought to play an important role in the production of the cerebrospinal fluid.

The fine connective tissue surrounding the vessels within the folds of the choroid plexus contains phagocytic cells (histiocytes) which readily ingest foreign material such as trypan blue. This is not true of the connective tissue of the pia in general.

THE SPINAL CORD

In transverse section (Fig. 159), the spinal cord appears oval in shape and slightly more flattened on its ventral than on its dorsal surface. It is surrounded by the pia mater spinalis, which extends into the deep longitudinal *ventral median fissure*. Dorsally, the cord is partitioned longitudinally by the *dorsal median septum*, which is composed principally of neuroglia and over which the pia mater passes without entering. At the entrance of the dorsal root fibers on either side, there is a *dorsolateral groove* or *sulcus*.

The *gray matter* occupies the central part of the section, where it is arranged somewhat in the form of the letter H. Dorsally the gray matter extends almost

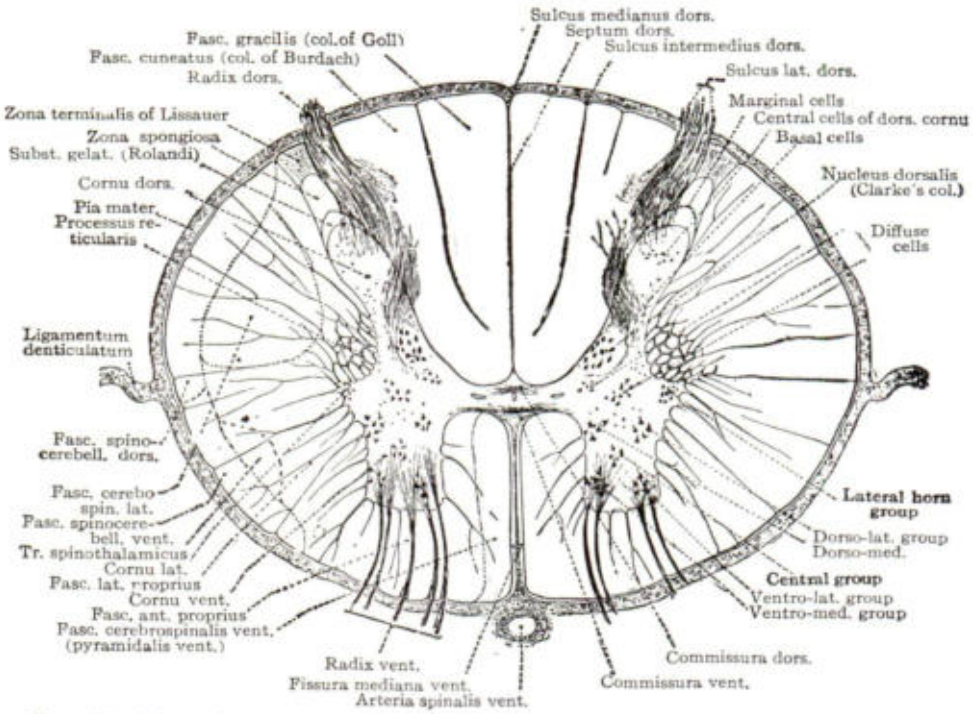

FIG. 159. Schematic transverse section of the spinal cord with pia mater and ligamentum denticulatum. On the left the positions of some of the fiber tracts of the cord are indicated. (Rauber-Kopsch, slightly modified.)

to the surface of the cord as the *dorsal gray columns* (*posterior horns* or *cornua*). The *ventral gray columns* (*anterior horns*) are shorter and broader and do not so nearly approach the surface of the cord. Surrounding the gray matter is the *white matter*, which in each lateral half of the cord is divided by the dorsal column into two parts. The part lying between the horn and the dorsal median septum is the *dorsal funiculus* (*dorsal* or *posterior white column*); the other part, comprising the remainder of the white matter, is the *ventrolateral funiculus* (*ventrolateral* or *anterolateral white column*). Between the two and lying between the dorsal gray column and the periphery of the cord is the fine-fibered *zone of Lissauer*, which should be regarded as the lateral part of the dorsal white column. The ventrolateral white column is again divided, rather indefinitely, by the ventral horn and nerve roots into a *lateral funiculus* (*lateral white column*) and a *ventral funiculus* (*ventral* or *anterior white column*). In the concavity between the ventral and dorsal horns some processes of the gray matter extend out into the white matter where they interlace with the longitudinally directed fibers of the latter to form the *reticular process*, which is not well marked in the lumbar and thoracic cord. In the thoracic segments of the cord there is usually a lateral protrusion of the gray matter slightly dorsal to the dorsal boundary of the ventral horn and not to be confused with the lateral protrusions of the latter in the cervical and lumbar enlargements. This is called the *lateral horn* (Fig. 159).

Gray Matter. In the cross portion of the H is seen the *central canal*, usually obliterated in the adult and represented only by a group of epithelial cells (ependymal). The central canal divides the gray matter connecting the two sides of the cord into a *ventral gray commissure* and a *dorsal gray commissure*. Immediately surrounding the epithelial cells is a light granular area composed mainly of neuroglia and known as the *central gelatinous substance*. Toward the surface of the cord the dorsal horn expands into a *head* or *caput*, external to which is an area similar in general appearance, but not in structure to that surrounding the central canal. This is the *gelatinous substance of Rolando*. The head is connected with the base of the dorsal horn by a narrower *neck* or *cervix*. External to the gelatinous substance of Rolando is a thin zone containing a plexus of fine medullated fibers known as the *zona spongiosa*, and external to this, occupying the space between it and the periphery, is a zone composed of fine longitudinal medullated fibers rather sparsely arranged. This is the zona terminalis or zone of Lissauer mentioned above, which belongs obviously to the white matter of the cord. The portion of the gray matter connecting the dorsal and ventral horns may be termed the *intermediate* or *middle* gray. There are well-defined groups of large nerve cells in the ventral horns, and coarse nerve fibers passing out from them to the surface of the cord are the *ventral (anterior) nerve root* fibers.

White Matter. The white matter of the cord is composed of myelinated and amyelinated nerve fibers, together with neuroglia, blood vessels and inward continuations of the pia mater. Among the nerve fibers, which constitute the bulk of the white matter and which are arranged mainly in a longitudinal direction, there are strands of tissue of different appearance which run inward from the periphery. They often contain blood vessels and are composed of continuations of the pia mater and of neuroglia. Just beneath the pia mater, the neuroglia forms a fairly thick layer from which trabeculae pass in among the fibers, the broadest strand forming the dorsal median septum. If the section has been cut through a *dorsal (posterior) nerve root*, a strong bundle of *dorsal root fibers* can be seen entering the white matter of the cord along the dorsal and mesial side of the posterior horn. Ventral to the anterior gray commissure is a bundle of transversely disposed medullated fibers—the *ventral white commissure*. In the dorsal part of the dorsal gray commissure there are also fine similarly disposed medullated nerve fibers —the *dorsal white commissure*.

General Structure of Gray and White Matter (Substantia Grisea and Substantia Alba). The gray matter and white matter are composed of nervous elements and neuroglia.

The Nervous Elements. The nerve cells of the spinal cord are multipolar neurons. The axons of some of these neurons, the *root cells*, emerge from the cord as ventral root fibers. Other neurons send their axons into the white matter of the cord where, by bending or bifurcating, they form the longitudinal fibers of the white columns. These are the *column cells*. Still other neurons, *Golgi's second type*, have their axons terminating in the gray matter near their origin.

In the nervous system as a whole, those neurons entirely confined to the central nervous system may be termed the *central* or *intermediate neurons*, in contrast to those which form parts of the peripheral nervous system (*peripheral neurons*). They coördinate or adjust in various ways the impulses carried out to the effectors by the efferent peripheral

neurons with the impulses brought in from the receptors by the afferent peripheral neurons. The column cells and second type cells of the cord are thus central neurons. Together with terminations of afferent root fibers, terminations of their axons in the gray matter of the cord constitute the central apparatus of the spinal cord reflexes. Ascending fibers from afferent roots and from column cells constitute conduction pathways to higher coördinating or integrating centers in the brain. In the gray matter are all the nerve cell bodies and dendrites together with portions of medullated and nonmedullated nerve fibers.

The white matter, as a rule, contains no neuron bodies nor dendrites, the nervous elements being represented by portions of medullated and nonmedullated fibers and their branches. There is a thin external layer containing no nerve fibers and known as the marginal glia. The fibers in the cord are mainly longitudinally disposed but there are also many transverse fibers running to and from the gray matter.

The Neuroglia. This has been seen (Chapter X) to consist of branching cells with their nuclei and cytoplasm and fine, unbranched neuroglia fibers. The various kinds of neuroglia cells (astrocytes, oligodendroglia and microglia and the ependyma cells) have already been described.

THE CEREBELLAR CORTEX

The cerebellum, connected with the rest of the brain by its three peduncles, consists of two lateral lobes or hemispheres connected by a median lobe, the vermis. These are divided by transverse fissures into lobules, each lobule consisting of a median portion, belonging to the vermis, and two wing-like extensions belonging to the hemispheres. The surfaces of the lobules are marked by folds (*laminae* or *folia*) running approximately parallel to the fissures and thus transversely to the longitudinal axis of the brain. When these folds are cut across it is seen that they give off secondary or tertiary laminae, the whole producing the appearance known as the arbor vitae.

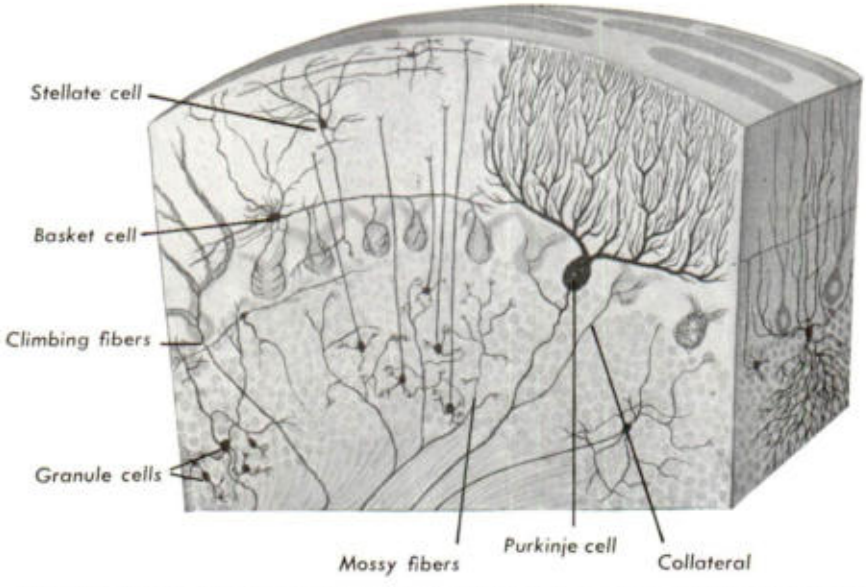

FIG. 160. Schematic diagram of structure of cerebellar cortex. (After Bargmann.)

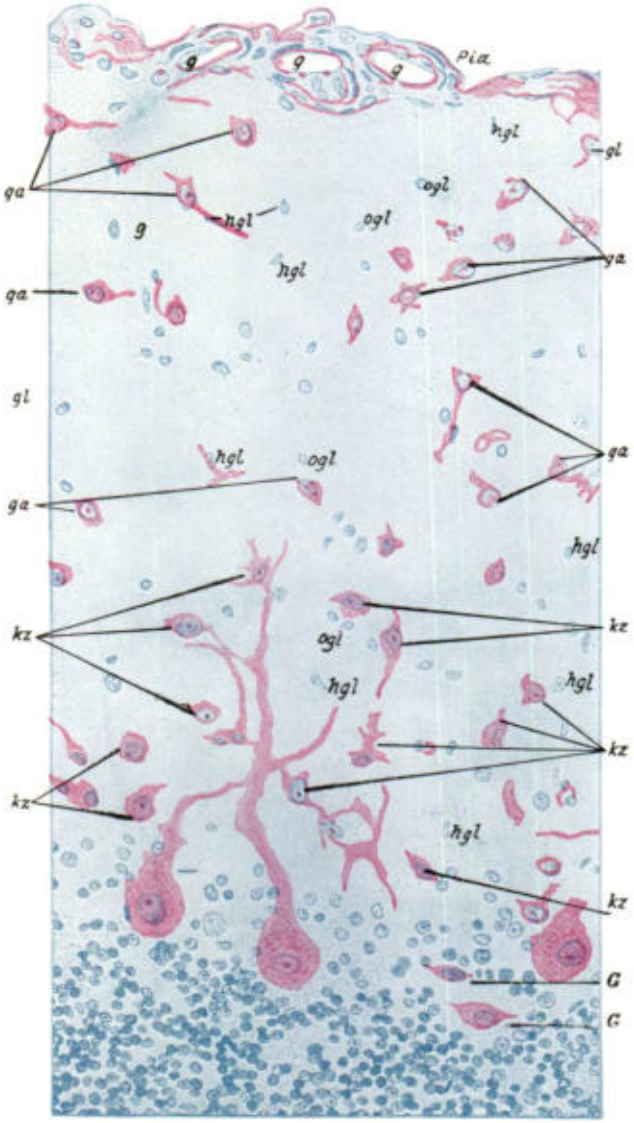

FIG. 161. Vertical section of the human cerebellar cortex. Toluidine blue preparation. *G*, Golgi cell, second type; *g*, blood vessel; *ga*, large stellate cell *kz*, basket cells; *gl*, macroglia; *hgl*, Hortega glia (microglia); *ogl*, oligodendroglia. (Jacob, from Möllendorff, Handbuch der mikroskopishen Anatomie des Menschen.)

The surface of the cerebellum is composed of gray matter, the cortex, which envelops the white matter.

In the *cerebellar cortex* there can be distinguished with ordinary stains (hematoxylin-eosin, Nissl) an outer or *molecular* or *plexiform layer* with few cells and few medullated fibers, an inner, *granular* or *nuclear layer*, and between the two a single row of large flask-shaped cells, the *cells of Purkinje* (Figs. 160 and 161). These latter give off several main dendrites, which enter the molecular layer and form a remarkably rich arborization extending to the surface. The dendritic arborization is fan-shaped, extending at right angles to the laminae. The axon is given off from the end of the cell opposite to the dendrites and passes through the granular layer into the white matter, either to one of the internal cerebellar nuclei where it terminates, or to some other part of the cortex. The Purkinje cells are almost the only cells the axons of which enter the white matter. It is evident that all intracortical connections must ultimately converge on these cells to reach the efferent cerebellar paths. The axons of the Purkinje cells give off collaterals not far from their origin, which pass into the molecular layer and appear to terminate there in end "buttons" upon the bodies of adjacent Purkinje cells. The medullated axons form the coarser fibers traversing the granular layer. The cell body is fairly well filled with small chromophilic bodies of uniform size, often showing a slightly concentric arrangement (Fig. 161).

The cells in the molecular layer (stellate cells) are either *superficial stellate cells* with irregular branching dendrites and a short axon, or *deep stellate (basket) cells*. Axons of the latter extend at right angles to the laminae for a distance of several Purkinje cells, giving off to each Purkinje cell one or more collaterals which pass toward the granular layer and envelop, with their terminal arborizations, the body and proximal, nonmedullated portion of the axon of the Purkinje cell. Collaterals of other basket cell axons may terminate around the same Purkinje cell, forming the "basket."

The granular layer with ordinary stains presents the appearance of closely packed nuclei with clear spaces here and there ("*islands*" or "*glomeruli*") and also a few larger cells (Fig. 161). Most of these nuclei belong to the *granule cells*, which are caryochrome cells. The granule cells are small and possess three to six dendrites which are comparatively short and terminate in the glomeruli with a compact arborization, each branch of which ends in a small varicosity (Fig. 160). The axons of the granule cells, which are nonmedullated, ascend into the molecular layer where each divides into two branches running longitudinally along the laminae and terminating in varicosities (Fig. 160). These are the *parallel fibers* of the molecular layer. They thus run at right angles to and through the dendritic expansions of the Purkinje cells and their cross sections together with the terminal dendritic arborizations of the Purkinje cells give the molecular layer its punctate appearance.

In the cortex there are also the terminations of the afferent cerebellar fibers already mentioned. These are of two types, *mossy fibers* and *climbing fibers*. The mossy fibers, so called from the appearance of their terminations in embryos, are the coarsest fibers of the white matter. While in the latter they bifurcate, branches going to different laminae. These main branches give off secondary branches which enter the granular layer and there arborize.

The climbing fibers pass from the white matter through the granular layer

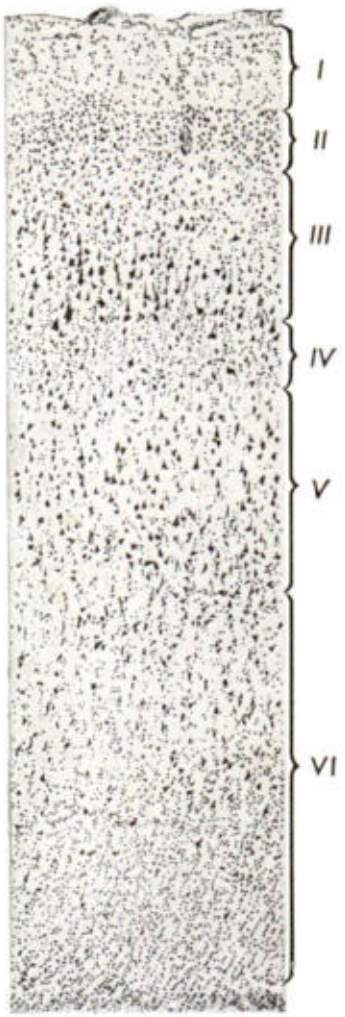

FIG. 162. Photograph of cell layers of Nissl-stained preparations of cerebral cortex. Note that there are six layers of pyramidal cells. (After Bargmann.)

to the cells of Purkinje (Fig. 130). Passing by the bodies of the latter they arborize into terminals which envelop the smooth dendritic branches of the Purkinje cells; i.e., all but the terminal dendritic arborizations (Fig. 160). Three kinds of fibers thus terminate around the Purkinje cells; the granule axons, probably in contact with its terminal dendritic arborizations; the climbing fibers around its coarser dendritic branches; and the basket fibers around its body. The respective sources of the mossy and climbing fibers are still not fully known.

It is evident from the above that all of the cells of the cerebellar cortex except the Purkinje cells are *association cells* of the cortex.

The medullated fibers of the cerebellum pass from the white matter into the granular layer and ramify throughout the latter, forming quite a dense plexus separating groups of granule cells.

The cerebellum appears to be, so to speak, a superadded mechanism for the ultimate regulation of all kinds of movements involving the striated voluntary musculature. Injury results in an incoördination, both in maintaining posture and, perhaps more strikingly, in the performance of movement. This is known as cerebellar ataxia, the most striking and perhaps most characteristic peculiarity of which is an inability properly to check movement (dysmetria), a peculiarity due to a lack of proper coördination or synergy between protagonist and antagonist muscles (dyssynergia).

There is no disturbance of sensation in cerebellar lesions; the sensory path to the cerebral cortex passes by, not through, the cerebellum.

THE CEREBRAL CORTEX

The cerebral cortex is the external layer of gray matter covering the convolutions and fissures of the cerebral hemispheres. It has an area of about 200,000 sq. mm. and varies in thickness from about 1.55 to 3.5 mm. It contains, besides nerve fibers, neuroglia and blood vessels, the bodies of nearly 14,000,000,000 neurons. The older olfactory cortex is termed the *allocortex*, the rest the *isocortex*.

The following types of nerve cells are found in the cerebral cortex: (1)

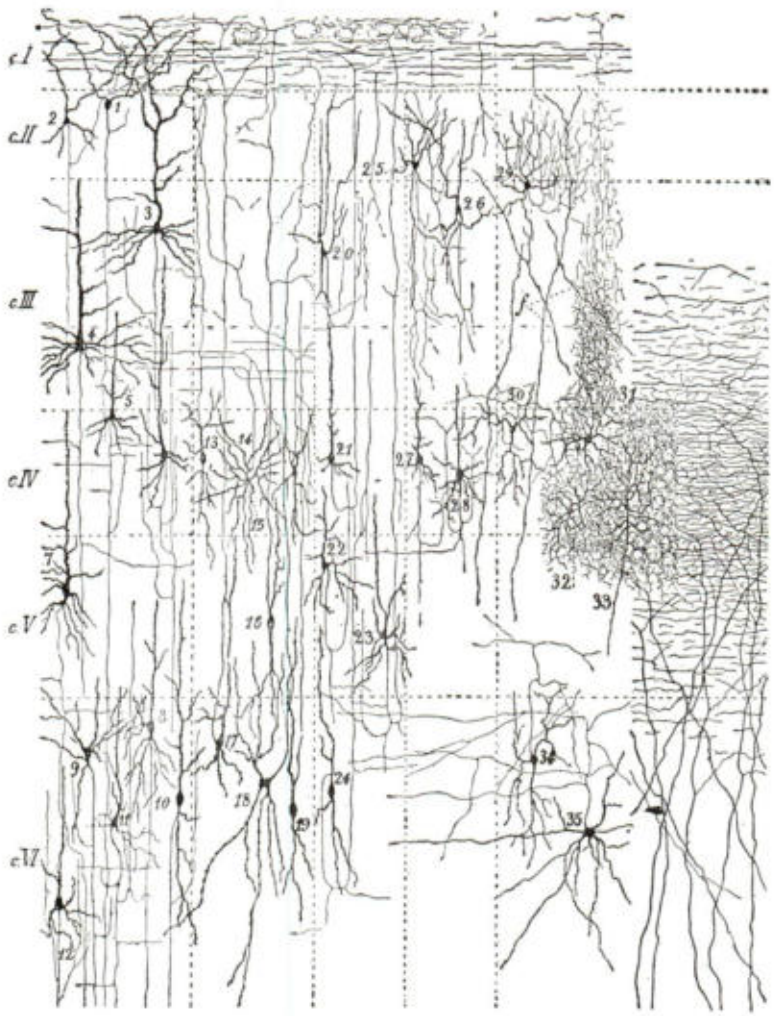

FIG. 163. Various forms of nerve cells of the cerebral cortex. From various figures of Cajal, especially of the temporal cortex. (Bonne.) *cI*, Zonal layer of cortex; *cII*, external granular layer; *cIII*, pyramidal layer; *cIV*, internal granular layer; *cV*, ganglionic layer; *cVI*, multiform layer. *1* to *12*, cells, mostly pyramidal, whose axons enter the white matter (corticifugal cells); *13* to *35*, cells whose axons do not leave the gray matter (or cortex); *13* to *19*, cells with directly ascending axons; *20* to *24*, cells with arciform ascending axons; *25* to *28*, cells with short descending axons, with or without ascending collaterals; *29*, *30*, short axon cells properly speaking (Golgi's type II); *31* to *33*, cells with bushy axons (bipenicillate and neuroglia form), some forming terminal nests around the small granule cells; *34*, *35*, large stellate cells of layer *VI* with extensively branching axons, especially extending horizontally; *f*, fibers of unknown origin. On the right are shown corticipetal fibers and their terminal plexus, especially dense in layer *IV*.

Pyramidal cells (Figs. 110 and 162). This is the prevailing type and is characterized by having a long apical dendrite usually directed toward the surface of the brain. This dendrite gives off branches and usually reaches the outer cortical layer, there to break up into a number of branches. From the cell body are also given off a number of basal dendrites. By the Golgi method, gemmules can be demonstrated on the dendrites. The axon proceeds from the base of the cell (opposite to the apical dendrite) and usually passes into the white matter. It gives off several collaterals on its way to the white matter. (2) *Stellate cells.* These have dendrites passing in various directions but no apical dendrite. Many, especially the smaller ones (*granule cells*), may have short axons (Golgi's second type). (3) *Fungiform* or *spindle cells* which are usually found in the deepest layers of the cortex and send their axons into the white matter. (4) *Horizontal cells* (of Cajal), found in the outer layer, with long horizontal dendrites and axons confined to the outer layer. (5) *Inverted cells of Martinotti*, triangular or polygonal in shape, with axons directed toward the surface. There are many variations in form and size of each of these principal types.

The largest cells of the cortex (giant cells of Betz) are very rich in chromophil substance arranged similarly to that in the somatic motor root cells of cord and brain. The medium and small cells have fewer chromophil bodies which are often in the shape of irregular masses, either near the periphery of the cell or around the nucleus. The neurofibrils vary in their arrangement according to the shape of the cell.

The neuroglia cells and fibers are in general similar to those in other parts of the nervous system.

The nerve cells of the isocortex are arranged in layers which have the same general character throughout, but in various regions exhibit variations such as suppression, diminution, enlargement, or subdivision of certain layers (Fig. 163). The cell layers of the cortex are: (1) *Zonal layer* (molecular layer, plexiform layer of Cajal). This contains the horizontal cells and other cells with short axons and also receives the axons of the Martinotti cells. Besides this, it contains the terminal branches of the apical dendrites of the pyramidal cells. (2) *External granular layer*, very often termed the layer of small pyramids. The dendrites of the cells of this layer mostly enter the first layer, their axons pass downward into the white matter. (3) *Pyramidal layer*, often called the layer of superficial medium and large pyramids. This is composed principally of typical pyramids sending dendritic branches into the first layer and axons into the white matter. The larger cells are in the deeper part (sublayer of large pyramids). This layer also contains many granule cells with short axons and cells of Martinotti. (4) *Internal granular layer.* Here the predominating elements are stellate cells, the larger usually sending their axons into the white matter. Among these are many short axon granules, the axons of which end in the same layer or in more superficial layers. (5) *Ganglionic layer* or deep layer of large and medium sized pyramids. These send their axons into the white matter. Mingled with them are short axon and Martinotti cells. (6) *Multiform layer* or layer of spindle shaped cells which usually send their axons into the white matter. This layer may be subdivided into an outer zone of more densely packed larger cells and an inner zone of smaller cells which are more loosely arranged. Mingled with them are short axon and Martinotti cells. Many cells

of the cortex send their axons upward instead of into the white matter.

By virtue of its capacity for associating sensory impulses of various kinds, the cortex enables a response originally aroused by one kind of stimulus to be subsequently elicited by another kind of stimulus previously cortically associated with the original stimulus, e.g., food placed in the mouth causes reflexly a salivary secretory reaction and is also felt (e.g., tasted) by the cortex. If this cortical stimulus is at the time associated with the sight of food the latter alone may subsequently cause the salivary secretion. Such reactions are known as conditioned or associated reflexes (Pavlov). Similar reactions in the vegetative sphere, aroused however by associated sensory impulses of a non-vegetative character, constitute the emotional type of reaction. These reactions are not skilled. In skilled reactions involving the voluntary musculature there are also present guiding or correcting proprioceptive impulses arising from the muscles and tendons involved which constitute perhaps the principal factor in the acquisition of skill. It is also obvious that any defect causing a lack of proprioceptive sensation will also cause an uncertainty or incoördination of voluntary performance which differs from cerebellar ataxia in the fact that here it is due to a sensory defect. By its extensive cerebellar connections, the pallium secures also, so to speak, the coöperation of the cerebellar regulative mechanism.

In the acquisition of speech, however, sound appears to play the principal rôle rather than proprioceptive stimuli. In disturbances of speech due to defects in its cortical mechanism (aphasia) there is usually, in a broad way, a distinction between those due to defects on the afferent or receptive side (sensory aphasia, lack of understanding of spoken speech) and those due to defects on the efferent or emissive side where spoken speech is understood but cannot be spoken ("motor" aphasia).

Looked at from a comparative point of view, the leading factors in the development of the pallium are two: reception of different kinds of stimuli other than its original olfactory ones and the great increase in its association neurons. A further change, seen in higher mammals and man, is the increase of the frontal lobes. The development of the pallium (neopallium) thus has two aspects: (*a*) The bringing together in association of many kinds of stimuli from all parts of the body. The pallial associative mechanism not only associates many stimuli previous to the performance of the act but also dissociates, i.e., discriminates, and thereby enables the act, by inhibition, to be more narrowly limited to the movements necessary for its performance. (*b*) The plasticity of the pallial associative mechanism whereby it is changed in some way by its activities. Whatever its nature, the acquired changes of the pallium, due to its plasticity and consequent capacity of "learning," affect the action of subsequent stimuli reaching the pallial cortex and furnish the basis of "memory," of personal experience and of individually acquired neural mechanisms, as opposed to germinal, inherited, and slowly evolved racial neural mechanisms. The pallial neural mechanisms increase efficiency by giving the act greater precision and thus avoiding waste of energy, by utilizing past individual experience and thereby increasing the efficiency and adaptability of the neural reaction to present and future situations. Finally, the transmission, by means of complex symbols, of this acquired and ever varying experience to the plastic pallia of other individuals, it may be of subsequent generations, constitutes an abbreviated *neopallial* evolution as compared with the slow process of *germinal* evolution. Other animals than man "learn," i.e. acquire and utilize individual experience, but it is doubtful if any other animal than man *summates* experience, i.e., transmits it to other individuals and generations in ever varying amounts and combinations. The pallium is thus the organ of human culture and conduct. The symbolization necessary for this summation undoubtedly requires a great complexity of the mnemonic pallial apparatus.

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XII

THE CIRCULATORY SYSTEM

The circulatory apparatus consists of a blood vascular system and a lymph vascular system.

The blood vascular system consists of (1) a central propelling organ, the *heart*; (2) a series of efferent tubes, the *arteries*, which by branching constantly increase in number and decrease in caliber, and which serve to carry the blood from the heart to the tissues; (3) minute anastomosing tubules, the *capillaries*, into which the arteries empty and through the walls of which the interchange of elements between the blood and the other tissues takes place; (4) a system of converging tubes, the *veins*, which receive the blood from the capillaries, unite to form fewer and larger vessels as they approach the heart, and serve for the return of blood to that organ.

The lymph vascular system consists of lymphatic capillaries and various sized lymphatic vessels which ultimately drain into two main trunks—the thoracic duct and the right lymphatic duct.

THE BLOOD VASCULAR SYSTEM

The entire system—heart, arteries, veins, capillaries—has a common and continuous lining which consists of a single layer of endothelial cells. In the capillaries this single layer of cells forms the entire wall. In the heart, arteries and veins, the endothelium is invested with accessory coats of muscle and connective tissue. Since the structure of the capillary is simpler than that of any of the other parts of the vascular system, it has become customary to describe the capillaries first instead of following the system in its functional order of heart, arteries, capillaries and veins.

Capillaries

The capillaries are delicate tubes whose average diameter is about 7 μ to 9 μ. Their diameter varies, however, to some extent in different organs and tissues, and in the sinusoidal capillaries of the liver may reach a diameter of 25 μ or 30 μ. The capillaries branch extensively without any or with only a negligible change in caliber, and these branches anastomose to form extensive networks with meshes which vary in size and shape in the different tissues and organs. The greater the metabolic activity of the tissue, the denser is the capillary network. Very narrow-meshed nets are found in the lungs, liver, kidney, most glands and mucous membranes. The nets are likewise dense in skeletal muscle and in the gray matter of the brain, while in smooth muscle, tendons, nerves, and serous membranes the network is large-meshed and sparser. The part of a capillary which is nearest to the arteriole supplying it is called an arterial capillary and that part nearest to the venule is designated as a venous capillary. This division of the capillary bed into two parts is based on topography and function and not on structural differences.

The relationship of the capillaries to

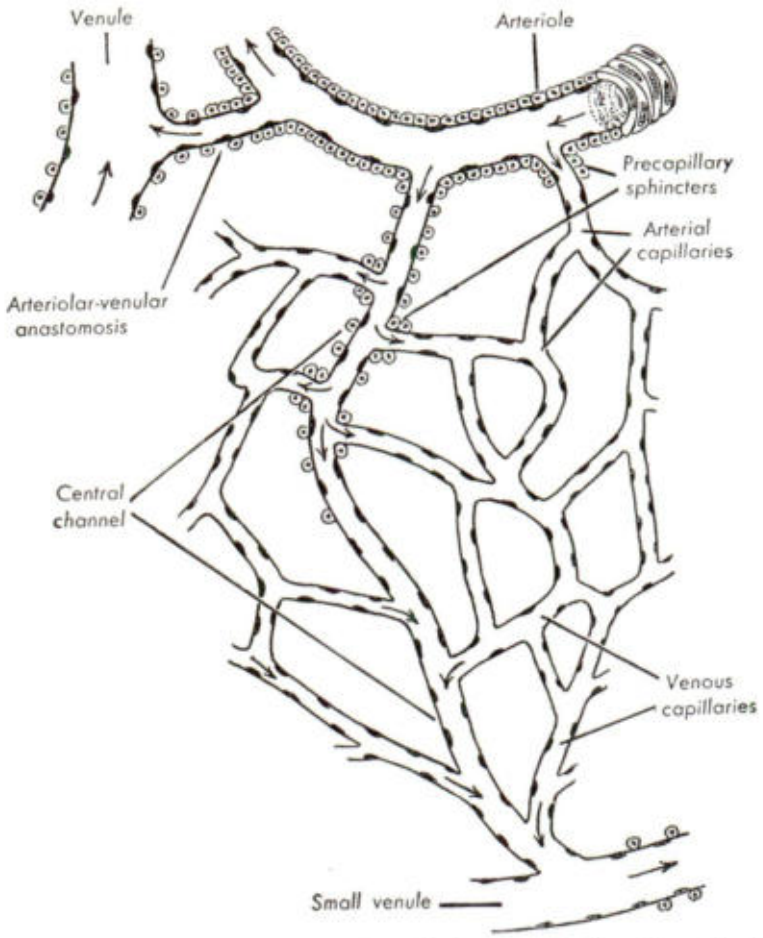


FIG. 164. Diagram showing relationship of capillaries to arteriole and venule. The proximal portion of the central channel through a capillary bed is surrounded by scattered smooth muscle fibers and has been named the "metarteriole" (Chambers and Zweifach, 1944); the distal portion of the central channel is structurally a true capillary.

the arterioles and venules is illustrated diagrammatically in Figure 164. The central, thoroughfare channel which is shown passing across the capillary network is an important functional component of the system. The proximal portion of this channel is a modified arteriole with a wall which contains smooth muscle cells which are widely separated from each other. It has been named the *metarteriole* (Greek, meta-, beyond). Arterioles with an incomplete layer of smooth muscle are also known as *precapillary arterioles*. The distal segment of the central channel is devoid of muscle and is structurally a true capillary, although it is broader than most other capillaries.

The thoroughfare channels and branched capillaries differ from each other in their functional behavior and they can be differentiated more readily in living than in fixed preparations. The thorough-

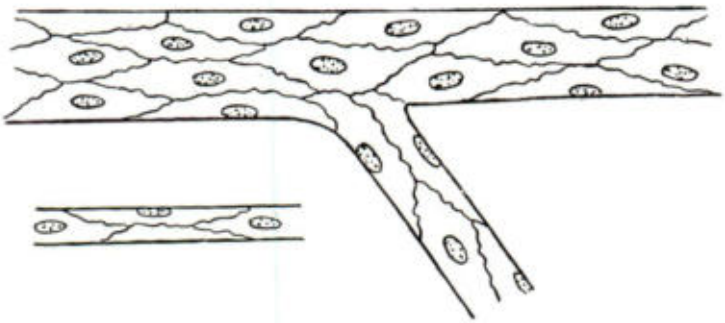


FIG. 165. Surface view of large and small capillaries stained with silver nitrate and hematoxylin to show outlines of endothelial cells and their nuclei.

fare channels convey an active flow of blood at all times although the actual amount of the flow is varied by vasoconstriction and vasodilation of the metarteriolar segment. Flow in the branched capillaries is intermittent in resting tissues; in other words, the capillary branches do not all function all of the time. The amount of blood entering the branches is controlled by the state of contraction of smooth muscle cells of the *precapillary sphincters* which are located wherever arterial capillaries arise from any of the arterioles—whether from metarterioles or from arterioles proper. The number of thoroughfare channels in proportion to the number of branched capillaries differs for different regions of the body; in skin, for example, the direct channels are numerous whereas in skeletal muscle they are relatively infrequent in comparison with the branches of the meshwork.

The wall of the capillary consists of a single layer of flat endothelial cells which rest on a very thin homogeneous basement membrane. In surface view the endothelial cells appear as a delicate mosaic which can be demonstrated by the precipitation of silver in the intercellular clefts (Fig. 165). The cell borders are usually serrated or wavy. The cells are arranged with their long axes parallel with the long axis of the tube. In capillaries of small caliber the cells are very narrow and their ends are pointed. In wider capillaries, the cells are oval and have blunter ends. The cytoplasm is clear or finely granular and the nucleus is elongated and centrally placed. The cells are thicker in the region of the nucleus. In fixed preparations this condition may be accentuated and there is frequently a more pronounced bulging of the nuclei into the lumen, due probably to the shrinking of the cytoplasm. Two endothelial cells—and occasionally even one—suffice to form the complete circumference of small capillaries. For the larger ones three to five cells may be required.

Electron micrographs show interesting details on the topography of the cells at their junctions (Fig. 166). They also show numerous vesicles within the cytoplasm of the endothelial cells. Some of the vesicles appear to be open either at the luminal or at the exterior surface of the capillary. It has been suggested that the cells form the vesicles by taking in fluids by a process named *pinocytosis*, and that substances may be transported by the vesicles to the opposite cell border where they are discharged.

Various types of *pericapillary cells* are closely applied to the outer surface of the endothelial walls of the capillaries. These cells include the ordinary connective tis-

FIG. 166. Electron micrographs of blood capillaries showing variations in the type of junction of the endothelial cells. *A*, Capillary with overlapping of ends of the endothelial cells (*En.*). Three red blood corpuscles (*R.b.c.*) are seen within the lumen of the vessel. *B*, Enlargement of portion of section shown in *A*. *C*, Capillary with an infolding of the ends of the endothelial cells at their junction. Note numerous vesicles within the cytoplasm of the endothelium. *A*, × 8,775; *B*, × 26,675; *C*, × 48,000. (Courtesy of Dr. Dan Moore.)

sue fibroblasts and histiocytes. The literature also contains frequent reference to cells which have been named in honor of Rouget who, in 1875, described them as "contractile" elements along certain capillaries of the frog. The Rouget cells are oriented spirally to the long axis of the vessel and have processes which encircle the vessel. Using a chrome silver impregnation method, Zimmerman demonstrated similarly shaped cells, which he called pericytes, in the human heart. The cells which have been described as "contractile Rouget cells" can probably be identified now as modified smooth muscle cells on the precapillary (metarteriolar)

portions of the thoroughfare channels in the capillary bed. Other so-called Rouget cells of true capillaries probably represent connective tissue cells. True capillaries (vessels completely devoid of smooth muscle) exhibit a "passive" contractility dependent on the amount of blood delivered by the arterioles but they apparently do not exhibit "active" contraction independent of the flow from the precapillary arterioles. (For further details, see Clark and Clark, 1943 and Chambers and Zweifach, 1944.)

The *sinusoids* or sinusoidal capillaries of the liver, blood-forming organs, adrenal cortex and other structures, differ in many respects from typical capillaries. Their caliber is larger and far more variable than that of ordinary capillaries. Some of the lining cells have processes and may extend considerably into the lumen. The stellate cells of Kupffer in the liver are of this type and similar cells are found in the sinusoids of other organs. The connective tissue which usually surrounds capillaries is greatly reduced in sinusoids and the lining cells are closely applied to the parenchyma of the organs, being separated from the latter only by a fine network of reticular fibers. In certain of the blood-forming organs, such as the spleen, openings in the walls of the sinusoids, through which cellular elements can enter or leave the blood stream, have been described. The lining cells of the sinusoids readily store injected dyestuffs and are included as part of the "reticulo-endothelial" system (see Chapter V).

The capillary walls furnish the membranes for the diffusion, filtration and osmosis of fluids to and from the blood stream. The passage of fluid through the capillary wall is partially dependent on the blood pressure within the capillaries

FIG. 167. Pericytes and muscle fibers from blood vessels of human heart. (After Zimmerman.) *1*, Artery with spirally coursing smooth muscle fibers; *2*, single fiber winding twice around lumen; *3*, muscle fiber from artery showing irregular processes; *4*, muscle fiber with more prominent processes in arterial fork; *5*, pericyte at junction of capillary and precapillary arteriole; *6*, pericytes on surface of precapillary.

and on the colloid osmotic pressure of the blood (Landis, 1937). The former factor promotes passage from the vessels to the tissues and the latter favors reabsorption. Landis found that the blood pressure in the arterial capillaries is higher than in the venous capillaries and that in the former it exceeds the colloid osmotic pressure. Hence, fluids tend to pass from the blood vessels to the tissues in the arterial part of the capillary bed and to be reabsorbed in the venous part (Fig. 182). The permeability of the capillary wall is also quite variable and is increased by agents which produce dilation. Changes in the physical consistency of the capillary walls which favor emigration of leucocytes were observed in living animals by the Clarks (1935). They showed that various mechanical and chemical stimuli make the endothelial wall sticky and that, as the stickiness is increased, the leucocytes adhere to the endothelial wall and later migrate through it.

Arteries

The wall of an artery is composed of three tunics or coats which are most distinct in vessels of medium caliber. (1) The innermost coat, the *intima*, consists of an inner endothelial lining continuous with and similar to that found in the walls of the capillaries; an intermediate layer of delicate connective tissue, which is absent in the smaller vessels; and an external band of elastic fibers, the *membrana elastica interna*, which marks the boundary between the intima and media. (2) The middle coat, or *media*, consists mainly of smooth muscle cells with varying amounts of elastic and collagenous tissue. (3) The outer coat, *adventitia* or *externa* is composed chiefly of connective tissue.

The structure of these three coats varies according to the size of the artery, and while the transition between them is never abrupt, it is convenient for purposes of description, to distinguish (1) small arteries or arterioles, (2) medium-sized arteries, and (3) large, elastic arteries.

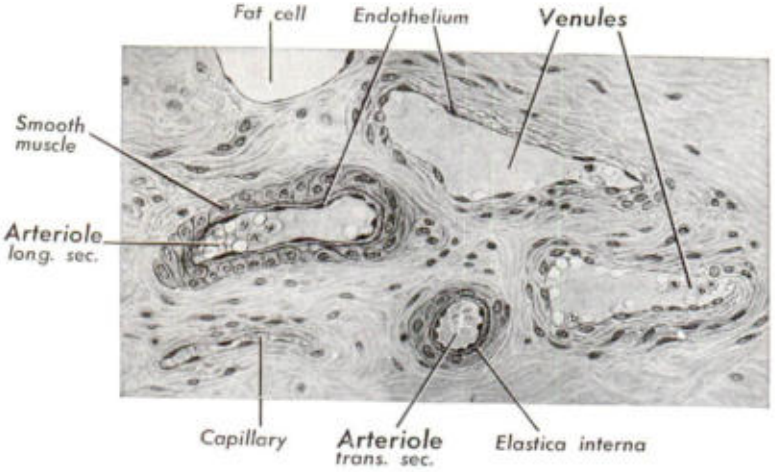


Fig. 168. Arterioles and accompanying venules from the submucosa of a human rectum. The arteriole at the left is cut longitudinally in the central part of its course where its walls show the circularly arranged muscle fibers cut across, but farther to the left, where it passes out of the plane of the section, the wall of the arteriole is cut tangentially and shows muscle fibers extending over the lumen. The vacuoles in the blood plasma just beneath the endothelium of the vessels are artifacts produced by shrinkage. × 250.

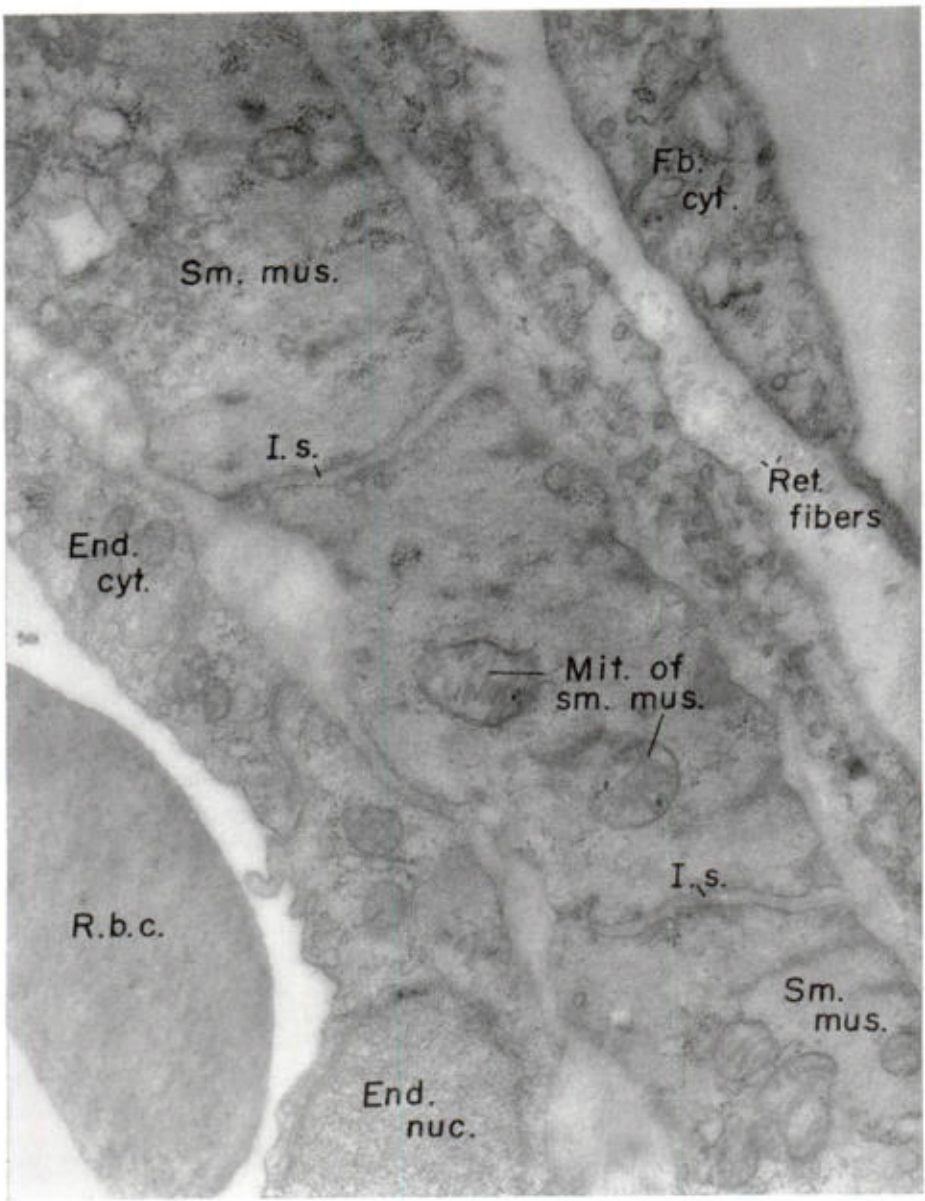


FIG. 169. Electron micrograph of a portion of an arteriole. A red blood cell (*R.b.c.*) is seen within the lumen. The wall of the vessel consists of: (1) an inner lining of endothelium (*End. cyt.* denotes endothelial cytoplasm and *End. nuc.* marks an endothelial nucleus); (2) a middle layer of smooth muscle (*Sm. mus.* denotes smooth muscle cytoplasm, *I.s.*, intercellular junctions of the muscle cells, *Mit. of sm. mus.* denotes mitochondria of the muscle; (3) an outer layer of connective tissue composed of collagenous and reticular fibers (*Ret. fibers*) and fibroblasts (*Fb. cyt.*). The plane of the section did not pass through any nuclei of the muscle fibers. × 30,600. (Courtesy of Dr. G. D. Pappas.)

Small Arteries. Arterioles. The transition from a capillary to an arteriole is marked by the appearance of isolated smooth muscle fibers which are arranged spirally. These small arterioles of transitional type are called precapillary arterioles or metarterioles (Fig. 164). As the vessels become larger, the muscle cells are increased in number and form a complete coat of one or two layers of circularly arranged fibers (Fig. 168). Outside of the muscle, the connective tissue is condensed to form a fibrous layer which is composed of flattened fibroblasts and longitudinally arranged collagenous fibrils. Thus in the walls of the small arteries or *arterioles*, the three coats are already distinguishable —an endothelial intima, a muscular media, and an adventitia of connective tissue. Although a few scattered elastic fibers can be demonstrated in the walls of very small arterioles, an internal elastic membrane sufficiently thick to be visible under the light microscope does not begin until the arterioles have reached a diameter of about 40 μ.

The coats become thicker and more definitely organized as the blood vesse'ls increase in caliber. In arterioles of about 130 μ the media contains three or four layers of muscle cells among which are found scattered elastic fibrils. The more distinct internal elastic membrane is composed of a delicate net of elastic fibrils. The adventitia contains, besides collagenous fibers, longitudinally disposed elastic and reticular fibers. The elastic fibers are aggregated mainly in the inner portion of the coat, close to the media. As the arterioles become larger, the muscle and elastic tissue components increase so that in arteries of about 400 μ, the wall shows all the structural features described in medium-sized arteries.

The arterioles are able to regulate the distribution of blood to different capillary beds by vasoconstriction or vasodilation in localized regions. Widespread vasoconstriction or vasodilation of the arterioles alters the peripheral resistance to flow from the larger arteries and hence plays an important part in regulating blood pressure. The arterioles are structurally adapted for vasoconstriction and vasodilation since their walls are composed primarily of circularly arranged muscle fibers which are controlled by the autonomic nervous system. Elasticity is not as important in these vessels as in the larger arteries in which the blood pressure is much higher. Hence, the sparseness of elastic tissue in arterioles is another example of correlation between structure and function.

Medium-sized Arteries. These include all the named arteries except the very large ones. There is a gradual transition between the branches of the medium-sized arteries and the arterioles described above. Examples of medium-sized arteries are the radial, tibial, popliteal, axillary, splenic, mesenteric, and intercostal arteries. The walls of these blood vessels are relatively thick, due mainly to the large amount of muscle in the media (Fig. 170). They are therefore called *muscular* arteries in contrast with the *elastic* arteries like the aorta, in the wall of which elastic tissue predominates. The muscular arteries have also been called *distributing arteries* because they distribute the blood to different organs and, by contraction or relaxation, they aid in regulating the supply to different regions in response to different functional demands.

The *intima* exhibits three definite layers —endothelium, intermediate layer, and internal elastic membrane. The intermediate layer consists of delicate elastic and collagenous fibrils and a few fibroblasts. The *internal elastic membrane* is a thick fenestrated band formed of closely interwoven elastic fibers. In certain arteries it splits in places and appears in section as a double membrane. It is inti-

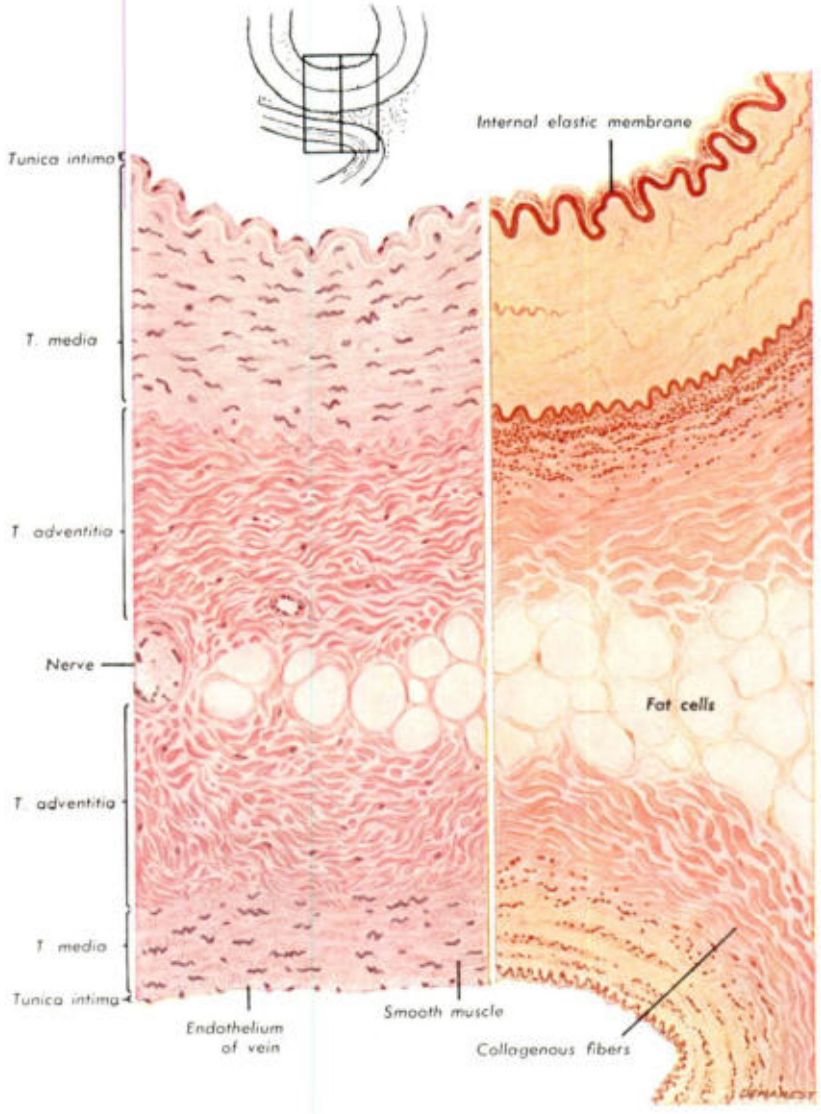


FIG. 170. Two cross sections of the same medium-sized artery and vein (intercostal artery above, vein below). The line drawing at upper left corner gives orientation of regions drawn. Section on the left is stained with hematoxylin-eosin; that on the right with resorcin-fuchsin to show elastic tissue. × 215.

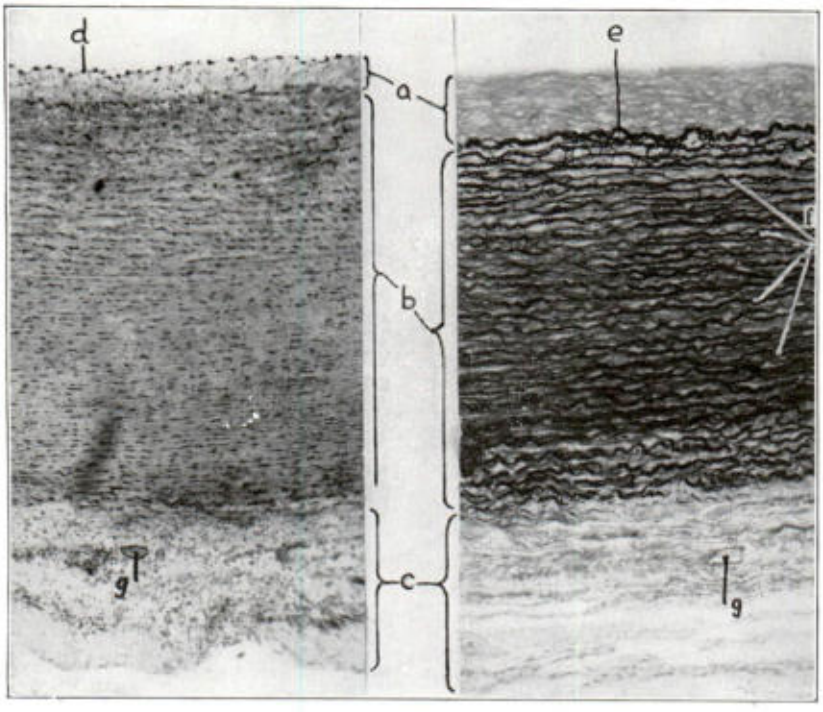
mately connected with the media and marks the boundary between the latter and the intima. In the smaller muscular arteries the intermediate layer is thin, and the endothelium is close to the elastic membrane.

Owing to the large amount of muscle in their walls the post-mortem contraction of arteries throws the elastic membrane into longitudinal folds, hence in transverse sections it has the appearance of a corrugated or wavy band.

Isolated longitudinal smooth muscle fibers are occasionally found in the intima, especially in places where the artery branches. Larger longitudinal bundles close to the elastic membrane are prominent in the coronary arteries and they have also been described in some of the larger muscular arteries (femoral, popliteal, axillary, hepatic, splenic, renal).

The *media* is the thickest coat and consists of 25 to 40 layers of circularly disposed muscle fibers. The thickness of the muscle coat is to some extent proportional to the size of the vessel, but there are considerable variations in arteries of the same size. Between the layers of muscle there are small amounts of connective tissue composed of elastic, collagenous and reticular fibers, and fibroblasts.

The amount and distribution of elastic tissue in the media are closely correlated with the caliber of the vessel. In the smaller vessels the elastic fibers are scattered between the muscle cells but in the larger vessels they form circularly ori-



A B

FIG. 171. Cross section through internal carotid artery. Retouched photograph. × 85. *A*, Stained with hematoxylin-eosin; *B*, with resorcin-fuchsin; *a*, intima; *b*, media; *c*, adventitia; *d*, endothelium; *e*, internal elastic membrane; *f*, elastic membranes in media; *g*, vasa vasorum.

ented elastic nets which permeate the entire media. The largest vessels of the group contain circularly disposed fenestrated membranes of elastic tissue (Fig. 171); hence these vessels are transitional between the muscular and elastic types in structure as well as in position.

Radial elastic fibers likewise occur in some arteries. In transverse section they appear as vertical fibers which penetrate the media for varying depths, sometimes extending from the adventitia to the internal elastic membrane.

The *adventitia* is a coat of considerable thickness and may occasionally be as thick as the media. It is composed of connective tissue containing collagenous and elastic fibers, most of which course longitudinally. The elastic fibers are concentrated in the inner layer of the coat, where they form a coarse network. In some of the larger arteries the elastic tissue immediately in contact with the media forms an especially well defined layer, the *membrana elastica externa*. The outer layer of the adventitia blends gradually with the surrounding connective tissue which attaches the artery to other structures.

A few longitudinal smooth muscle fibers are occasionally found in the inner layer of the adventitia, between the elastic fibers. In some arteries (splenic, dorsalis penis) bundles of longitudinally disposed muscle fibers occur in close proximity to the media.

Large Arteries. The large arteries belong to the *elastic* type. They have also been called *conducting arteries* because they conduct the blood from the heart to the medium-sized distributing arteries. The wall is relatively thin for the size of the vessel. There is a tremendous accretion of elastic tissue, and a great reduction in the amount of smooth muscle. While in the medium-sized arteries the various elements are arranged circularly or longitudinally, the muscle fibers, elastic and collagenous tissue, have in the main a spiral disposition in the large arteries. The chief representative of this group is the aorta (Fig. 172).

The endothelial cells of the *intima* are short and polygonal in shape. Below the endothelium there is a layer of connective tissue containing fine collagenous and elastic fibers and a few fibroblasts. The deeper portion of the intima contains coarser collagenous fibers, some longitudinally oriented smooth muscle cells and many elastic fibers which are also arranged longitudinally (Fig. 172). The amount of elastic tissue and the character of the internal elastic membrane change with age. The inner elastic membrane is usually split into two or more lamellae which merge with other similar membranes, both in the intima and in the media. Hence it is generally difficult to identify this membrane in the aorta with exactness.

The *media* is distinguished by numerous distinct elastic membranes which course spirally and which anastomose to form complex elastic nets. The narrow spaces between the lamellae are permeated by a finer elastic network in the meshes of which the muscle fibers are contained. The muscle tissue is greatly reduced in amount and its fibers are short flat cells of irregular outline. They unite to form branching bands which, like the elastic lamellae, pursue a spiral course. The muscle fibers are surrounded and supported by a small amount of collagenous and reticular fibers.

The *adventitia* is a thin coat and consists of connective tissue composed mostly of collagenous fibers arranged in longitudinal spirals. It contains relatively few elastic fibers. The elastic layer at the junction of the adventitia and media does not differ from the numerous elastic membranes found throughout the media

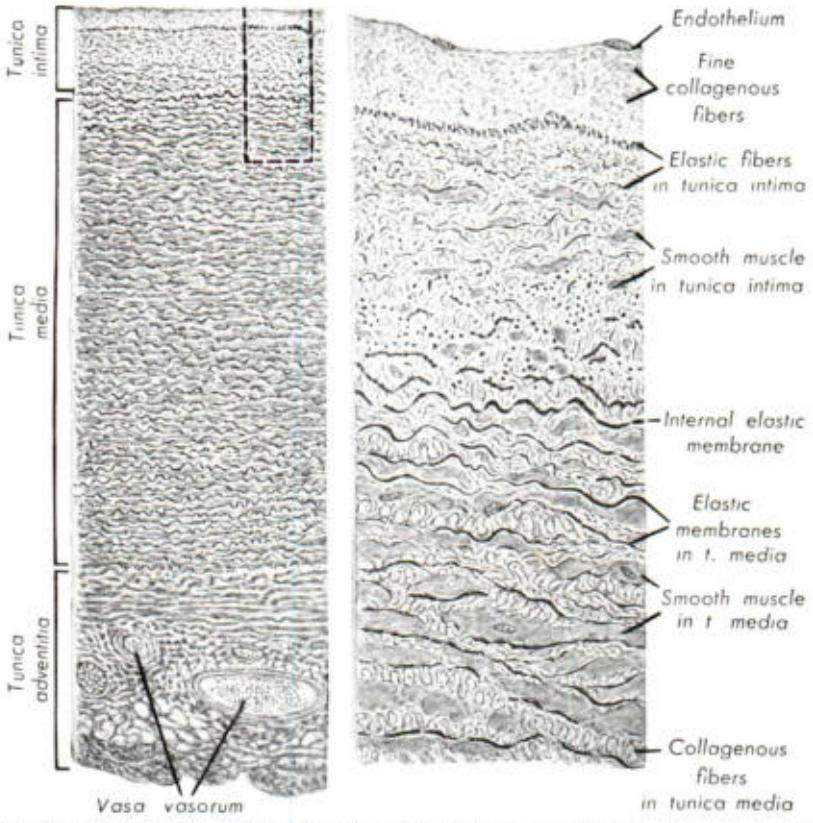


FIG. 172. Cross section through ascending aorta, human, age 23 years. Low power view at left shows layers of tissue present in total thickness of vessel. Region outlined by broken line is enlarged in the figure at the right to show structure of tunica intima and a portion of the adjacent media. Weigert's elastic tissue and Van Gieson's stains to differentiate elastic fibers, collagenous fibers and smooth muscle. Figure at left, × 60; at right, × 285.

and hence it is not as clearly demarcated as the external elastic membrane of medium-sized arteries. A few longitudinally arranged smooth muscle fibers are occasionally found in the adventitia of some of the large arteries.

Besides the aorta, the large arteries include the pulmonary, innominate, common carotid, subclavian, vertebral, and common iliac. These resemble the aorta in structure, but they have less elastic tissue and more smooth muscle. As these arteries branch and become smaller they gradually assume the structure of a medium-sized artery.

Since the blood is propelled through the blood vessels by the rhythmic contractions of the heart, the rate of flow is not uniform. When the heart contracts and forces blood into the aorta, the walls of the elastic arteries stretch and a part of the force of the beat is converted into potential energy in the form of increased elastic tension. During diastole of the ventricles, the potential energy of the expanded arterial walls is transformed into kinetic energy which keeps the blood moving forward. Thus, the elasticity of the large arteries makes the blood flow less irregularly than it would if the vessels

were rigid tubes. The most marked irregularity is found at the beginning of the arterial system; the flow becomes more uniform in the terminal parts of the arterial system.

Special Forms of Arteries. There are certain arteries which exhibit pronounced structural peculiarities. The *cerebral* and *dural* arteries which are protected from external mechanical forces, are thin-walled for their caliber. They have a well developed internal elastic membrane, but almost no elastic fibers in the media. The adventitia is poorly developed and consists mainly of collagenous fiber bundles.

The arteries of the *lung* have thin walls, due to a reduction of both muscle and elastic tissue. This is probably associated with the lower blood pressure in the pulmonary circulation.

In the *penile* (and *pudic*) arteries, a hyperplasia of the intima and media manifests itself after puberty, while the adventitia remains relatively thin. The intima especially becomes greatly thickened, and contains many longitudinal muscle fibers in its outer layer.

The *umbilical* arteries have a media composed of two muscle layers, an inner longitudinal and an outer circular. The internal elastic membrane is indistinct and entirely missing in some places. A true adventitia is lacking in the segment which is in the umbilical cord, and is but poorly developed in the intra-abdominal portion of the artery.

Ageing of the Arteries. The arteries undergo age changes which differ in type and in degree in different vessels of the same individual. The elastic arteries, especially the aorta, show more changes than the muscular arteries of the extremities such as the femoral or brachial. Particularly pronounced and early changes occur in the arteries of the brain and heart.

In a four months' human fetus, the aorta has a tunica intima composed only of endothelium and one elastica interna. By the end of fetal life, the internal elastic membrane has become thicker and after birth it splits into two or more layers. Additional elastic and collagenous fibers develop between the endothelium and elastica interna and the elastic layers of the media also increase in number. The layers of the aorta are not completely differentiated until about twenty-five years of age. It is difficult to separate some of the final stages of differentiation from regressive changes due to use. In fact, some authors think that arteriosclerosis may be a physiological rather than a pathological process. In the ageing or wearing out process, the tunica intima becomes thicker, the elastic layers of the media change chemically and become less elastic, and fat gradually accumulates between the elastic and collagenous fibers.

The anterior descending branch of the left coronary artery furnishes another interesting example of age changes. In this vessel, the internal elastic membrane is already split at the time of birth, definite hyperplasia of the elastic tissue occurs during the first decade, and calcification of the media begins in the third decade. It is interesting to note that similar changes in another branch of the coronary arteries—the posterior descending branch of the right coronary—do not occur until considerably later. The calcification of the media is one of the main changes in the arteries of muscular type.

The Carotid and Aortic Bodies. These structures were formerly included with the paraganglia as a part of the endocrine system. They were erroneously thought to contain chromaffin cells similar to those of the adrenal medulla.

The *carotid bodies* are small, paired organs which lie in or near the bifurcation of each common carotid. Each one is frequently divided, a half lying on either side of the bifurcation. They are very vascular and contain numerous sinusoidal capillaries. The parenchyma is composed of epithelial-like cells which are pale staining and show no particular characteristics in the usual hematoxylin and eosin preparations. For cytological details which can be observed with special technical methods, reference should be made to the work of Hollinshead (1943). The cells contain cytoplasmic granules which can be particularly well stained with neutral red and other basic dyes in supravital preparations. They do not give the chromaffin reaction and no fat, cholesterol or glycogen can be demonstrated in them. They exhibit no Nissl substance, neurofibrils, or other evidence of similarity to neurons.

Afferent nerve fibers from the carotid body form the sinus branch of the glossopharyngeal nerve or sinus nerve. The fibers have delicate endings on the epithelioid cells which are in close contact with the sinusoidal capillaries. The mechanism is spoken of as a *chemoreceptor*, since nerve impulses are initiated in response to changes in the pH of the circulating blood.

The *carotid sinus* is an enlargement at the bifurcation of each common carotid. In this region, the wall of the artery is relatively thin and the adventitial layer has a rich supply of specialized sensory nerve endings derived from the carotid sinus branch of the glossopharyngeal nerve. The nerves have reticulated swollen endings in contact with the cells of the adventitial layer and appear as terminal menisci. They are stimulated by distention of the vessel wall (by an increase in blood pressure) and bring about reflex vasodilation, slowing of the heart and a fall in blood pressure.

The *aortic bodies* and *aortic nerves*. The position of the aortic bodies varies somewhat for different species. In the rabbit, where their structure and innervation have been described in detail by Nonidez (1935), the right aortic body is in the angle formed by the subclavian and common carotid, whereas the left aortic body is closely applied to the roof of the arch of the aorta. Histologically, they appear to be similar to the carotid bodies and they are thought to have a similar function, that is, they are responsive to changes in the chemical composition of the blood, and stimuli initiated by these changes are carried to the central nervous system by the afferent nerves. These nerves are derived from branches of the aortic nerves and end as delicate rings or swellings in contact with the epithelioid cells. The *aortic nerves* (*depressor nerves*) are branches of the vagus which send numerous fibers into the right subclavian artery and the arch of the aorta (that is, into the walls of the vessels adjacent to the aortic bodies). In these arteries, the sensory fibers have reticulated swollen endings similar to those of the carotid sinus but they differ from the latter by penetrating the tunica media as well as the adventitia. Like the fibers of the carotid sinus, they are stimulated by changes in blood pressure.

Veins

The caliber of veins is as a rule larger than that of arteries, but their walls are much thinner, due to a great reduction of the muscular and elastic elements. The collagenous connective tissue, on the other hand, is present in much larger amounts and constitutes the bulk of the wall. The relatively sparse circular muscle of the media is more loosely arranged and separated into layers by abundant collagenous fibers. The internal elastic membrane is not a compact fenestrated layer, but consists of a network of elastic fibers, which becomes distinct only in the larger veins. The three coats—intima, media and adventitia—are present, but their boundaries are often indistinct for lack of definite limiting membranes. The whole wall is flabbier and more loosely organized than in arteries, and tends to collapse when not filled with blood.

A histological classification of veins is quite difficult as their structure varies extensively. The variations are not always related to the size of the vessels but depend on local mechanical conditions. The structure may be quite different in veins of the same caliber and even in different portions of the same vein. A description of the venous wall can therefore enumerate only the most general features.

Small Veins. The transition from capillary to vein is a very gradual one, the connective tissue elements appearing first and the smooth muscle fibers somewhat later. The smallest veins (*venules*) are endothelial tubes surrounded by an outer sheath of collagenous fibrils with a few fibroblasts (Fig. 168).

Isolated, circularly disposed muscle fibers make their appearance in vessels of 40 μ to 50 μ, though the presence of muscle fibers is variable and is not always dependent on caliber. In venules of 0.2–0.3 mm. the circular muscle fibers form a continuous layer and the adventitia is a relatively thick coat of longitudinally disposed collagenous fibers. In its inner portion, just outside the muscle, are scattered elastic fibrils.

As the veins increase in size the muscle becomes many-layered, and the individual layers are separated by loose collagenous tissue. The elastic fibers gradually invade the whole media and form longitudinal elastic nets between the muscle layers. The innermost elastic net comes to

lie directly underneath the endothelium and constitutes a poorly defined inner elastic membrane.

Medium-sized Veins. These include practically all the named veins and their branches, excepting the main trunks found in the thoracic and abdominal cavities (Fig. 170).

The *intima* is thin. The endothelial cells are short and polygonal in shape. A subendothelial layer of delicate collagenous and elastic fibers is present in the larger veins, but missing in many of the smaller ones. The internal elastic layer consists of a dense network of longitudinal fibers. Often the layer is so indistinct that there is no demarcation between media and intima.

The *media* is thin as compared with that of arteries of the same size. It is composed of circular muscle fibers, and collagenous and elastic connective tissue. In the larger veins the muscle is arranged in bundles or layers separated by collagenous fibers. The muscle layers alternate with nets of longitudinal elastic fibers which are especially distinct in the outer portions of the media.

The media is thickest in the veins of the lower extremity, where it resembles more nearly that of an artery. In the veins of the head and abdomen it is very thin, and in some it is missing entirely.

The *adventitia* is well developed and forms the bulk of the wall. It consists of collagenous and elastic tissue, and often

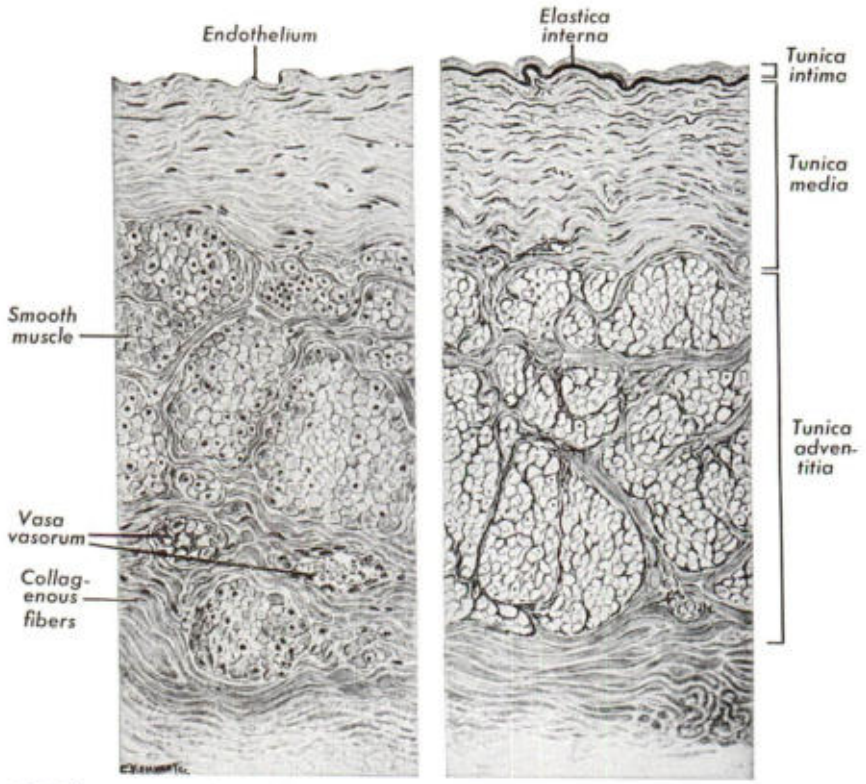


FIG. 173. Two cross sections of the same internal jugular vein; left, stained with hematoxylin-eosin; right, stained with resorcin-fuchsin and picro-fuchsin to differentiate elastic fibers, collagenous fibers and smooth muscle. × 180.

contains a few bundles of longitudinal muscle fibers.

Large Veins. In the large venous trunks of the thoracic and abdominal cavities, the circular muscle is greatly reduced, while the longitudinal muscle bundles of the adventitia are strongly developed (Fig. 173). Unusually stout bundles are found in the portal vein and hepatic portion of the inferior vena cava. The media is very thin and in some places is lacking altogether, as in the thoracic portion of the superior vena cava. To this group belong the superior and inferior venae cavae, the innominates, the internal jugulars, portal, splenic, azygos, superior mesenteric, renal, adrenal and external iliac veins.

Special Features of Certain Veins. Very little or no muscle whatever is found in the following veins: the subpapillary veins ("giant capillaries") of the skin and nailbed, the trabecular veins of the spleen, the dural sinuses, most pial and cerebral veins, the veins of the retina and of the bones, and the deeper veins of the maternal placenta (v. Ebner, Benninghoff).

Especially rich in muscle are the veins of the *gravid uterus*, which contain muscle fibers in all three coats. The *umbilical* vein has an inner longitudinal and outer circular muscle layer. Occasionally longitudinal fibers are also found outside the circular coat.

Longitudinal smooth muscle fibers are found in the intima of the saphenous, popliteal, femoral, basilic, cephalic, median, internal jugular, umbilical, and some mesenteric veins; also in the veins of the gravid uterus (v. Ebner).

Near their entrance into the heart, the adventitia of the venae cavae and pulmonary veins is invested with a layer of cardiac muscle, the fibers coursing spirally or circularly around the tube.

Valves. Veins over 2 mm. in diameter are provided at intervals with valves (Figs. 174 and 175). These are semilunar flaps which project into the lumen, their free margin being directed towards the heart. As the blood flows towards the heart it flattens the flaps against the wall and thus passes without obstruction towards the heart, but if it starts to flow

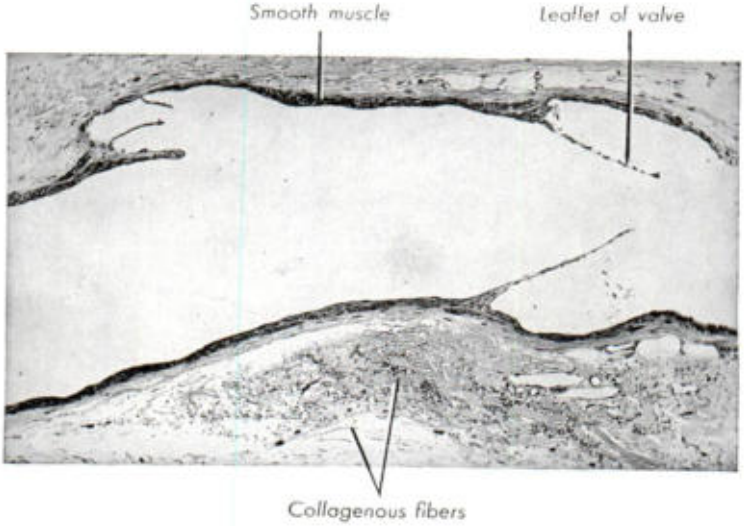


FIG. 174. Longitudinal section of a vein from human subcutaneous tissue showing valves. The valve at the upper left is in a small tributary which curves out of the plane of the section. A part of the lower leaflet of the valve at the right is enlarged in Figure 175. Photomicrograph. × 84.

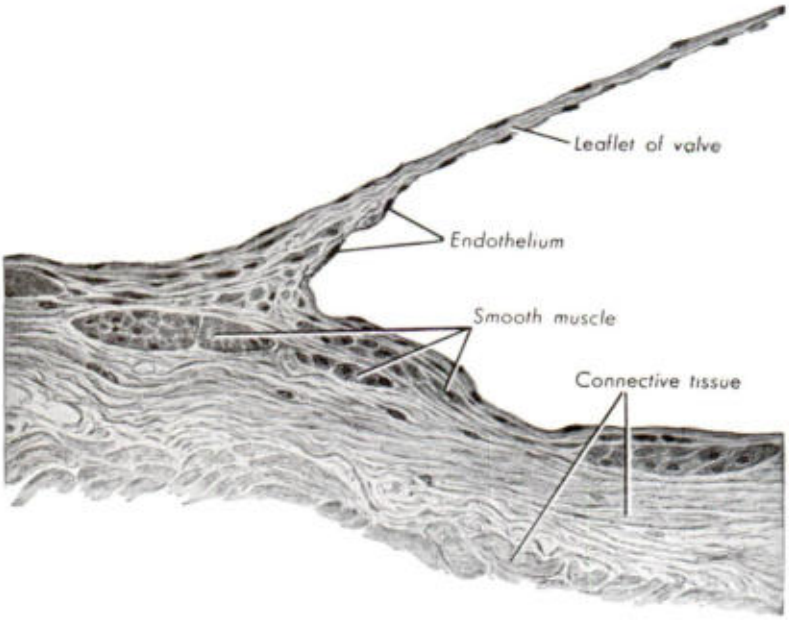


FIG. 175. Higher magnification of a part of the valve of the vein shown in Figure 174. × 447

in the reverse direction, the valves float up, approximate each other and occlude the cavity.

The valves are derived from the intima and consist of loose connective tissue covered by a layer of endothelium. Beneath the endothelium of the surface of the valve directed against the blood current is a rich network of elastic fibers continuous with the elastic tissue of the intima. The connective tissue of the side facing the wall of the vein is entirely free from or contains but few elastic fibers. Adjacent to the valve, on the side toward the heart, the wall of the vein is usually distended and thin; this region is called the *sinus of the valve*. The smooth muscle of the vein at the base of the valve and along the sinus region runs mostly in a longitudinal or spiral direction. Valves are especially numerous and strong in the larger veins of the lower extremities. They are absent in the veins of the brain and cord and their membranes, in the umbilical vein, in most of the visceral veins with the exception of some branches of the portal, and in the superior and inferior venae cavae and their branches.

Arterio-venous Anastomoses. In addition to the capillary and sinusoidal connections of vessels already described, arteries sometimes empty directly into veins—*arterio-venous anastomoses*. Such connections have been found in pathological conditions resulting from injury, in vascular neoplasms, and in developmental anomalies. They also occur normally in certain parts of the body. They are especially numerous in the sole of the foot, in the palm of the hand, in the skin of the terminal phalanges, and in the nail bed. They have been studied in considerable detail in the living condition in the rabbit's ear (see review by Clark, 1938). The arterio-venous anastomoses are usually surrounded by a connective tissue sheath and the arterioles generally follow

a convoluted course, forming a structure known as a *glomus*. The smooth muscle fibers are modified in shape and structure and are epithelioid in appearance.

When the arterio-venous anastomoses are open, they shunt a considerable amount of blood directly into the veins and decrease the flow through adjacent arterioles leading to the capillary bed, but in the normal behavior of the peripheral vessels, they are contracted a large part of the time.

Blood Vessels, Lymphatics and Nerves of the Blood Vessels

Vasa Vasorum. Arteries and veins with a diameter over 1 mm. are supplied with small nutrient blood vessels, the *vasa vasorum*. These vessels enter and branch in the adventitia and terminate in a capillary network which penetrates the media, probably reaching its deepest layers. No capillaries are found in the intima.

Lymphatics. Lymphatics have been found in the adventitia of many of the larger arteries and veins. Extensive *perivascular lymph spaces* surround the thin walled blood vessels of the pia-arachnoid of the brain and spinal cord.

Nerves. The walls of blood vessels have a rich nerve supply. The nerve fibers are mainly unmyelinated axons from sympathetic ganglia and are known as vasomotor nerves, since they control the caliber of the blood vessel. They form a plexus in the adventitia, from which are given off secondary plexuses which permeate the entire media. Here the fibers branch extensively and end on the muscle fibers with delicate knob-like terminations.

Scattered ganglion cells have been found in the adventitia of the aorta.

Besides the vasomotor nerves, the blood vessels receive myelinated sensory nerve fibers which are the peripheral arms of spinal or cranial ganglion cells. The larger fibers run in the connective tissue surrounding the blood vessel. They enter the adventitia, divide repeatedly, lose their medullary sheath, and terminate in free sensory endings. In some blood vessels they can be traced into the intima.

The Heart

The heart is the part of the blood vascular system which propels the blood through the blood vessels. Its wall consists of three layers: an inner layer or *endocardium*, a middle layer or *myocardium*, and an outer layer or *epicardium*. The myocardium forms the main mass of the heart.

Endocardium. The endocardium is a thin glistening membrane covering the inner surface of the atria and ventricles. It is thick in the atria, especially in the left atrium, and thin in the ventricles; this explains the whiter color of the inside of the atria as contrasted with the red appearance of the inside of the ventricles, where the color of the cardiac muscle shows readily through the thin endocardium. At the arterial and venous orifices it becomes continuous with the intima of the vessels, with which it is comparable in structure. It is lined by an endothelium of irregularly shaped polygonal cells with oval or round nuclei. Beneath the endothelium is a thin layer of exceedingly fine collagenous fibrils, and outside of this, a stouter layer containing abundant elastic tissue and varying numbers of smooth muscle cells (Fig. 177). In most parts of the heart, the deepest layer of the endocardium is composed of loose connective tissue which binds the endocardium proper to the myocardium. This layer, known as the *subendocardium*, contains collagenous fibers, elastic fibers and blood vessels. In the ventricles, it also contains some of the specialized muscle fibers of the impulse conducting system. This layer is absent

from the papillary muscles and chordae tendinae.

Myocardium. The myocardium consists of a special form of striated muscle tissue already described as cardiac muscle (Chapter VIII). Its thickness varies in different parts of the heart, being thinnest in the atria, and thickest in the left ventricle. The atrial muscle tends to be arranged in bundles in a sort of lattice work with spaces where the connective tissue of the subendocardium joins with that of

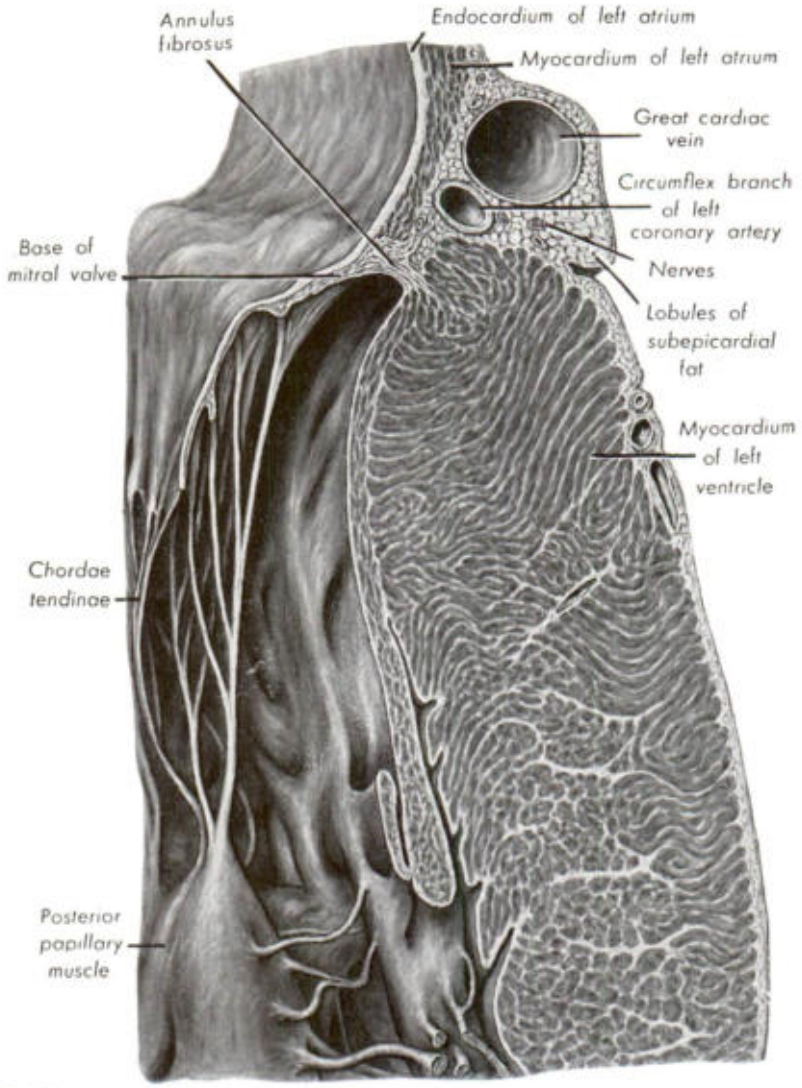


FIG. 176. Low power, three dimensional view of the zone of juncture of the left atrium with the left ventricle in the human heart. The region illustrated is from the posterior part of the heart and shows the posterior papillary muscle and the posterior cusp of the bicuspid (mitral) valve. × 3.5.

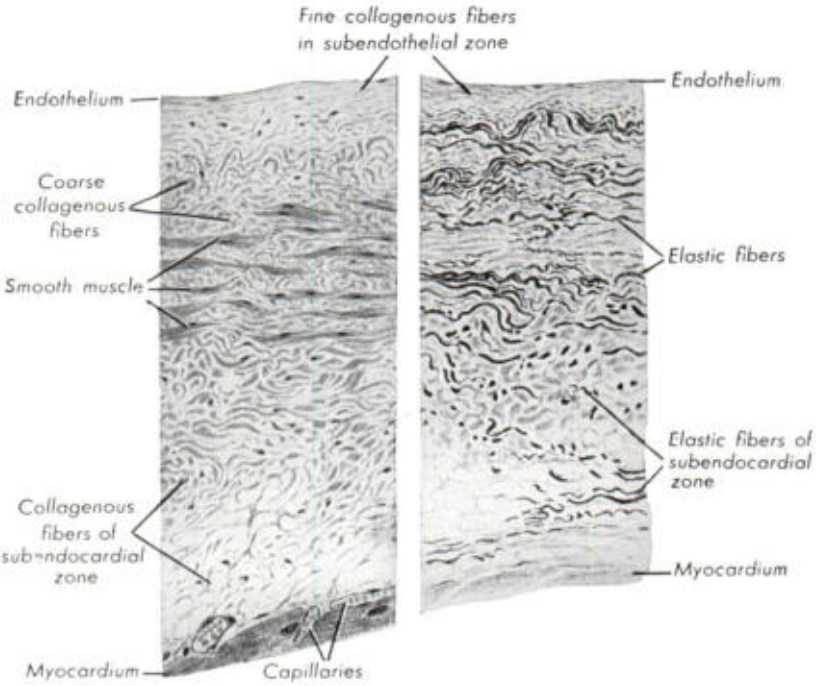


FIG. 177. Cross sections through the endocardium of the left atrium of an adult. Section on the left is stained with hematoxylin and eosin; the one on the right is stained for elastic fibers. × 206.

the subepicardium. The muscle bundles of the outer part of the atrial wall are oriented chiefly in a transverse or oblique direction and continue over both atria. The bundles in the deeper portions of the atrial wall are more independent for each atrium and are oriented approximately at right angles to the bundles of the outer layer. The inner bundles stand out as ridges (pectinate muscles) in the auricular portions of the atria.

The disposition of the muscle tissue of the ventricles is much more complicated. It is usually described as composed of several layers, the fibers of which run in different directions. The arrangement of these fiber layers can be readily determined by dissecting hearts in which the connective tissue has been broken down by maceration. The muscle of the ventricles consists mainly of two sets of fibers, a *superficial* set and a *deep* set. These run at approximately right angles to each other. Both sets of fibers begin at the atrioventricular rings. The superficial fibers follow a spiral course from the base of the ventricles to the apex of the heart, where they turn inward to terminate in the papillary muscles. The deeper layers follow a circular course on each ventricle, with some of the fibers making an S-shaped pattern as they pass from one ventricle to the other by way of the interventricular septum. (The arrangement of these layers has been particularly well illustrated by Robb et al., 1935.)

The muscle of the atria is completely separated from that of the ventricles by strong fibrous rings, the *annuli fibrosi*, which surround the atrioventricular ostia. The fibrous rings are composed mainly of dense bundles of collagenous fibers. They

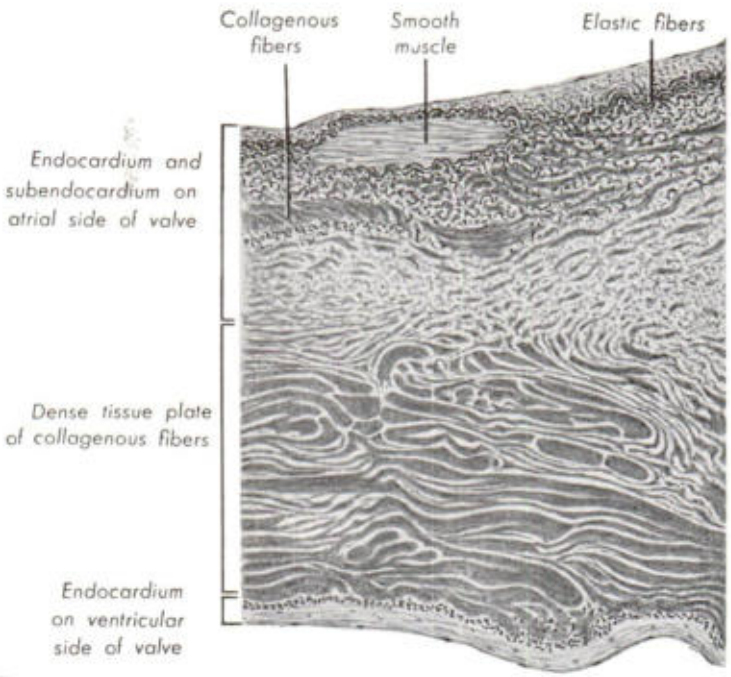


FIG. 178. Cross section through a human mitral valve. The region shown is from the base of the valve, with the right side of the illustration facing the A-V junction and annulus fibrosus. (Compare with Fig. 176 for orientation.) Weigert's elastic tissue and Van Gieson's stains to differentiate elastic fibers, collagenous fibers and smooth muscle. × 110.

also contain some elastic fibers, fibroblasts and fat cells which become continuous with the subepicardial adipose tissue in the region of the coronary sulcus (Fig. 176).

The fibrous rings show structural variations in different persons and at different ages. They exhibit more marked variations in different species, e.g., they contain hyaline cartilage in sheep and bone in the ox.

The *annuli fibrosi of the atrioventricular ostia* form a part of a dense connective tissue supporting structure known as the *cardiac skeleton*. Other parts of this system are: the *annuli fibrosi at the arterial foramina*, the *trigona fibrosa*, and the *septum membranaceum*.

Epicardium. The epicardium is the visceral layer of the pericardial sac in which the heart is placed. It is lined by a single layer of mesothelial cells which may be flat or cubical depending on the contraction state of the heart. Below the mesothelium is a layer of connective tissue containing a considerable number of elastic fibers in its deeper portion. At the venous and arterial openings, the elastic fibers pass over into the adventitia of the blood vessels. The epicardium is attached to the myocardium by the *subepicardial layer* of areolar tissue containing blood vessels, nerves, and varying amounts of fat.

Valves of the Heart. The atrioventricular valves (tricuspid and mitral) are attached at their bases to the annuli fibrosi (Fig. 176). They consist of folds of endocardium covering a central plate of dense bundles of collagenous fibers which are continuous with the fibrous tissue of the annuli fibrosi and chordae

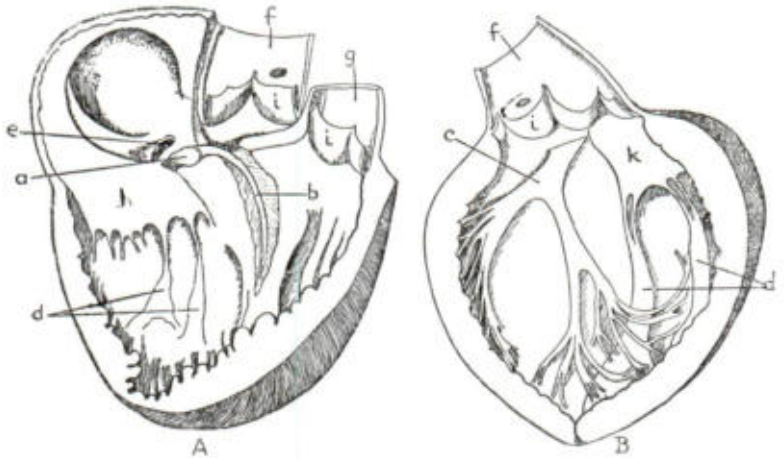


FIG. 179. Atrioventricular bundle of human heart. *A*, View of right heart; *B*, view of left heart. *a*, Atrioventricular node; *b*, right trunk of bundle; *c*, left trunk of bundle; *d*, papillary muscles; *e*, coronary sinus; *f*, aorta; *g*, pulmonary artery; *h*, flap of tricuspid valve; *i*, semilunar valve; *k*, flap of mitral valve. (After Tandler.)

tendinae. The endocardium is thicker on the atrial than on the ventricular side and contains more elastic tissue. Smooth muscle fibers are also present on the atrial side but these are rarely distinguishable except in specially prepared slides.

The semilunar valves are similar in structure to the atrioventricular valves, but contain no muscle fibers.

Impulse Conducting System. Besides its ordinary musculature which furnishes the energy for the movement of the blood, the heart possesses a system of peculiar muscle fibers whose function is to regulate the proper succession of contractions of atria and ventricles. It is known as the impulse conducting system. A part of the system extending from the right atrium into the ventricles may be easily demonstrated by gross dissection and is known as the *atrioventricular bundle*, or *bundle of His*. This bundle has its origin in the *atrioventricular node* (node of Tawara) which is found in the subendocardium of the median wall of the right atrium close to the termination of the coronary sinus (Fig. 179). From the node a common bundle or stem, the *crus commune*, is continued into the membranous septum of the ventricles where it divides into two trunks which go respectively to the left and right ventricles. Each trunk ultimately breaks up into a large number of fine branches which radiate to all parts of the ventricle and form an extensive network of fibers in the subendocardial tissue. From the subendocardial plexus numerous branches extend into the myocardium and eventually terminate by connecting with the fibers of the surrounding cardiac muscle.

The atrioventricular bundle and its branches consist of modified muscle fibers, *Purkinje fibers*, which surpass the ordinary cardiac fibers in their rate of conduction. Although the histological differences between Purkinje fibers and ordinary cardiac muscle fibers are less pronounced in human hearts than in ungulate hearts, they are sufficient for identification of the fibers in the majority of human cases studied (Fig. 180). In comparison with ordinary cardiac fibers, the distinguishing characteristics of Pur-

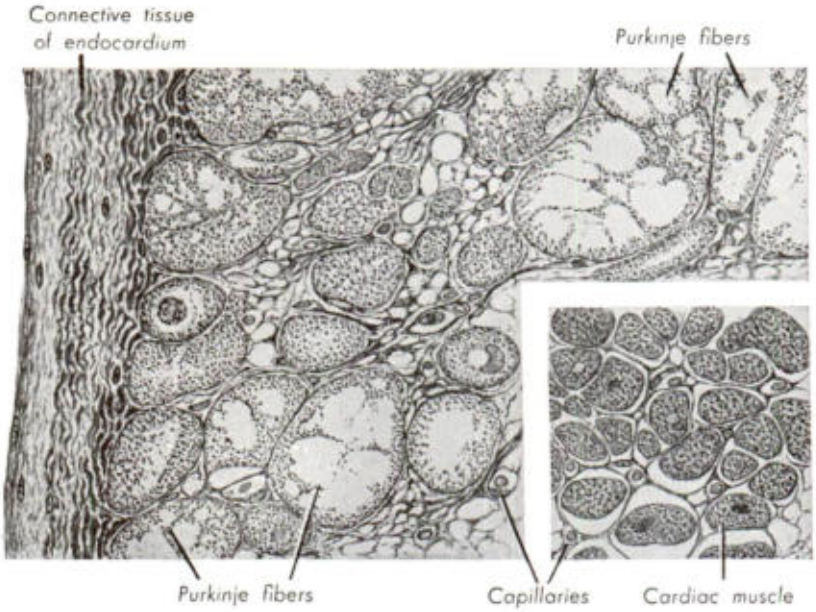


FIG. 180. Purkinje fibers from the subendocardial region of a human moderator band cut transversely. The cardiac muscle illustrated in the insert is taken from the same section as the Purkinje fibers but is located at a greater distance beneath the endocardium. The magnification of the two figures is the same. × 500. (Redrawn from preparations of Truex and Copenhaver, Am. J. Anat., vol. 80, 1947.)

kinje fibers are: their myofibrils are reduced in number and usually limited to the periphery of the fiber; they contain relatively more sarcoplasm; their nuclei are more rounded and more often in groups of two or more; they usually have a larger diameter, particularly in the peripheral branches of the system; and they generally have more glycogen. The myofibrils resemble those of ordinary fibers in that they are cross-striated and occasionally have intercalated discs. The Purkinje fibers ultimately lose their specific structure and become continuous with ordinary fibers of the myocardium (Fig. 181).

The atrioventricular node is composed of a group of irregularly arranged, branching fibers (*nodal fibers*) which have a smaller diameter and fewer myofibrils than ordinary cardiac muscle fibers. On the side of the A-V node adjacent to the A-V fibrous ring, the nodal fibers become continuous with Purkinje fibers of the A-V bundle; on the opposite side of the node, they are continuous with ordinary cardiac muscle fibers of the atrium.

Another division of the specialized system, the *sino-atrial node* of Keith and Flack, is found in the subepicardium at the junction of the superior vena cava and right atrium in the region of the terminal sulcus. This node is composed of slender, fusiform fibers which have the general structure of the fibers of the atrioventricular node. A plexus of fibers extends from the S-A node into the myocardium of the right atrium.

There is evidence to show that the stimuli for cardiac contraction are normally initiated in the sino-atrial node. Since some of the fibers of this node are

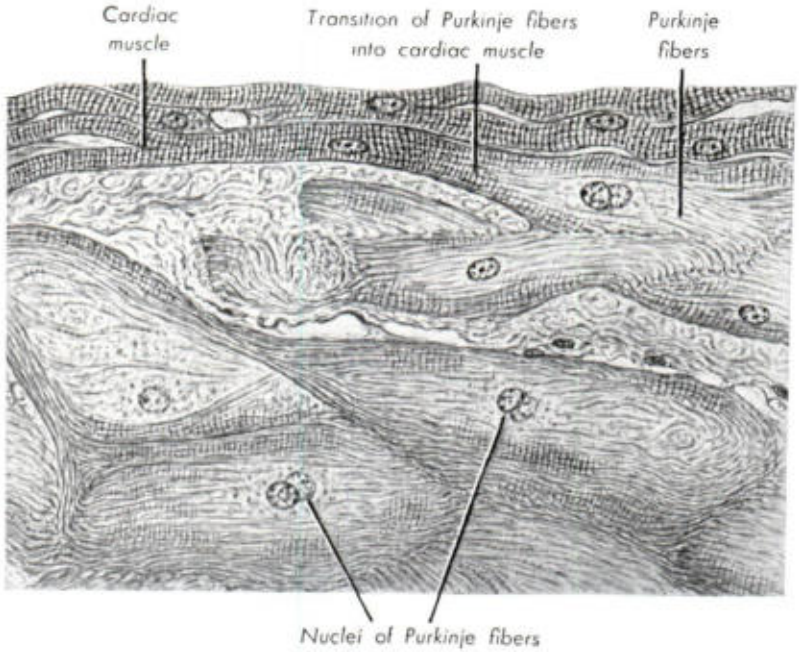


FIG. 181. Longitudinal section of a beef moderator band showing transition of Purkinje fibers into cardiac muscle fibers in the myocardial portion of the band. × 500. (Redrawn from preparation of Truex and Copenhaver, Am. J. Anat., vol. 80, 1947.)

continuous with the typical cardiac fibers of the right atrium and since, as already described, cardiac muscle fibers are continuous between the two atria, the impulse started in the sino-atrial node spreads as a contraction wave (Lewis) over the typical cardiac fibers of both atria and also into the special fibers of the atrioventricular node. Some authors claim that there are Purkinje fibers in all parts of the myocardium of the atria and that these specialized fibers conduct the impulse from the sino-atrial node to all regions of the atria and into the atrioventricular node (see review by Todd). However, no bundles or pathways comparable to those of the ventricles have been demonstrated and it seems more likely that the impulse spreads over the atria by way of typical cardiac fibers. After the impulse has reached the atrioventricular node, it is conducted across the fibrous ring by the Purkinje fibers of the bundle of His and by other accessory bundles. Then it spreads rapidly over all of the branches of the bundle and by their terminations into the typical cardiac fibers of both ventricles. If the bundle is injured or experimentally destroyed, the normal rhythm in the succession of the atrial and ventricular beats is lost.

Blood Vessels. Blood for the nutrition of the heart is supplied through the two coronary arteries. Each coronary artery sends a branch to its respective atrium and then courses between the ventricles to the apex of the heart, giving numerous branches to both ventricles. These branches subdivide again and break up in the myocardium into a rich capillary network with elongated meshes. From the myocardium capillaries penetrate the connective tissue of the epicardium and endocardium. The blood in the capillaries

is collected by veins which unite to form the cardiac veins. These empty into the right atrium by way of the coronary sinus. A number of small veins, the *venae minimae*, empty directly into the lumen of the heart, especially in the right atrium. Additional direct connections between the vessels and the heart chambers have been described (see review by Wearn, 1940).

The atrioventricular valves apparently have only a few or no vessels in the dense fibrous, central plates. The supply in the subendothelial layers differs for the different leaflets of the valve—it is richer in the aortic cusp of the mitral valve than in others. The supply is also richer in infants than in normal adults.

The bundle of His is, according to some investigators, supplied by special fine branches of the coronary arteries. The capillary net is less dense than in the ordinary musculature of the heart.

Lymphatics. The heart is richly supplied with lymph channels which form networks in the subendocardial and subepicardial tissue. In the myocardium the exact distribution of lymphatics is not definitely established. According to some, lymph capillaries occur in close association with the blood capillaries. Others maintain that lymphatics are found only in the interfascicular connective tissue, and that the lymph capillaries between the individual muscle fibers are merely tissue clefts unlined by endothelium.

Nerves. The heart receives nerve fibers from the vagus and the sympathetic division of the autonomic system. These fibers form an extensive cardiac plexus at the base of the heart. The vagus and sympathetic fibers have antagonistic functions, the former inhibiting, the latter accelerating the action of the heart.

The efferent vagus fibers do not go directly to the cardiac muscle but arborize around ganglion cells scattered in the wall of the heart, chiefly in plexuses in the subepicardium. These cells are especially numerous in the dorsal wall of the atria, in the coronary sulcus near the larger coronary vessels and at the base of the aorta and pulmonary artery. The ganglion cells send out delicate nonmyelinated nerve fibers which branch and end in terminal varicosities on the muscle fibers.

Some of the sensory nerve fibers are derived from the vagus; others have their cell bodies in the spinal ganglia of the first to the fourth thoracic nerves. The fibers of the latter group pass through the white rami, up the sympathetic trunk to the cervical sympathetic ganglia, and thence to the heart by way of the cardiac nerves.

THE LYMPH VASCULAR SYSTEM

Besides the blood vessels the body contains a collateral system of channels, lined by endothelium, which collect the tissue fluid and return it by a circuitous route to the blood stream. The fluid in these vessels is called lymph. Unlike the blood, the lymph circulates in one direction only, from the periphery toward the heart. The *lymphatic capillaries* end blindly in the tissues from which the lymph is collected (Fig. 182). They, as well as the larger vessels which conduct the lymph to the blood stream, freely anastomose along their course, gradually fuse to form fewer lymph channels, and are ultimately gathered into two main trunks, the large *thoracic duct* and the smaller *right lymphatic duct*, which open into the large veins of the neck. In the pathways of the lymph vessels there are groups of lymph nodes containing lymph sinuses in which the lymph is filtered before reaching the thoracic and lymphatic ducts.

Lymphatic capillaries and vessels occur in most tissues and organs. They

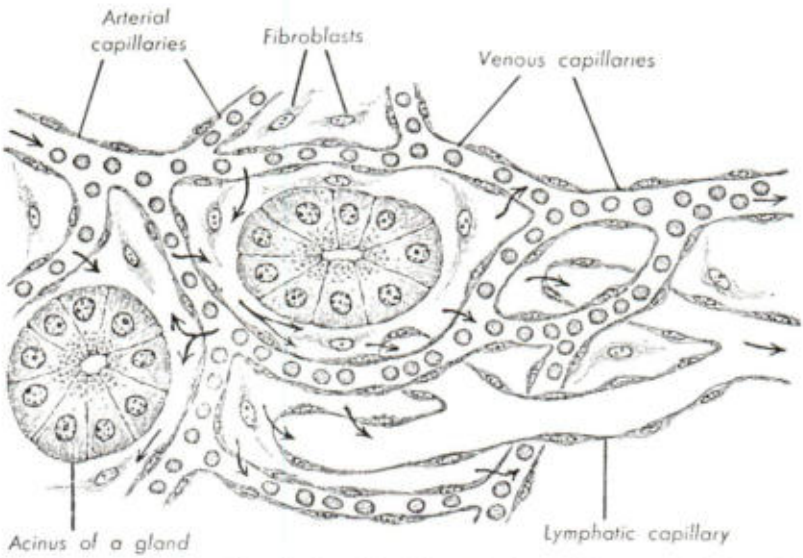


FIG. 182. Diagram showing the relationship of lymphatic capillaries to blood capillaries and to the tissue fluids around the acini of a gland. Similar relationships exist in most of the organs of the body. Arrows indicate the direction of flow of fluid leaving the arterial capillaries, permeating the connective tissue spaces as tissue fluid and reentering the blood capillaries on the venous side. The lymphatic capillaries supplement the venous capillaries in the drainage of fluid from the tissues to the circulatory system.

have not been demonstrated in the central nervous system, the bone marrow, the intra-lobular portion of the liver, the coats of the eyeball, the internal ear, and the fetal placenta.

Lymph Capillaries. The lymph capillaries, like those of the blood, are delicate tubes with walls which consist of a single layer of endothelial cells. The tubes are however larger, and instead of having a uniform diameter, vary greatly in caliber within short distances. The exceedingly thin cells have a flattened oval nucleus and a very irregular outline which can be demonstrated with silver impregnations.

The lymph capillaries anastomose to form extensive networks which run in the spaces between the blood capillaries. In the skin and in the mucous and serous membranes the lymph capillaries are usually more deeply placed than the blood capillaries. Toward the surface the lymph capillaries often end in blind swollen or looped projections.

Lymph Vessels. The lymph vessels resemble the veins in structure, but their walls are as a rule thinner than those of veins of a corresponding caliber. In the smaller lymph vessels the endothelium is surrounded by collagenous and elastic fibers and a few muscle cells. In the larger ones three coats may be distinguished—intima, media, and adventitia. The intima is composed of the endothelial lining underneath which is a delicate network of elastic fibers disposed longitudinally. The media consists mainly of circularly disposed smooth muscle fibers and a few longitudinal ones. Between the muscle fibers are relatively few delicate elastic fibrils. The adventitia, which is the thickest coat, is composed of longitudinally coursing collagenous fibers, in which are bundles of longitudinal muscle and elastic fibers.

The lymph vessels contain numerous valves which are usually arranged in pairs and whose free margins are always directed centrally, i.e., in the direction of the lymph flow. The valves are folds of the intima.

Thoracic Duct. The thoracic duct has a considerable amount of muscle tissue. The *intima* consists of an endothelial lining, a thin intermediate layer of fibroelastic tissue in which are bundles of longitudinal muscle fibers, and an internal elastic membrane composed of a longitudinal network of elastic fibers. The elastic membrane is best developed in the caudal portion of the duct, and is much thinner or missing altogether in the cervical portion.

The *media* is the thickest coat and consists of longitudinal and circular muscle bundles, the former predominating. The muscle bundles are separated by abundant connective tissue composed mainly of collagenous fibers. Elastic fibrils are scarce in the inner layer of the media, but become more numerous in the outer portion.

The *adventitia* is poorly defined. Near the media is a layer of coarse collagenous fibers, mainly longitudinally disposed, and containing considerable elastic tissue and occasional longitudinal muscle fibers. The outer layer is more finely fibrillar and merges with the surrounding areolar connective tissue.

DEVELOPMENT OF THE CIRCULATORY SYSTEM

Blood Vessels and Heart. The blood vessels and the heart begin their development separately and afterward become united. Both are derived from mesoderm, but while the heart develops within the embryo, the earliest blood vessels and blood are developed from extra-embryonic mesoderm. The earliest vessels to be formed are the capillaries. These make their appearance in the mesodermic tissue near the periphery of the area vasculosa which surrounds the developing embryo. Here groups of cells known as *"blood islands"* differentiate from the rest of the mesodermic cells, appearing in the chick by the end of the first day of incubation. The superficial cells of these islands become flattened to form the endothelium and the remaining, more central cells, develop into the primitive blood cells. These represent the earliest blood vessels. The channels, which are at first unconnected, anastomose and give rise to a network of channels which are the earliest capillaries. These develop rapidly in the area vasculosa and some of them increase in size to become arteries and veins, the smooth muscle and connective tissue of their walls being differentiated from the surrounding mesoderm. These grow toward, and finally into, the embryo where they unite with intra-embryonic vessels which have developed from mesoderm *in situ*. After a primary system of closed vessels has been established and after the embryonic circulation has been initiated, new vessels develop as outgrowths from pre-existing vessels. New vessels arise by a similar method in the adult, e.g., the outgrowth of new vessels into granulation tissue.

The *heart* in the earliest human embryos (2–3 mm.) consists of an *endothelial tube* surrounded by a layer of splanchnic mesoderm which forms the *myo-epicardial mantle*. The two layers are at first separated by a considerable space filled with a gelatinous fluid, connected to each other only by occasional protoplasmic strands. As development proceeds the two layers become firmly united, the endothelium now forming the lining of the myo-epicardial mantle. The endothelium gives rise to the endocardium. From the myo-epicardial mantle are formed both myocardium and epicardium.

Lymphatics. According to one view the lymph vessels arise originally as evaginations or buddings from veins. By further sprouting of the original anlagen the whole system of lymphatics is ultimately formed.

According to the more widely accepted second view, the lymphatics first appear as tissue clefts in the mesenchyme. These clefts elongate and become dilated by pressure of accumulated fluid and finally fuse to form the primary lymph vessels which later on establish communication with the veins.

Whatever the primary origin, it is certain that in later development, formation of lymph vessels takes place in large part by budding from the walls of already existing lymphatics.

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XIII

LYMPHOID ORGANS

LYMPHOID TISSUE

Lymphoid (lymphatic) tissue is not one of the fundamental tissue types of the body but is merely a particular variety of connective tissue. It consists of a framework of reticular cells and reticular fibers infiltrated with lymphocytes. In many parts of the body, such as the gastrointestinal and respiratory tracts, some of the so-called lymphoid tissue is not sharply outlined from the surrounding connective tissues and the lymphocytes are not packed closely together. This is often spoken of as diffuse lymphoid tissue in contrast to the denser form (*lymph nodules*) in which the lymphocytes are densely aggregated. Various gradations exist between these two types and also between lymphoid tissue and an infiltration of lymphocytes into the connective and epithelial tissues of the mucosa.

The more or less circumscribed spherical aggregations of densely packed lymphocytes forming the *lymph nodules* have been called the structural units of lymphoid tissue. Each nodule may contain a lighter staining central area, *germinal center*, where there is active proliferation of lymphocytes. The solitary nodules or follicles of the intestinal tract are examples of isolated lymph nodules. The lymph nodules may also be found in aggregations in definitely encapsulated organs having a characteristic lymphatic and blood vascular system as in the lymph nodes. Nodules may also be aggregated into less highly organized structures which are intermediate in complexity between the isolated nodules and the lymph nodes. The nonencapsulated aggregations forming the Peyer's patches of the intestine and the partially encapsulated aggregations forming the tonsils are examples of this intermediate type. The various circumscribed aggregations of lymphoid tissue forming the lymphoid organs include the lymph nodes, tonsils, thymus and spleen.

The chief characteristic common to all lymphoid organs is the presence of large numbers of lymphocytes in a stroma of reticular cells and fibers. The lymph nodes are the only lymphoid or lymphatic organs which are located in the course of lymphatic vessels; that is, they are the only ones which have both afferent and efferent lymphatic vessels. They are also the only ones which contain lymphatic sinuses and the only structures for filtering the lymph. The spleen, thymus and tonsils resemble most other organs in their relationship to lymphatic vessels. They have efferent lymph vessels draining from them but they have neither afferent lymphatic vessels nor lymphatic sinuses.

THE LYMPH NODES

The lymph nodes are variable in number but are more or less constantly

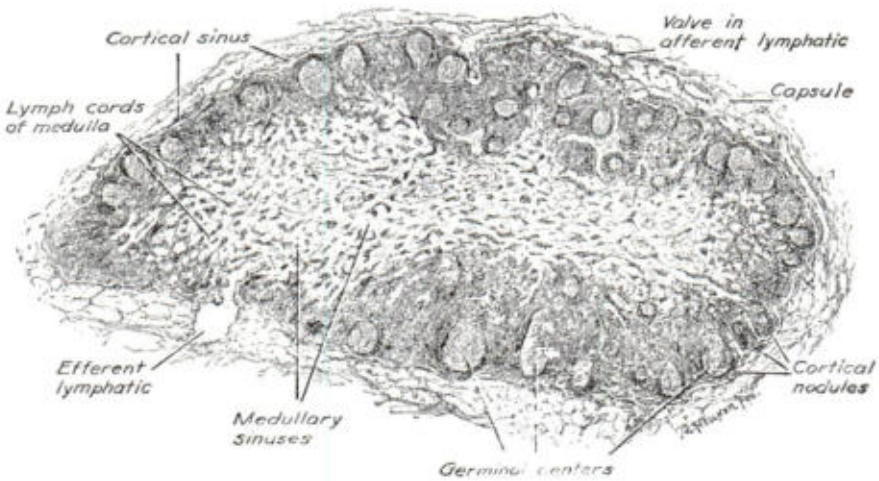

FIG. 183. Section through a human lymph node. × 10

found in certain definite regions of the body such as the mesentery, the axilla, groin, etc. Frequently they are found in groups or in a series as in the inguinal and axillary regions. They vary considerably in size ranging from very minute bodies to as much as an inch in length. They are usually oval or bean-shaped, with an indentation, the *hilum*, on one side where the blood vessels enter and leave the node. The lymphatic vessels coming from the node, the *vasa efferentia*, are also found at the hilum but the entering vessels, the *vasa afferentia*, are found at various points along the convex surface of the node.

Lymph nodes are covered by a very definite *capsule* of connective tissue. At its outer surface the capsule blends with the surrounding connective tissue and in this way the organ is attached in position. The capsule consists of rather closely packed bundles of white fibrous connective tissue and scattered elastic fibers, which are more numerous in its inner layer. A few smooth muscle fibers can be found in the capsule around the points of entrance and exit of lymphatic vessels. At the hilum there is a depression where the capsule is thickened and extends deep into the node. At various points over the surface of the node the capsule gives off septa or *trabeculae* which extend into the substance of the organ. Both the trabeculae of connective tissue and the elements of the lymphoid tissue are arranged differently in the outer or cortical and the inner or medullary part of the node.

The Cortex. In the cortex the trabeculae are more or less perpendicular to the surface and partly subdivide this region into compartments (Figs. 183 and 184) which are continuous centrally with the more irregularly arranged anastomosing subdivisions of the medulla. The cortical compartments also communicate laterally with each other through spaces between the trabeculae. The degree of development of the trabeculae and separation into compartments varies in nodes taken from different parts of the body and in nodes of different animals. In some of the lower animals (e.g., ox) the trabeculae are more highly developed and more definitely mark off compartments than in man.

In the cortical compartments, most of

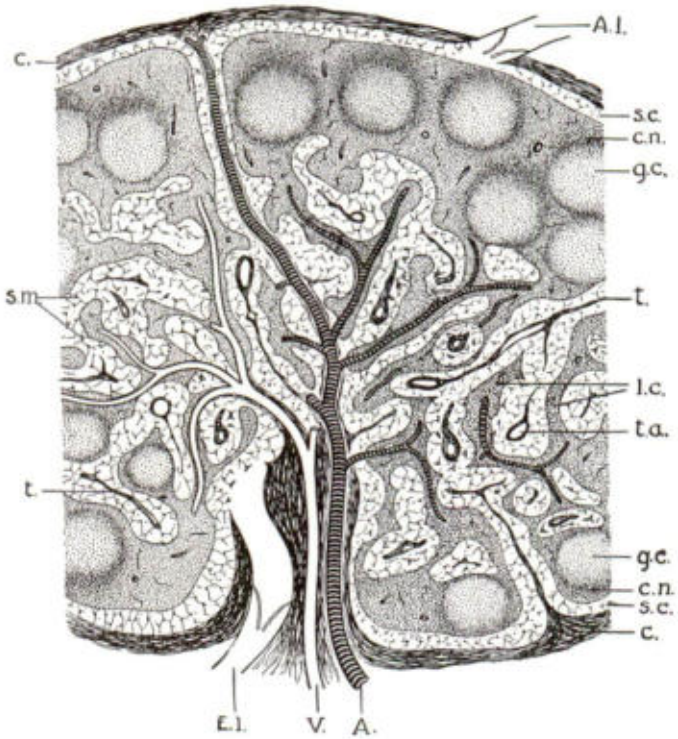

FIG. 184. Diagram of a lymph node. *A*, Artery, entering at hilum; *A.l*, afferent lymphatic with valve; *c*, capsule; *c.n*, cortical nodules; *E.l*, efferent lymphatic with valve; *g.c*, germinal centers (secondary nodules); *l.c*, lymph cords of medulla; *s.c*, lymph sinus of cortex; *s.m*, lymph sinuses of medulla; *t*, trabeculae; *t.a*, artery in trabecula; *V*, vein, leaving node at hilum. (Modified after a drawing by M. Heidenhain, from Heudorfer.)

the lymphocytes are closely packed together to form *cortical nodules* or *primary nodules* which are continuous centrally with the cords of lymphoid tissue found in the medulla. When the cortical areas are well separated by trabeculae the lymph nodules are more or less spheroidal or pear-shaped as shown in Figure 188, *D*, but when trabeculae are not well developed the cortical nodules are more irregular in shape and are frequently continuous with each other laterally, forming a more continuous mass of lymphoid tissue as shown in Figures 183 and 184.

The cortical nodules often contain lighter staining central areas (Figs. 184 and 185) which are called *germinal centers* because lymphocytes are formed in them. It should be noted, however, that proliferation of lymphocytes is not restricted to these regions and that no germinal centers are found in lymph nodes until after birth. The pale centers are also called *secondary nodules* because they are spherical zones within the primary nodules. They usually contain many large, undifferentiated lymphocytes which have pale, vesicular nuclei. On account of the active proliferation of new cells in the germinal centers, surrounding cells are pushed outward and a darker staining zone is produced immediately surrounding each center where small lymphocytes are

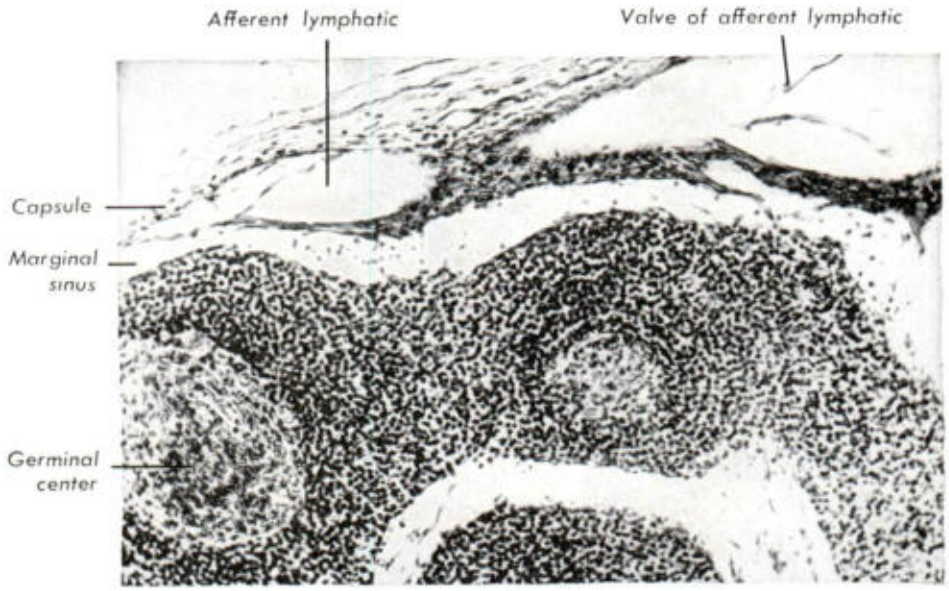

FIG. 185. Portion of cortex of human lymph node. Photomicrograph. × 260. (After Petersen.)

particularly abundant and more closely packed together than elsewhere in the cortical nodules. Under certain pathological conditions, some of the pale centers contain numerous free macrophages; such areas have been called *reaction centers*.

The cortical nodules are separated from the capsule and trabeculae by channel like spaces, *lymph sinuses*, through which the lymph circulates. The peripheral or marginal sinus (Figs. 184, 185), between the capsule and the nodules, receives the lymph from the afferent lymphatics. From the peripheral sinus the lymph flows centrally through sinuses passing around the nodules to enter the sinuses of the medulla.

Although the cortical tissue is usually found completely surrounding the medulla except at the hilum, it varies considerably in thickness. At times it may extend for a considerable depth into the node while at other times medullary substance may approach more closely to the surface. Frequently the cortical nodules appear to form a single layer at the periphery but where the cortex is thickened they may be found in layers and be more deeply situated.

Medulla. The cytological components of the medulla and cortex are very similar except for the difference in arrangement. Contrasted to the more regular arrangement in the cortex, the trabeculae in the medulla are very irregularly arranged and anastomose frequently. The aggregated masses of lymphocytes do not form nodules as in the cortex but anastomosing cords, *lymph cords*, which run through the communicating divisions outlined by the meshwork of trabeculae (Figs. 184 and 186). Lymph sinuses, which are relatively broad and numerous in the medulla, separate the lymph cords and trabeculae

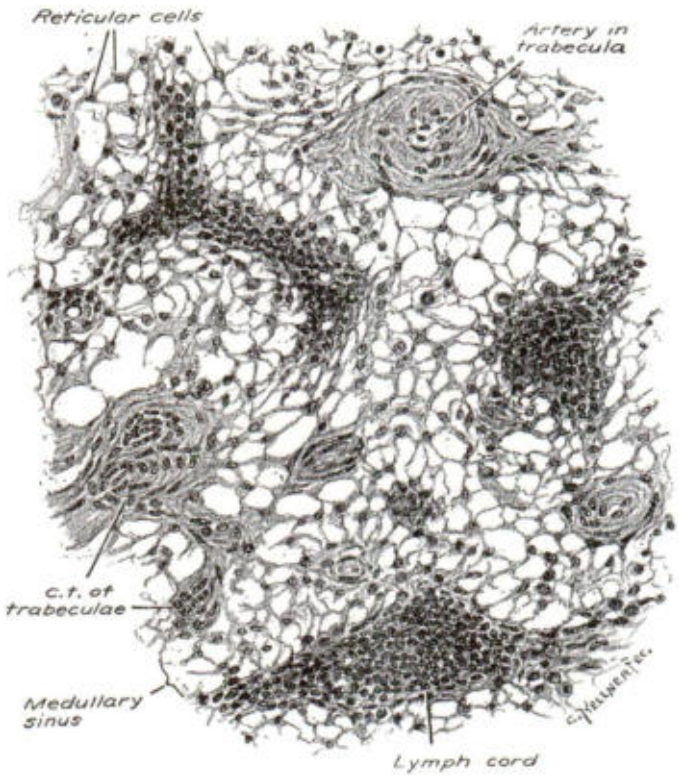

FIG. 186. A portion of the medulla of a human lymph node showing arrangement of connective tissue (*c.t.*) trabeculae, lymph cords, and medullary sinuses. × 200.

at all points. The relationship of these three parts—dense lymphoid tissue, sinuses and trabeculae—is therefore similar throughout the node.

Components of the Lymphoid Tissue. The lymphoid tissue of the node consists of reticular cells and fibers which form the framework or stroma and free cells contained in the meshwork. The reticular meshwork (Fig. 186) extends throughout the node although it varies in density in different parts. Its reticular fibers are continuous with the collagenous fibers at the trabeculae. Its reticular cells form a syncytium and have pale staining nuclei with very little chromatin (Fig. 187). They form a part of the reticulo-endothelial system (Chapter V). They readily take up vital dyes such as trypan blue and normally may be found with engulfed materials such as degenerated lymphocytes. They may become detached from the syncytium and form free macrophages. The reticulum also contains some cells which remain free of phagocytosed material, even after prolonged vital staining (Maximow). These cells are supposed to be less differentiated than the more numerous phagocytic cells of the reticulum. By proliferation, the undifferentiated cells give rise to daughter cells which may follow any one of the following courses: some remain undifferentiated as a reserve line, others differentiate into phagocytic cells, and still others into lymphocytes.

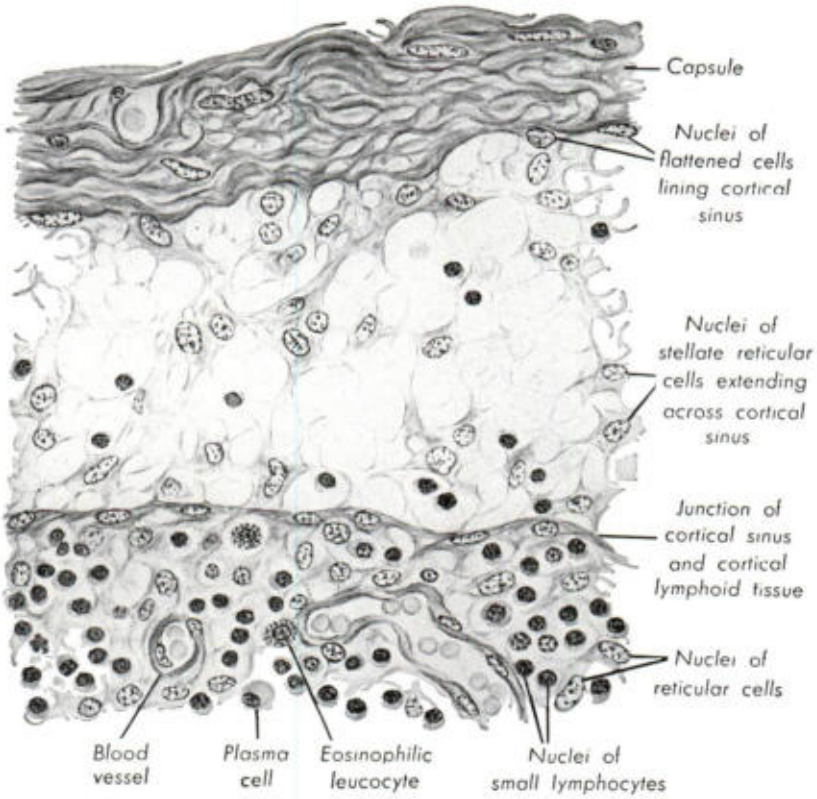

FIG. 187. A portion of the cortex of a human lymph node showing cortical (marginal) lymphatic sinus and adjacent dense cortical lymphoid tissue. × 725.

The majority of the free cells are lymphocytes. Free macrophages and plasma cells are also fairly numerous. Various types of granulocytes which have migrated from the blood vessels are found in small numbers. Occasionally a few red blood cells may be found which have escaped from the blood vessels by diapedesis. Usually they are soon engulfed by the macrophages.

The lymphocytes of the node consist of small, medium-sized and large forms. These same types are also found in the other lymphoid organs and are illustrated in a germinal center of the spleen (Fig. 203). The small lymphocytes are the most numerous and are the typical lymphocytes of adult blood and lymph. They are found throughout the node but are especially numerous and closely packed at the periphery of the germinal centers. The medium-sized lymphocytes contain more cytoplasm than the small ones and their nuclei contain less chromatin and are clearer staining. In structure as well as in size they are intermediate between the small and large lymphocytes. The large lymphocytes are not as numerous as the other forms. They are found throughout the node but are most numerous in the germinal centers. They may be as much as 15 or 20μ in diameter. They have a wider zone of cytoplasm which is usually more basophilic than that of the small lymphocyte on account of its greater content of RNA. The

nucleus is quite different from that of the small lymphocyte; it is larger, more vesicular, clearer staining, and contains one or more large nucleoli. The medium-sized and large lymphocytes are frequently found in stages of mitosis. In addition to the proliferation of pre-existing lymphocytes which occurs abundantly in the germinal centers and throughout the node, lymphocytes also form in the germinal centers from the undifferentiated cells of the reticulum.

The germinal centers undergo constant changes, alternating between active and resting phases. During the resting phase they may be difficult to locate since they become smaller and less clearly outlined from the remainder of the cortical tissue. When a new active phase begins some of the undifferentiated cells of the reticulum proliferate and lose their connections with the syncytium. These primitive free cell types gradually differentiate into large and medium-sized lymphocytes which undergo frequent divisions and eventually give rise to the small lymphocytes.

The cell which has been described as the large lymphocyte of the lymph node is frequently called a "lymphoblast." Under normal conditions this cell is usually found only in the blood-forming organs but under pathological conditions, e.g., lymphatic leukemia, it may be found in considerable numbers in the circulating blood. It has been called a "lymphoblast" since, in accordance with the dualistic and polyphyletic theories of blood cell origin, it is the specific stem cell for the small lymphocyte and represents the earliest cell in the lymphocyte line, which has become definitely restricted in potencies for lymphocyte differentiation. On the other hand, it has been found that the large lymphocyte is not necessarily the specific stem cell, since undifferentiated germinal center reticulum may give rise to medium-sized lymphocytes that proliferate and form the small forms without passing through the large lymphocyte stage. Furthermore, in accordance with the unitarian theory of blood cell development neither the large lymphocyte nor any of the cells of the lymphocyte series are restricted to lymphocyte development; all lymphocytes are said to retain unrestricted potencies for blood cell development and to form the hemocytoblasts of all blood-forming organs.

Lymphatic Vessels and Sinuses. The afferent lymphatic vessels pierce the capsule on the convex side of the node and open into the marginal or peripheral sinus. The lymph circulates slowly through the sinuses of the cortex and medulla which afford a greatly enlarged area for the circulating lymph as compared with the afferent vessels. From the medullary sinuses a plexus of vessels arises which penetrates the connective tissue of the hilum and forms the efferent lymphatic vessels. The efferent lymphatic vessels are usually wider but less numerous than the afferent vessels. The afferents possess valves which open toward the node while the efferent lymphatics have valves which open outward from the node.

In the walls of the sinuses there are flattened cells (Fig. 187), sometimes described as endothelium, which become continuous at the periphery of the node with the endothelial cells lining the afferent and efferent lymphatic vessels. Like the cells lining the venous sinuses of the spleen they differ from the ordinary endothelial cells of blood and lymph vessels. They have many similarities to reticular cells and are classified by most authors as reticular cells rather than as endothelium. They are associated with reticular fibers and are joined with the syncytium of reticular cells of the lymph cords and nodules and also with the cells and fibers which bridge across the sinuses. They are very phagocytic and belong to the reticulo-endothelial system (Chapter V).

Blood Vessels. An artery enters the lymph node at the hilum and gives off

some branches which go directly to the medullary cords of lymphoid tissue while other branches enter the trabeculae (Fig. 184). The branches entering the lymph cords, giving off capillaries along the way, continue into the cortex where an arterial branch penetrates each cortical nodule and breaks up into diverging capillaries to form a plexus around the germinal center and the peripheral part of the nodule. The arteries entering the trabeculae supply the trabecular connective tissue and continue into the capsule. They also give off branches which cross the lymph sinuses to join in the supply of the lymph cords and nodules. From the capillaries the blood is collected into veins which follow the same general course as the arteries and leave the node at the hilum.

Nerves. The nerves, which are not abundant, enter the node at the hilum and accompany the blood vessels. Some of them terminate in the trabeculae and capsule while others form perivascular networks which follow the vessels into the lymphoid tissue.

Functions. One of the primary functions of lymph nodes is the production of lymphocytes. The lymph of the efferent vessels contains many more lymphocytes than that of the afferent vessels. The smallest lymphatic vessels, whose lymph has not yet passed through any lymph nodes, contain very few lymphocytes. The development of new lymphocytes from undifferentiated cells in the germinal centers as well as the active proliferation of existing lymphocytes in all parts of the node has already been described. Many of the newly formed lymphocytes pass into the lymph sinuses to be carried along by the lymph and out the efferent vessels. Others, mostly small lymphocytes, go directly into the blood by passing through the endothelial walls of the capillaries of the lymph nodules.

As already pointed out, the lymph nodes contain numerous phagocytic reticular cells and free macrophages. In this way the nodes act as filters to remove degenerating cells and other particulate matter from the lymph. The bronchial lymph nodes are good examples of this function. Inhaled carbon particles are removed in the lungs and eventually reach the bronchial lymph nodes where they are found accumulated in phagocytic cells, especially in the medullary sinuses. Eventually such accumulations become very extensive and blacken the entire node.

The lymph nodes also appear to play an important part in the formation of antibodies and in the production of immunity.

Development. The first indications of lymph node formation are found in the axilla and groin, toward the end of the third month of development. In connective tissue closely associated with plexuses of lymphatic vessels, condensations of mesenchymal cells occur around capillary loops as shown in Figure 188, *A*. Since the cellular condensation is very vascular from the beginning, it is difficult to determine with certainty whether the lymphocytes migrate from the blood vessels or whether they develop in situ from the mesenchymal cells, although the latter is now the generally accepted manner of origin. In addition to lymphatic plexuses which are in continuity from the beginning with lymphatics that become the afferent and efferent vessels there are apparently other blind or isolated lymph spaces which develop *in situ* in the mesenchyme. The isolated lymph spaces and the different parts of the plexuses gradually all fuse together to form lymph sinuses. The marginal or peripheral sinus forms first (Fig. 188, *B*). Meanwhile the lymphocytes have been proliferating rapidly, the artery has formed new branches and more capillary loops, and the node has increased rapidly in size. From the marginal sinus subdivisions penetrate centrally into the lymphoid tissue as central sinuses (Fig. 188, *B*), which subdivide and eventually form a system of anastomosing sinuses throughout the node (Fig. 188, *C*, *D*). The connective tissue outside the marginal sinus becomes thickened to form the capsule. From the capsule projections of

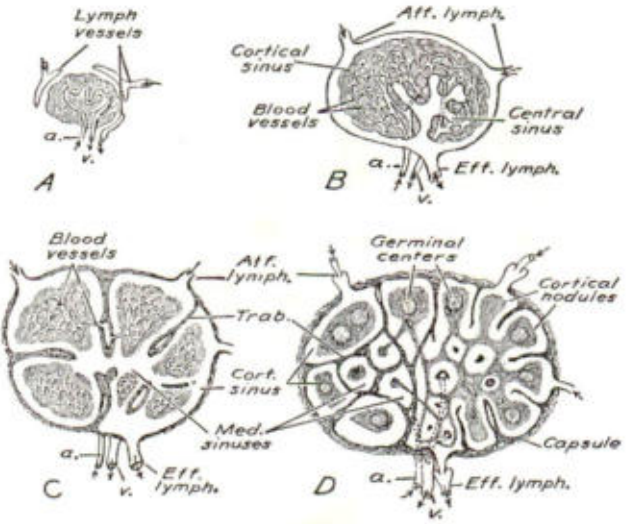

FIG. 188. Diagrams illustrating stages in the development of a lymph node. *A*, Early stage, showing aggregation of lymphoid tissue around a capillary loop and surrounded by a plexus of lymphatic vessels; *B*, *C*, and *D*, Later stages showing development of sinuses, etc. *a*, Artery; *Aff. lymph*, afferent lymphatics; *Cort. sinus*, cortical sinus; *Eff. lymph*, efferent lymphatics; *Med. sinus*, medullary sinuses; *v*, vein. (Slightly modified after Braus.)

connective tissue extend into the marginal sinus and later into the other sinuses to form the trabeculae. The trabeculae penetrate the central parts of the sinuses and always remain separated from the lymphoid tissue by the sinuses.

HEMOLYMPH NODES

In certain animals, structures have been described which are very similar to lymph nodes except that afferent and efferent lymphatic vessels are absent and the sinuses contain blood instead of lymph. True hemal nodes of this type occur in the sheep. In the pig, hemolymph nodes have been described which have sinuses connected with both lymphatic and blood vessels and so they appear to be intermediate between lymph nodes and the hemal nodes of sheep. In man, the occurrence of organs of this type has been questioned and seems very doubtful. Normal lymph nodes usually contain a few red blood cells, some of which may have been brought in by the afferent lymphatics while others probably enter from the blood vessels within the node by diapedesis. It is true that nodes may also be found in man with considerable numbers of red blood cells but there have been no clear descriptions of any connections between the sinuses and the blood vessels. It seems very probable that they are merely modified lymph nodes and that the presence of an unusual number of erythrocytes may be the result of hemorrhage from vessels either within the node or in the neighborhood of the afferent lymphatics, and in pathological conditions they may also be due to formation of red blood cells within the node.

THE TONSILS

The Palatine Tonsils. The palatine tonsils are paired, oval-shaped bodies located in the oropharynx between the glossopalatine and pharyngopalatine arches. They consist of dense accumu-

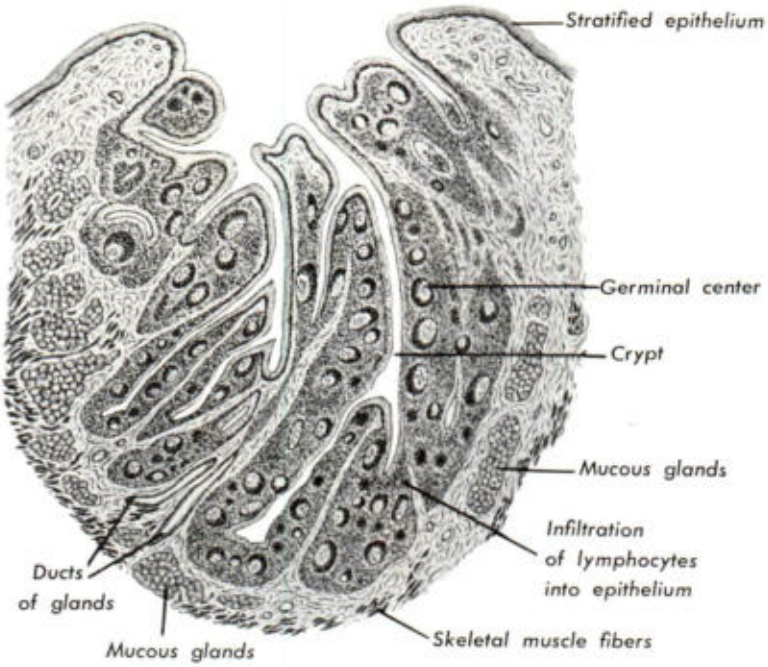

FIG. 189. Section through the palatine tonsil of man. × 9

lations of lymphoid tissue in the connective tissue of the mucosa. They are covered on their free surface by a stratified squamous epithelium which is continuous with the epithelium of the rest of the pharynx. This epithelium has the same structure as elsewhere in the pharynx, flat surface cells, beneath which are irregular cells, while the deepest cells are cuboidal or more or less columnar and rest upon a basement membrane. A very thin layer of fibrous connective tissue with papillae is usually found between the basement membrane and the underlying lymphoid tissue. At various places on the surface of the tonsil deep indentations or pockets occur. These depressions, ten to twenty in number, are known as the *crypts* of the tonsil (Fig. 189), and are lined by a continuation of the surface epithelium which becomes thinner as the deeper part of the crypt is reached. Passing off from the bottoms and sides of the main or primary crypts are frequently several secondary crypts, also lined with the same type of epithelium.

Surrounding each crypt there is a zone of varying thickness consisting of a rather diffuse lymphoid tissue in which are embedded *nodules* of compact lymphoid tissue similar to those of the lymph nodes. The nodules are frequently more or less fused together. The nodules, like those of lymph nodes, may contain *germinal centers* which consist of a lighter staining central area and a surrounding zone of more closely packed cells.

There is a *capsule* of connective tissue present over the attached or basal surface of the lymphoid tissue which is firmly adherent on the one side to the tonsillar tissue and on the other to the surrounding structures from which it separates the tonsils. From the capsule there are *septa* of loose connective tissue which separate

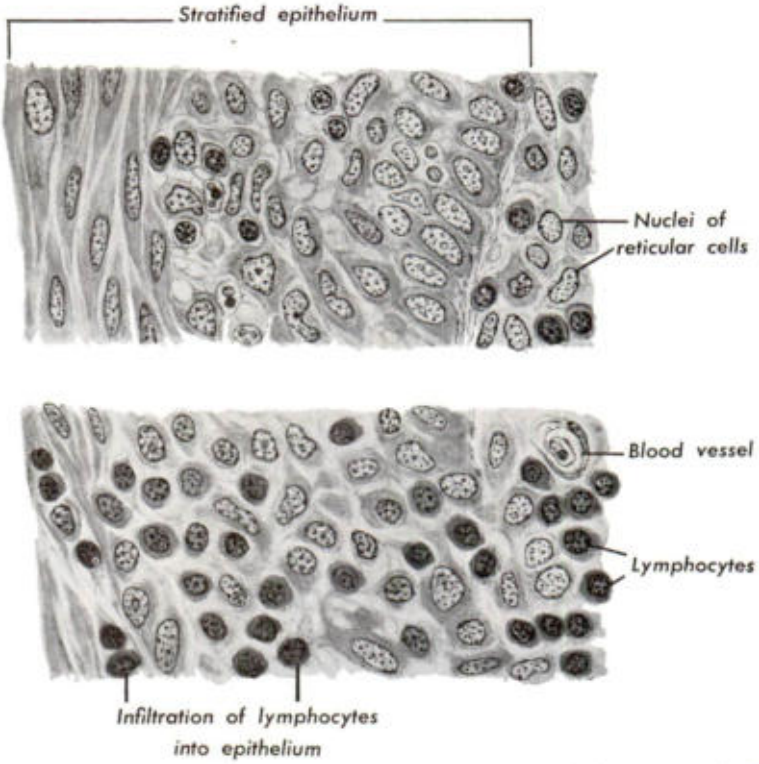

FIG. 190. Two fields from different depths along the crypts of a human tonsil. The squamous epithelial cells, facing the lumen of the crypt, are toward the left. The *upper* figure shows only slight lymphocytic infiltration of the epithelium. The *lower* figure shows an extensive infiltration which makes it difficult to distinguish the junction of epithelium and underlying connective tissue. × 932.

the various crypts with their surrounding zones of lymphoid tissue from one another. Infiltrated into this connective tissue are various sized lymphocytes, plasma cells, mast cells and frequently neutrophil leucocytes.

At various points on the surface of the tonsil, and especially in the crypts, there occurs what is known as *lymphoid infiltration of the epithelium* (Fig. 190). This consists of an invasion of the epithelium by the underlying lymphocytes. It varies from only a few lymphocytes scattered in the epithelium, to an almost complete replacement of epithelium by lymphoid tissue. In this way the latter reaches the surface and lymphocytes are discharged upon the surface of the tonsil and into the crypts. These cells probably form the bulk of the so-called salivary corpuscles. Surface regions of tonsillar tissue may also contain cells with polymorphic nuclei (Kingsbury, 1945). These cells differ from the blood neutrophiles since their cytoplasm usually lacks granules. They may arise from lymphocytoid (mononuclear) cells either through partial nuclear fragmentation or through differentiation from a "blast" stage. In inflammation, the tonsillar tissue also contains numerous polymorphonuclears which emigrate from the blood.

Small mucous glands, similar to those

found in other parts of the pharynx, are numerous in the connective tissue adjacent to the tonsil. The bodies of these glands are separated from the tonsils by the capsule. Their excretory ducts usually open on the free surface, but occasionally they may open into the tonsillar crypts.

The Lingual Tonsils. These are spherical aggregations of lymphoid tissue situated on the dorsum and sides of the back part of the tongue between the circumvallate papillae and the epiglottis (Fig. 231). They contain rather wide-mouthed deep crypts which may be branched and which are lined with a continuation of the surface stratified squamous epithelium. As in the palatine tonsils, each crypt is surrounded by an aggregation of lymph nodules containing distinct germinal centers, or secondary nodules. In most crypts there is marked lymphoid infiltration of the epithelium with free salivary corpuscles. Ducts of some of the mucous glands of the tongue frequently open into the crypts (Fig. 232).

The Pharyngeal Tonsil. There is a median aggregation of lymphoid tissue in the posterior wall of the nasopharynx which forms the pharyngeal tonsil. The lymphoid tissue is similar to that of the palatine tonsils. The epithelium over the free surface, as is characteristic for the nasopharynx and other respiratory passages, is largely pseudostratified columnar ciliated. There are patches of stratified squamous epithelium, which become more numerous in the adult. Hypertrophy of the pharyngeal tonsil, with consequent obstruction of the nasal openings, is common especially in children, constituting what are known as adenoids.

Blood Vessels of Tonsils. They have a distribution similar to those of the lymph nodes but enter the organ along its entire attached side and not at a definite hilum.

Lymphatic Vessels. The tonsils have no afferent lymphatic vessels and do not have any lymph sinuses. Surrounding the outer surface of the lymphoid tissue there are plexuses of lymph capillaries which form the beginnings of efferent lymphatic vessels. The tonsils, therefore, unlike the lymph nodes which are situated in the course of lymphatic vessels, are situated at the beginnings of lymphatic vessels.

Nerves. The nerves of the palatine tonsils are derived from the glossopharyngeal nerve and from the sphenopalatine ganglion and enter the organ along its attached side.

Functions. The mitoses in the germinal centers show that the tonsils participate in the development of lymphocytes. No other function has been definitely established. The lymphoid tissue of the tonsils, along with that of other parts of the digestive tract such as the Peyer's patches of the intestine, is believed to aid in some manner in the destruction of microörganisms and to have a local detoxifying action. The tonsils apparently aid in the protection of the body. On the other hand, the deep tonsillar crypts which are often imperfectly lined by epithelium can serve as regions where microörganisms invade and proliferate leading to general infections.

Development. The palatine tonsils make their first appearance during the third month of development. Evaginations of entoderm begin to grow down into the underlying mesenchyme at the site of the second entodermal pouch and at the same time, there is a subepithelial condensation of mesenchyme. The cells of the inner part of the mesenchymal condensation become arranged around the epithelium of the evaginations, and gradually develop the reticulum and lymphocytes of the lymphoid tissue, while the entodermal evaginations become the crypts of the tonsil. At the outer part of the mesenchymal condensation, fibrous connective tissue develops to form the capsule separating the lymphoid tissue from the underlying structures.

The lingual and pharyngeal tonsils begin their development during the later months of fetal life. In the pharyngeal tonsil, definite nodules appear at about the time of birth or during the first or second year. In the lingual tonsil the nodules are not fully formed until the fifth or sixth year.

THE THYMUS

The thymus varies in size and development with the age of the individual. It grows very rapidly until the end of the second year, after which there is a much slower growth and only a slight increase in weight until about the twelfth to the fourteenth year. Following this the thymus begins to decrease in size (undergoes "age involution") and gradually becomes replaced by fat and connective tissue until in old age very little thymic tissue is left. (Compare Figures 191 and 192.)

As a rule the thymus consists of two lobes which are closely applied to each other and joined in the midline by connective tissue. The lobes are surrounded by a connective tissue *capsule* which gives off numerous *trabeculae* to subdivide each lobe into many lobules (Fig. 191). Each lobule consists of a *cortex* and *medulla*. In individual sections, some of the lobules appear to be completely separated from adjacent lobules and to have the medulla entirely surrounded by the cortex. From a study of serial sections

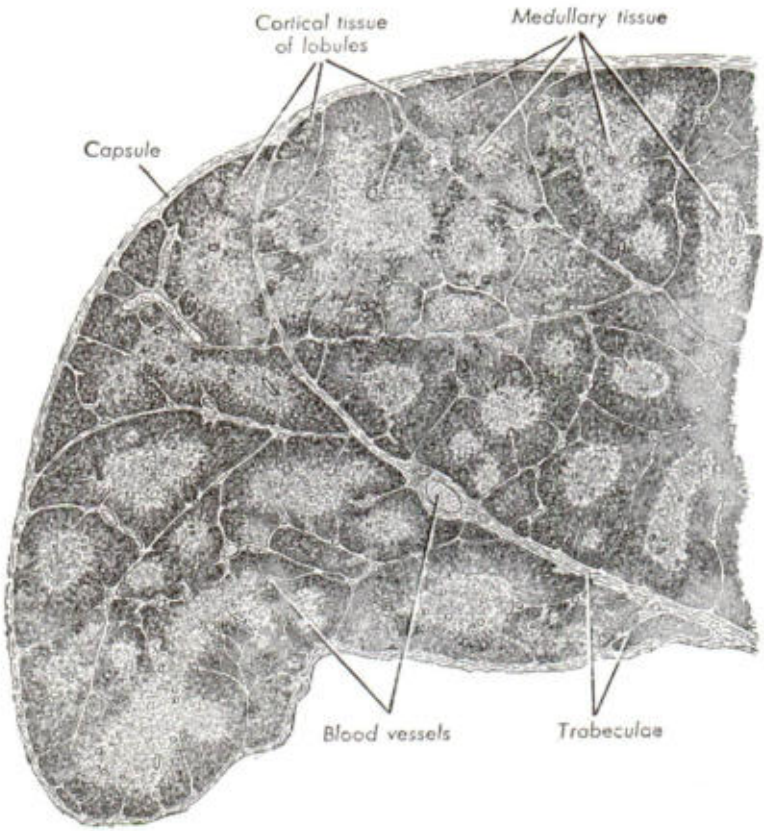

FIG. 191. Section through a part of the human thymus at birth. × 12

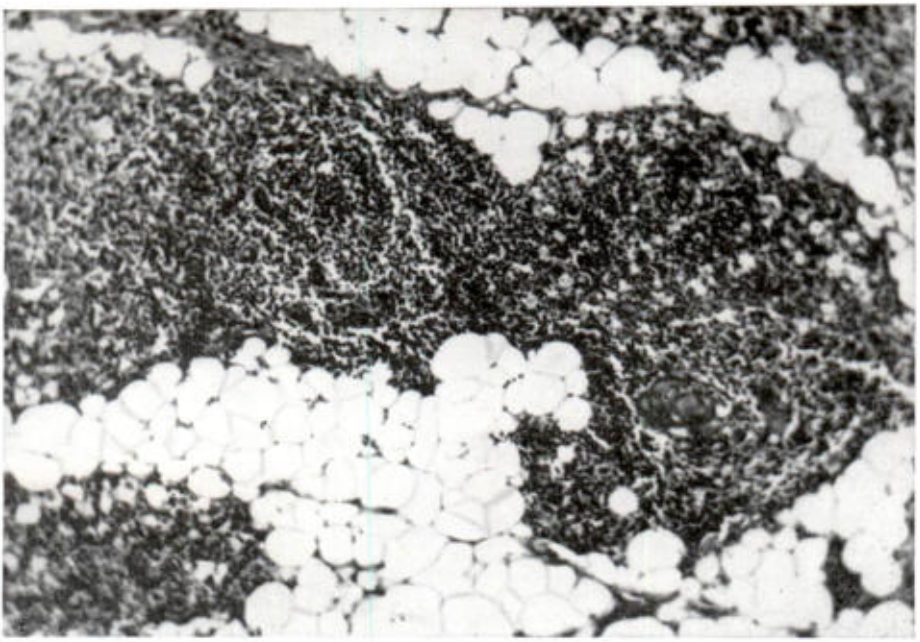

FIG. 192. Photomicrograph of a portion of thymus from a man 36 years of age. Note that portions of the organ have undergone involution and have been replaced by fat. × 110.

it has been found that, at least until involution is well advanced, the lobules are only partially separated and that, by means of medullary cords, the medullary tissue is continuous from one lobule to another throughout each lobe.

The cortex consists of a compact, dense lymphoid tissue in which the reticular syncytium is obscured by the abundance of closely packed free cells. These cells, sometimes called thymocytes, seem to be identical with small lymphocytes. The lymphoid tissue, unlike that of the lymph nodes, is not arranged in nodules, and although mitoses occur, there are no germinal centers. In the medulla the thymocytes or lymphocytes are less numerous and not so closely packed together. The medulla contains a number of spherical or oval bodies which are composed of concentrically arranged cells (Fig. 193) and known as *thymic corpuscles* (*Hassall's corpuscles*). They are characteristic of the thymus. Their average diameter in the fully developed thymus is from 20 μ to 50 μ, but they vary considerably in size and much smaller as well as much larger corpuscles may be found. The cells composing the corpuscles are polygonal or flattened, are concentrically arranged, and are frequently hyalinized and take a bright red stain with eosin-hematoxylin. In the center of the corpuscle the cells may be completely degenerated, while at the periphery some of the cells usually retain protoplasmic connections with cells of the surrounding reticulum.

The reticular connective tissue of the thymus differs in some respects from that of the other lymphoid organs. Reticular fibers are sparse and are found chiefly in the connective tissue around blood vessels. The reticular cells have an unusual embryonic origin, arising from entoderm rather than from mesenchyme.

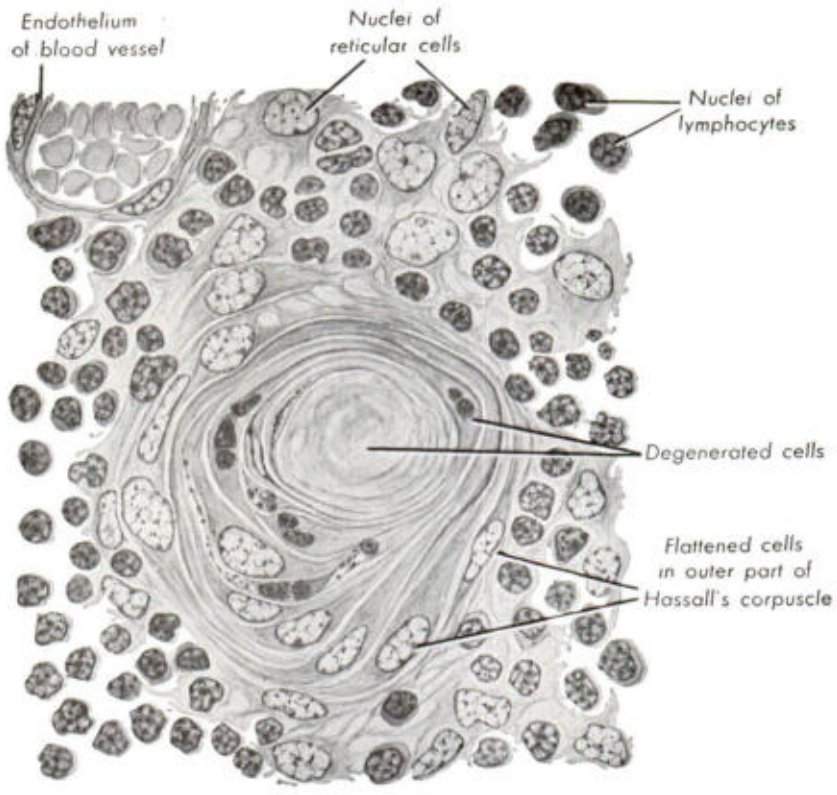

FIG. 193. Portion of medulla of thymus at birth showing Hassall's corpuscle. × 1134.

They do not phagocytose injected colloidal dyes as readily as other reticular cells do. In tissue culture preparations from the thymus, the reticular cells reassume epithelioid characteristics in keeping with their entodermal origin.

Blood Vessels. The blood vessels supplying the thymus are branches of the internal mammary and inferior thyroid arteries. The larger vessels follow the connective tissue septa and give off branches which penetrate the cortex of the lobules and break up into capillary networks in the cortex and medulla. The medulla is more vascular than the cortex. The capillaries collect into thin walled veins in the medullary tissue which converge to form larger veins that pass into the connective tissue septa to accompany the arteries. They empty into the left innominate and thyroid veins.

Lymphatics. There are no lymph sinuses. Lymphatic vessels are not found penetrating the lymphoid tissue. They arise around the lymphoid tissue of the lobules and fuse to form larger vessels which accompany the arteries in the connective tissue septa.

Nerves. The nerves are branches of the vagus and cervical sympathetics. They are distributed mainly to the walls of the blood vessels. A few fine fibers, terminating freely in the lymphatic tissue of the cortex and medulla, have been described.

Functions. Apparently the thymus is able to produce lymphocytes, but otherwise its functions are not clear. It is interesting to note that there are in-

terrelationships of some kind between the thymus and the thyroids, adrenals, and gonads. As already stated, the thymus reaches its greatest development during childhood and begins to undergo involution as the reproductive organs become developed at the time of puberty. When the gonads are removed the thymic involution is delayed. Removal of the thyroid hastens involution, while removal of the adrenals, on the other hand, is said to not only delay involution but also to bring about thymic regeneration. Removal of the thymus is not followed by any definite symptoms.

Development. The thymus arises as a paired entodermal outgrowth from the median and ventral portions of the third pair of pharyngeal pouches. Each outgrowth contains a narrow cleft-like lumen at first, but this is soon obliterated by proliferation of the epithelial cells. In the thymus most of the reticular cells are derived from epithelial cells and not from mesenchyme as in the other lymphoid organs. Transformation of epithelial cells into reticulum begins at about the end of the second month in the central portion of the outgrowth. In this transformation the epithelial cells become more loosely arranged and form protoplasmic connections. Small thymic cells similar to small lymphocytes also appear at about this same time. Different theories have been held with regard to the origin of these cells. Some authors believe that they arise from epithelial cells and that they are not identical with true lymphocytes. Many investigators, on the other hand, believe that the thymocytes are true lymphocytes and find that they arise, not from entodermal epithelial cells, but by an immigration of cells into the gland from the surrounding mesenchyme. The latter appears to be the more generally accepted view. In embryos of 50–60 mm. length, the medulla begins to become differentiated from the cortex as the lymphocytes become more densely aggregated in the peripheral regions of the gland and less numerous at the center. At about this same time the lobules begin to form and become separated by the septa of connective tissue. As already stated, the connective tissue septa subdivide the cortical tissue but do not completely subdivide the medullary tissue.

Hassall's corpuscles make their first appearance during the first half of fetal life. At first they are few in number and small in size, but they increase rapidly in diameter, and new ones continue to form until the time when thymic involution begins. The Hassall's corpuscles apparently are formed from hypertrophic and degenerating entodermal reticular cells.

THE SPLEEN

The spleen is the largest lymphoid organ in the body. Unlike the lymph nodes, however, it has no afferent lymphatic vessels and no lymph sinuses; its sinuses, like those of hemolymph nodes, are filled with blood instead of lymph.

Except at the hilum, the spleen is covered by a serous membrane, the peritoneum. Beneath this is a *capsule* of fibrous tissue containing numerous elastic fibers and some smooth muscle. From the capsule (Figs. 194 and 195), strong connective tissue septa, similar to the capsule in structure, extend into the interior of the organ. These branch and unite with one another to form very incomplete anastomosing chambers. At one point on the surface of the spleen known as the *hilum* a deep indentation occurs. This marks the entrance and exit of the splenic vessels. Accompanying the vessels broad strands of capsular tissue extend deep into the organ where they radiate and subdivide, to form with the smaller trabeculae which extend in from other parts of the capsule, the connective tissue framework of the organ. Due to the abundance of elastic tissue together with some smooth muscle, and the arrangement of fibrous connective tissue in wavy bundles, the organ is distensible and capable of considerable change in volume.

The spleen is divided into lobules, about 1 mm. in diameter, in accordance with the distribution of the trabeculae and blood vessels. Each lobule is supplied by a central artery which runs along its axis and is drained by veins which enter the trabeculae at the periph-

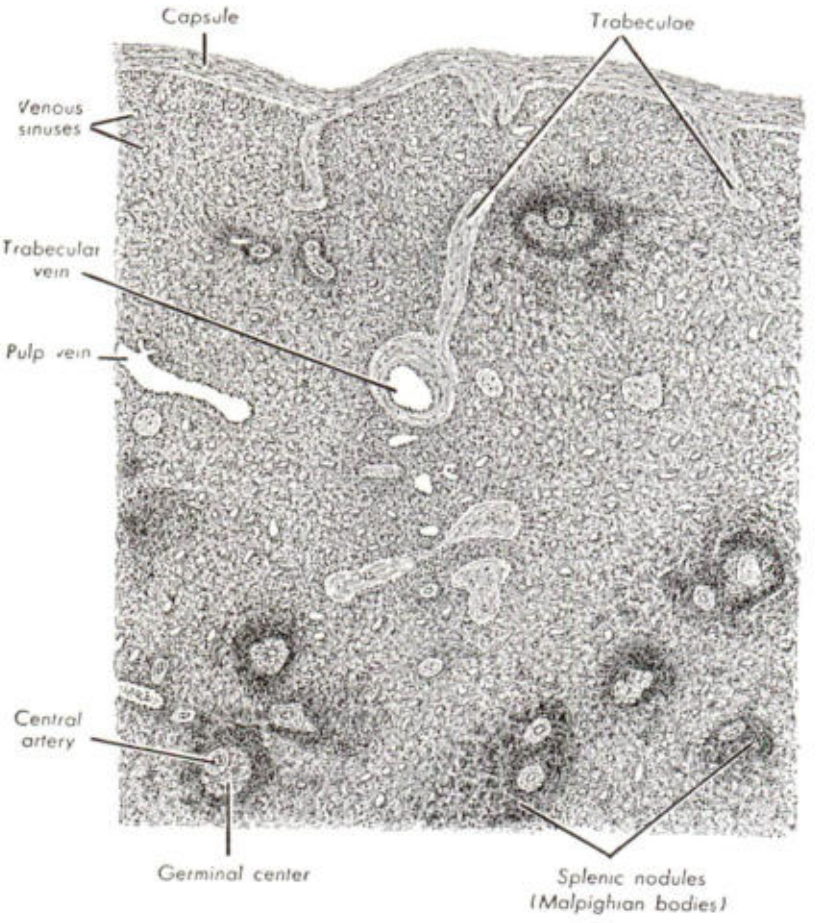

FIG. 194. Section through a portion of human spleen to show general topography. × 40.

ery of the lobule to become the trabecular or interlobular veins. Since the artery and veins are in different planes and have numerous branches only a part of this general plan of the vascular arrangement can be seen in any one section. The division of the spleen into lobules appears to be somewhat artificial, for the lobules are only very imperfectly separated and outlined by trabeculae; over a considerable part of its surface a lobule is in continuity with adjacent lobules.

The spaces within the connective tissue framework shown in Figure 195 are filled in with a soft, sponge-like tissue known as the *splenic pulp*. On the basis of color differences seen in fresh preparations, different regions of the splenic pulp have been named *red pulp* and *white pulp*. Both types consist of lymphoid tissue (that is, reticular connective tissue and lymphocytes) together with other cell types described on page 270.

The red pulp is traversed by a plexus of *venous sinuses* (Figs. 196, 197) by which it is broken up into anastomosing cords, known as *pulp cords* (*cords of Billroth*). The lymphoid tissue of the pulp cords is almost always infiltrated with some erythrocytes, the number varying under different conditions. The venous sinuses

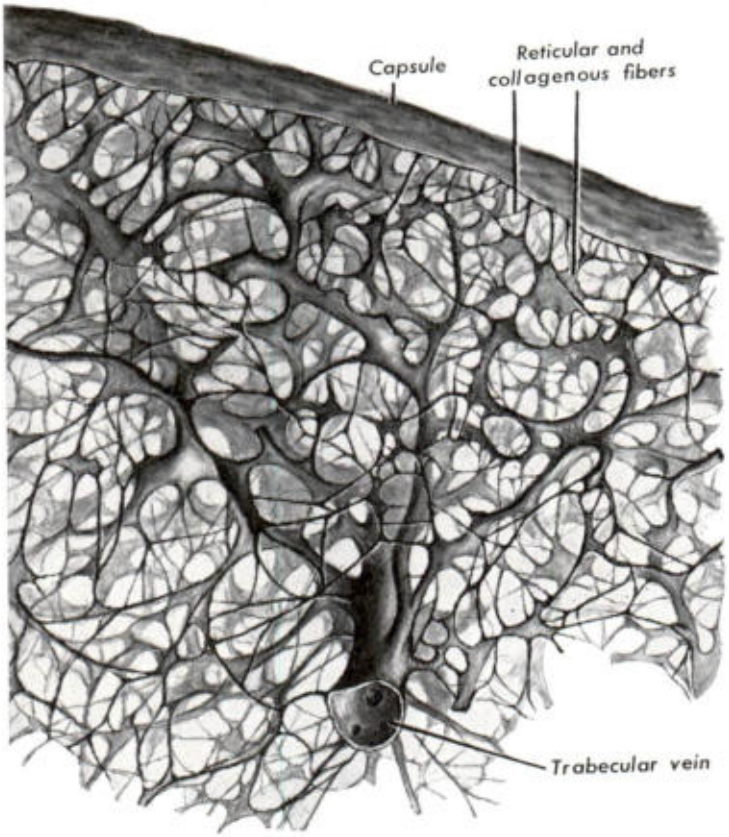

FIG. 195. Connective tissue framework of a portion of the spleen after a maceration technique which has removed all of the cells of the red and white pulp. × 15. (Preparation and drawing by Mr. Kellner.)

contain erythrocytes which are packed particularly close together when the sinuses are in a storage phase (page 270). Thus the red pulp as a whole (venous sinuses plus pulp cords), contains relatively large numbers of erythrocytes responsible for its color in fresh preparations.

The white pulp is composed of a compact type of lymphoid tissue arranged around certain divisions of the arteries. Most of the white pulp tissue is in the form of spherical or ovoid aggregations which are known as *Malpighian corpuscles* or *splenic corpuscles* (Figs. 194, 196). Except for their larger blood vessels, the splenic corpuscles are very similar to the lymph nodules found in lymph nodes and, like the latter, they may contain germinal centers. In children, a germinal center is usually found in each splenic corpuscle but in the adult spleen the germinal centers are less numerous.

The structure of the spleen depends largely upon the characteristic arrangement of the blood vessels, which will be described before considering further the minute structure of the organ.

Blood Vessels. The arteries enter the spleen at the hilum and divide into

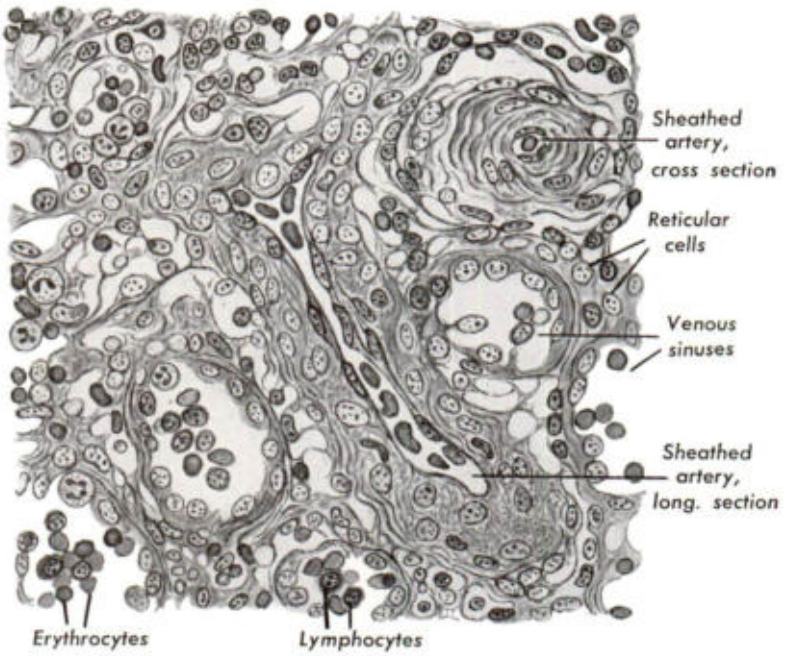

FIG. 197. Section of human spleen showing sheathed arteries and venous sinuses. × 619. (Redrawn after Hartmann.)

branches which enter the trabeculae to become the *trabecular* or *interlobular arteries* (Fig. 196). These are accompanied by the trabecular branches of the splenic veins. After following the trabeculae for a short distance, the arteries leave the veins and the septa and pursue an entirely separate course through the splenic pulp. The adventitial coat of these smaller arteries takes on the character of reticular tissue and becomes infiltrated with lymphocytes. At various points along the course of the vessels the lymphoid tissue is increased in amount and forms the splenic corpuscles, already mentioned. These arteries are called the *central arteries*, although they are eccentrically located with reference to the splenic corpuscles. When a corpuscle is located at a point where the artery divides, as frequently happens, two or more arteries will be seen in a cross section of the corpuscle. As they pass through the white pulp, the arteries give off numerous capillaries which nourish the lymphoid tissue. The capillaries of the white pulp extend into the red pulp where they anastomose with other vessels which drain into the venous sinuses (Fig. 200).

After a number of divisions, the central arteries become reduced in size, lose their surrounding coat of compact lymphoid tissue and enter the cords of the red pulp. At this point each artery divides into a number of rather straight branches which lie close together like the bristles of a brush or *penicillus*. The arteries of the penicilli consist of three successive portions: *pulp arteries, sheathed arteries,* and *terminal arterial capillaries.* The pulp arteries are the longest of the three divisions and possess a thin tunica of smooth muscle which is immediately surrounded

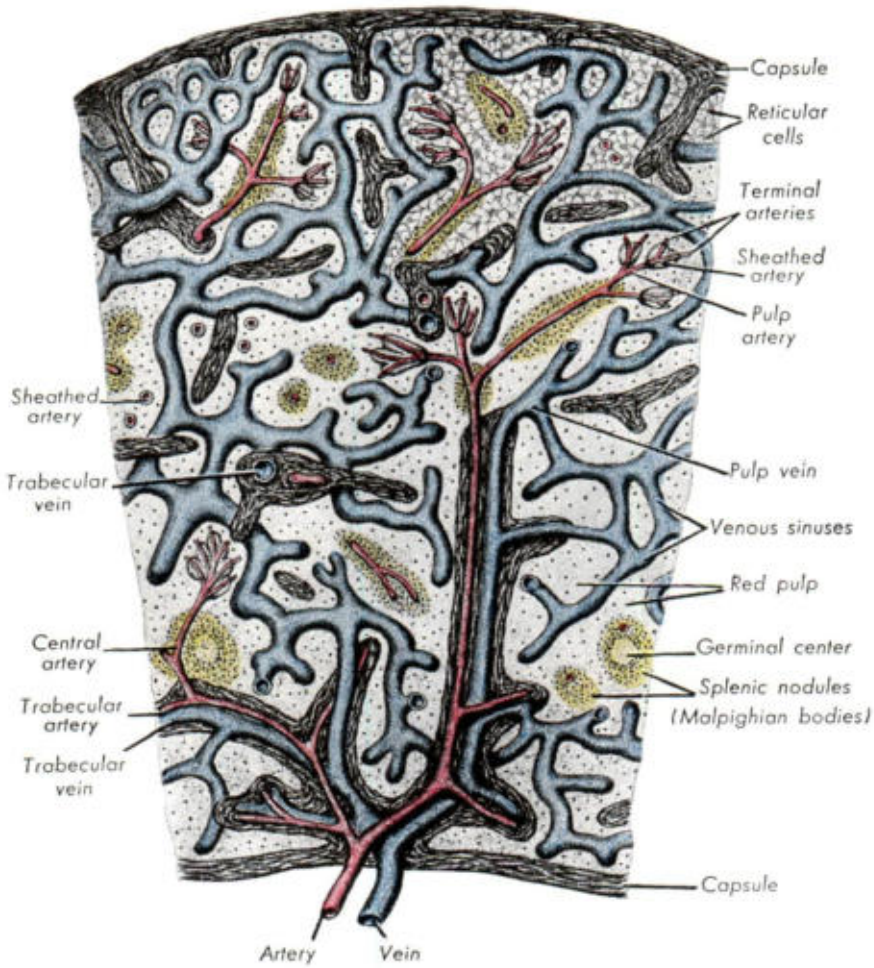

FIG. 196. Schema of the structure of the spleen. The diagram shows a portion of the organ extending from the hilum to the opposite (convex) surface. The structural components (vessels, nodules, etc.), which would be contained in such a large section, have not been drawn to scale, but have been increased in size and decreased in numbers for clearer illustration. (Redrawn and modified from Hartmann.)

by the tissue of the red pulp. The pulp arteries become smaller and divide into the sheathed arteries (Figs. 196, 197) which have an unusually thickened wall, the *Schweigger-Seidel sheath*. The sheath is not as well developed in man as in some of the lower animals like the pig and dog. These arteries have no muscular layer. The sheath or thickening consists of a compact mass of concentrically arranged cells and fibers continuous peripherally with the reticulum of the red pulp. The sheath apparently represents a local modification and condensation of the reticulum. The sheathed arteries have a lumen of about 6–8 microns and are very uniform in size.

Each of the sheathed arteries divides into two or more arterial capillaries which may have conical shaped enlargements (ampullae) at their terminations. The exact manner in which these vessels terminate has been a controversial subject. Some authors claim that the capillaries empty into intercellular spaces of the red pulp reticulum and that the blood finds its way from the pulp spaces into the venous sinuses through perforations in the walls of the sinuses. According to this view, the initial pulp space into which the capillary opens may represent the so-called ampulla. Other authors deny the existence of openings into the reticulum and believe that the capillaries empty directly into the venous sinuses.

The *venous sinuses* (terminal veins, cavernous veins) form an anastomosing plexus throughout the red pulp breaking it up into the pulp cords. The outer part of the wall of the venous sinus contains thick anastomosing circular fibers. They are continuous with the fibers of the surrounding reticulum of the red pulp and are evidently reticular fibers. The cells

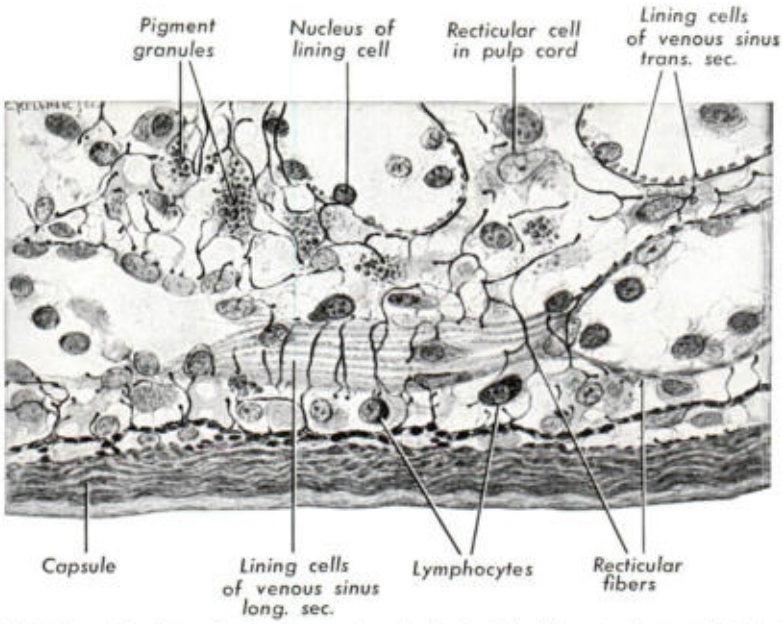

FIG. 198. Section of spleen of rhesus monkey stained with silver to show reticular fibers and with hematoxylin-eosin-azure to show cell types. The venous sinus in the lower part of the field is cut longitudinally and tangentially so that the section gives a surface view of part of the sinus wall. The two upper sinuses are cut transversely. Granules of hemosiderin pigment were unusually abundant in this particular field. × 950.

which line the sinuses are rod-shaped and run longitudinally in the vessel wall (Fig. 198). The cell bodies usually protrude into the vessel more than ordinary endothelial cells and are particularly enlarged at the location of the nucleus where they bulge in a characteristic manner relatively far into the lumen of the vessel. The bulging into the lumen becomes especially pronounced when the sinuses are contracted.

Some investigators believe the sinuses open to the pulp by clefts between the lining cells; others think that a thin homogeneous membrane extends between the cells and partly or completely closes the wall. The lining cells have protoplasmic connections with the reticular cells of the pulp. They are very phagocytic and belong to the reticulo-endothelial system (Chapter V). They are frequently described as endothelial cells but they have many similarities to reticular cells and are sometimes classified as reticular cells which differ from ordinary stellate reticular cells only in that they have become more elongated, somewhat flattened, and are more regularly arranged as structural adaptations to their position. They have been variously named special endothelial cells, lining reticular cells, and littoral cells.

The terminal veins or venous sinuses unite to form larger *pulp veins*, or *collecting venules*, lined by endothelium. The collecting venules enter the trabeculae to become the *trabecular* or *interlobular veins* which follow the trabeculae toward

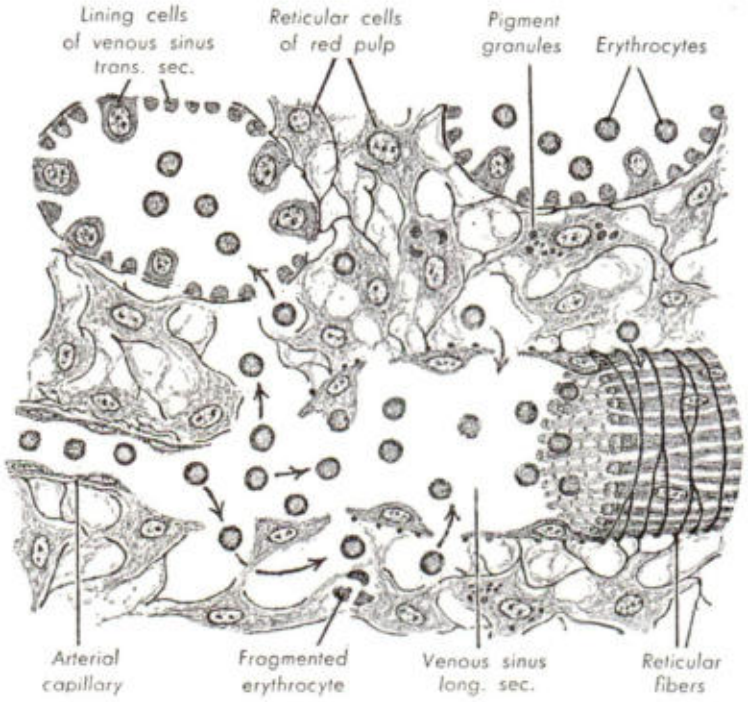

FIG. 199. Schema of union of arteries and veins in splenic pulp showing an "open" type of circulation. The venous sinus at lower right is drawn to give a three dimensional view to show the longitudinal orientation of the lining cells surrounded by circularly arranged reticular fibers. Granules in the reticular cells represent hemosiderin resulting from the phagocytosis of senile erythrocytes.

the hilum where they unite to form the splenic veins.

Union of Arteries and Veins. The union of the terminal arterial capillaries and venous sinuses requires a more detailed discussion in relation to the problem of whether the circulation is "open" or "closed." As already stated, some authors think all of the arterial capillaries open into the intercellular spaces of the red pulp (Fig. 199); others believe that all capillaries connect with the venous sinuses (Fig. 200). According to a third view—a compromise between the opposing theories of "open" and "closed" vascular systems—some of the capillaries open into the intercellular spaces while others open directly into the sinuses.

Sections of fixed spleens usually show a number of red corpuscles scattered through the pulp cords. When the spleen is experimentally congested and distended by ligation of the splenic veins, there is a marked increase in the number of erythrocytes found in the pulp. These findings have been interpreted as evidence for an "open" circulation, although Knisely's more recent studies of living spleens suggest that the erythrocyte distribution observed in fixed spleens may be produced by extremely rapid post-mortem changes. Most of the evidence obtained from injections of the vascular system supports the "open" theory, although here again it is difficult to rule out post-mortem changes.

Using a quartz rod method of transillumination, Knisely (1936) made some interesting observations on the circulation of the spleen in living mice, rats and cats. He observed arterial capillaries connecting with the venous sinuses and also with the collecting venules or pulp veins (Fig. 200). He did not find any vessels "open" to the pulp spaces. The vessels which open directly into collecting venules pass lengthwise through the pulp cords between the sinuses and nourish the tissue of the cords. The arterial capillaries which enter the sinuses

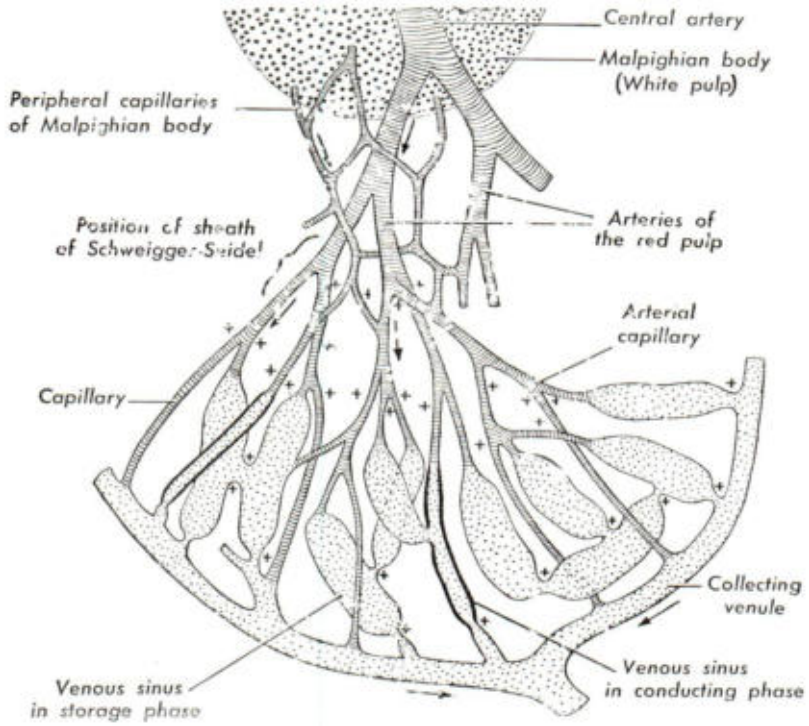

FIG. 200. Diagram of the different types of vascular connections which have been observed in the spleens of living animals. (Plus signs indicate positions of physiological sphincters.) (Redrawn and slightly modified from Knisely.)

are shorter. Most of them open into the afferent ends of sinuses; a few enter laterally. Sinuses are frequently joined together to form multiple sinus systems (Fig. 200).

Knisely found that the amount of blood flowing into different parts of the vascular plexus is controlled by three sets of physiological sphincters. Their positions are indicated by the plus signs in Figure 200. He also has found that the venous sinuses show cyclic activity in which four phases may be recognized. In the *conducting phase*, all of the sphincters are open, the wall of the sinus looks thin, and whole blood enters at the afferent end and leaves at the efferent end. At the beginning of the *filtration-filling phase*, the sphincter at the efferent end of the sinus closes. Whole blood continues to enter at the afferent end, blood fluid passes rapidly through the sinus wall, and the sinus becomes a distended cucumber-shaped structure packed with blood corpuscles. When the sinus is nearly full of blood cells, the afferent sphincter constricts and the *storage phase* begins. During this phase the sinus maintains its distended condition. This phase lasts from a few minutes to several hours. The sinus walls are very thin during this phase, and a few erythrocytes have been seen passing through the walls into the pulp cords. The corpuscles are distorted and "snap" forward as they pass through a visible membrane-like wall. At the end of the storage phase, the efferent sphincter opens and masses of cells move into the collecting venule. Presently, the afferent sphincter opens, the packed cells are washed out of the sinuses, and another conducting phase begins.

Further investigations on the spleens of living animals by Knisely's method have given conflicting results. One group of investigators (MacKenzie et al., 1941) has described an open circulation, whereas another group (Peck and Hoerr, 1951) has supported most of Knisely's findings as outlined above. Thus, an interpretation of the observations on the splenic circulation of living animals leads to the same controversy as the studies of fixed material.

An intermediate view—i.e., that some capillaries connect directly with the venous sinuses and that others open into the pulp spaces—has been supported by a number of studies on fixed spleens, particularly by those on silver-stained serial sections of the guinea pig spleen (Snook, 1944).

It seems likely that there is a fairly direct connection from the capillary to the venous sinus in most cases, but that the system is "open" in the sense that the lining membrane changes from endothelial cells to flattened reticular cells, and contains perforations through which erythrocytes may readily pass (Fig. 199).

The Splenic Pulp. As already stated, the splenic pulp fills in all the spaces between the connective tissue trabeculae and has been subdivided into white pulp which consists of compact lymphoid tissue surrounding the central arteries, and red pulp containing an abundance of erythrocytes and broken up into pulp cords by the venous sinuses. A meshwork of reticular connective tissue extends throughout both the red and white pulp, although its density and arrangement vary in different parts. The reticular cells (Fig. 201) and also the fibers, are more numerous and more closely arranged around the arteries and at the periphery of the splenic corpuscles than elsewhere.

The *cells of the reticulum*, like those of lymph node reticulum, can be subdivided into reticular cells with phagocytic properties and the more undifferentiated mesenchyme-like cells. The former belong to the reticulo-endothelial system and represent fixed or attached histiocytes. They frequently lose their connections with the reticulum and become free histiocytes or macrophages. Both the free and attached histiocytes may be found containing various foreign particles, degenerating leucocytes, fragmented and whole erythrocytes, etc. Frequently they contain reddish or brownish pigment granules (hemosiderin) derived from the hemoglobin of erythrocytes which have been phagocytosed (Fig. 198).

The undifferentiated cells of the reticulum easily transform themselves into reticular cells with phagocytic properties or they may become primitive free cells which develop into lymphocytes in the germinal centers of the white pulp just as in the germinal centers of lymph nodes.

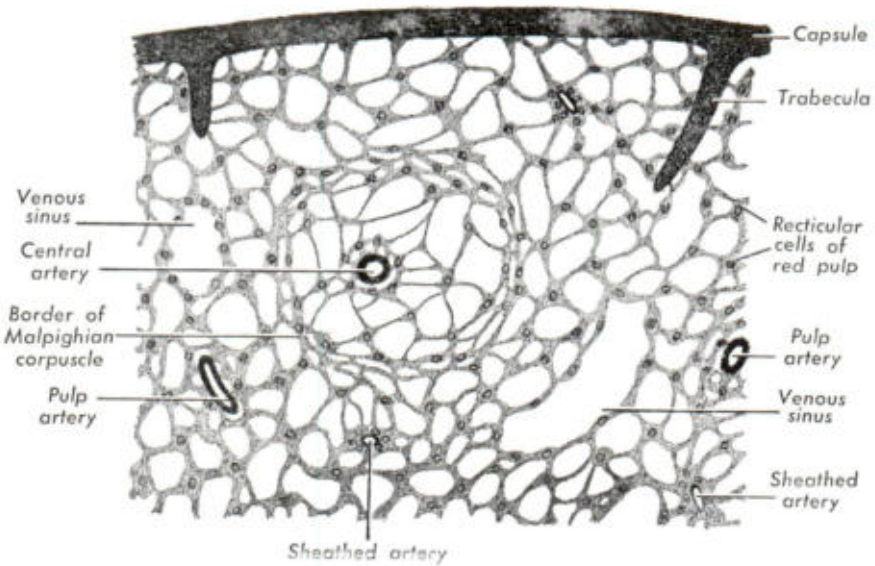

FIG. 201. Schema of the arrangement of the reticular cells of the spleen. Reticular fibers are omitted. No structural details are shown for the capsule, trabeculae and walls of arteries, but the positions of these structures are shown as the darkest parts of the schema. (Redrawn from Hartmann.)

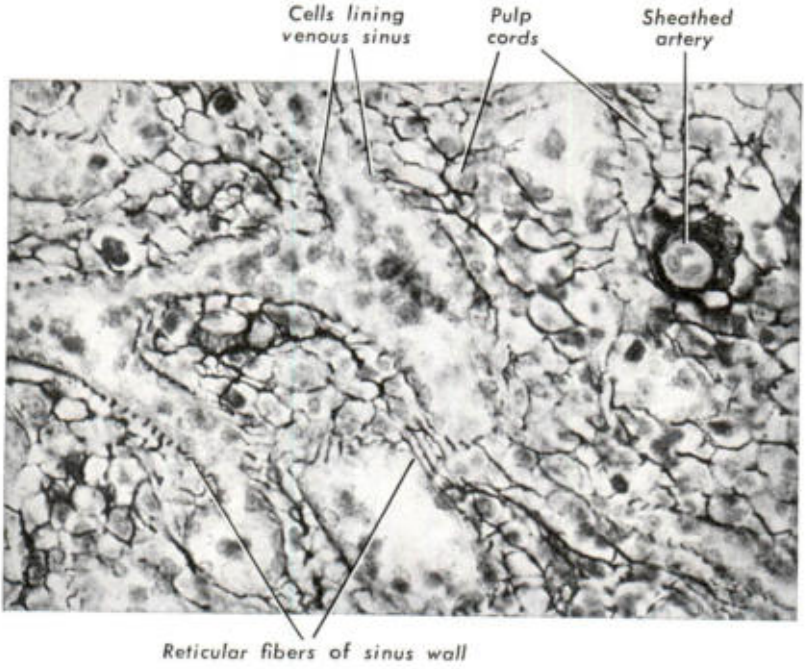

FIG. 202. Photomicrograph of spleen of rhesus monkey. Stained with Bielschowsky-Foot silver for reticular fibers and with eosin-azure for cell types. × 410.

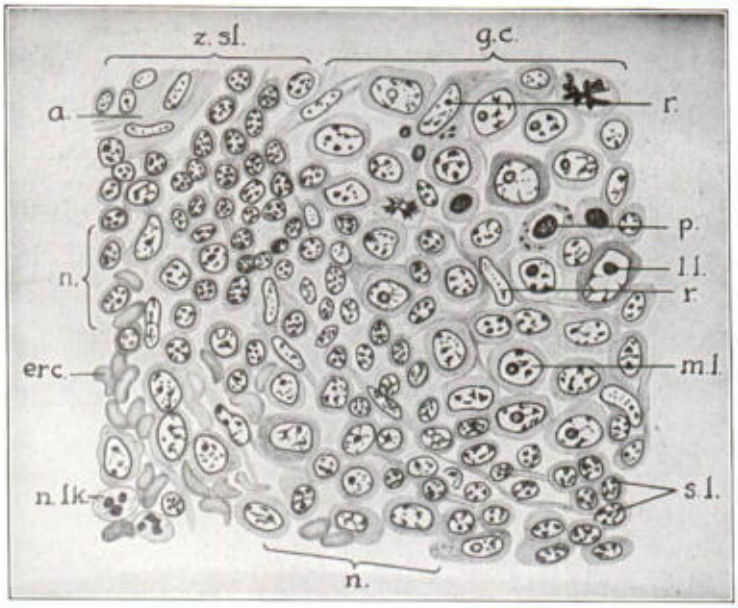

FIG. 203. Portion of Malpighian corpuscle of cat's spleen showing a part of a germinal center and various types of lymphocytes. *a*, Central artery; *erc*, erythrocytes in red pulp; *g.c*, germinal center; *ll.*, large lymphocytes; *m.l*, medium size lymphocytes; *n*, outer border of splenic nodule (junction of red and white pulp); *n.lk*, neutrophile leucocytes; *p*, phagocyte; *r*, nuclei of reticular cells; *s.l*, small lymphocytes; *z.sl*, compact zone of small lymphocytes around germinal center. × 570. (Redrawn from Hartmann.)

Lymphocytes of large, medium and small sizes are numerous and compactly arranged in the white pulp, the first two types being especially numerous in the germinal centers (Fig. 203). In the red pulp, the lymphocytes are less numerous and more loosely arranged.

Monocytes and plasma cells are also fairly numerous. Monocytes are brought into the spleen by the circulating blood and are also formed within the spleen, both by proliferation of existing monocytes and by differentiation from a more embryonic cell type. According to some authors they arise from primitive free cells by way of a "monoblast" type but according to the unitarian theory of blood formation which derives all types of blood cells from lymphocytes, the monocytes are derived from and are closely related to the cells of the lymphoid series. The monocytes have phagocytic properties and when very active may enlarge and become similar in appearance to the free macrophages already described which arise from the reticular cells of the pulp cords and from the cells lining the sinuses.

The red pulp also contains the various types of *granular leucocytes* and a variable number of *erythrocytes*. Due to the thinness of the walls of the sinuses and the presence of some erythrocytes outside the sinuses it is frequently difficult to distinguish in ordinary sections between the sinuses and pulp cords.

Giant cells or *megakaryocytes*, similar to those of bone marrow, are found in the splenic pulp of a number of animals (e.g., cat, rat, etc.). They are present in man during fetal life but are usually absent in the adult.

Lymphatics. Efferent lymphatic ves-

sels are present in the connective tissue of the capsule and trabeculae. Contrary to previous views, it has been found that deep efferent lymphatic vessels are present also in the white pulp, coursing parallel with the arteries (Snook, 1946).

Nerves. These are mainly nonmedullated, although a few medullated fibers are present. The latter are probably sensory in function. The nonmedullated fibers—axons of sympathetic neurons—accompany the arteries, around which they form plexuses. From these plexuses terminals pass to the muscle cells of the arteries, to the septa, to the capsule, and also to the splenic pulp.

Functions of the Spleen. The spleen is not essential for life and can be removed without giving very definite evidence as to what its functions are since various other organs, particularly the bone marrow, readily take over its functions.

The spleen acts as a filtering organ for the blood in much the same way as the lymph nodes function as organs of lymph filtration. The phagocytic cells of the spleen, both the free and attached histiocytes, remove foreign particles, including bacteria, degenerating leucocytes, etc., from the circulating blood.

The phagocytic reticular cells also remove fragmented and whole erythrocytes and in this way the spleen functions as an organ for blood destruction. Many of the cells engulfed have already begun to fragment in the peripheral circulation, but the spleen is thought to also play a part in making the cells more fragile. Regardless of what initiates the fragmentation or determines which erythrocytes are to be destroyed, the spleen is of importance in blood destruction as an organ for the removal and phagocytosis of the cells.

Engulfed erythrocytes are digested by the phagocytic cells and iron is recovered from the hemoglobin and temporarily stored in the cells. In this way the spleen plays a part in the iron metabolism of the body. The stored iron is given up as needed and is utilized by the body in the formation of new hemoglobin.

The spleen functions as an organ for blood development during a part of fetal life but after birth the only blood cells normally formed in the spleen are lymphocytes and monocytes. In certain pathological conditions the splenic pulp reassumes its earlier function as a blood-forming organ for all types of cells and becomes similar to bone marrow in appearance, containing all types of myelocytes, erythroblasts, and megakaryocytes.

As already described, the framework of the spleen contains elastic fibers and smooth muscle and undergoes pronounced and rapid changes in volume. Contraction of the spleen expels erythrocytes and increases the number of these corpuscles in the general circulation. Contraction and decrease in volume occurs under many different conditions (e.g., during exercise, following hemorrhage, etc.) and although this reservoir function of the spleen is not necessary for life there are a great variety of conditions, both normal and pathological in which it may be of considerable value (Barcroft and Stephens, 1927).

The spleen also appears to play an important part in the formation of antibodies and in the production of immunity.

Development. The spleen appears as a thickening of the mesenchyme in the left side of the mesogastrium (greater omentum) during the fifth week of embryonic development. When the primordium of the spleen first appears, the cells of the mesothelium are not sharply separated from the underlying mesenchyme, and it is thought that some of the mesothelial cells are added to the mesenchymal thickening. By continued growth the primordium becomes a larger elevation from the mesentery and extends into the body cavity. The attachment to the mesentery gradually becomes constricted, and finally forms only a narrow band of tissue surrounding the splenic vessels.

The mesenchymal cells differentiate into the reticulum and into primitive free cells resembling lymphocytes. Slightly later the tissue becomes myeloid in type, containing all stages in the development of erythrocytes, granulocytes and megakaryocytes. The development of lymphocytes continues throughout life but the formation of erythrocytes and granulocytes normally ceases shortly after birth.

In its early stages of development the primordium of the spleen is supplied with a capillary plexus connecting the afferent and efferent vessels. Following the development of the characteristic distribution of vessels, lymphocytes gradually become more compactly arranged around the arteries to form the beginning of the white pulp. The enlargements of the white pulp forming definite splenic corpuscles are not found until toward the end of fetal development. The characteristic adult structure of the red pulp is not attained until after birth, when the myeloid characteristics of the tissue are lost as the development of erythrocytes and granulocytes ceases.

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XIV

THE INTEGUMENT

The integument comprises the skin which covers the entire body together with certain accessory organs which are derivatives of the skin, such as nails, hair, and glands of various kinds.

The skin performs many important functions. It protects the body from injurious substances; helps in the regulation of the body temperature; excretes water, fat and some other substances; and constitutes the most extensive sense organ of the body for the reception of tactile, thermal and painful stimuli.

THE SKIN

The skin or cutis consists of two main parts: (1) the *epidermis*, a stout epithelial layer derived from the ectoderm, and underneath this, (2) the *derma, corium*, or cutis vera, a connective tissue derivative of the mesoderm. Below the corium is a layer of loose connective tissue, the *subcutaneous tissue* (superficial fascia), which attaches the skin to the underlying organs (Fig. 204). In certain places this layer is so richly infiltrated with fat as to be called the *panniculus adiposus*. The subcutaneous tissue makes possible an easy movement of the skin, and where such mobility is slight or absent, as for instance in the soles, palms and finger tips, this tissue is more dense.

The thickness of the skin varies considerably in different parts of the body. The relative proportions of epidermis and dermis vary also, and a thick skin is found in regions where there is a thickening of either layer or of both layers. On the interscapular region of the back, where the dermis is particularly thick, the skin may be more than 5 mm. in thickness whereas on the eyelids it may be less than 0.5 mm.; the usual thickness is 1 to 2 mm. The skin is generally thicker on the dorsal or extensor surfaces of the body than on the ventral or flexor surfaces but this is not true for the hands and feet where the skin of the volar surface is thicker than on any dorsal surface except the interscapular region. The palms and soles have a characteristically thickened epidermis, in addition to a thick dermis (Fig. 204). The epidermis of these regions is not only thicker than that of any other region but it also differs structurally from the thin epidermis present elsewhere.

The whole surface of the skin is traversed by numerous fine furrows which run in definite directions and cross each other to bound small fields of a rhomboid or rectangular form. These furrows correspond to similar ones on the surface of the corium, so that in section the boundary line between epidermis and corium appears wavy. On the thick skin of the palms and soles the fields form long narrow ridges separated by parallel running furrows, and in the finger tips these ridges are arranged in the complicated loops, whorls and spirals which give the fingerprints characteristic for each individual. In those regions where the epidermis is thickest these ridges are more prominent.

In addition to the ridges seen on the

surface of the skin, there are dermal elevations, or *papillae*, which indent the inner surface of the epidermis (Figs. 204, 206). Between the dermal papillae, the downward projections of the epidermis appear peg-like in sections and are known as *rete pegs*. In the palms and soles the dermal papillae are numerous, tall and often branched, varying in height from 0.05–0.2 mm. They are likewise numerous and tall in the lips, clitoris, penis, labia minora and nipples. Where the mechanical demands are slight and the epidermis is thinner, as in the skin of the abdomen, chin and face, the papillae are low and few in number.

The Epidermis

The epidermis is composed of stratified squamous epithelium whose thickness varies in different parts of the body.

Epidermis of Palms and Soles. The epidermis of the palms and soles, and the volar surfaces of the digits, is particularly thick and highly differentiated. In these regions, four components are recognized: (1) the stratum germinativum (stratum mucosum, stratum Malpighii); (2) stra-

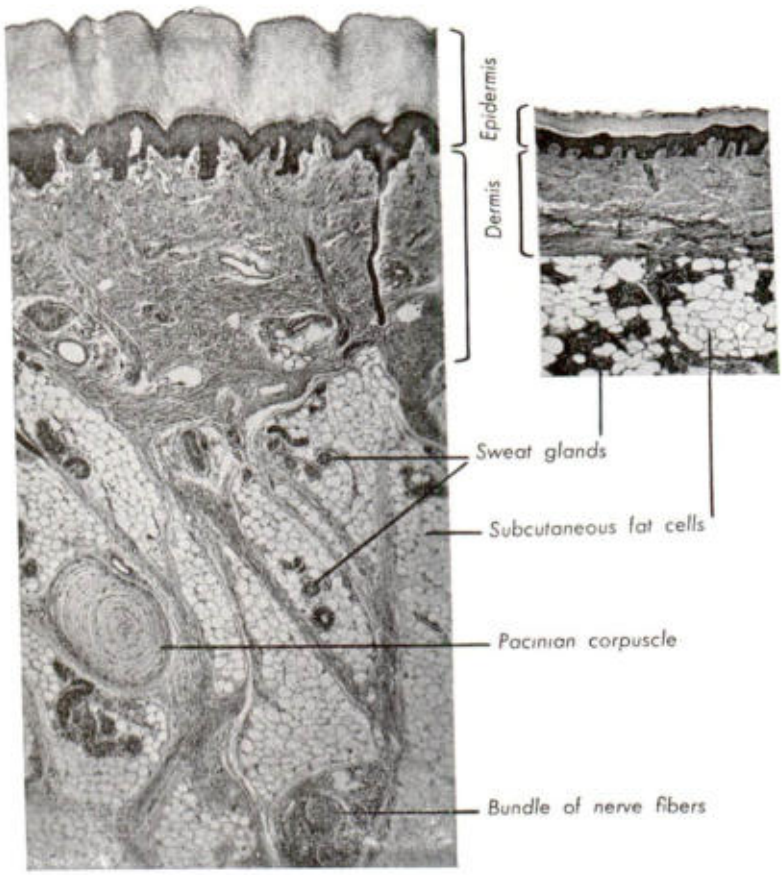

Fig. 204. Low power photomicrographs of vertical sections of skin. Thick skin from finger tip, at left; skin of medium thickness from dorsal surface of finger, at right. The fields shown do not include the total thickness of the subcutaneous tissue. × 30.

tum granulosum; (3) stratum lucidum; and (4) stratum corneum (Figs. 205, 206).

(1) The *stratum germinativum* is composed of a number of cell layers. The deepest layer consists of columnar or high cuboidal cells arranged in a single row (*stratum cylindricum*) which rests on a thin basement membrane which follows the depressions and elevations of the corium. The basement membrane of adult human skin is so thin that its existence has been questioned by some who have looked for it under the light microscope; it is seen as a well-defined membrane in electron micrographs. It is thicker and structurally more complex in many of the lower animals than in man.

The cells of the basal layer of the stratum germinativum, i.e., the stratum cylindricum, have short and thin protoplasmic processes which appear to anchor the cells in the underlying connective tissue (Fig. 206). Electron micrographs of human skin indicate that the processes fit into pockets of the basement (dermal) membrane and that they probably do not pierce the membrane.

Above the stratum cylindricum, the cells of the stratum germinativum are usually polygonal in shape. They have well-defined "intercellular bridges" which appear as delicate spines projecting from the surfaces of the cells. For this reason, the cells are often called "prickle cells," and the whole region the "*stratum spinosum*." Electron micrographs show that the short spinous processes of neighboring cells make intimate contact but they do not represent bridges of protoplasmic continuity. The relatively dense thickened knobs at the contiguous points of the processes are known as desmosomes (Fig. 24).

The nuclei of the cells of the stratum germinativum are deeply chromatic. Their shape varies with that of the cells, being ovoid or cylindrical in the stratum cylindricum and round in the stratum spinosum. Some of the nuclei of the deeper layers are found in various stages of mitosis. This is the means by which new cells are formed to replace those which migrate outward and eventually slough from the superficial layer of the stratum corneum.

The cytoplasm of the cells of the stratum germinativum is basophilic in its staining reaction particularly in the deeper cell layers. There is evidence that this is correlated with the RNA content of the cytoplasm and that it is associated with active protein synthesis, in this case with growth and division of the cells. The cytoplasm of the cells of the stratum germinativum also contains numerous fine filaments composed of polypeptide chains of keratin-precursor proteins. In fixed and stained preparations, the submicroscopic filaments are precipitated and clumped in the form of *tonofibrils* which are visible under the light microscope. Although the tonofibrils course through the cytoplasm in all directions, they tend to predominate in a direction towards cell processes, i.e., toward the basement membrane in the cells of the stratum cylindricum and into "intercellular bridges" in the stratum spinosum. They do not extend across cell membranes. Histochemical studies show that the tonofibrils have a relatively high content of sulfhydryl groups. Electron micrographs of epidermis also support the view that tonofibrils are precursors of the keratin found in the stratum corneum.

(2) The *stratum granulosum* consists of two to five rows of flattened rhombic or spindle-shaped cells whose long axis is parallel with the surface of the skin (Figs. 206, 207). The cytoplasm contains deeply staining granules of a substance known as *keratohyalin*. Since the keratohyalin granules lack sulfhydryl groups characteristic of tonofibrils and disulfide

groups characteristic of keratin, it seems doubtful that they have any direct role in the process of keratinization. The nuclei are pale and show disintegrative changes. The tonofibrils and intercellular bridges are present, but are indistinct and difficult to recognize.

(3) The *stratum lucidum* consists of several rows of flattened cells which are shiny and refractile, so that the whole layer appears as a homogeneous translucent band in which the cell boundaries are difficult to make out (Figs. 205, 206). The cytoplasm of the cells is filled with a semi-fluid substance, *eleidin*. The nuclei have degenerated and are not visible in most cases. With special technique they appear as vacuoles in the centers of the cells.

(4) The *stratum corneum* is very thick

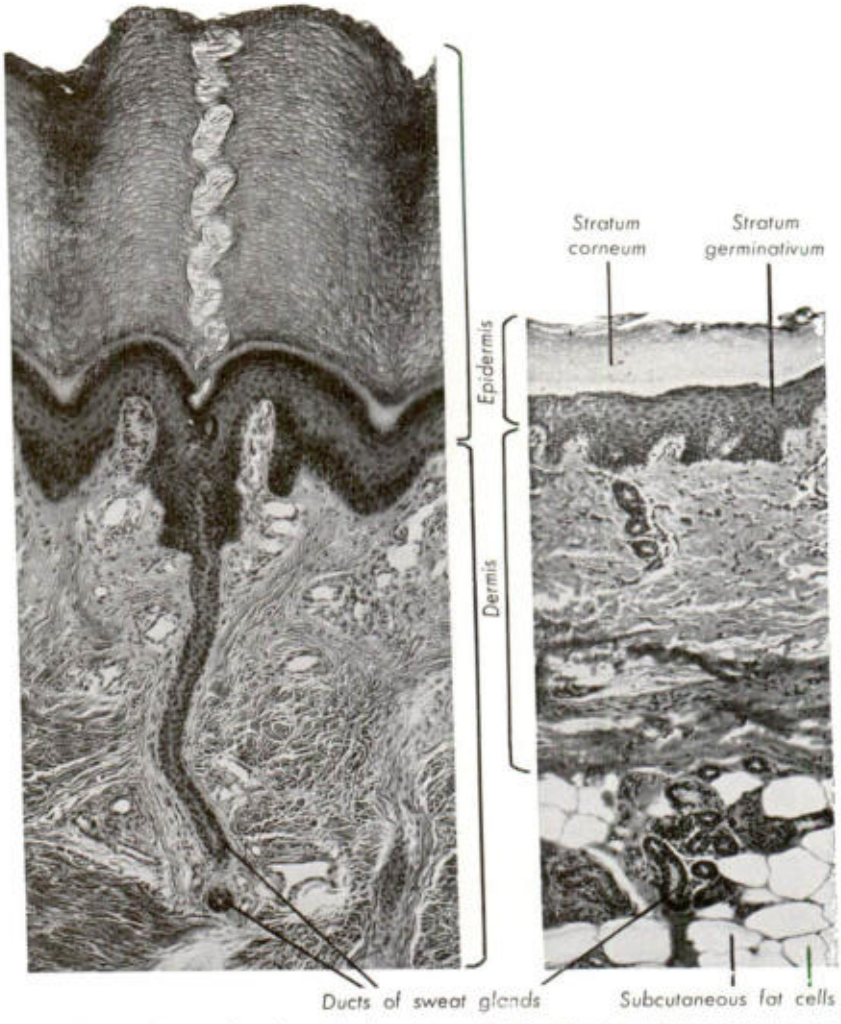

FIG. 205. Photomicrographs of vertical sections of skin showing thick epidermis from finger tip, at left, and thinner epidermis from dorsal surface of finger, at right. × 94.

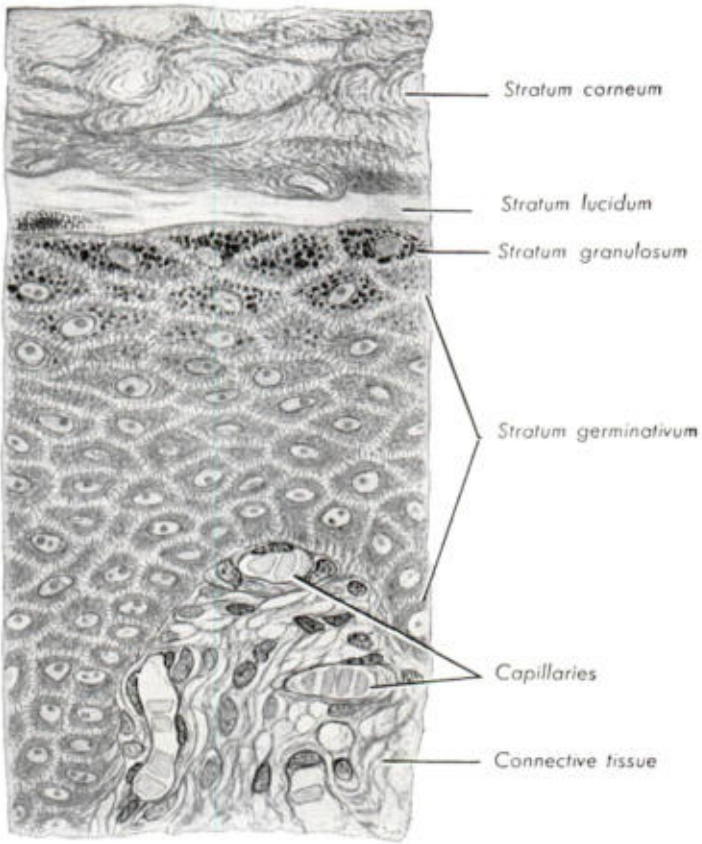

FIG. 206. Vertical section through skin of human finger tip showing structure of epidermis. The field drawn does not include the total thickness of the stratum germinativum at the sides of the connective tissue papilla and includes only a fraction of the total thickness of the stratum corneum. × 654.

in the palms and soles and is composed of clear, dead, scale-like cells which become more and more flattened as the surface is approached, the most peripheral layer containing flat horny plates which are constantly desquamated. The cells have a definite horny membrane and are closely packed, so that no intercellular bridges are present. The nuclei have disappeared but the spaces which they occupied can be seen in many cells.

The cytoplasm contains many fine filaments composed of polypeptide chains of the fibrous protein which is known as *keratin*. The submicroscopic filaments clump to form fibrils which are observable under the light microscope. Histochemical studies show that the fibrils of the cornified layer contain disulfide groups and the evidence indicates that they are derived from the sulfhydryl-containing tonofibrils of the deeper layers of the epidermis.

The cornified layer of the epidermis is composed of "*soft keratin*" which contrasts with "*hard keratin*" found in the nails and in the cortex of the hairs. Hard keratin contains relatively more sulfur, is

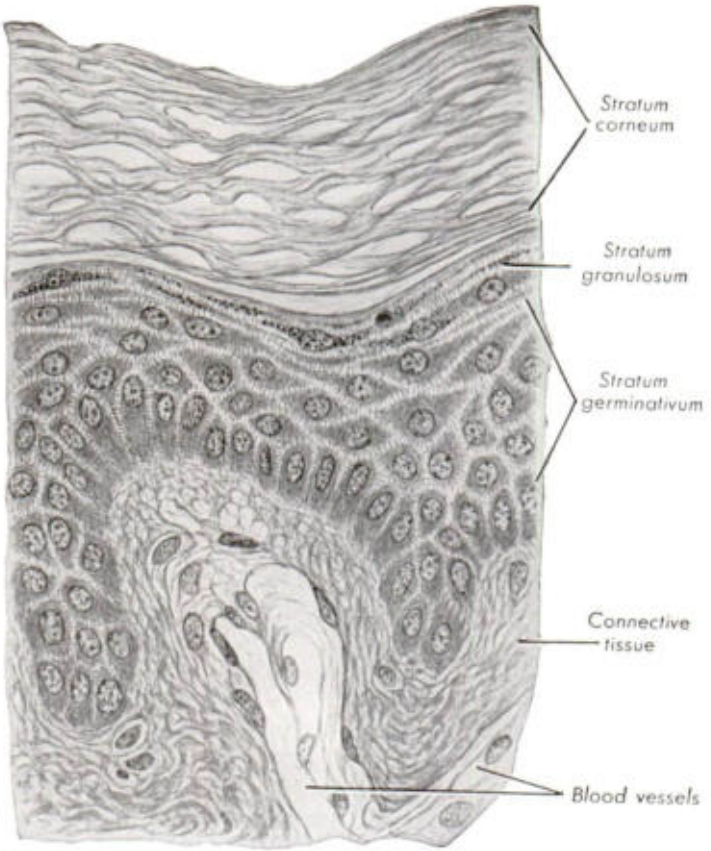

FIG. 207. Vertical section through skin, median side of lower leg. Field drawn includes total thickness of stratum corneum. Stratum granulosum is thin in comparison with that shown in Figure 206; the stratum lucidum is barely discernible. × 654.

less elastic, and is more permanent in the sense that it does not desquamate as the epidermis does.

The desquamated cells of the stratum corneum are constantly replaced by new cells which are formed by mitoses in the germinative layer and are pushed toward the surface in the process of keratinization.

Epidermis of the General Body Surface. The epidermis of the rest of the body is considerably thinner than that of the palms, soles, and volar surfaces of the digits. All layers of the epidermis are reduced and the stratum corneum and stratum germinativum are the only layers that are constantly present in all parts of the body. A thin stratum granulosum, composed of only one or two cell rows is frequently present, but a definite stratum lucidum is generally absent. The structure varies with the region studied. On the leg, for example (Fig. 207), where the epidermis is thicker than that of abdominal or pubic skin yet much thinner than that of the finger tip, a faint stratum lucidum may be found. The reduction in the thickness of layers in thin epidermis is probably due to the fact that keratinization is far less marked

and occurs not as a continuous process but only at certain times.

Color of the Skin. The color of the skin is determined by the presence of varying amounts of *melanin* pigment. Certain patches of skin are especially rich in pigment, such as the circumanal region, the areolae and nipples, the axilla, the labia majora, the penis and scrotum, while practically no pigment is present in the palms and soles. The pigment is stored as fine granules within the cells of the germinative layer, though some granules may be deposited between the cells. In the white races the granules occur only in the deepest cell layers and chiefly in the cylindrical cells of the basal row. In colored races pigment is found throughout the entire stratum germinativum and even in the stratum granulosum.

The origin of pigment is not fully understood but it is thought that all melanin is elaborated in specialized cells called *melanoblasts* and then distributed to the epithelial cells. The melanoblasts are scattered in the basal layers of the epidermis and send numerous processes for considerable distances between the epithelial cells. The melanoblasts are identified by their reaction to the "dopa" reagent — 3,4-dioxyphenylalanine; they oxidize the solution and stain black. The intracellular substance responsible for the reaction is an oxidase, *tyrosinase*, which is an enzyme normally involved in the formation of melanin from a precursor, presumably *tyrosine*. It should be noted that the dopa reaction does not occur for fully formed melanin present in the pigmented epithelial cells and the dermal chromatophores to be described later. The melanoblasts apparently differentiate and migrate from the neural crest during embryonic development. The melanoblasts supposedly wear out and slough off with the epidermal scales but their number is maintained by proliferation of persisting basal melanoblasts. By the use of a gold chloride technique, it is possible to demonstrate in the stratum germinativum a few branched cells, the so-called cells of Langerhans, which may represent worn-out melanoblasts.

The fate of the pigment within the epithelial cells is not understood but some of it is probably broken down and eliminated with the epidermal scales. As the cells of the deeper layers are gradually pushed to the surface, the pigment becomes diffuse and is observed in only small amounts in the stratum corneum.

The Derma or Corium

The dermis varies from 0.2 mm. to 4 mm. in thickness and is composed of dense irregularly arranged connective tissue. It contains the three types of connective tissue fibers and fibroblasts and histiocytes. Two layers can be distinguished although they blend without distinct demarcation. The deeper one is relatively thick and is known as the reticular layer, the superficial layer is thinner and is named the subepithelial or papillary layer.

The *reticular layer* is characterized by coarse collagenous fibers and fiber bundles which often unite to form secondary bundles of considerable thickness (nearly 100 μ in diameter). The fibers cross each other to form an extensive feltwork with rhomboid meshes, the direction of the fibers in the main being parallel to the surface of the skin. The elastic fibers form complex elastic nets permeating the entire corium. Here too the course of the main fibers is parallel with the surface, though vertical and oblique fibers are present in considerable number. The elastic fibers form basket-like, capsular condensations around the hair bulbs, sweat and sebaceous glands.

The *papillary or subepithelial layer* is similar in structure to the preceding, but

the fibers are finer and more closely arranged. Directly underneath the basal epithelial row is a fine feltwork with many reticular as well as elastic and collagenous fibers. In ordinary preparations it appears as a homogeneous narrow zone in which the tooth-like processes of the epithelial cells are anchored. This is the region which can be demonstrated as a basement membrane in electron micrographs.

Although the connective tissue fibers of the corium form complex nets and meshes, those bundles which course parallel with the lines of tension of the skin are more numerous and better developed than the others. The lines of skin tension, due to the direction of the predominant fibers are known as *Langer's lines*. These lines have different directions in the various parts of the body. Their direction is of surgical importance because incisions made parallel with the lines gape less and heal with less scar tissue than incisions made across the lines.

As had been mentioned the surface of the corium is beset by numerous papillae which penetrate into the epidermis. These papillae vary in structure and content. Some are simple, others branched. Some contain loops of capillary blood vessels (vascular papillae), others contain special nerve terminations (nervous papillae) (Fig. 147).

In addition to the usual types of connective tissue cells, the corium of certain regions may contain a few branched, pigmented connective tissue cells, the *dermal chromatophores*, which resemble the pigmented cells of the choroid coat of the eye. The dermal chromatophores are normally scarce in the white race. They occur chiefly in regions where the epidermis itself is richly pigmented, and their pigment is apparently obtained from melanogenic cells of neural crest origin.

Smooth muscle is found in the skin in connection with the hair (arrector pili muscles). Smooth muscle fibers also occur in considerable number in the skin of the nipple, prepuce, glans penis, scrotum (tunica dartos) and parts of the perineum

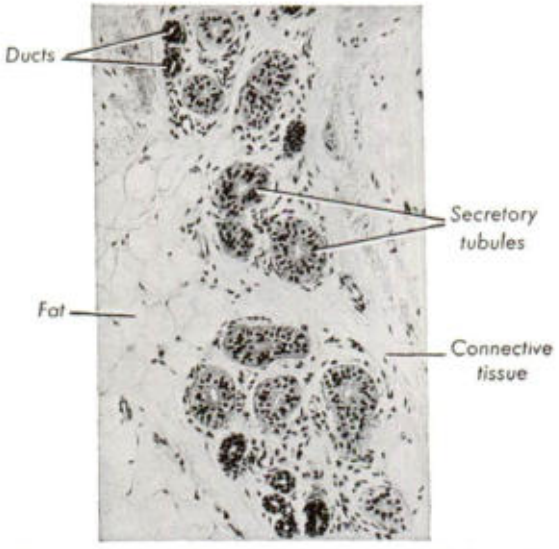

FIG. 208. Section of sweat glands of human finger tip. Field shown is at junction of dermis and subcutaneous tissue. Photomicrograph. × 110.

The fibers are arranged in a network parallel to the surface, and contraction of the fibers gives the skin of these regions its wrinkled appearance. In the face and neck skeletal muscle fibers from the mimic musculature likewise penetrate the derma. Both smooth and skeletal fibers end in delicate elastic bands which are continuous with the general elastic network of the corium.

GLANDS OF THE SKIN

Two kinds of glands occur in the skin—sweat glands and sebaceous glands.

Sweat Glands (Glandulae sudoriparae). These are found over the entire body surface excepting the margin of the lips, the ear drum, the inner surface of the prepuce, and the glans penis. They are most numerous in the palms and soles and are the only glands found in these places. They are simple coiled tubular glands. The coiled, ball-shaped portion usually lies in the subcutis, though it may lie partly or wholly in the deeper portion of the reticular layer. The excretory duct runs a straight or oblique course through the derma, and enters the epidermis between two papillae. In the epidermis the duct takes a spiral course to the surface where it opens into a minute pit just visible to the naked eye, the *sweat pore* (Fig. 205).

The coiled secretory portion of the gland is lined with a simple columnar or high cuboidal epithelium (Fig. 209), the height varying with the activity of the cells. The nuclei are round and stain deeply; the protoplasm contains secretory granules, fat droplets, and occasionally pigment granules. Distinct intracellular secretory channels can be demonstrated with proper technique (Fig. 209). A definite basement membrane is present but is separated in places from the secretory cells by *myoepithelial* cells which wind in longitudinal spirals around the tube.

The first part of the duct forms a part of the coil of the sweat gland and may be thinner than the secretory portion. The wall of the duct consists of a stratified cuboidal epithelium, resting upon a delicate basement membrane, outside of which is a network of longitudinally disposed elastic and collagenous connective tissue fibers (Fig. 209). The cells which form the inner layer of the duct wall have a refractile, cuticular-like zone along their inner or free surfaces where the cytoplasm is homogeneous and stains more intensely. On reaching the epidermis the

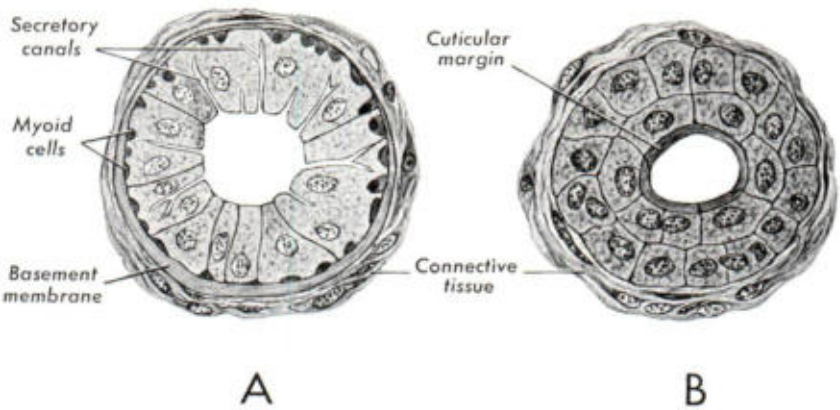

FIG. 209. Sections through a terminal secretory tubule (*A*) and a duct (*B*) of a sweat gland. × 650. (Redrawn and slightly modified from Schaffer.)

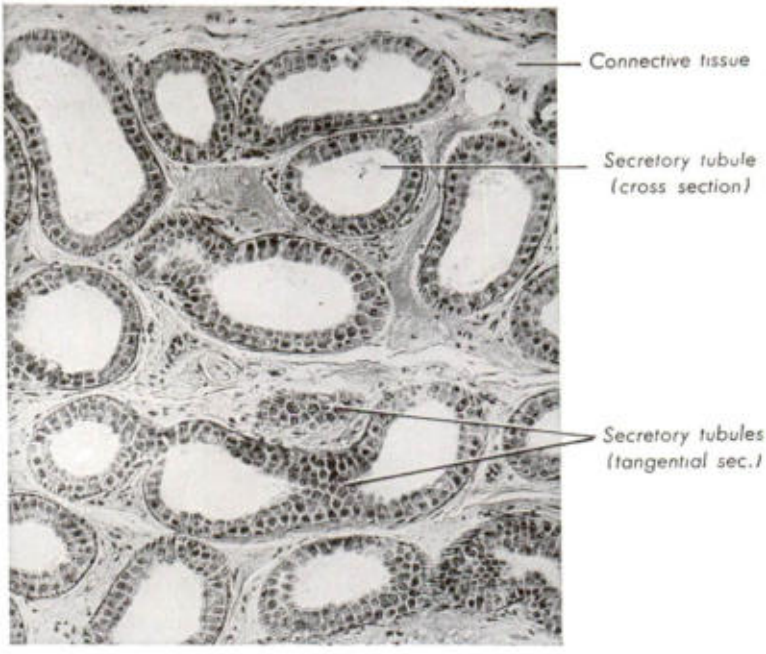

FIG. 210. Section of human circumanal glands. Note that these glands are much larger than the typical sweat glands illustrated in Figure 208. Photomicrograph × 110.

duct loses its own wall and becomes a mere channel through the epithelium (Fig. 205).

Especially large sweat glands are found in the axilla, mammary areola, labia majora and circumanal region (Figs. 210, 211). They yield a thicker secretion than the sweat formed by the smaller glands. To this group belong also the wax-secreting *ceruminous* glands of the external auditory canal, and the glands of Moll in the margin of the eyelid.

In glands of the axillary type the apical ends of the cells are frequently broken off in the process of secretion. This is known as an *apocrine* method of secretion and contrasts with the *merocrine* or *eccrine* method of the ordinary sweat glands in which the secretory materials are given out without an actual loss of cell cytoplasm.

Sebaceous Glands. These are usually associated with the hair follicles and will be described in that connection.

THE HAIR

The hairs are elastic horny threads developed from the epidermis. They are placed in deep narrow pits or pockets which traverse the derma to varying depths and usually extend into the subcutaneous tissue (Fig. 212). Each hair consists of a *shaft* which projects above the surface and a *root* which is imbedded within the skin. At its lower end the root presents a knob-like expansion the *hair bulb* in the under surface of which is a flask-shaped indentation of the connective tissue the *papilla* of the hair. Enclosing the hair root is the *hair follicle* which consists of an epidermal (epithelial) and dermal (connective tissue) portion.

Structure of the Hair. The hair is composed entirely of epithelial cells which are arranged in three definite layers; the medulla, cortex and cuticle.

(1) The *medulla* forms the central axis of the hair varying in thickness from 16–20 μ. It consists of two or three layers

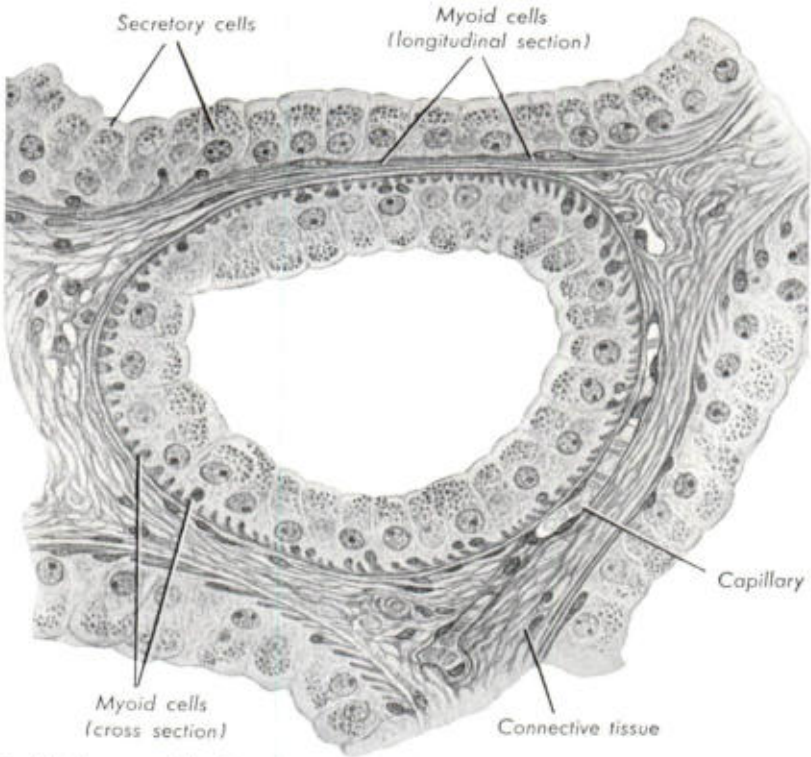

FIG. 211. Higher magnification of a portion of the circumanal gland shown in Figure 210. × 420.

of cells which vary in appearance in different parts of the hair. In the lower portion of the root of the hair the cells are cuboidal and have rounded nuclei (Fig. 214). In the shaft the cells of the medulla are cornified and shrunken and the nuclei are rudimentary or absent (Fig. 213). The intercellular spaces are usually filled with air. The medulla is absent from the finer, shorter (lanugo) hairs and is absent also from some of the hairs of the scalp. It frequently fails to extend the whole length of the hair.

(2) The *cortex* makes up the main bulk of the hair and consists of several layers of cells. In the lower part of the root of the hair, the cortex is composed of cuboidal cells with nuclei of normal appearance (Fig. 214). The cells become progressively flattened and modified at higher levels. In the upper part of the root of the hair and in the shaft, the cortex is composed of cornified, elongated cells with longitudinally striated cytoplasm and shrunken, degenerated nuclei (Fig. 213). In colored hair, pigment granules are found in and between the cells. Air also accumulates in the intercellular spaces and modifies the hair color.

(3) The *cuticle* of the hair is exceedingly thin and is composed of a single layer of clear cells. In the deeper part of the hair root, the cuticular cells are nucleated (Fig. 214). In the upper part of the root and on the shaft, the cuticular cells are clear scale-like and nonnucleated (Fig. 213). The cells overlap like shingles on a roof giving the surface of the hair a serrated appearance.

The color of the hair is determined

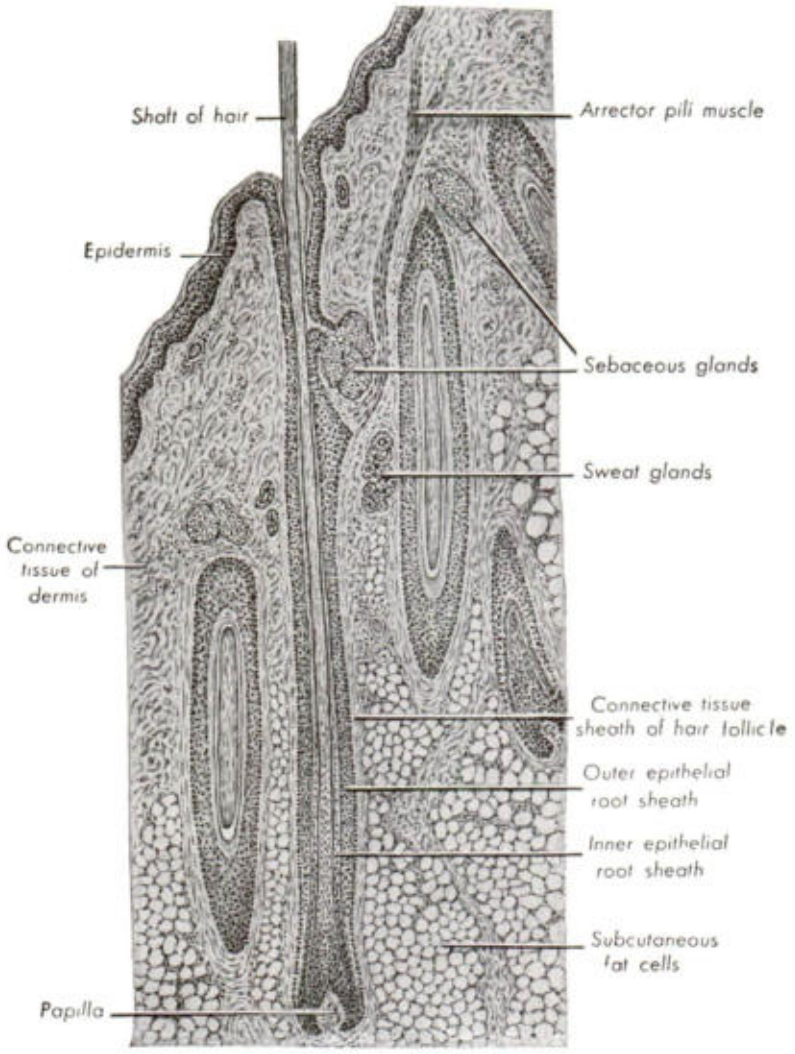

FIG. 212. Vertical section of human scalp showing a longitudinal section of a hair and its follicle. Reconstructed from serial sections to show a complete follicle cut through its longitudinal axis. × 36.

primarily by the amount of pigment, but to some extent also by the presence of air since the latter appears white in reflected light. Hair in which the pigment has faded and the medulla has become filled with air appears silvery white.

Hair Follicle. The hair follicle consists of the inner and outer epithelial root sheaths derived from the epidermis, and the connective tissue sheaths derived from the derma.

(1) The *inner epithelial root sheath* is composed of three distinct layers—the cuticle of the root sheath, Huxley's layer and Henle's layer.

The *cuticle of the root sheath* lies against the cuticle of the hair and is similar to the latter in structure (Fig. 214). It con-

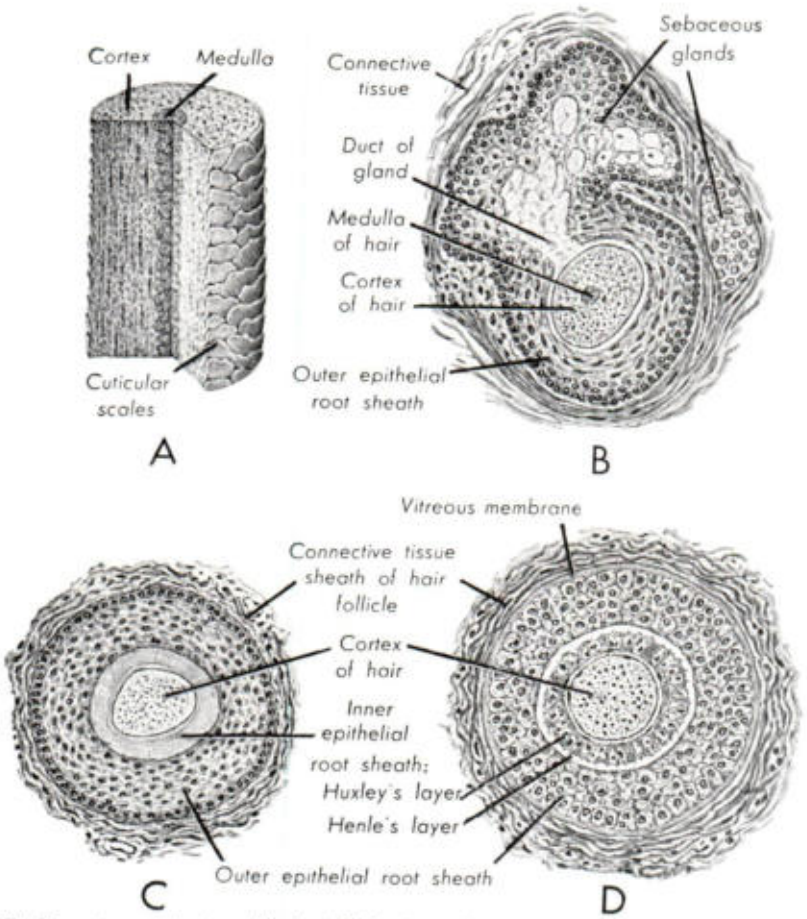

FIG. 213. Structure of hair and hair follicles from human scalp. *A*, A portion of the shaft of a hair reconstructed from a surface view and from longitudinal sections. *B*, *C*, and *D*, Cross sections of hairs and their follicles at various levels: *B*, at the level of the sebaceous glands; *C*, midway between epidermis of scalp and papilla of hair root; *D*, through the lower third of follicle. No medulla was present in the hairs illustrated in *C* and *D*. *A*, × 280; *B*, *C*, and *D*, × 175.

sists of thin scale-like overlapping cells, nucleated in the deeper parts of the sheath, nonnucleated nearer the surface. The free edges of the scales project downward and interdigitate with the upward projecting edges of the hair cuticle.

Huxley's layer lies immediately outside the cuticle of the root sheath, and consists of several rows of elongated cells whose protoplasm contains eleidin-like granules (trichohyalin). In the deeper portion of the hair follicle these cells contain nuclei (Fig. 215). Nearer the surface the nuclei are rudimentary or absent (Fig. 213, *C*).

Henle's layer is a row of rectangular somewhat flattened clear cells. The cytoplasm contains longitudinal horny fibrils, and nuclei are present only in the deepest portions of the follicle (Figs. 214, 215). Between the cells are sometimes seen

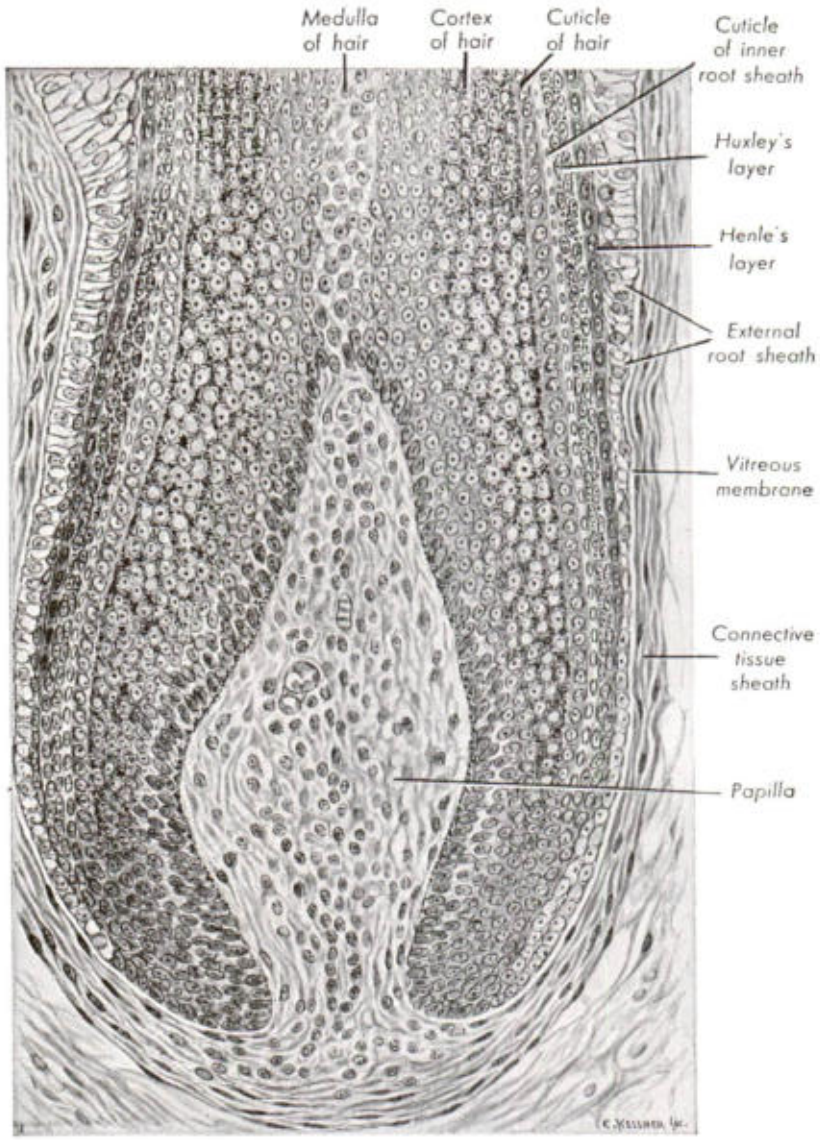

FIG. 214. Longitudinal section of lower end of root of hair, including hair follicle and connective tissue papilla. × 310.

short wedge-like processes which extend from the cells of Huxley's layer.

(2) The *outer epithelial root sheath* is a direct continuation of the stratum germinativum to which it corresponds in structure. The outermost cells, adjacent to the connective tissue, are tall and they are arranged in a single row (the stratum cylindricum). The rest of the cells are more polygonal in shape. They have distinct intercellular bridges and resemble the "prickle cells" already described for the stratum germinativum of the skin.

(3) The *connective tissue sheath* is de-

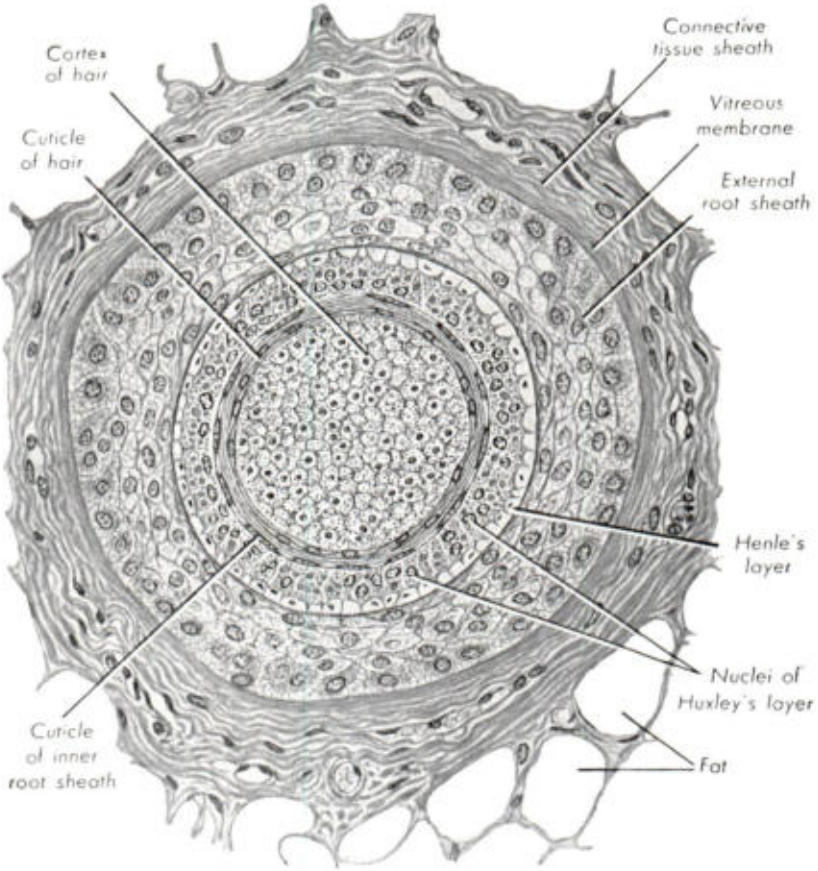

FIG. 215. Transverse section through root of hair and hair follicle. × 334

rived from the derma and consists of an inner, middle and outer layer.

The inner layer is a homogeneous narrow band, the hyaline or *vitreous membrane*, and is closely applied to the cylindrical cells of the outer root sheath. According to Stöhr two lamellae may be distinguished, an inner one formed by the epithelium, and an outer formed by the derma.

The middle layer is thickest and is composed of fine connective tissue fibers which are arranged circularly. The outer layer is poorly defined, and consists of rather coarse loosely woven bundles of white fibers which run in a longitudinal direction.

In the deeper portion of the root, some little distance above the bulb, all the layers of the hair and its follicles can be distinctly seen. The differentiation of the layers becomes less marked as one passes in either direction. At the level of entrance of the ducts of the sebaceous glands (Fig. 212) the inner epithelial root sheath disappears and the outer root sheath passes over into the stratum germinativum of the skin. The connective tissue follicle ceases as a distinct structure at about the level of insertion of the *arrector pili* muscles.

Marked changes are also noted as the bulb is approached. The outer root sheath thins down to two and finally to one layer of rather flat cells and then dis-

appears. The layers of the inner root sheath retain their identity until the neck of the papilla is reached, at which point the different layers coalesce.

The bulbous thickening of the hair root, which surrounds the papilla, is not organized into layers but constitutes a matrix of growing multiplying cells which superficially become transformed into the horny cells of the hair and the inner root sheath. Laterally the cells of the bulb become continuous with the outer root sheath which like the stratum germinativum grows by mitosis of cells in the deeper layers, i.e., the external layers of the root sheath. Thus the growth of the outer root sheath is radial, while the hair and inner root sheath grow upward from the thickened base of the follicle. The hair papilla, though much larger, is similar in structure to other dermal papillae and contains delicate elastic and collagenous fibrils, cellular elements, blood vessels and nerves.

Muscles and Glands of the Hair Follicle. The erectors of the hairs (*arrectores pilorum*) are oblique bands of smooth muscle fibers, from 50–220 μ in diameter, which arise in the sub-epithelial tissue and are usually inserted in the connective tissue follicle of the hair, about the middle of the follicle or a little above. Each muscle, at its origin, is attached to several delicate connective tissue strands, runs for a distance as a compact bundle and then divides into several bundles which go to the individual hairs of a hair group. The muscles usually arch around the sebaceous glands which fill the angle between the muscle and hair, though large sebaceous glands occasionally penetrate through the muscle. The thickness of the muscle bands corresponds roughly to the thickness and length of the hair. They are poorly developed in the hairs of the axilla and in certain parts of the face where the mimic musculature apparently takes over the function of arrectors. The eyebrows, eyelids and lashes have no arrectors.

The hairs and hair follicles are not perpendicular to the skin, but slope distinctly. The *arrector pili* muscle is situated in the obtuse angle between the hair follicle and surface. When the muscle contracts the hair becomes more vertical to the surface, and at the same time a small groove appears in the skin at the place where the muscle is attached. This gives rise to the so-called "goose flesh."

The *sebaceous* glands are with few exceptions connected with the hair follicles (Fig. 216). They are simple or branched alveolar glands. Their size varies considerably and bears no relation to the size of the hair, the largest glands being frequently connected with the smallest hairs. The glands are spherical or ovoid in shape and each gland is encapsulated in connective tissue. The excretory duct is wide and empties into the neck of the follicle. It is lined with stratified squamous epithelium continuous with the outer root sheath and stratum germinativum. The lower end of the duct opens into several simple or branched alveoli, at the mouths of which the epithelium becomes thinner. The alveoli themselves are completely filled with a stratified epithelium.

The most peripheral cells are rather small and cubical in shape and occasionally show mitotic figures. Towards the interior the polyhedral or spheroidal cells become progressively larger, due to the accumulation of numerous fat droplets in their cytoplasm. Their secretion, an oily substance called *sebum*, appears to be the direct product of disintegration of the alveolar cells (holocrine mode of secretion), and all the stages of the process are seen in the various layers of cells. The smaller peripheral cells contain but few small fat droplets or none at all. The most central ones and those in the lumen of the duct show the most marked changes.

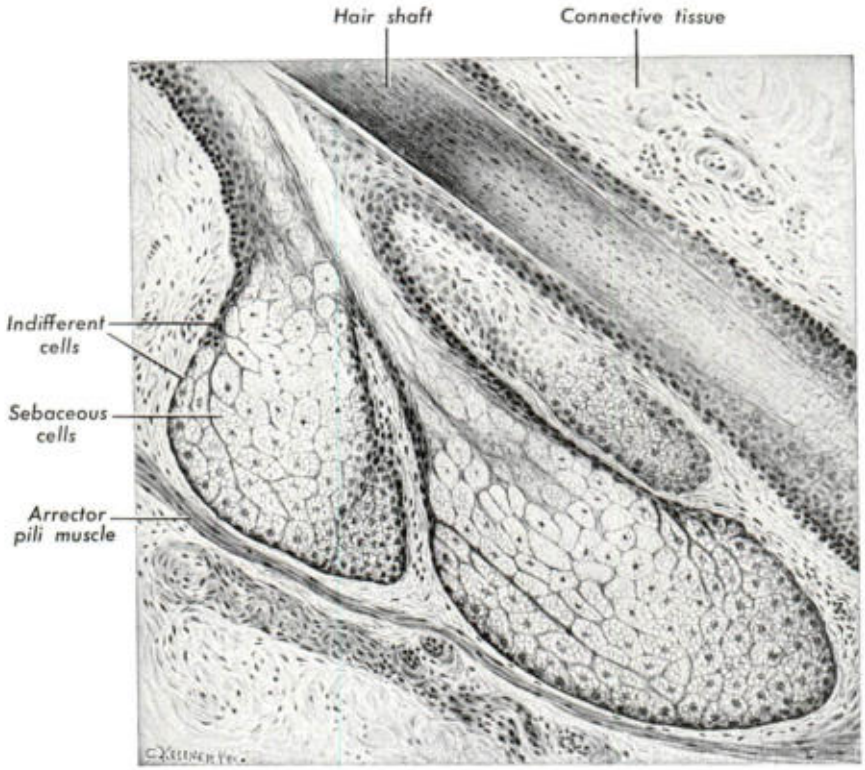

FIG. 216. Sebaceous gland from scalp. × 130

Their cytoplasm is almost wholly converted into fat and their nuclei are shrunken or disintegrated. In the middle zone are cells showing intermediate stages.

The replacement of cells lost in secretion is accomplished chiefly by mitotic divisions of the indifferent cells at the periphery of the gland (Fig. 216). The stages in this process as well as the proliferation of new alveoli from the cells of the excretory ducts can be demonstrated in animals in which the growth of the sebaceous glands is stimulated experimentally (Montagna and Kenyon, 1949). In fetal development, the glands and their ducts arise by proliferation and differentiation from the outer epithelial root sheath of the hair follicle (Fig. 221).

Sebaceous glands unconnected with hair follicles occur along the margin of the lips, in the nipple, in the glans and prepuce of the penis, and in the labia minora.

Replacement of Hairs. Shedding of hair takes place in most mammals at regularly recurring periods. In man there is constant though gradual loss and replacement of hairs. The scalp hairs have the longest duration of life, from two to five years, while those of the eyebrows and ears last only from three to five months. Even shorter is the age of the eyelashes.

When a hair is about to be shed, proliferation of cells above the papilla slows down and finally ceases. The bulb develops into a solid club-shaped mass and becomes completely keratinized, its lower end splitting brush-like into numerous fibers. The club-shaped bulb, firmly fused with the lower ends of the inner

root sheath which likewise cornifies, becomes detached from the papilla and is slowly shifted towards the surface of the skin, to about the level of the entrance of the sebaceous ducts. There it may remain for some time, until pulled out or shed or pushed out by a replacing hair. Such hairs are called *club hairs* to distinguish them from hairs which possess papillae.

The papilla atrophies and may completely disappear. The outer root sheath collapses and forms a cord of cells extending between the atrophied papilla and the lower end of the shedding hair.

The formation of a new hair starts with the proliferation of cells of the outer root sheath in the region of the old papilla. The papilla becomes larger and invaginates the cell mass, or, according to some, a new papilla is formed. From this new matrix or "hair germ" the new hair develops in a manner similar to embryonal hair formation. The new hair grows towards the surface, under or to one side of the dead hair which it finally replaces.

THE NAILS

The nails are composed of flat, horny scales which form protective coverings for the distal phalanges of the fingers and toes. Each nail consists of (a) a *body*, the attached uncovered portion of the nail; (b) a *free edge*, the anterior unattached extension of the body; (c) the *nail root*, the posterior or proximal part of the nail which lies beneath a fold of the skin (Fig. 217). Most of the body of the nail is pink because it is sufficiently translucent to transmit the color from the underlying vascular tissue. The proximal part of the nail is whitish and is called the *lunula* because of its shape.

The fold of skin which extends around the proximal and lateral borders of the nail constitutes the *nail fold* and the skin which lies beneath the nail forms the *nail bed*. The furrow between the nail bed and nail fold is the *nail groove* (Figs. 217 and 218).

The nail itself is hard and horny and consists of several layers of clear, flat cells which contain shrunken and degenerated nuclei. The striated appearance observed in sections cut perpendicular to the surface is produced by the arrangement of the cells in layers.

The nail bed consists of epithelium and corium continuous with the epidermis

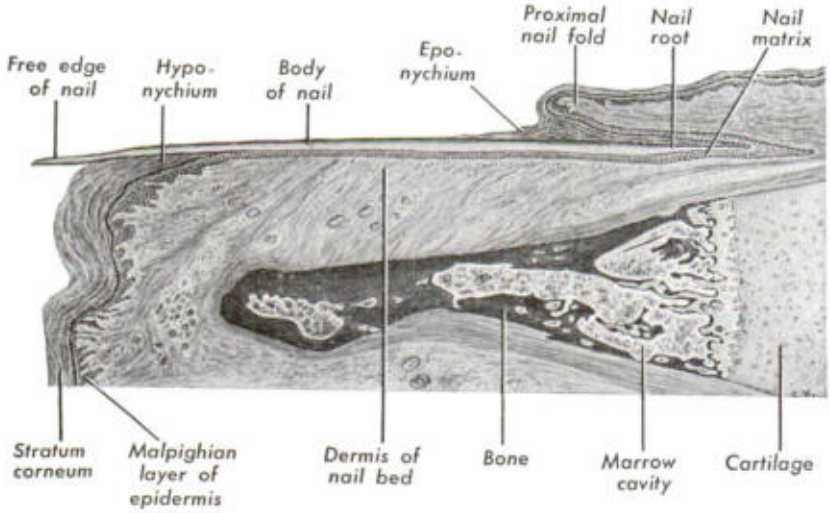

FIG. 217. Longitudinal section of a finger nail of a newborn infant. × 18

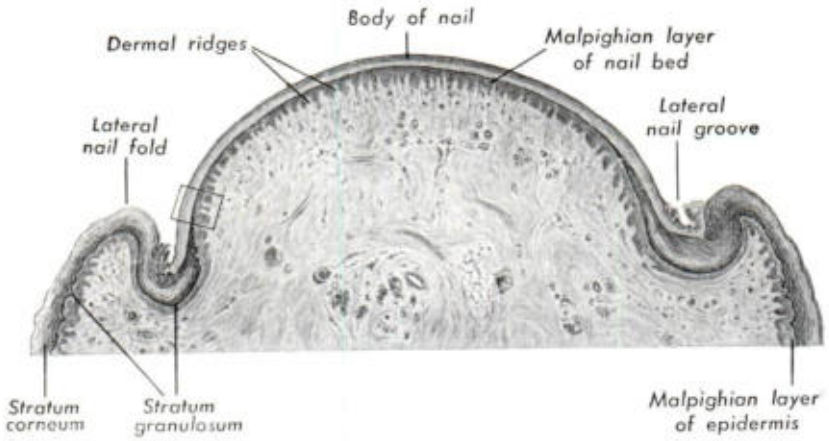

FIG. 218. Transverse section of nail and nail bed. Human, adult. The area marked by the square is enlarged in Figure 219. × 19.

and dermis of the skin of the nail folds. The epidermis of the nail folds usually has the four zones characteristic of palmar skin although the stratum lucidum may be thin or absent in some cases. The stratum corneum of the proximal nail fold turns into the nail groove, spreads over the upper surface of the nail root, and continues for a short distance onto the surface of the body of the nail as the *eponychium* (Fig. 217). The stratum corneum of the lateral folds ends in the lateral grooves in contact with the borders of the nail (Figs. 218 and 219). The stratum granulosum and lucidum terminate in the lower part of the grooves.

The epithelium of the nail bed corresponds to the stratum germinativum of the skin and, like the latter, consists of polygonal "prickle" cells and a stratum cylindricum resting upon a basement membrane. The epithelium of the posterior part of the nail bed, the part which lies beneath the root and the proximal portion of the body corresponding to the lunula, is thicker than elsewhere and is called the *matrix* because it functions for nail growth. Growth of the nail takes place by a transformation of the more superficial cells of the matrix into true nail cells. In this process the outer harder layer is pushed forward over the stratum germinativum, the latter remaining always in the same position.

Under the anterior free edge of the nail, the epithelium of the nail bed becomes continuous with the stratum germinativum of the skin and the other layers characteristic of the epidermis begin. The stratum corneum of the skin beneath the free edge of the nail is thickened and is known as the *hyponychium* (Fig. 217).

The corium of the nail bed differs somewhat from that of ordinary skin. Its connective tissue fibers are arranged partly longitudinal to the long axis of the nail and partly in a vertical plane extending from the periosteum to the nail. Dermal papillae are found beneath the posterior part of the nail root but disappear beneath the anterior part of the root to be replaced by longitudinal dermal ridges which, increasing in height as they pass forward, continue to the anterior end of the nail bed. Since the dermal ridges run longitudinally, the boundary between the epithelium and connective tissue appears smooth in longitudinal sections and irregular and papilla-like in cross sections (Fig. 218).

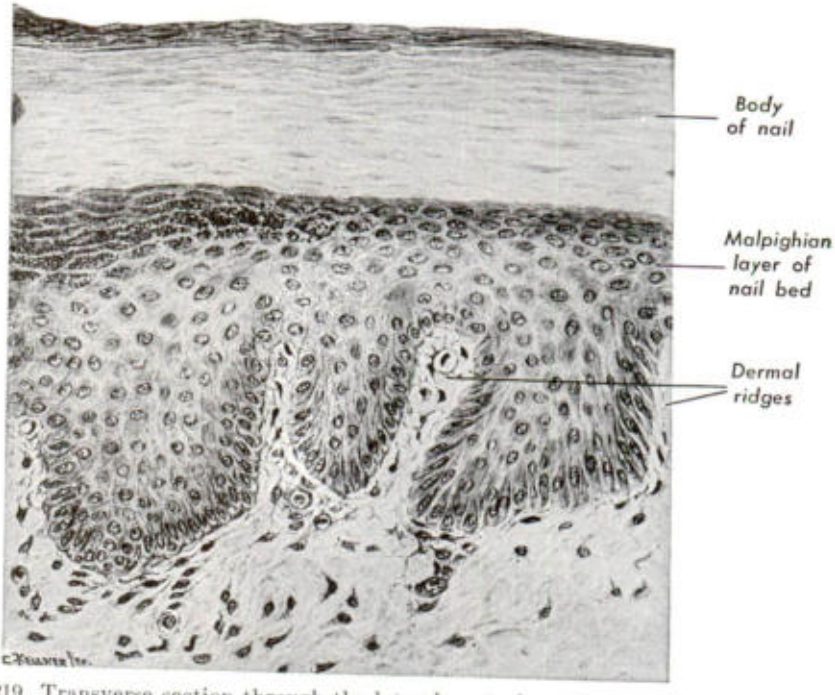

FIG. 219. Transverse section through the lateral part of a nail, near the lateral nail groove, as outlined in Figure 218. × 365.

BLOOD VESSELS, LYMPHATICS AND NERVES OF THE SKIN

Blood Vessels of the Skin. From the larger arteries in the subcutaneous tissue branches penetrate the reticular layer of the derma, where they anastomose to form cutaneous networks. The latter give off branches, which pass to the papillary layer of the derma and there form a second series of networks, the subpapillary, just beneath the papillae. From the cutaneous networks arise two sets of capillaries, one supplying the fat lobules, the other supplying the region of the sweat glands. From the subpapillary networks are given off small arteries which break up into capillary networks for the supply of the papillae, sebaceous glands and hair follicles. The return blood from these capillaries first enters a horizontal plexus of veins just under the papillae. This communicates with a second plexus just beneath the first. Small veins from this second plexus pass alongside the arteries of the deeper part of the corium, where they form a third plexus with larger, more irregular meshes. Into this plexus pass most of the veins from the fat lobules and sweat glands, although one or two small veins from the sweat glands usually follow the duct and empty into the subpapillary plexus. The blood next passes into a fourth plexus in the subcutaneous tissue, from which arise veins of considerable size. These accompany the arteries (Fig. 220).

Small arteries from the plexuses of the skin and subcutis pass to the hair follicle. The larger arterioles run longitudinally in the outer layer of the follicle. From these are given off branches which form a rich plexus of small arterioles and capillaries in the middle vascular layer of the follicle. Capillaries from this plexus also

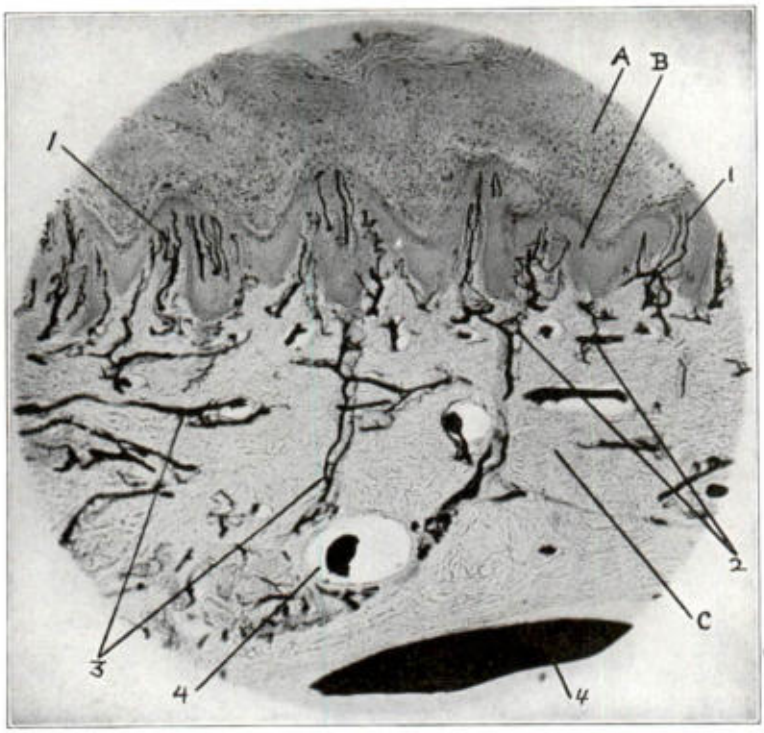

FIG. 220. Section of skin with injected blood vessels. Photograph. *A*, stratum corneum; *B*, stratum germinativum of epidermis; *C*, derma; *1*, papillary vessels; *2*, subpapillary vessels; *3*, blood vessels of reticular layer; *4*, larger veins of derma.

pass to the sebaceous glands, the arrectores pilorum muscles and the papillae.

The Lymphatics of the Skin. These begin as clefts in the papillae which open into a horizontal network of lymph capillaries in the papillary layer. This communicates with a network of larger lymph capillaries with wider meshes in the subcutaneous tissue. The latter also receives lymph capillaries from plexuses which surround the sebaceous glands, the sweat glands, and the hair follicles.

The Nerves of the Skin. These are mainly sensory. Efferent sympathetic axons supply the smooth muscle of the walls of the blood vessels, the arrectores pilorum, and the secretory cells of the sweat glands. The sensory nerves are peripheral processes of somatic ganglion cells. The larger trunks lie in the subcutis, giving off branches which pass to the corium, where they form a rich subpapillary plexus of both medullated and nonmedullated fibers. From the subcutaneous nerve trunks and from the subpapillary plexus are given off fibers which terminate in more or less elaborate special nerve endings (Chap. X). Their location is as follows: (1) *In the subcutis:* Vater-Pacinian corpuscles. They are most numerous in the palms and soles. (2) *In the derma:* tactile corpuscles of Meissner are found in the papillae, especially of the finger tip, palm and sole. Krause's end bulbs are usually in the derma just beneath the papillae, more rarely in the papillae themselves. (3) *In the epithelium:*

free nerve endings among the epithelial cells.

Branches of the cutaneous nerves supply the hair follicles which are important in sensory reception. As a rule but one nerve passes to each follicle, entering it just below the entrance of the duct of the sebaceous gland. As it enters the follicle the nerve fiber loses its medullary sheath and divides into two branches, which further subdivide to form a ring-like plexus of fine fibers encircling the follicle. From this ring, small varicose fibrils run for a short distance up the follicle, terminating mainly in slight expansions on the vitreous membrane.

DEVELOPMENT OF THE SKIN AND ITS APPENDAGES

The *epidermis* develops from the ectoderm and consists at first of a single row of cuboidal

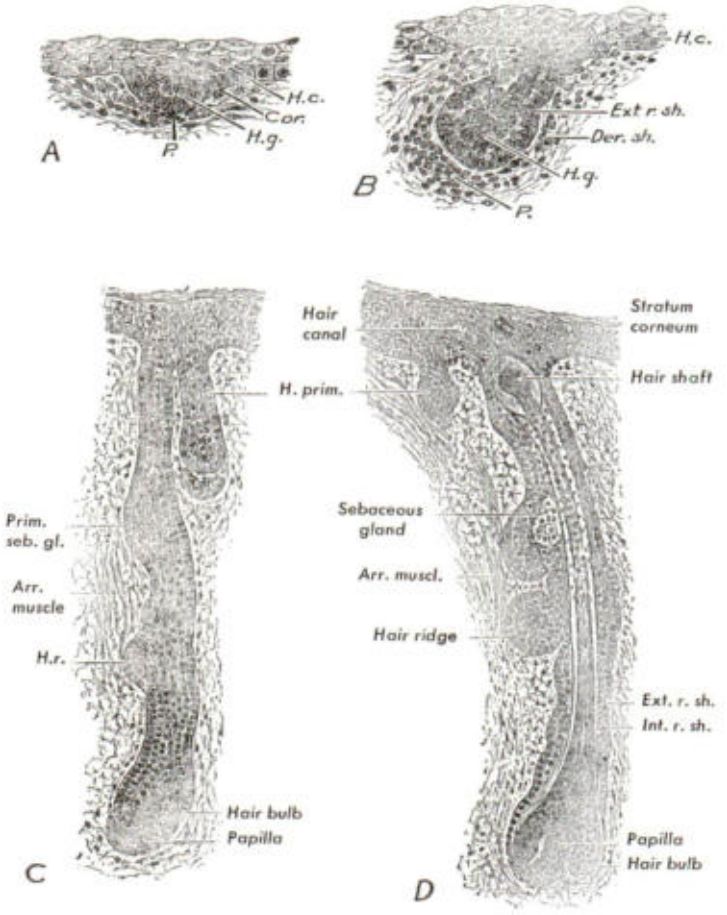

FIG. 221. Four stages in the development of a human hair. *A* and *B*, Early stages from frontal skin of a three month embryo. *C* and *D*, Later stages from chin of a five month embryo. *Arr. muscl*, Primordium of arrector muscle; *Cor*, border of the corium; *Der.sh*, primordium of dermal sheath (connective tissue follicle); *Ext.r.sh*, external root sheath; *H.c*, hair canal cells; *H.g*, hair germ; *H.prim*, primordia of young hairs; *H.r*, hair ridge for attachment of arrector muscle; *Int.r.sh*, internal root sheath; *P*, primordium of papilla; *Prim.seb.gl*, primordium of sebaceous gland. (Redrawn after Schaffer.)

cells. By the second month it has differentiated into two layers. The outer layer, the periderm or epitrichium, consisting of flattened cells, is a transient structure and is lost shortly before or after birth. The inner layer, composed of irregular cells with large nuclei and abundant protoplasm, gives rise to the entire epidermis. The cells multiply by mitosis and soon form a stratified epithelium. True keratinization, however, begins only in the fifth month.

The *derma* develops from the parietal mesoderm and consists at first of typical mesenchyme. In the second month cell differentiation begins, some of the cells becoming fibroblasts. Towards the end of the second month fibrils make their appearance, at first collagenous, and soon after elastic fibrils as well. Some time later the whole mass shows a differentiation into two layers, an upper denser one giving rise to the corium proper, and a deeper, looser one which forms the subcutaneous tissue.

Hair appears in human embryos at the end of the second month, the first places being the brows, upper lips and chin. By the seventh month lanugo hairs are distributed all over the body.

Each hair develops as a thickening of the germinative layer, which grows down obliquely into the derma, forming a slender solid cord of cells, the *hair germ* or *hair plug* (Fig. 221). The connective tissue cells of the corium surrounding the cord condense to form the dermal follicle, while an invagination of connective tissue into the lower end of the hair germ forms the papilla.

The cells surrounding the papilla become differentiated into two zones, a central conical mass whose apex is directed toward the surface (the hair bulb), and an outer layer of epithelial cells which becomes the outer root sheath. The cells of the hair bulb grow towards the skin, the axial cells forming the hair, the peripheral cells giving rise to the inner root sheath. The various sub-layers are formed from these by subsequent differentiation. Above the apex of the cone the axial cells of the hair plug cornify and disintegrate, and a channel is formed which penetrates into the epidermis. The hair grows into this channel, and when first formed lies wholly beneath the surface. As the hair reaches the surface its pointed extremity pierces the surface epithelium and emerges as the hair shaft.

The *sebaceous* gland develops from the outer root sheath and appears first as a thickening in the upper portion of the hair germ (see above). It soon develops into a flask-shaped solid mass of cells which later differentiate to form the ducts and alveoli of the gland.

The *sweat* glands first appear as solid ingrowths of the stratum germinativum into the underlying corium. The lower end of the ingrowth becomes thickened and convoluted to form the coiled portion of the gland, and somewhat later the central portion becomes channeled out to form the lumen. The myoepithelial cells which lie between the epithelium and the basement membrane, are derived from the ectodermal cells of the ingrowth, and not from mesoderm.

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XV

GLANDS

STRUCTURE AND CLASSIFICATION

All cells of the body take up, from the surrounding blood and lymph, substances required for their own nutrition and give off waste products. In this sense all cells secrete and excrete. Certain cells of the body, in addition to carrying on these metabolic processes necessary for their own existence, also manufacture specific substances not for their own use but to be extruded from the cells and used elsewhere in the body (secretions, e.g., gastric juice), or discarded (excretions, e.g., urine). Such cells are known as *gland cells* or *glandular epithelium*, and an aggregation of these cells to form a definite structure for the purpose of carrying on secretion or excretion is known as a *gland*.

A gland may consist of a single cell, as for example, the goblet cell (p. 52) or the *unicellular glands* of invertebrates. Such a cell produces within itself a substance which is to be used outside the cell. The appearance which this cell presents depends upon the stage of secretion. It is thus possible to differentiate between a "resting" and an "active" cell or between an "empty" and a "loaded" cell.

Most glands are composed of more than one cell (*multicellular glands*). Usually there is a large number of cells, and these cells, instead of lying directly upon the surface, line more or less extensive invaginations into which they pour their secretions.

General Structure of Secretory Cells. The goblet cell of the intestine is one of the simple columnar cells which constitute the surface epithelium of the mucous membrane. It is distinguishable as a mucous cell only after the formation of secretion has begun. When filled with secretion, the apical portion of the cell becomes dilated while the basal portion remains slender, giving the cell a goblet shape. The swollen apical portion in the living cell contains droplets of premucin or mucigen. The mucigen droplets are usually not stained in sections of fixed tissues, since most of the methods of histological technique dissolve the mucigen, leaving a loose filamentous network in the cytoplasm.

At the height of the "active" phase of secretion, the mucigen droplets are extruded through an opening at the apical end of the cell (Fig. 222), and are dissolved and immediately converted into mucin. In some cases the droplets are extruded gradually while new droplets are formed and the goblet shape of the cell is retained for a considerable length of time. More often the droplets are extruded in mass and the cell immediately loses the goblet shape and reverts to a more slender form. After a period of rest the same cell may again become active and pass through the same stages of secretion.

The exact processes involved in the formation of secretory materials are not

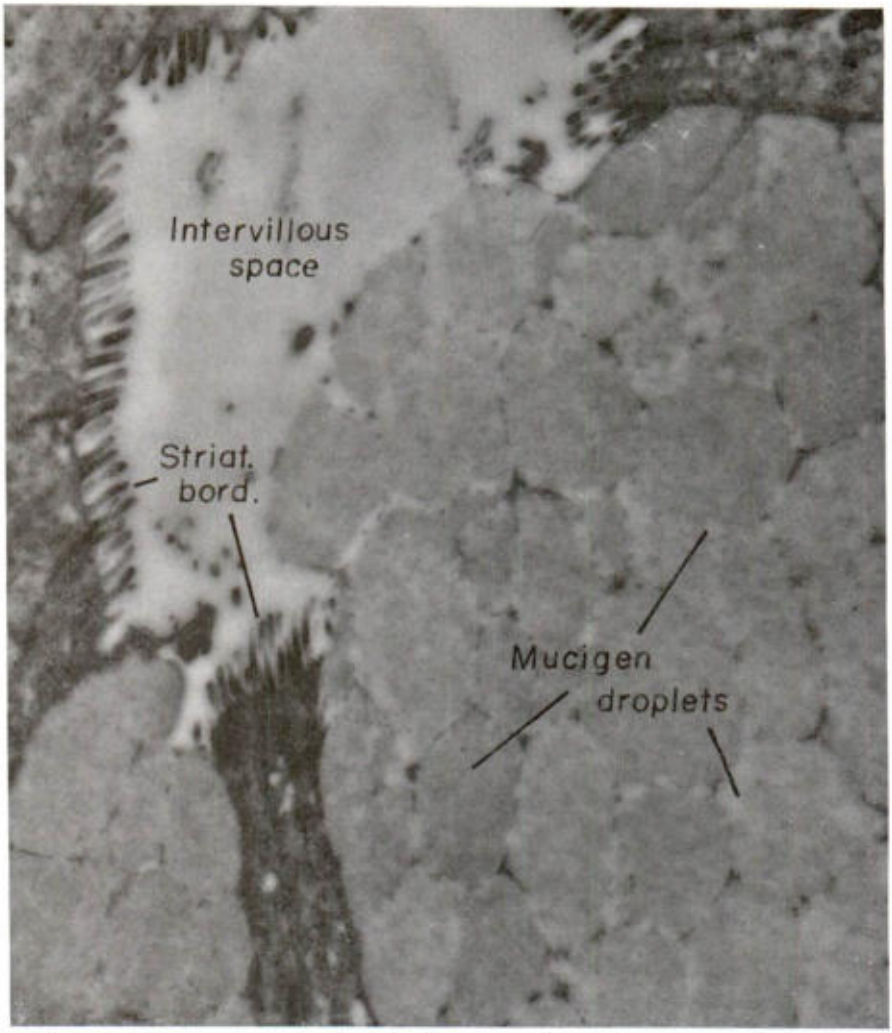

FIG. 222. Electron micrograph of apical portion of goblet cell of jejunum releasing secretory material into intervillous space. The goblet cell is flanked on either side by columnar absorbing cells showing striated free border. × 19,500. (Courtesy of Dr. S. L. Palay.)

entirely understood, but certain cytoplasmic structures such as the Golgi complex, mitochondria and endoplasmic reticulum appear to play important, although perhaps indirect, roles in the process (pp. 14 and 17). In electron micrographs, secretory granules or droplets may be identified in very close association with or even within vesicular elements of the Golgi complex (Fig. 223) or of the endoplasmic reticulum (Fig. 224).

In the basal portion of serous gland cells (e.g., cells of salivary glands, pancreas, etc.) there is a characteristic basophilic or *chromophilic material* (Figs. 225, 287). Its appearance is due to the presence of a high concentration of ribose nucleic acid (RNA) associated with a

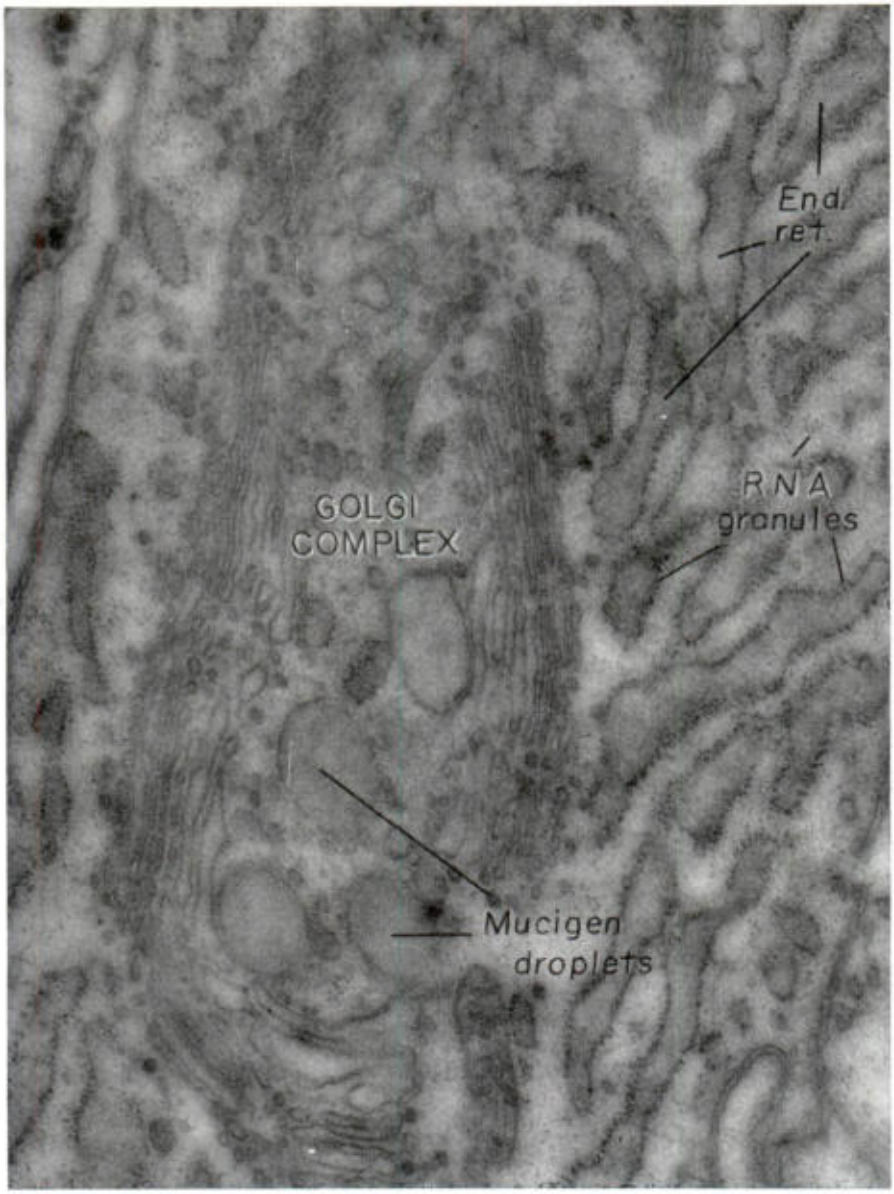

FIG. 223. Electron micrograph of Golgi area of goblet cell of jejunum. Mucigen droplets are enclosed within the cisternae of the Golgi complex. Vesicles of endoplasmic reticulum (*End. ret.*) are covered with RNA granules. Cell membranes can be seen at upper left and lower right. × 40,600. (Courtesy of Dr. S. L. Palay.)

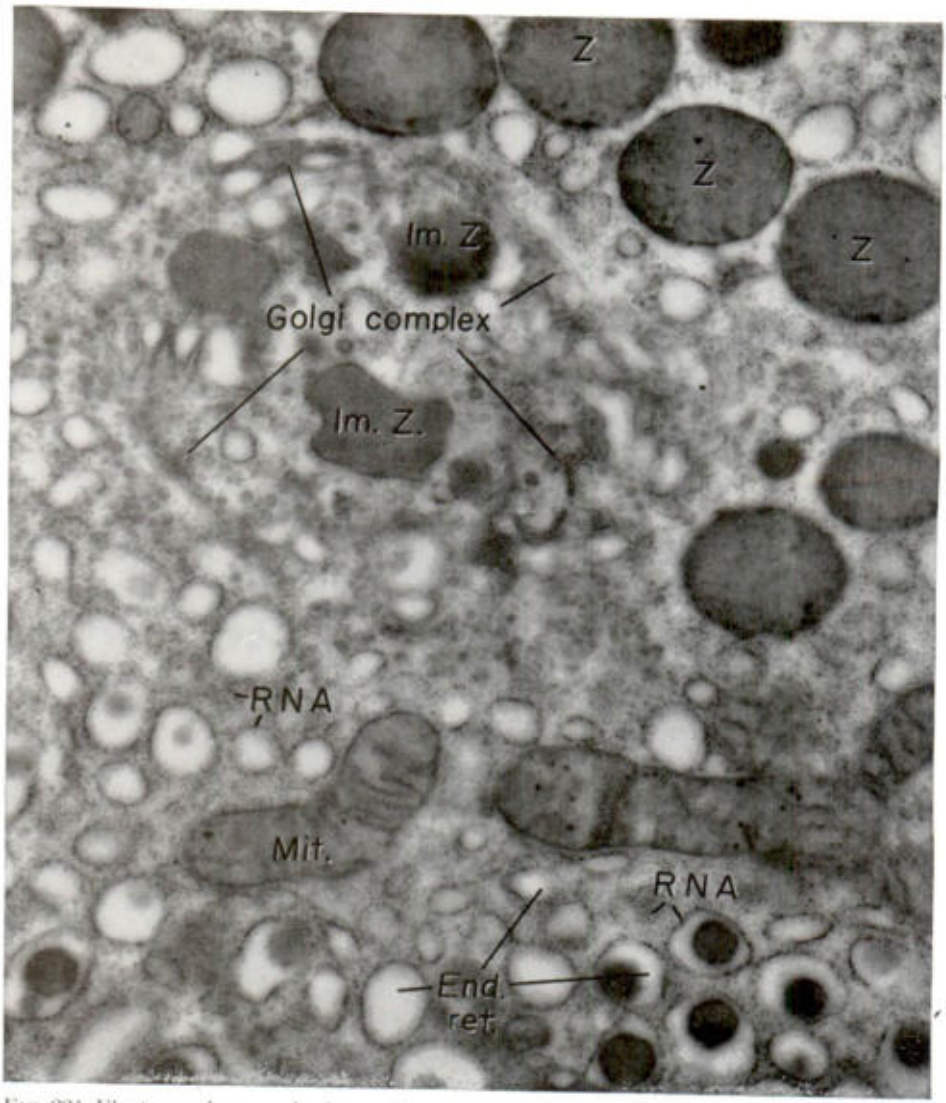

FIG. 224. Electron micrograph of a portion of guinea pig pancreatic acinar cell showing relationship of secretory granules to cytoplasmic components. Large zymogen granules (*Z*) are present in the apical cytoplasm (upper part of figure), immature zymogen granules (*Im. Z.*) in the region of the Golgi complex, and in addition, smaller granules of somewhat different density within some of the vesicles of the endoplasmic reticulum (*End. ret.*). These are particularly numerous in the basal portion of the cell. The surfaces of the vesicles as well as the intervening cytoplasm show many RNA granules. The nucleus was not included in the plane of this section. × 25,000. (Courtesy of Dr. G. E. Palade.)

highly developed endoplasmic reticulum (Fig. 288). It is now known that RNA is important in the synthesis of cytoplasmic proteins, including secretory granules of protein nature.

The nucleus of the cell is also of importance in the secretory processes, but like the cytoplasmic structures mentioned above, the exact manner in which it participates is not clear. It is thought by some that the nucleolus, with its high content of RNA, may contribute to the formation of protein materials both inside and outside the nucleus. Certainly the amount of nucleolar material is in general greatest in those cells which are actively engaged in the synthesis of protein substances.

Classification of Glands. As has been mentioned, a gland may consist of a single secretory cell (unicellular gland), or it may be composed of many cells (multicellular gland), the secretory cells usually lining an invagination from the free surface. In the simplest form of a glandular invagination all the cells lining the lumen are secreting cells. In more highly developed glands only the deeper cells secrete, the remainder of the gland serving merely to carry the secretion to the surface. This latter part is then known as the *duct*, in contradistinction to the deeper *secreting portion*. In both the duct portion and secreting portion of a gland the epithelium rests upon a more or less definite *basement membrane* or *membrana propria* (p. 50). Beneath the basement membrane, separating and supporting the glandular elements, is a fine vascular connective tissue.

Glands are sometimes classified according to the nature of their secretion into *mucous* (producing a viscous, slimy secretion), *serous* (producing a thin, watery secretion) and *mixed glands* (producing both types of secretion). Although this classification is particularly useful for some of the glands, such as those of the oral cavity, it cannot be satisfactorily used for all glands of the body, such as the sebaceous and mammary glands, the kidney, etc.

The cells of mucous and serous glands differ in structure. The structure of the unicellular mucous gland or goblet cell has already been described. In the multicellular mucous glands or *mucous alveoli* (e.g., of the palatine glands) the cells are usually more or less pyramidal in shape due to their arrangement around the lumen of the terminal tubule. When the cell is filled with secretion the nucleus is flattened against the basal part of the cell. As already described for the goblet cell, the mucigen of these cells is not well preserved with ordinary techniques, and is dissolved leaving a loose network which, with hematoxylin and eosin, remains unstained or stains very faintly with hematoxylin.

The cells of *serous alveoli* secrete a clear, watery, albuminous product. The secretion of many serous glands (e.g., pancreas and parotid gland) contains digestive enzymes. In these cells, the secretory granules are the enzyme precursors, or *zymogen granules* (Figs. 225, 226), and the cells are therefore known as

FIG. 225. Acinus of human pancreas. Acinar enlargement produced by the increase in height of the secretory cells while the lumen remains narrow. All secretory cells in same functional stage. Zymogen granules in apical portion of cells; chromophilic material in basal portion of cells. Chrome hematoxylin and phloxine stain. × 1100.

FIG. 226. Serous alveolus of human submaxillary gland. Cells are shown in different functional stages; the cells containing zymogen granules are taller than those without. Myoepithelial cells are seen between the secretory cells and the basement membrane. Hemalum-mucicarmine-aurantia. (Redrawn after Zimmerman.)

serozymogenic cells. Serous cells are usually pyramidal with rounded nuclei lying in the basal half of the cells. The cells pass through active and resting phases of secretion, similar to the mucous cells. During the resting phase, the secretory granules may become so numerous as to almost fill the cell, but the nucleus does not become flattened as in the mucous cell. The secretory granules, if they are preserved, are acidophilic. Chromophilic material (RNA) is found in high concentration in the basal portion of serozymogenic cells, giving that part of the cell a strongly basophilic, striated appearance (Fig. 225).

The alveoli of mixed glands (e.g., submaxillary and sublingual) contain both mucous and serous cells.

According to whether the secretion is merely a product of the cell or whether it consists of gland cells, the glands may be classified as *merocrine*, *apocrine* and *holocrine*. The majority of the glands are merocrine. The secretion is a product of the cell but the cell itself is not extruded with the secretion. In the sebaceous glands, entire cells laden with secretory material are extruded as the secretion. This is the holocrine type of secretion. An intermediate type of secretion known as apocrine may be found in the active mammary gland. During periods of marked activity the apical ends of these cells with their accumulated secretory materials are sometimes broken off to form a part of the secretion. The nucleus and most of the cytoplasm remains, and from this a complete cell is reformed to again pass through the same stages. The gonads (ovary and testis) produce very highly specialized secretions consisting of living cells (ova and sperm) which continue to develop after extrusion from the gland. Since they secrete living cells, the ovary and testis are sometimes called *cytogenic* glands.

Glands may also be classified according to whether they possess ducts which carry their secretions to an epithelial surface (*exocrine glands, glands of external secretion*) or whether they are ductless and pour their secretions into the blood or lymph (*endocrine glands, glands of internal secretion*).

The exocrine glands may further be subdivided and classified in accordance with the type of duct system contained. When a gland consists of a single secretory passage or a single system of secretory passages opening into an unbranched duct it is called a *simple gland* (Fig. 227, *A–G*). When a gland contains a duct system which is elaborate and branched, it is called a *compound gland* (Fig. 227, *H–J*). Both the simple and compound glands may be subdivided in accordance with the form of the terminal secretory portions. When the secreting portion is a tubule, the lumen of which is of fairly uniform diameter, the gland is known as a *tubular gland* (Fig. 227, *A–C*). When the secreting

portion is dilated in the form of a sac or alveolus the gland is known as a *saccular, alveolar* or *acinar gland* (Fig. 227, *E*, *F*, *G*, *J*). In many glands the secretory passages are neither typically tubular nor alveolar but intermediate in type and combine certain characteristics of both; such glands are called *tubulo-alveolar* or *tubulo-acinar* (Fig. 227 *D*, *I*). The serous salivary glands and pancreas are typical examples of this type and have acinar enlargements which are produced by an increase in the height of the secretory epithelium while the lumen remains undilated and tubular (Figs. 225 and 227 *I*, *a*, *e*, *f* and *g*). In some of the glands usually included in this group, such as the mucous salivary glands, mucous portions of the mixed salivary glands, and Brunner's glands of the duodenum, the secretory passages vary in type of lumen from tubular forms to dilations resembling elongated alveoli (Fig. 227, *I*, *b*, *c*).

According to some authors the terms "acinus" and "alveolus" should be differentiated and acinus should be restricted to an enlargement which has a narrow lumen. In this sense, the serous salivary glands are tubulo-acinar and not tubulo-alveolar, while Brunner's glands and many of the mucous terminations of the sublingual, etc., are tubulo-alveolar and not tubulo-acinar. Most authors, however, have not followed this differentiation of terms. In the present text alveolus and acinus are used interchangeably and accordingly tubulo-alveolar is used in the classification as synonymous with tubulo-acinar.

Glands may thus be classified as follows:

A. Exocrine glands (or glands with ducts)
  1. Simple glands
    (a) Tubular { straight / coiled / branched }
    (b) Tubulo-alveolar (tubulo-acinar)
    (c) Alveolar (acinar, saccular)
  2. Compound glands
    (a) Tubular
    (b) Tubulo-alveolar (tubulo-acinar)
    (c) Alveolar
B. Endocrine glands (or glands without ducts)

EXOCRINE GLANDS

Simple Tubular Glands. These glands consist of simple epithelial-lined tubules which open on the surface. All the cells may be secreting cells, or only the more deeply situated ones. In the latter case the more superficial portion of the tubule serves merely as a duct. In the more highly developed of the simple tubular glands we distinguish a mouth, opening upon the surface, a neck, usually somewhat constricted, and a fundus, or deep secreting portion of the gland.

Simple tubular glands are divided according to the behavior of the fundus into (1) straight, (2) coiled, and (3) branched.

(1) A *straight tubular gland* is one in which the entire tubule runs a straight unbranched course, e.g., the glands of the large intestine (Fig. 227, *A*).

(2) A *coiled tubular gland* is one in which the deeper portion of the tubule is coiled or convoluted (Fig. 227, *B*). The sudoriferous glands of the skin are the most typical examples.

(3) A *branched tubular gland* is a simple tubular gland in which the deeper portion of the tubule branches, the several branches being lined with secreting cells and opening into a superficial portion which serves as a duct (Fig. 227, *C*). Examples of branched tubular glands are the fundic, pyloric, and cardiac glands of the stomach and the glands of the mucous membrane of the uterus. Many of the pyloric glands are also slightly enlarged and coiled at their terminations so that they resemble to some extent both the convoluted tubular and the tubulo-alveolar glands.

Simple Tubulo-alveolar Glands.

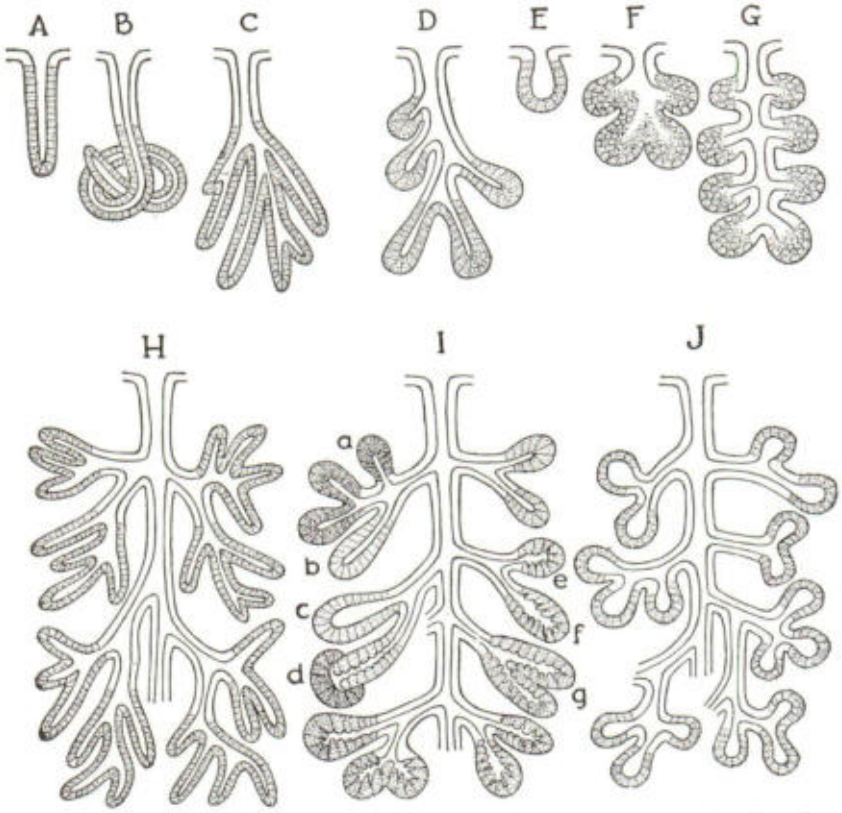

FIG. 227. Schema of various types of exocrine glands. *A* to *G*, simple glands; *H* to *J*, compound glands. *A*, simple, nonbranched tubular; *B*, simple coiled tubular; *C*, branched tubular; *D*, branched tubulo-acinar (tubulo-alveolar); *E*, simple, nonbranched alveolar; *F* and *G*, branched alveolar as in sebaceous glands; *H*, compound tubular; *I*, compound tubulo-acinar, showing some of the various types of terminations. *a*, Serous acinus with simple, nonbranched canal; *b* and *c*, variations in size of lumen of mucous terminations, as in sublingual, etc.; *d*, tubulo-alveolus of mucous tubule and serous demilune; *e*, *f*, and *g*, serous terminations with branched canals, or intercellular secretory canaliculi as in parotid, etc.; *J*, compound alveolar gland.

Simple tubulo-alveolar or tubulo-acinar glands are found only in the branched form (Fig. 227, *D*). Included in this group are the smaller glands of the following types: salivary glands of the oral cavity, seromucous glands of the respiratory tract, mucous glands of the esophagus and the Brunner's glands of the duodenum. Many of these, such as the esophageal and Brunner's glands, are classified by some authors as branched tubular but since they are frequently enlarged at their terminations, they may be included in the branched tubulo-alveolar group.

Simple Alveolar Glands. The simplest form of alveolar gland, consisting of a single sac with a dilated lumen and connected with the surface by a constricted portion, the neck, is shown in Figure 227, *E*. This simple form of alveolar gland is found in the skin of certain amphibians but does not occur in man. Simple alveolar glands in which there are several saccules are represented by the smaller sebaceous glands (Fig. 227,

F). In the sebaceous glands, as already pointed out (p. 290), the secreting cells undergo fatty degeneration, are pushed centrally by new cells, and are finally extruded as the secretion. Consequently, there are a number of layers of cells filling the space which would otherwise represent the lumen of the alveolus. Simple branched alveolar glands (Fig. 227, *G*), in which a common duct gives rise to a number of saccules are seen in the larger sebaceous glands and in the Meibomian glands.

Compound Tubular Glands. The compound tubular glands consist of a number, often of a large number, of distinct duct systems, which open into a common or main excretory duct (Fig. 227, *H*). The kidney and testis are examples of compound tubular glands. Some authors, as already stated, classify the smaller mucous glands of the esophagus, etc., as branched tubular glands and accordingly classify the larger of the mucous glands of the esophagus, mucous terminations of the salivary glands, and Brunner's glands of the duodenum as compound tubular, but since these glands frequently terminate in enlargements they are usually classified as compound tubulo-alveolar.

Compound Tubulo-alveolar Glands. The glands of this type (Fig. 227, *I*) are numerous and widely distributed and include the parotid, pancreas, submaxillary, sublingual, the larger of the mucous glands of the esophagus and seromucous glands of the respiratory tract, many of the duodenal (Brunner's) glands, etc. The structure of the terminal tubules and duct systems vary somewhat in the different glands and for the finer structure reference may be made to the special sections dealing with some of the more typical glands of the group such as the parotid and the pancreas, Chapter XVI.

Compound Alveolar Glands. The compound alveolar glands resemble the compound tubular and compound tubulo-alveolar glands in having a large number of duct systems, but the terminal ducts, instead of ending in tubular and tubulo-acinar secreting passages, end in alveoli with dilated sac-like lumina (Fig. 227, *J*). The mammary gland is the best example of a compound alveolar gland.

Architecture of Compound Glands. All compound glands are surrounded by connective tissue which forms a more or less definite *capsule*. From the capsule connective tissue *septa* or *trabeculae* extend into the gland. The broadest septa usually divide the gland into a number of compartments or *lobes*. Smaller septa from the capsule and from the interlobar septa divide the lobes into smaller compartments, usually microscopic in size—the *lobules*. A lobule is not only a definite portion of the gland separated from the rest of the gland by connective tissue, but represents a definite grouping of tubules or alveoli with reference to one or more terminal ducts. The glandular (epithelial) tissue is known as the *parenchyma* of the gland, in contradistinction to the connective tissue or *stroma*.

The relations of the glandular tissue proper to the connective tissue are best understood by reference to development. Glands originate as ingrowths from a surface lined with epithelium. The epithelial invagination grows down into the underlying connective tissue. As the invagination grows and subdivides, the main portion, connected with the epithelium at the original point of outgrowth, becomes the excretory duct, while the subdivisions form the larger and smaller ducts and finally the secreting tubules or alveoli. During the development of the gland tubules, the connective tissue is also developing, but is being largely replaced by the more rapidly growing tubules. The gland tubules do not develop irregularly, but in definite groups, each group being dependent upon the tubule (duct) from which it originates. Thus the main excretory duct gives rise to a few large branches which may lie either between or within the developing lobes (inter-

lobar and intralobar ducts respectively), a lobe being formed by all of the subdivisions of one of the lobar branches. From each intralobar duct there arise within the lobe a large number of smaller branches, each of which gives rise to subdivisions which make up a lobule of the gland. These branches lie first between lobules (interlobular ducts) and finally within the lobules to which they give rise (intralobular ducts). As groups of tubules develop into lobes and lobules, the largest strands of connective tissue are left between adjacent lobes (interlobar connective tissue) smaller strands between lobules (interlobular connective tissue), and the finest connective tissue between the tubules or alveoli within the lobule (intralobular connective tissue).

ENDOCRINE GLANDS

Certain glands, as already stated, are lacking in ducts and are called *endocrine glands* or glands of internal secretion in contrast to those with ducts, the exocrine or external secreting glands. Some glands, such as the pancreas and testis secrete both externally by way of ducts and internally by way of the blood stream. The endocrine glands secrete specific substances called *hormones* which have specific effects on the other tissues or organs of the body. The endocrine glands include the thyroid, parathyroid, adrenal, hypophysis, islands of Langerhans of the pancreas, and parts of the ovary and testis. The pineal gland is also usually included in this group. For a detailed description of the endocrine glands, see Chapter XXI.

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XVI

THE DIGESTIVE SYSTEM

The *digestive system* consists of the alimentary tract and such structures as the tongue, teeth and accessory glands which are associated with it. The alimentary tract is conveniently divided by structural variations and topographical locations into a series of regions: mouth, pharynx, esophagus, stomach, small intestine and large intestine, including the rectum and anal canal. The structural modifications of the various regions are associated with the function of the tract, namely, the forwarding of the food through the tube, where in transit it can be mechanically altered and acted on by enzymes, a portion of it absorbed, and the residue eliminated as feces.

General Features of the Alimentary Canal. The innermost layer of the digestive tract is a *mucous membrane* or *mucosa* (p. 57). This has two constantly occurring components, an *epithelial lining* and a stratum of connective tissue, the *lamina propria*. The lamina propria is formed of interlacing connective tissue fibers, which are usually fine. It contains fibroblasts and macrophages, is frequently infiltrated with lymphocytes and may also contain plasma cells and eosinophiles. Beginning with the esophagus, a thin stratum of smooth muscle (*muscularis mucosae*) appears subjacent to the lamina propria and forms a third component of the mucosa.

A *submucosa*, formed of loose connective tissue, is invariably present beneath the mucosa from the beginning of the esophagus to the lower end of the anal canal. This layer is absent from parts of the mouth and pharynx. It contains rather coarse collagenous fibers which are usually loosely interwoven, and among which are elastic and reticular fibers and connective tissue cells. It attaches the mucosa to the underlying firm structures but allows considerable movement in much the same way that superficial fascia allows movement of the skin. In the regions where no definite submucosa is present, the mucosa attaches directly to the firm underlying structures as, for example, in the gums.

Throughout most of the alimentary canal, there is a rather thick layer of muscle (*muscularis externa*) which has a very regular arrangement. In the mouth the muscle layer has no uniform arrangement and is absent in certain regions.

Beginning with the pharynx there is an external layer composed of connective tissue (*fibrosa*) or of connective tissue and mesothelium (*serosa*, *serous membrane*, p. 57).

THE MOUTH

The *mouth* or oral cavity is an irregular shaped structure which is bounded by and contains a number of different parts, such as the lips, cheeks, teeth, gums, tongue and palate. Except over the surface of the

teeth, the mouth is lined throughout by stratified squamous epithelium; the lamina propria is rather dense, and a submucosa is present only in certain regions.

Lips and Cheeks. The lips may be divided into three rather distinct regions: the cutaneous area, the red area, and the oral mucosa (Fig. 228). The cutaneous area of the lips is covered by typical thin skin, with cornified epithelium, hair follicles, sebaceous and sweat glands. Over the red area, the stratified squamous epithelium is not cornified; the superficial cells, however, do contain eleidin which makes them translucent. The epithelium is quite thick and is deeply indented by vascular connective tissue papillae (Figs. 228, 229). The translucency of the epithelium and the deeply indenting vascular papillae are responsible for the red color. Glands are absent in the red area except for an occasional sebaceous gland. The red area is continuous with the skin externally and with the mucous membrane of the lips internally.

The inner surfaces of the lips and cheeks are similar in structure. The epithelium is not cornified and connective tissue papillae of moderate length indent it. The lamina propria is rather compact and connected by a submucosa to the underlying skeletal muscle (orbicularis oris in the lips, buccinator in the cheeks). The submucosal fibers are thick and are so arranged that they closely bind the mucosa to the underlying structures, preventing the formation of folds and thus reducing the chance of biting the mucous membrane during mastication. In the area where the mucosa of the lips and cheeks becomes continuous with that

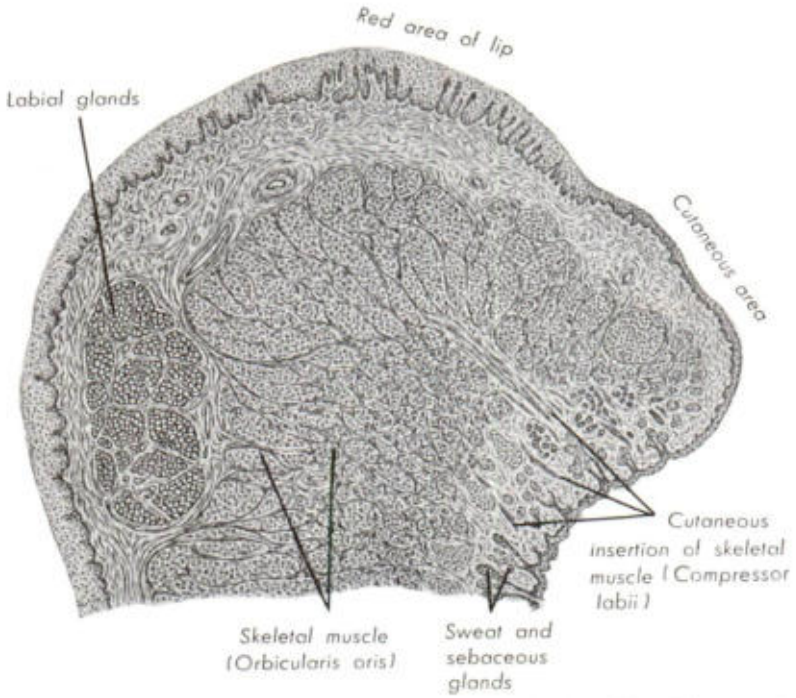


FIG. 228. Transverse section through lower lip of newborn infant. Van Gieson stain. The oral surface of the lip is at the left. × 18.

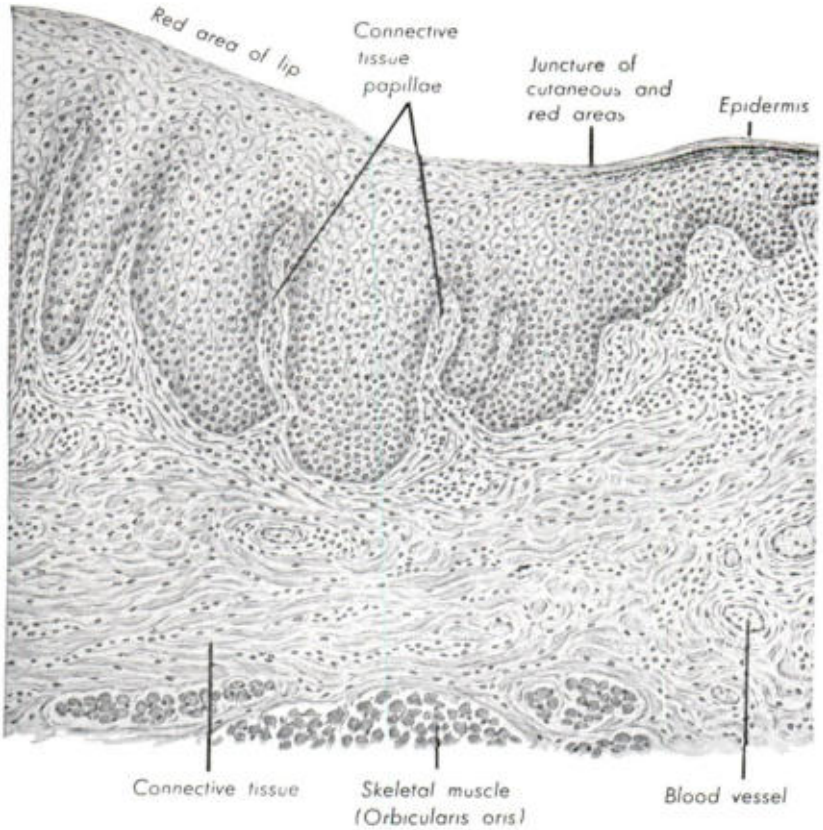


FIG. 229. Transverse section through the contiguous cutaneous and red areas of lip of newborn infant. × 110.

of the gums (fornix vestibuli) the submucosa is very loose, thus allowing movement.

Numerous glands of a mixed (mucous and serous) type and a mucous type are present in the submucosa of the lips and cheeks and they also penetrate into the buccinator muscle.

Gums. The epithelium of the *gums* or *gingivae* is cornified to a variable degree. Cornification is most pronounced in those areas which are subject to the greatest amount of abrasion from mastication or brushing, i.e., on the free margin of the gums. Numerous long vascular papillae deeply indent the epithelium and are responsible for its pink color. At the gingival sulcus the epithelium of the gums is continuous with the epithelial attachment of the tooth (p. 329 and Fig. 236). The lamina propria of the gums is formed of coarse interweaving collagenous fibers which bind it closely to the periosteum of the alveolar processes. The lamina propria is also attached to the gingival fibers of the periodontal membrane. No submucosa and no glands are present in the gingiva.

Hard Palate. The epithelium of the *hard palate* is much like that of the gums. It has a cornified layer in which the cells are hard and scale-like (Fig. 230), and it usually has a stratum granulosum. Long vascular papillae deeply indent it giving a

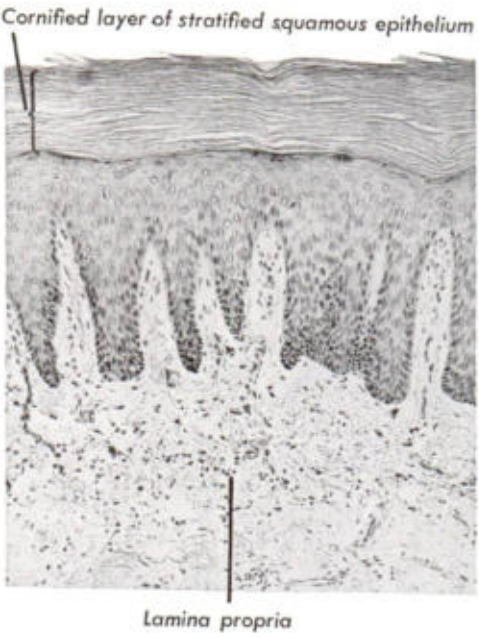


FIG. 230. Mucous membrane from anterior region of hard palate. Human. Photomicrograph by Mr. L. Koster. × 110.

pinkish color. Except in the area adjacent to the gums and in the midline, a submucosa is present. Its fibers are coarse and run largely in a vertical direction, thus binding the lamina propria firmly to the periosteum of the hard palate. In the anterior region of the hard palate, a considerable amount of fat is present in the submucosa (the fatty zone); in the posterior two-thirds, are many mucous glands (the glandular zone). In the narrow longitudinal zone of the raphe, glands are absent. Spherical or ovoid aggregations of flattened, concentrically arranged epithelial cells occasionally occur near the midline. They are remnants of the embryonic fusion of the palatine processes. Structures of this type are called epithelial pearls.

Soft Palate. The oral surface of the *soft palate* and *uvula* is lined by noncornified stratified squamous epithelium. This type of epithelium extends over the free margin and for a variable distance onto the pharyngeal surface where it becomes continuous with pseudostratified ciliated columnar epithelium. The submucosa is loose and contains many glands: mucous on the oral side and mixed (mucous and serous) on the upper (respiratory) side. A number of small skeletal muscles enter into the formation of the soft palate and uvula.

Floor of the Mouth. The *floor* of the *mouth* is lined by a noncornified epithelium. The submucosa is loose and contains the sublingual glands.

THE TONGUE

The main bulk of the tongue, particularly of the anterior two-thirds, is skeletal muscle. The interlacing muscle fibers course chiefly in three directions, longitudinally, transversely, and vertically, an arrangement which gives maximum mobility and physical control. In the posterior third of the tongue there are aggregations of lymphoid tissue, the lingual tonsils (Figs. 231, 232).

Lower Surface of the Tongue. The *lower surface* of the tongue is covered by a stratified squamous epithelium which is not cornified. The lamina propria is thin and closely bound down to the underlying muscle.

Dorsum of the Tongue. The *dorsal surface* of the tongue is divided into an anterior two-thirds and a posterior one-third by a V-shaped row of circumvallate papillae (Fig. 231). Some structural features are very different in the two regions. On the *anterior two-thirds*, there are numerous projections, the *lingual papillae*. These papillae are virtually small organs and should not be confused with the connective tissue papillae which indent stratified squamous epithelium. The lingual papillae are formed of a central core of connective tissue and a covering

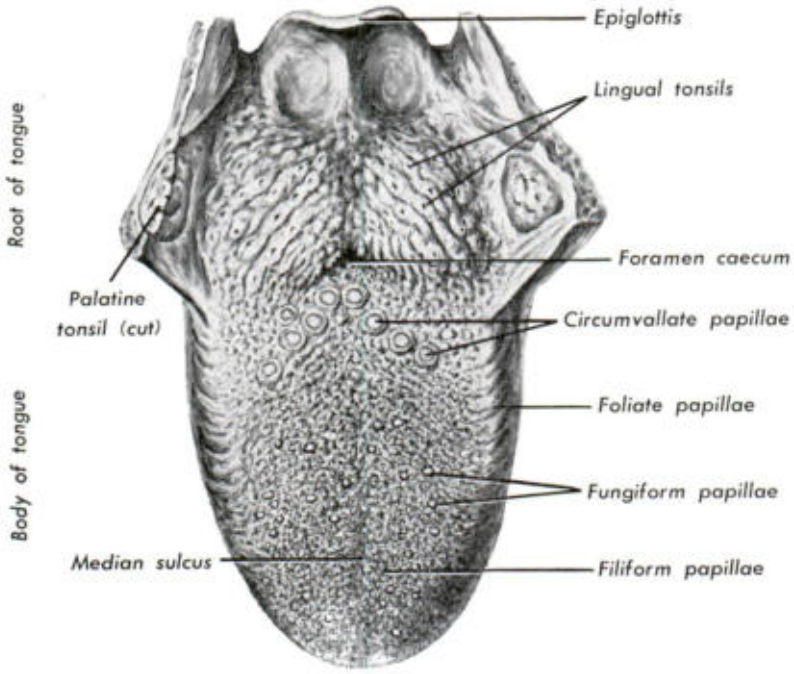


FIG. 231. Dorsum of human tongue. (Redrawn after Spalteholz.)

layer of stratified squamous epithelium, (Figs. 232, 233, 234). The connective tissue core may give rise to small (connective tissue) papillae which indent the epithelium. According to their shape, the lingual papillae are divided into three types: *filiform*, *fungiform* and *circumvallate*.

The *filiform papillae* are much the most numerous and are quite evenly distributed over the dorsal surface of the anterior two-thirds of the tongue. Each consists of a slender vascular core of connective tissue covered by stratified squamous epithelium which is cornified. The epithelium forms one or more secondary projections which taper into thread-like points (Fig. 234).

The *fungiform papillae* are relatively few in number and are interspersed among the filiform papillae. Their summits are rounded and are broader than the bases. They are covered by noncornified epithelium which is indented with connective tissue papillae. The connective tissue core is highly vascular. This and the thinness of the epithelium are responsible for their red color.

The *circumvallate papillae*, usually 9–12 in number, are arranged along a V-shaped line, the apex of the V pointing posteriorly. They resemble the fungiform papillae but are much larger and are surrounded by a trench and a wall, hence their name vallate or circumvallate. The wall is somewhat lower than the papilla, thus allowing the latter to project slightly above the surface. Connective tissue papillae, indenting the epithelium, are limited to the upper surface, the sides being free of them. The circumvallate papillae, the trench and the wall are covered by noncornified stratified squamous epithelium. In the epithelium of the lateral wall and sometimes in that of the trench also, are small oval bodies, *taste*

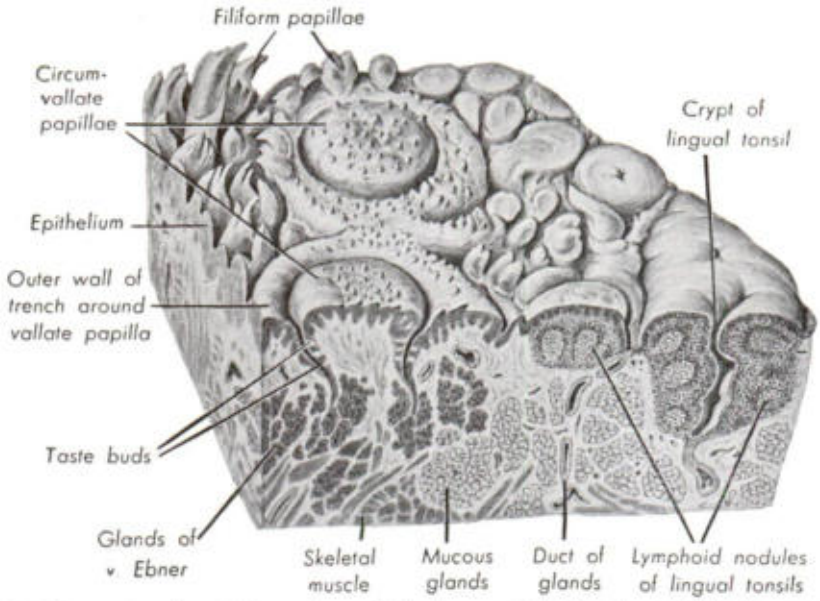


FIG. 232. Reconstruction of the surface of the tongue at the juncture of the dorsum and root. × 13. (Redrawn from Braus.)

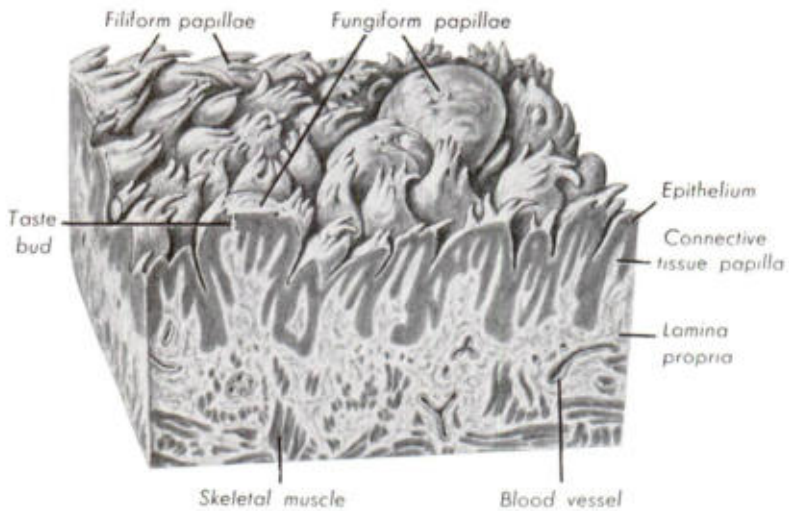


FIG. 233. Reconstruction of the surface of the dorsum of the tongue. The front surface represents a sagittal section, with the root of the tongue at the reader's right. × 16. (Redrawn from Braus.)

buds (Figs. 232, 235), which serve as receptor organs of taste (see Chapter XXII).

Along the posterolateral border of the tongue there are folds of the mucous membrane, sometimes called the foliate papillae (Fig. 231). They are not well developed in man.

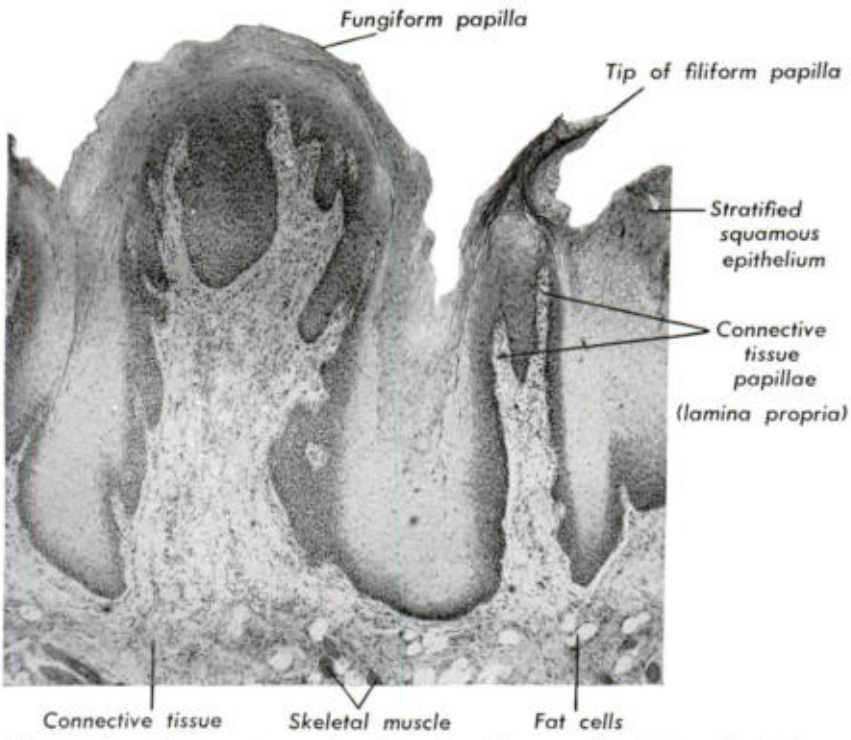


FIG. 234. Section of human tongue showing a fungiform papilla (left) and a filiform papilla (right). Photomicrograph. × 56.

The *dorsal surface* of the *posterior third* of the tongue is free of papillae but has mucosal ridges and *lingual tonsils*. The latter appear as low eminences caused by the underlying aggregations of lymphoid nodules (Figs. 231, 232). Each tonsil usually has a centrally placed pit or crypt lined by stratified squamous epithelium. The epithelium is infiltrated with lymphocytes.

No submucosa is distinguishable on the dorsum of the tongue.

Glands of the Tongue. The glands of the tongue can be divided into three main groups according to their structure and location.

A paired group of mixed mucous and serous glands are located in the anterior part of the tongue near the apex (*glands of Nühn*). They are embedded in the muscle but are closer to the ventral than the dorsal surface. They have several ducts which open on the ventral surface.

A group of serous glands located in the region of the vallate papillae are known as the *glands of von Ebner*. They extend into the muscle. Their ducts open into the trenches of the vallate papillae (Fig. 232).

Mucous glands of the root of the tongue are the most numerous. They lie in the posterior third of the tongue and extend far enough forward to mingle with the serous (von Ebner's) glands (Fig. 232). Their ducts open into the crypts of the lingual tonsils and into depressions between the tonsils (Fig. 232).

Nerve Supply. The *nerve supply* of the oral cavity is quite complex. The skeletal muscle of the lips and cheeks is supplied by the VII cranial nerve; that of the

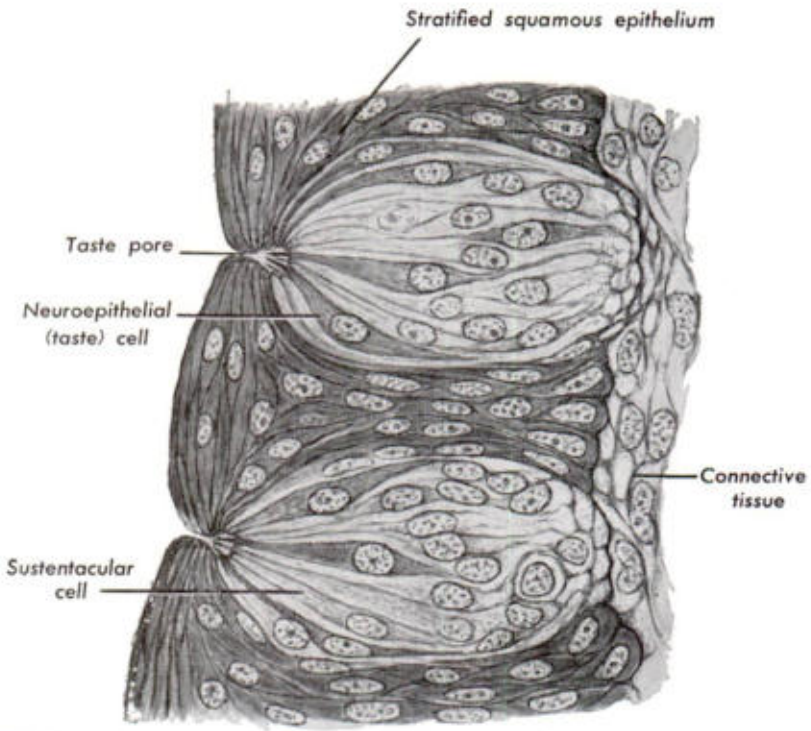


FIG. 235. Taste buds from wall of circumvallate papilla of a rhesus monkey. Masson's trichrome stain. × 800.

tongue by the XII nerve. The fibers carrying ordinary sensation are from the lingual branch of the V nerve and from the IX nerve. The fibers carrying the special sense of taste are from the VII nerve (through the chorda tympani) and from the IX nerve.

THE TEETH

A *tooth* has three anatomic divisions, *crown*, *root* and *neck* or *cervix*. (Fig. 236).

The *clinical crown* refers to that part which is visible with the tooth *in situ*. In early life the gums cover a part of the enamel so that the clinical crown consists of only a part of the anatomic crown. More of the enamel normally becomes exposed with the ageing process so that later in life the clinical crown includes all of the anatomic crown and even a part of the anatomic root.

The root is embedded in a cavity (the *alveolus* or *socket*) in the alveolar process of either the mandible or the maxilla and it is firmly attached to the bony wall of its socket by connective tissue, the *periodontal membrane* or *ligament*.

Structurally a tooth has four components: *enamel*, *dentin*, *cementum* and *pulp*.

Enamel. Enamel is the hardest substance in the body and, by weight, is composed largely (96 per cent) of inorganic salts of which the greater part (about 90 per cent) is calcium phosphate. By volume, however, the organic component of enamel is very considerable, nearly equalling the inorganic element. Enamel is somewhat brittle but due to the support of the underlying dentin and also to its internal structural arrangement it does not fracture with normal occlusal stress. Enamel is present in greatest amounts on the cusps of the permanent bicuspids and molars where it is 2 to 2.5 mm. in thickness.

Structurally, enamel is composed of

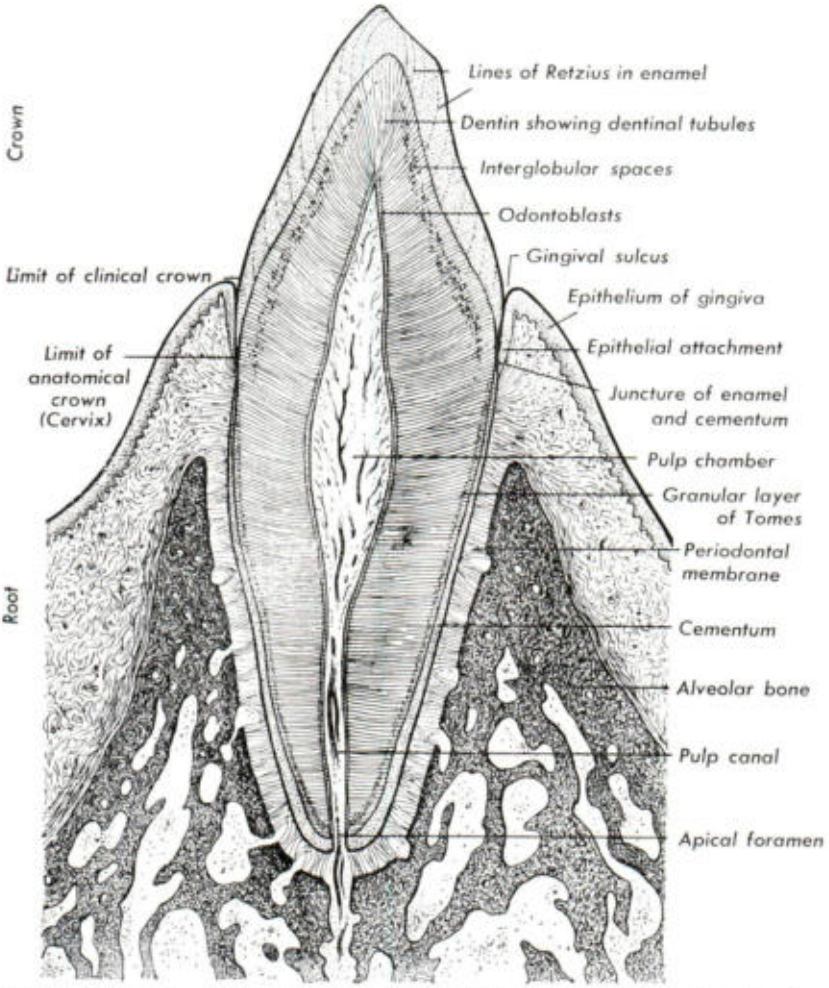


FIG. 236. Diagram of a section through an incisor tooth and surrounding structures. The enamel at the tip of the crown shows some abrasion.

enamel rods or *prisms* and *interprismatic substance*.

The *enamel rods* are elongated columns, each of which extends throughout the thickness of the enamel layer from the dentino-enamel juncture to the surface of the anatomical crown. When seen in cross section some rods are hexagonal, oval or polygonal, but most are arcade or scale-shaped with a depression on one side (Fig. 237). The average diameter of the enamel rods is about 4μ, i.e., about one-half that of a red blood cell. The diameter increases as they course toward the periphery, since the area of the outer surface of the enamel is greater than at the dentino-enamel juncture.

Each enamel rod is composed of submicroscopic crystals of inorganic material embedded in a sparse framework of organic material. A thin peripheral region, the *rod sheath*, contains a higher proportion of organic material than the bulk of the rod (Fig. 238). Between the rods is a small amount of a calcified organic substance, the *interprismatic* or *interrod substance* which appears to act as a cementing substance.

Enamel rods are transversely striated, the striations being evenly spaced at

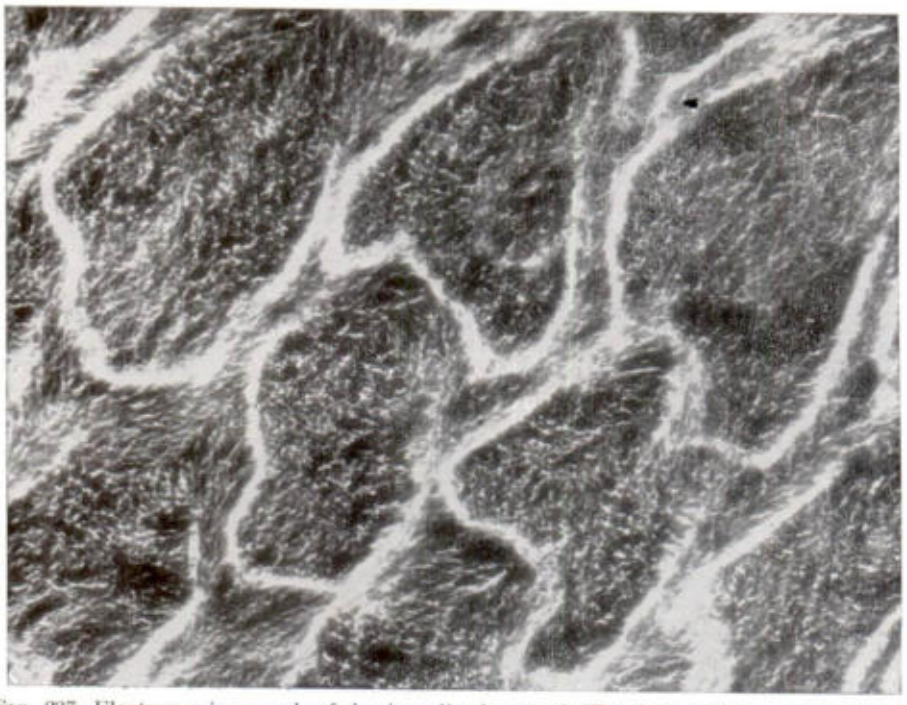


FIG. 237. Electron micrograph of demineralized enamel. The field includes cross sections of several enamel rods. Note the submicroscopic fibrillar network of the organic matrix and the more dense peripheral rod sheaths. Human. × 5000. (Courtesy of Dr. D. B. Scott.)

intervals of about 4μ (Fig. 239). This is due to the rhythmic longitudinal growth of the rods during development. In addition to the closely spaced striations on the individual rods, there are more widely and somewhat more variably spaced continuous lines in the enamel, the *incremental lines of Retzius* (Fig. 236). In coronal sections through the crown these appear as concentric circles. In longitudinal sections they form arches over the apex of the dentin. In the deciduous teeth and in the first permanent molar an especially prominent line, the *neonatal line*, marks the boundary between the enamel formed before and after birth. The incremental lines are due to variations in the rate of enamel deposition and are roughly analogous to the growth rings of a tree. The neonatal line is an accentuated incremental line resulting from a disturbance in the deposition of enamel at the time of birth.

The course of the enamel rods is usually wavy or, especially on the occlusal surfaces, they may entwine forming *gnarled enamel*. This is a functional adaptation which adds strength to the enamel by reducing the danger of cleavage between the rods.

An awareness of the direction and course of the enamel rods in different regions of a tooth is important in the technique of cavity preparation, both from the standpoint of ease in removing portions of the enamel, and also of maintaining the strength of the surrounding wall.

A membrane, *Nasmyth's membrane or enamel cuticle*, covers the exposed surface of the crown for a short time after eruption (see p. 327).

Dentin. Dentin is hard, yellowish and elastic. It forms the bulk of a tooth and gives the main strength to it, also. Chemically, it contains more mineral than bone but less than enamel (69 per cent by weight as compared to 46 per cent and 96 per cent, respectively). Morphologi-

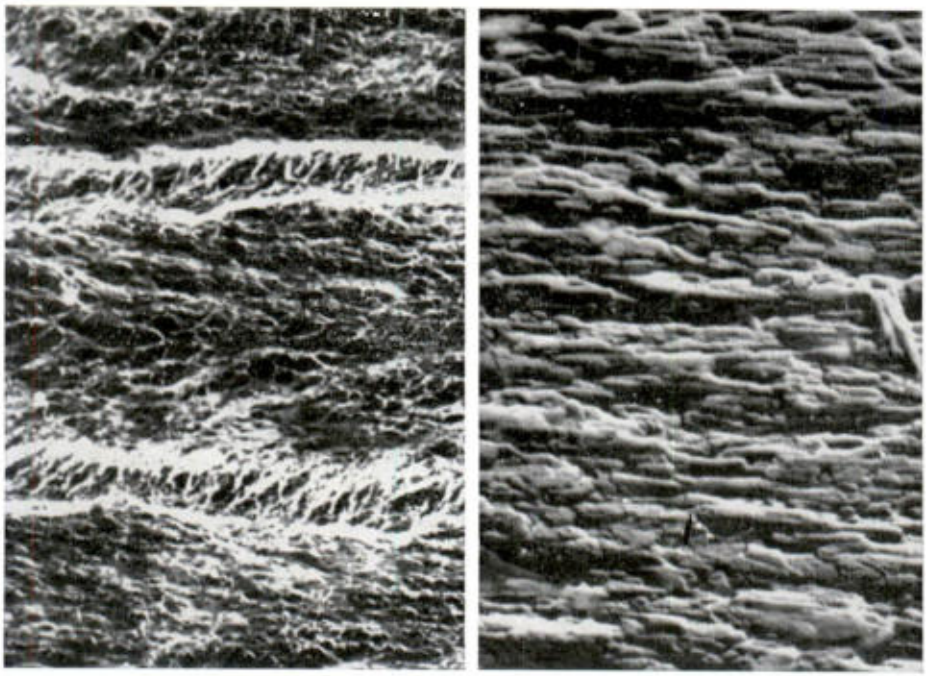


FIG. 238. Electron micrographs of longitudinal sections of mature enamel. (*Left*) Demineralized enamel. The field shows the entire width of one enamel rod and parts of two adjacent rods. Note the submicroscopic fibrillar network of enamel rods and rod sheaths. Extending crosswise between the rods (prisms) are interprismatic fibrils. × 10,000. (*Right*) Pseudoreplica of acid-etched ground longitudinal section of mature enamel. The field shows the surface layers of crystallites and the crystalline pattern within a single enamel rod. × 21,000. (Courtesy of Dr. D. B. Scott.)

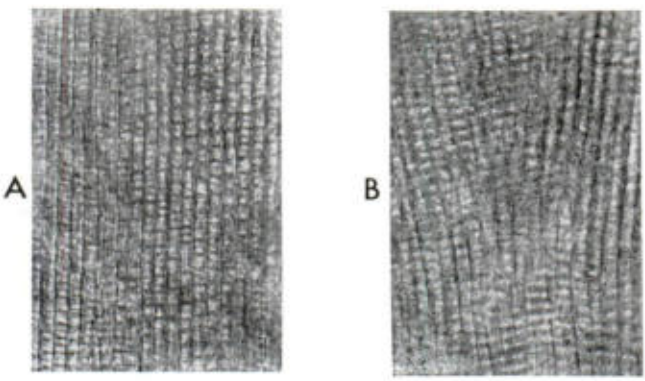


FIG. 239. Photomicrographs of ground section through enamel of human bicuspid tooth. *A*, Lateral area in which the prisms are regularly arranged. *B*, Cuspal area in which the prisms entwine. × 380.

cally, it resembles bone in that it is composed of collagenous fibers in a calcified ground substance. It differs from bone in that it contains no cells but has only processes of cells (odontoblasts) whose bodies lie adjacent to the dentin in the pulp cavity.

The cells responsible for the deposition of dentin are specialized mesodermal cells, the *odontoblasts*, which are arranged in an epithelial-like layer on the inner surface of the forming dentin. Unlike osteoblasts, the odontoblasts do not become imprisoned, but retreat progressively as the

layers of dentin are deposited, each one leaving a single branching process embedded in the dentin matrix. These *odontoblastic processes* or *dentinal fibers* (of Tomes) become increasingly elongated as the odontoblasts recede with the formation of successive layers of dentin. The dentinal fibers thus occupy narrow, tubular channels within the dentin, the *dentinal tubules*. The dentinal fibers branch and anastomose somewhat, but in general they run parallel to one another in a slightly wavy course through the dentin. This is well shown in ground sections of dentin in which the dentinal canals are filled with air and thus appear dark in transmitted light (Fig. 240). The odontoblastic processes probably completely fill the dentinal tubules in the living tooth; the spaces which appear between the processes and the walls of the tubules in fixed sections are very likely artifacts (Fig. 241). The rim of dentin matrix bordering on the tubule stains darker than the remainder of the dentin, and is known as *Neumann's sheath*.

In each layer of dentin, the meshwork of collagenous fibers in the matrix runs perpendicular to the long axis of the tubules, i.e., parallel or tangential to the outer surface of the tooth. The mineral salts are in the form of crystals and have two types of arrangement: (1) the long axes of the crystals are parallel to the collagenous fibers, and (2) the crystals radiate out from a center in spherulite arrangement. In all cases, as in bone, the crystals lie in the ground substance between the collagenous fibers. The ground substance of dentin is composed of mucopolysaccharides.

In certain regions of the tooth there

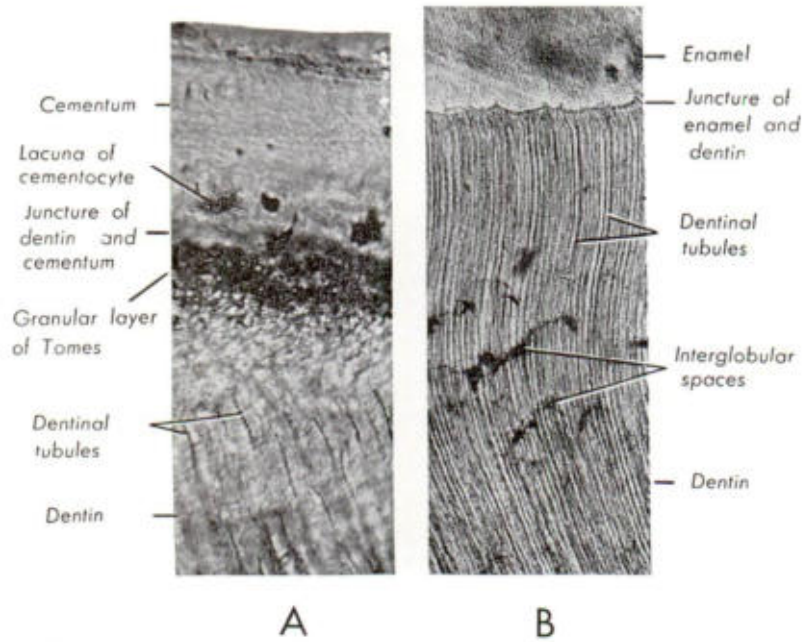


FIG. 240. Photomicrographs of ground section of human bicuspid tooth. *A*, Through root showing dentin and adjoining cementum. *B*, Through crown showing dentin and adjoining enamel. The dark areas in the enamel are not due to pigment or discoloration but to refraction of the rods. × 265.

FIG. 241. Electron micrograph of thin section of demineralized dentin showing organic matrix and cross sections of dentinal tubules with their enclosed odontoblastic processes (dentinal fibers). The space between each process and the rim of organic matrix (Neumann's sheath) is probably largely artifact. Human. × 5500. (Courtesy of Dr. D. B. Scott.)

are areas which have less inorganic material than elsewhere, due to a failure of the individual areas of calcification to meet and fuse. The matrix in such regions shrinks in ground (dried) sections, and a space is formed which becomes filled with air and so appears dark in transmitted light. One such constantly occurring region is in the root of the tooth close to the dentino-cementum juncture. It has a granular appearance, and is known as the *granular layer of Tomes* (Figs. 240, *A*; 236). A second location, the *interglobular areas* (Fig. 240, *B*), occurs chiefly in the crown (Fig. 236) but may be present in the root also. It lies a short distance from the dentino-enamel (or dentino-cementum) juncture. Each area is irregular in shape and much larger than the areas forming the granular layer of Tomes.

Parallel incremental growth lines (*contour lines of Owen, imbrication lines of von Ebner*) which are due to the deposition of dentin in successive lamella-like layers are present. In cross sections of a tooth, they appear like annual rings of a tree.

Dentin, unlike enamel, normally continues to be formed throughout life. In the dentin formed during the later period of life, *secondary dentin*, the dentinal tubules are more wavy and usually less numerous than in earlier formed dentin. It seems justifiable to assume that, as in the case of bone, stress is a factor stimulating its formation. More severe stimuli, such as caries or erosion, cause a localized deposition of dentin in which the elements are very irregularly arranged, *irregular* or *reparative dentin*. The deposition of dentin induced by stress may be so extensive that it obliterates the pulp chamber and even part of the root canal. It is an important protective response.

Dentin is sensitive to a number of painful stimuli. Despite this well-known fact, nerve fibers have not been demonstrated penetrating to any appreciable extent into its matrix. The odontoblastic processes presumably convey impulses to the pulp where many nerve endings are located.

Cementum. Cementum is similar to bone both in morphology and in composition. It is darker than enamel but lighter in color than dentin. It forms a thin sheath on the surface of the dentin of the anatomical root of the tooth (Fig. 236). It is usually somewhat thicker at the apex of the root, and sometimes covers the inner surface of the dentin for a short distance at the apical foramen.

Cementum may either be free of cells, *acellular cementum*, or it may be *cellular*, containing cells similar to osteocytes (*cementocytes*) which lie in irregular shaped lacunae in the matrix (Fig. 240, *A*). Except at the apex of the root the acellular type is usually adjacent to the

dentin. The ground substance of cementum resembles that of bone. Collagenous fibers of the matrix extend into the surrounding connective tissue (periodontal membrane) and, as in bone, are known as *Sharpey's fibers*. Cementoblasts on the surface continue to form cementum throughout life. The added layers are irregular in thickness and may be either cellular or acellular. Hypertrophy of cementum frequently occurs in response to unusual stress or movement of a tooth.

Although the bond between dentin and cementum is a firm one, the mode of attachment is not clear. Cementum forms a protective covering over the dentin and serves to attach the tooth to the surrounding structures. Movement of teeth without injury to the tooth structures is possible in orthodontic procedures because cementum is more resistant to resorption than is the alveolar bone.

Periodontal Membrane. The *periodontal membrane* or *ligament* is composed of connective tissue which surrounds the root of the tooth. It attaches the root to: (1) the wall of its alveolus; (2) the gingival connective tissue; (3) the more superficial parts of the roots of adjacent teeth. Fibers extending into the cementum of the tooth interweave with those extending into the alveolar bone, thus binding the tooth to the bone. The fibers do not course in the same direction at different levels (Fig. 236) but are arranged in a way which makes them most effective in maintaining the tooth in position and in serving as a suspensory ligament. The fibers are collagenous, but due to their waviness, some temporary physiological movement of a tooth is possible without morphological alterations in its root and socket.

In the periodontal membrane are fibroblasts, osteoblasts and cementoblasts. There are also groups of cells which are remnants of Hertwig's epithelial root sheath, a derivative of the enamel organ. Blood vessels and nerve fibers, particularly proprioceptive endings, are present.

The density and strength of a periodontal ligament varies with the stress to which a tooth has been subjected. Thus if there is no opposing tooth the fibers of the membrane become more delicate, a change which is accompanied by a rarefaction of the bone of the alveolus. When this is allowed to occur, restorative measures become more difficult.

Pulp Cavity and Dental Pulp. The shape of the *pulp cavity* is quite similar (in miniature) to that of the tooth in which it occurs. It consists of an expanded *pulp chamber* which lies in the crown portion and adjacent part of the root, and a narrow *pulp canal* or *root canal* in each root (Fig. 236). The pulp cavity is relatively much larger in teeth of young than of old individuals, for there is a continuous deposition of dentin throughout life. A root canal communicates with the periodontal tissue through one or more foramina at or near the apex, the *apical foramina*.

The *dental pulp* contains the dentin-forming cells and is essential to the nourishment and vitality of the tooth. It consists of fine connective tissue which fills the pulp cavity. In addition to fibroblasts and histiocytes, there are specialized connective tissue cells, the odontoblasts. These cells, which line the pulp cavity, are responsible for dentin formation. They resemble epithelium, being columnar in the pulp chamber and becoming more cuboidal toward the apex. They have "intercellular bridges" and, at their dentinal ends, terminal bars. Elongated cytoplasmic processes, the odontoblastic processes (Tomes' fibers), extend peripherally into the dentin in the dentinal tubules.

Both reticular (argyrophil) and collagenous fibers are present in the pulp.

From the region adjacent to the odontoblasts, where the reticular fibers are particularly numerous, coarse argyrophil fibers extend spirally between the odontoblasts. These are Korff's fibers (see p. 325).

The pulp is richly vascular. An arteriole entering at the apical foramen forms a profuse capillary network close to the odontoblast layer, the blood being returned by one or more venules.

The dental pulp contains both myelinated and amyelinated nerve fibers. The sensory fibers terminate as free nerve endings among the odontoblasts. They are pain receptors (eliciting a sensation of pain regardless of the type of stimulus). Since it has not been demonstrated conclusively that any nerve fibers enter the dentinal tubules, it seems probable that sensation is carried from the periphery of the dentin to the pulp by the odontoblastic processes.

Attachment of Gingiva to Teeth. The gums are attached to the teeth in two ways. One has been described under "Periodontal Membrane," namely, the attachment of the subepithelial connective tissue to the cementum. The other is a direct attachment of the gingival epithelium to the tooth, the *epithelial attachment* (Figs. 236, 248). At the gum line the epithelium turns inward and follows along the surface of the tooth and is attached to it. Although the structural features of this attachment are not clear, it is certain that there is a definite adhesion of the epithelium to the tooth. In contrast to the epithelium of the exposed part of the gums, the epithelium of this zone of attachment is not indented by connective tissue papillae.

With advancing age an increasing proportion of a tooth becomes exposed, i.e., the clinical crown increases in size. This is due to a continued but slow rate of eruption and also to a normal recession of the gums. As more of the crown becomes ex-

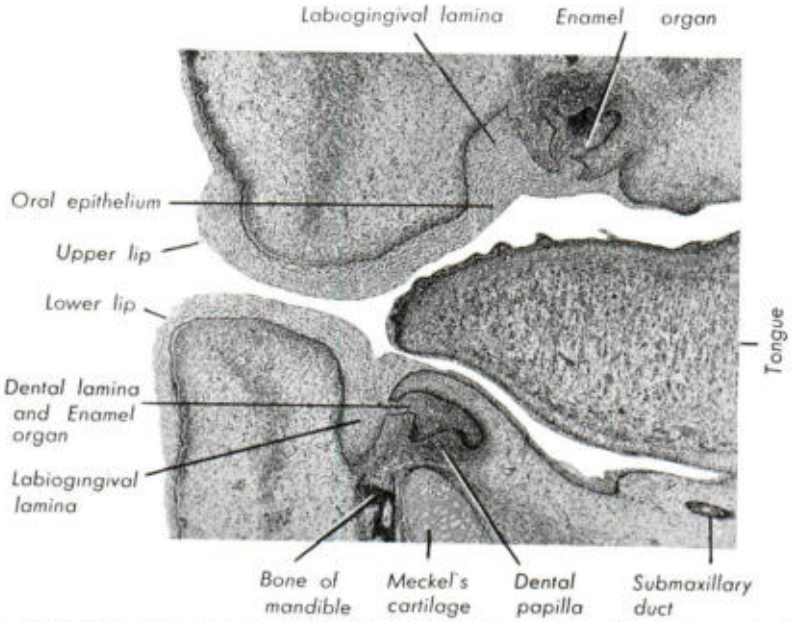


FIG. 242. Sagittal section through developing upper and lower medial deciduous incisor teeth of a 50-mm. (C–R) human embryo. Age about 10 weeks. Photomicrograph. × 34.

posed, the epithelial attachment gradually grows apically for a short distance over the surface of the anatomical root. Thus in young individuals the epithelial attachment is on the enamel only; with increased exposure of the crown it is partly on the cementum; late in life it may attach to the cementum only. Mild mechanical stimulation, such as massage and brushing, strengthen the epithelium at its attachment by increasing keratinization of its surface layers.

THE DEVELOPMENT OF THE TEETH

In man there are normally two sets of natural teeth, the *primary dentition* or deciduous teeth, and the *secondary dentition* or permanent teeth. Each tooth develops from a *tooth germ* which is derived from ectoderm and mesoderm. The enamel organ, derived from oral epithelium forms the enamel; the dental papilla, a condensation of mesoderm which becomes partially enclosed by the enamel organ, gives rise to the dentin and pulp, while the sac of mesoderm surrounding the enamel organ and papilla, the dental follicle, produces the cementum and periodontal membrane.

The first indication of tooth development, in man, occurs during the sixth or seventh week of intrauterine life at which time the embryo measures slightly over one centimeter in length. At this time an epithelial ingrowth into the underlying mesenchyme forms along the future dental arch of each jaw. This is the *dental lamina*. Slightly external to this lamina, but in close association with it, is a continuous epithelial ingrowth, the *labiogingival lamina*, in which a groove (Fig. 242) and then a deep separation will form (Fig. 243). This will divide the dental arch from the lips and cheeks. The dental lamina is of nearly uniform thickness at first but proliferations soon form at

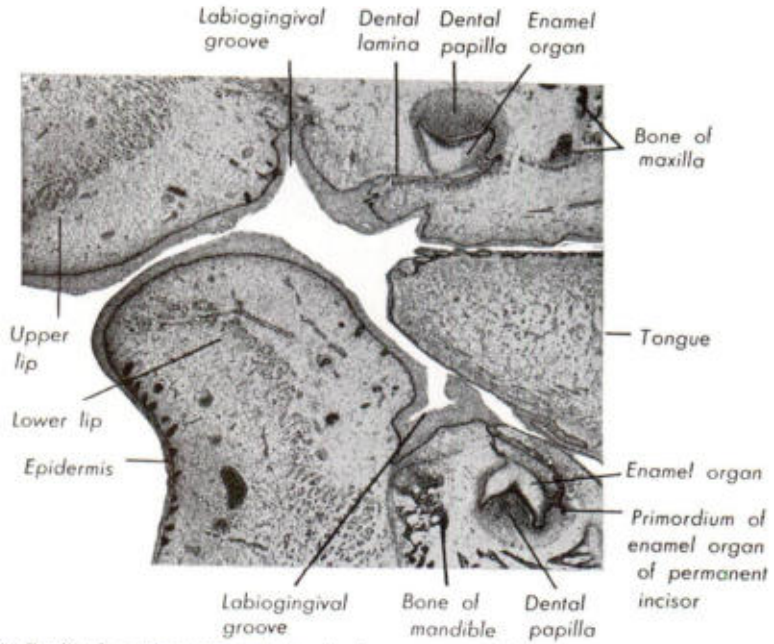


FIG. 243. Sagittal section through developing upper and lower medial deciduous incisor teeth of an 86-mm. (C–R) human embryo. Age about three months. Photomicrograph. × 34.

intervals on its outer side close to the oral epithelium. These proliferations are the primordia of the *enamel organs* of the deciduous teeth. They develop into cap-shaped and then into bell-shaped structures (Figs. 242, 243). Within and beneath the concavity of an enamel organ, a proliferation and condensation of the mesenchyme takes place (Figs. 242, 243) and forms the *dental papilla*. It is the primordium of the pulp. Its peripheral cells, i.e., those adjacent to the enamel organ, will become odontoblasts.

Somewhat later in development there appear the primordia of the enamel organs of those permanent teeth which correspond to the milk teeth. Each primordium arises as an inner or lingual growth from the dental lamina at a point coexistent with the enamel organ of a milk tooth. Later as the dental arch lengthens, the dental lamina also grows dorsally and from this dorsal extension arise the primordia of the enamel organs of the molars. The primordium of the last molar (wisdom tooth) does not form until the fourth or fifth year. The developmental pattern of the permanent teeth is the same as that of the deciduous teeth.

During the development of an enamel organ the dental lamina, which connected the enamel organ with the oral epithelium, disintegrates. The developing tooth thus becomes entirely separated from the oral epithelium and does not again come into relationship with it until eruption.

The mesenchyme surrounding the enamel organ and dental papilla plays a role in tooth development. It thickens and forms a capsule-like structure named the *dental sac* or *dental follicle*. From the relationship in the developing tooth it is evident that the segment of the dental sac adjacent to the papilla has the position of the future periodontal membrane. From it arise the cells associated with the deposition of cementum. Its peripheral part serves as the periosteum of the wall of the future alveolus.

All the teeth do not show the same degree of development at any one time. The most anterior ones are usually the most advanced in development. In any one tooth, the future occlusal area develops more rapidly than do the more apically situated regions. Thus at the time of eruption, the crown is fully formed but the root is still in the process of development.

Formation of Dentin. Dentin is the first hard substance formed in a developing tooth. Preceding its actual deposition, several changes occur in the dental papilla. Reticular fibers form in the papilla, more especially in the peripheral zone adjacent to the enamel organ. The outer portions of these fibers (Korff's fibers) fuse with the delicate basement membrane which separates the papilla from the enamel organ, and the thickened membrane thus formed is known as the *membrana preformativa*. The mesenchymal cells closest to the membrane enlarge and form a continuous layer of columnar cells, *odontoblasts*. The Korff's fibers which lie between the odontoblasts become collagenous and change their direction so that they become largely parallel to the membrana preformativa. Around them is deposited a gel-like ground substance. The final stage in the formation of dentin is the deposition of lime salts in the ground substance. Dentin is deposited in lamella-like layers. The incremental or growth lines are known as *contour lines of Owen* or *imbrication lines of von Ebner*.

The apical ends of the odontoblasts prior to the deposition of dentin were in contact with the membrana preformativa. As dentin is deposited, the odontoblasts are not imprisoned but remain on the advancing surface of the dentin. Each, however, leaves a process, *odontoblastic*

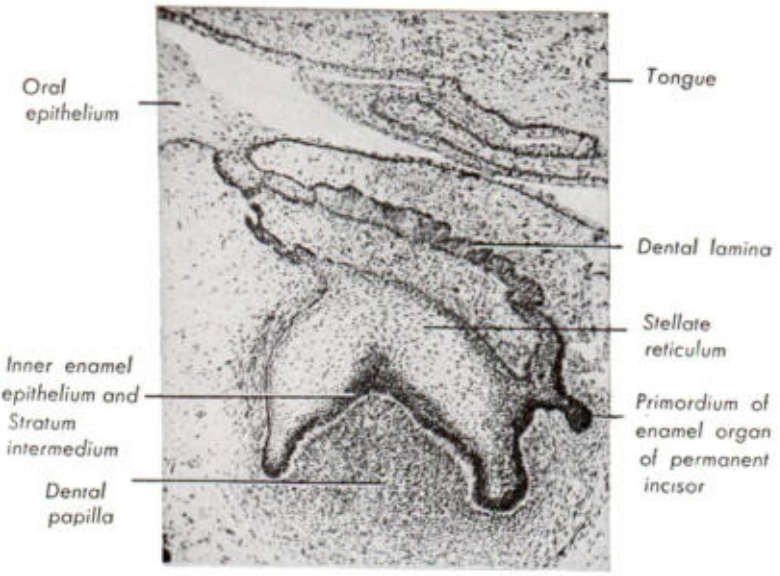


FIG. 244. Higher magnification of the developing lower incisor tooth shown in Fig. 243 (86-mm. human embryo). Photomicrograph. × 73.

process (fiber of Tomes), in the path of its retreat. As more and more dentin is deposited, these processes increase in length, for they extend through the entire thickness of the dentin. The odontoblasts remain functionally active throughout the life of a tooth and as a consequence, secondary dentin can be formed.

Enamel Organ and Deposition of Enamel. The enamel organ[2] is structurally rather complex (Figs. 244, 245). Four layers are usually described which from its concavity outward are: (1) *Inner enamel epithelium*. This is a single layer of columnar cells, the future *ameloblasts*. A basement membrane separates these from the dental papilla; (2) *Stratum intermedium*. This is composed of two or more layers of squamous or cuboidal cells; (3) *Stellate reticulum*, (*enamel pulp*). This is composed of loosely arranged branching cells; (4) *Outer enamel epithelium*. This is a single layer of cuboidal cells, adjacent to which is a rich vascular plexus in the connective tissue.

The margin of the bell-shaped enamel organ is called the *cervical loop* (Fig. 245). After completion of the crown, the inner and outer enamel epithelial layers of the cervical loop grow apically to form *Hertwig's epithelial root sheath*. This structure determines the shape of the future root or roots, and stimulates the differentiation of odontoblasts from the underlying mesoderm. The root sheath later disintegrates leaving nests of cells in the periodontal membrane.

Deposition of enamel begins only after a layer of dentin is formed. At this time the cells of the inner enamel epithelium

[2] Some authors prefer to substitute the term "*epithelial dental organ*" for "enamel organ," since this structure, although primarily concerned with enamel formation, does contribute to the form and development of the entire tooth, particularly in the formation of root dentin by means of Hertwig's epithelial root sheath. Similarly, there is some justification for the use of the terms *inner* and *outer dental epithelium* in place of inner and outer enamel epithelium.

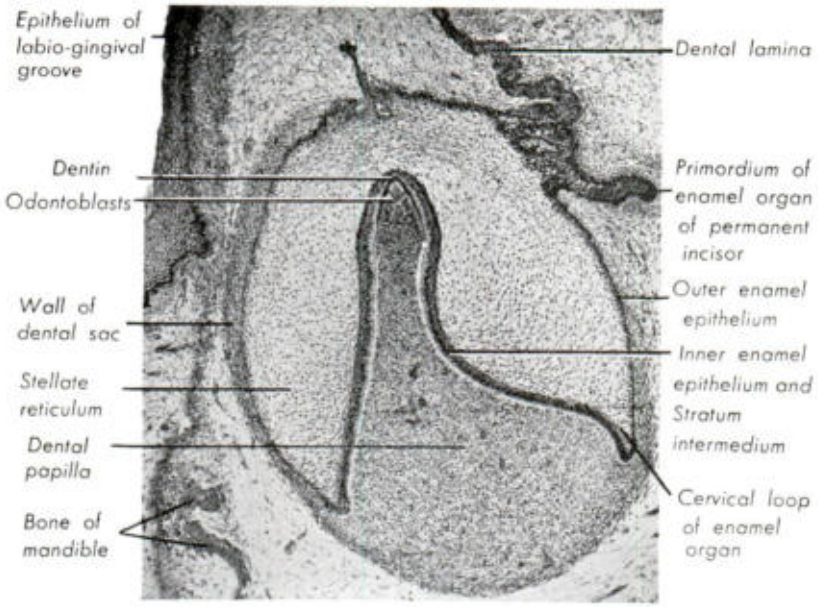


FIG. 245. Sagittal section of medial deciduous incisor tooth of an 111-mm. (C–R) human embryo. Age about fourteen and a half weeks. Photomicrograph. × 57.

elongate and differentiate into *ameloblasts*. Enamel is elaborated in the form of rods or prisms cemented together by an interprismatic substance. A short segment at the dentinal end of each ameloblast becomes more granular. This part of the cell is then known as *Tomes' process*. It is separated from the processes of adjacent cells by distinct terminal bars. As each process becomes transformed into enamel rod matrix, the ameloblast lengthens and recedes, and a new portion of cytoplasm becomes a process. In this way, Tomes' processes always remain the same length. As the enamel prisms increase in length, there is a progressive mineralization, an uncalcified segment always remaining next to the ameloblast. The interprismatic substance derived from the terminal bars between the processes also becomes calcified. The amount of mineral deposited at this time, however, is only about one fourth that of mature enamel. Complete calcification takes place after the rods have reached full length. At that time *maturation* of the enamel matrix takes place, water is withdrawn and there is a crystallization and further deposition of salts. Maturation always begins at the occlusal region and progresses toward the cervix.

After completion of enamel formation, the ameloblasts deposit on its surface a thin (0.2μ) homogeneous protective covering, the *primary enamel cuticle*. The enamel organ then regresses, but remains as a few layers of cuboidal cells, the *reduced enamel epithelium*. As the tooth erupts it is covered by a thicker (up to 10μ) keratinous layer, the *secondary enamel cuticle*, produced by the reduced enamel epithelium. These two cuticles together form *Nasmyth's membrane*, which covers the surface of the enamel until it is worn off by mastication or brushing. When the tooth erupts, the reduced enamel epithelium fuses with the oral epithelium at the gingival margin and

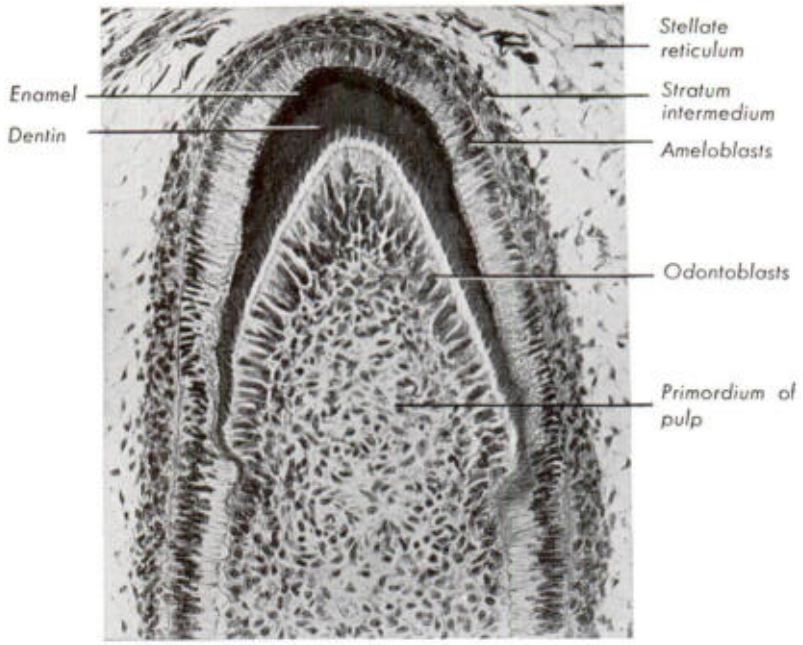


FIG. 246. Sagittal section through the developing crown of a medial deciduous incisor tooth of a 170-mm. (C–R) human fetus. Age about five months. Photomicrograph. × 180.

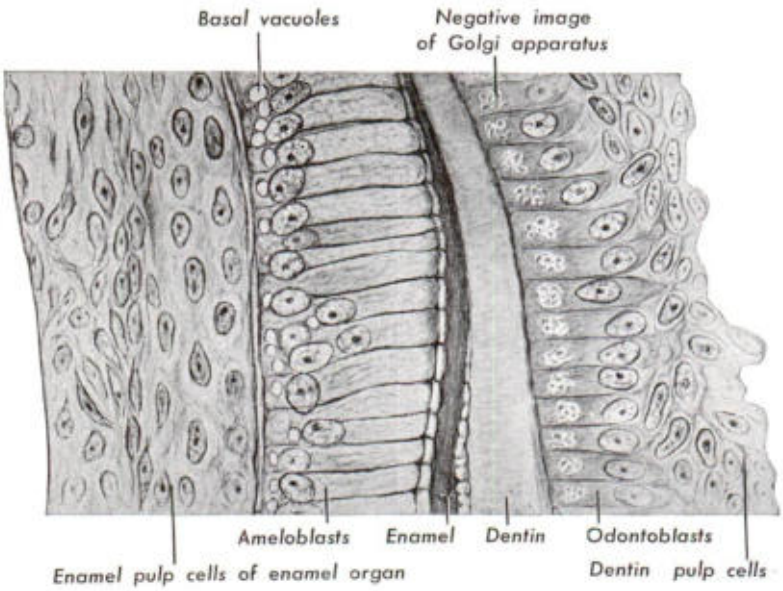


FIG. 247. Section through a developing molar tooth of a 5-day-old rat. (Beams.)

forms the *epithelial attachment* or *attached epithelial cuff*. As the tooth continues to erupt, and more of the crown becomes exposed, the epithelial attachment gradually separates from the exposed enamel. Concomitantly, the epithelial attachment grows apically on the tooth so that the extent of the attachment does not materially decrease.

Deposition of Cementum. The developing tooth is surrounded by a condensation of embryonal connective tissue which forms the *dental follicle* or *dental sac*. That portion of the follicle adjacent to the dental papilla occupies the position of the future periodontal membrane. It is separated, however, from the papilla by the apically directed extension of the enamel organ, Hertwig's epithelial root sheath. Following the first deposition of dentin of the root, the epithelial root sheath disintegrates. At this time cells of the dental sac differentiate into *cementoblasts*, following which cementum is deposited on the surface of the root. Cells in the outermost zone of the dental sac differentiate into osteoblasts of the periosteum of the alveolus. Collagenous fibers also develop in the follicular tissue. Some are attached to the cementum, others to the alveolar bone or gingival connective tissue. Together they form the fibrous component of the *periodontal membrane* or *ligament*.

THE PHARYNX

The *pharynx* extends from the base of the skull to the level of the cricoid cartilage where it becomes continuous with the esophagus. It is five to six inches in length. Its cavity is continuous with the cavities of the nose, mouth and larynx and continues caudally as the lumen of the esophagus. Superiorly and laterally the Eustachian tubes open into it. The cavity of the pharynx is incompletely divided by the soft palate and uvula into upper (pars nasalis) and lower (pars oralis and pars laryngea) regions.

The *wall of the pharynx* consists of three coats: mucosa, muscularis and fibrosa. There is no submucosa except in the superior lateral region and near the juncture with the esophagus.

The *epithelium* lining the pharynx is not the same throughout. That lining the nasopharynx is the ciliated pseudostratified columnar type, except near the juncture with the oropharynx where the soft palate and uvula comes in contact with the posterior wall. There the epithelium changes to a stratified squamous type which continues through the lower region. The *lamina propria* of the pharnyx is a tough fibroelastic layer subjacent to which is a strongly developed layer of elastic fibers which course mainly in a longitudinal direction. Fibers from it penetrate between the skeletal muscle bundles and thus bind the lamina propria to the muscularis.

The *muscle* of the pharynx is skeletal and is quite irregularly arranged. The *fibrosa* is a tough fibroelastic layer which, with varying degrees of firmness, attaches the pharynx to the surrounding structures.

In the *nasopharynx* lymphoid tissue is very abundant, being arranged both diffusely and as aggregations. These aggregations, the *pharyngeal tonsils*, frequently become enlarged and are then known as adenoids. Mixed glands which often penetrate deeply into the muscular layer are present. (See also page 396.)

In the *oral* and in the *laryngeal pharynx* scattered nodules of lymphoid tissue occur. The glands are a mucous type and are few in number.

PLAN OF THE ESOPHAGUS, STOMACH AND INTESTINES

Except for the mucosa, no layer has been constantly present throughout the

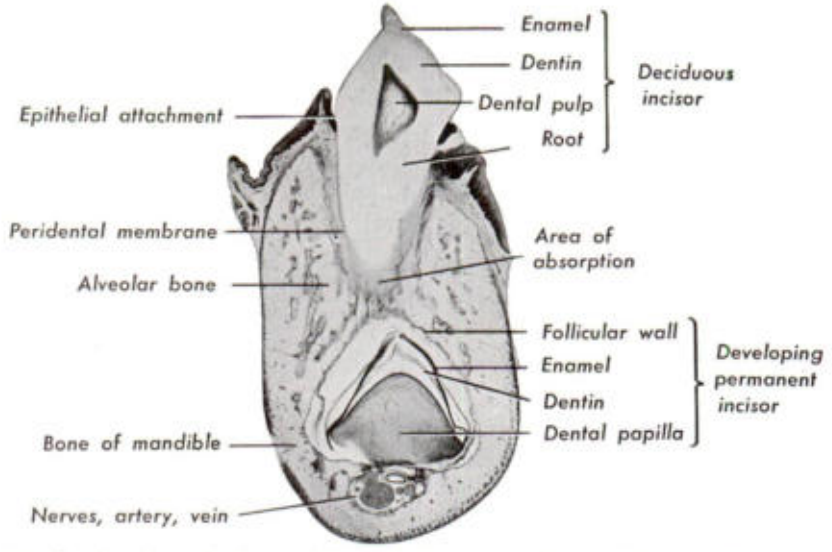


FIG. 248. Section through the mandible of a kitten showing deciduous tooth and developing permanent tooth germ.

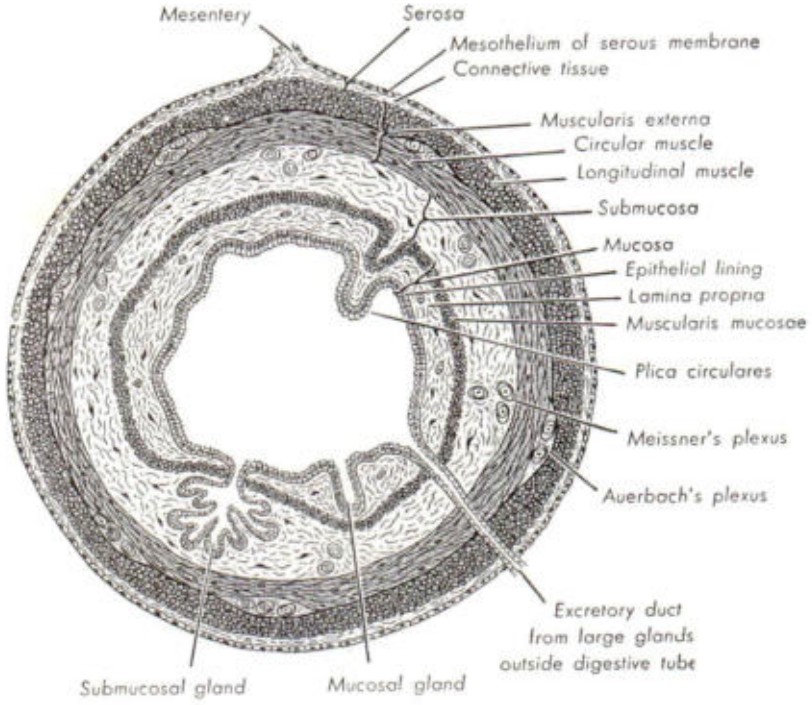


FIG. 249. Diagrammatic cross section to illustrate the structure of the wall of the alimentary tract.

mouth and pharynx. Beginning with the esophagus, however, four layers are constantly present through the remainder of the alimentary tract (Fig. 249): *mucosa*, *submucosa*, *muscularis externa* and *fibrosa* or *serosa* (peritoneum). The chief structural modifications in the different segments of the tract occur in the mucosa and these modifications are of extreme importance, functionally. In the mucosa, also, a third component, the *muscularis mucosae*, appears for the first time. This is composed of smooth muscle, most of the fibers running in a longitudinal direction. The submucosa differs somewhat in successive segments of the alimentary tract, chiefly as regards the presence or absence of glands. The muscularis externa (so named in contradistinction to the muscularis mucosae) is formed of two very regularly arranged layers, an *inner circular* and an *outer longitudinal*, except in the stomach. As in other saccular organs, the muscle of the stomach is irregularly arranged especially in the upper more saccular part. Three rather indistinct layers have been distinguished, largely by exposing the muscle by dissection.

Two nerve plexuses from the autonomic nervous system are present beginning with the esophagus. One of these, the *myenteric* (*Auerbach's*) *plexus*, is readily seen in most sections of the tract. It is located between the two layers of the muscularis externa. The other, the *submucous* (*Meissner's*) *plexus* is in the submucosa. It is more difficult to find.

A knowledge of these general features will be helpful in the more detailed descriptions which follow.

THE ESOPHAGUS

The *esophagus* begins at the level of the cricoid cartilage and extends to slightly below the diaphragm where it becomes

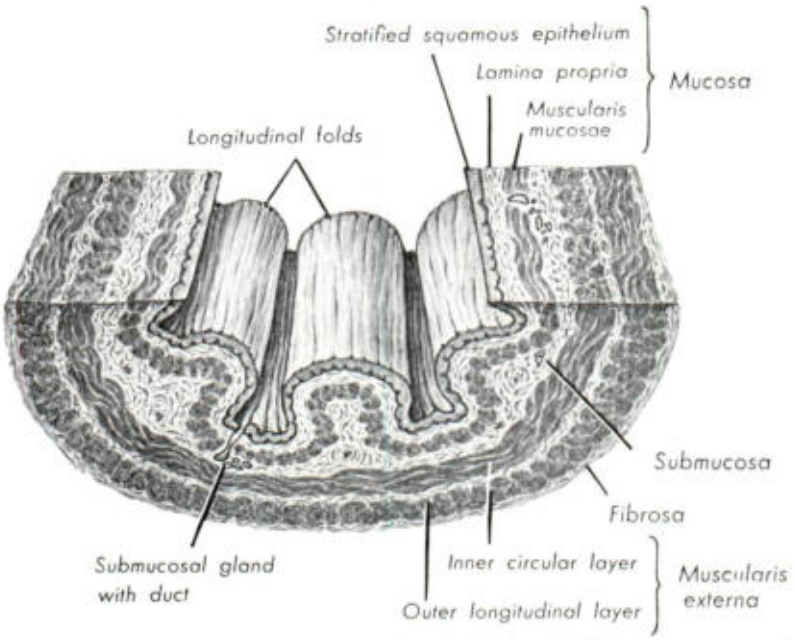


FIG. 250. Camera lucida drawing of a segment of the dorsal half of human esophagus. The entire esophagus had been in fixing solution before being cut open, thus preserving the internal longitudinal folds normally present. × 8.

continuous with the stomach, a distance of 10 to 12 inches.

There is no sharp structural demarcation between the esophagus and pharynx although a greater regularity in the muscularis externa and also the beginning of the muscularis mucosae soon become evident. The esophagus is the most muscular segment of the alimentary tract. Except during the passage of food or water the lumen is small and irregular in shape due to the contraction of the inner layer of the muscularis externa with a consequent formation of longitudinal folds (Fig. 250).

Mucosa. The *mucous membrane* (Fig. 251) is lined with a stratified squamous epithelium and, as in the pharynx, it is not cornified in man. The lamina propria is formed of fine interlacing connective tissue fibers with fibroblasts and histiocytes, and may have areas of infiltration with lymphocytes (Fig. 251). The *muscularis mucosae* is formed of smooth muscle running in a longitudinal direction, but with some inner circular fibers. It occupies a position corresponding to that of the elastic stratum of the pharynx, with which it is continuous. The muscularis mucosae is thicker in the esophagus

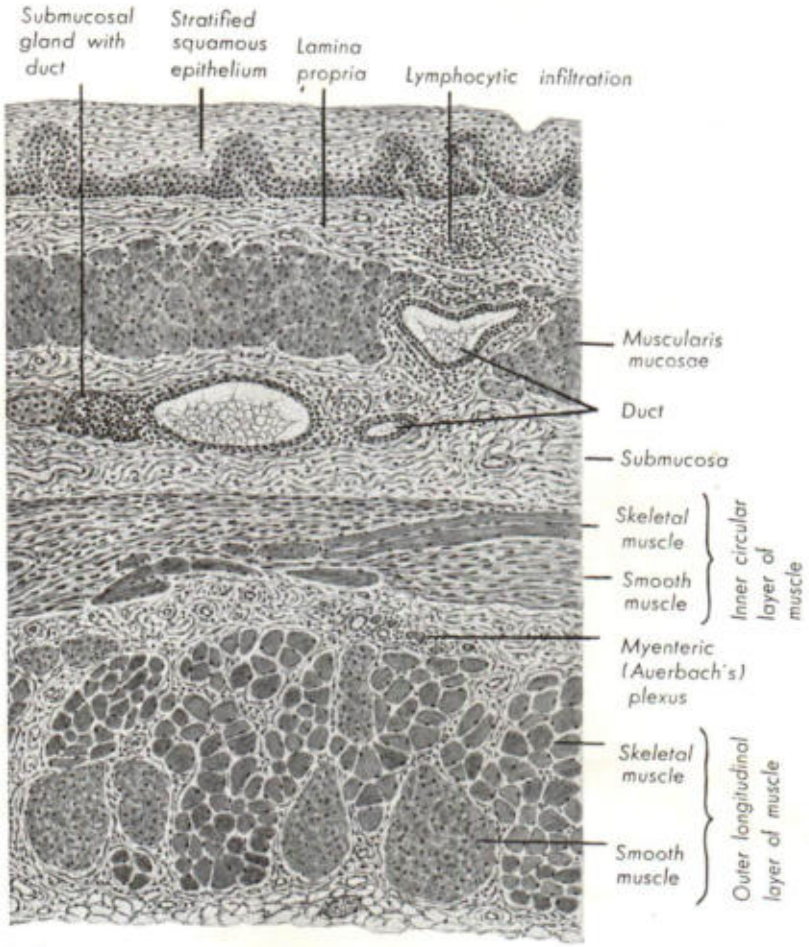


FIG. 251. Transverse section through wall of the upper third of human esophagus. × 65.

than in any other segment of the digestive tube.

Submucosa. The *submucosa* is composed mainly of coarse, loosely interweaving collagenous fibers which permit the formation of extensive folds of the mucous membrane. It contains a plexus of the larger blood vessels, lymphatics and occasional autonomic (parasympathetic) ganglion cells and nerve fibers.

Muscularis. The *muscularis externa* of the first quarter or even less of the esophagus is composed of skeletal muscle, and from the juncture with the pharynx it becomes progressively more regularly arranged into inner circular and outer longitudinal layers. Some smooth muscle soon appears in each of the layers (Fig. 251) and gradually increases in amount. Skeletal fibers extend for a variable distance but are rarely present below the juncture of the upper and lower halves of the organ and may be entirely replaced by smooth muscle at a higher level. Small groups of autonomic ganglion cells, part of Auerbach's plexus, are frequently found in the connective tissue between the inner and outer layers of muscle.

Fibrosa. The *fibrosa* is composed of loosely arranged connective tissue which binds the esophagus to surrounding structures. The short segment of esophagus which extends below the diaphragm lies in the peritoneal cavity and is covered by a *serosa*.

Glands of the Esophagus. Glands occur in the submucosa (*submucosal glands*) and in the mucosa (*mucosal* or *cardiac glands*). The number of *submucosal glands* is extremely variable in man. In some animals, as the dog, they are very numerous. The submucosal glands are composed of typical mucous alveoli such as those in the tongue. Several alveoli open by short ducts into a main duct which, in its course through the submucosa and muscularis mucosae, is dilated forming an ampulla. In the lamina propria, the walls of the duct change from a simple cuboidal to a stratified epithe-

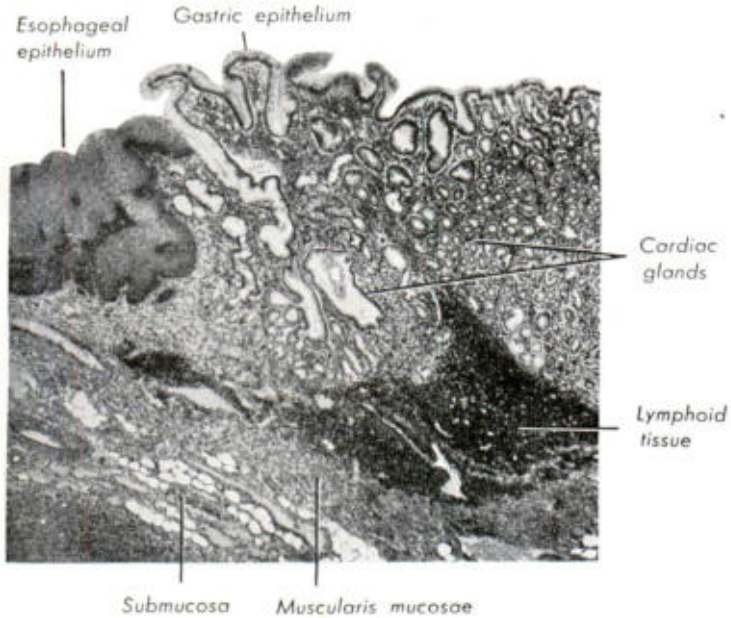


FIG. 252. Longitudinal section through juncture of human esophagus and stomach. Photomicrograph. × 26.

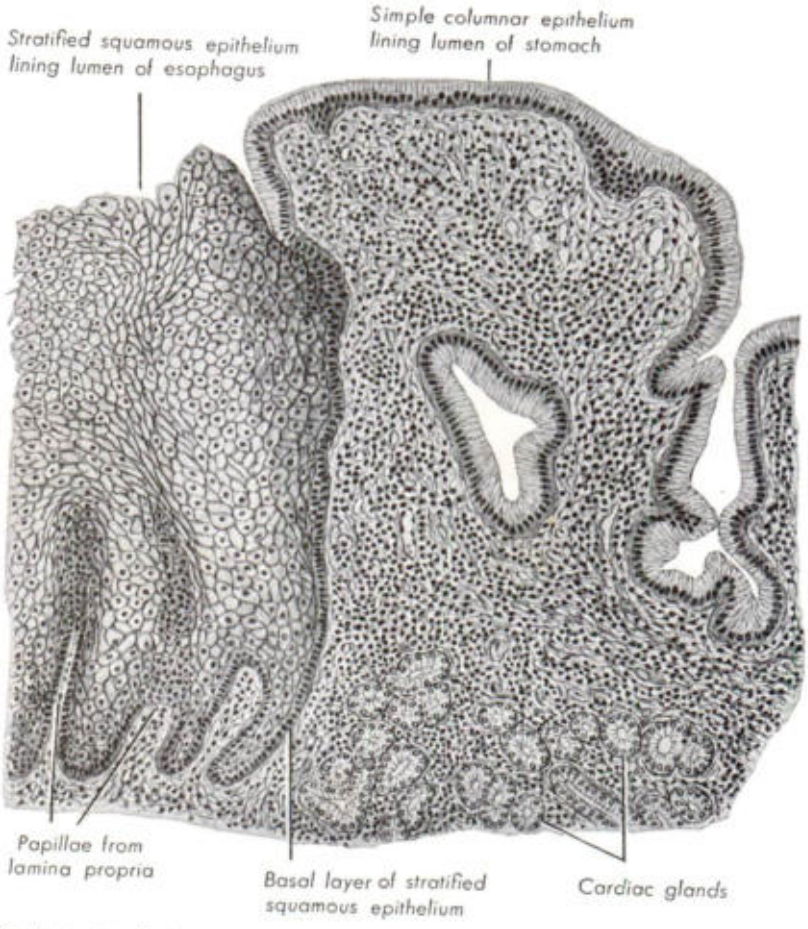


FIG. 253. A longitudinal section through the juncture of the esophagus and stomach in man. × 110.

lium, the surface cells being either squamous or columnar. The duct opens between two adjacent connective tissue papillae. In the connective tissue near the duct there is a lymphocytic infiltration (Fig. 251).

The *cardiac glands* of the esophagus occur in its uppermost and lowermost regions and lie in the lamina propria. The upper group is frequently absent. The cardiac glands are structurally similar to the glands in the upper or cardiac region of the stomach (Fig. 253). They are branched tubular glands which secrete a mucous substance.

THE STOMACH

The stomach extends from the esophagus to the duodenum. In the empty state it is almost tubular in shape except for the upper part which has a pear-shaped bulge superiorly and to the left. A frequently assumed shape when moderately distended is shown in Figure 254. As regards motor activity, the stomach is divisible into upper and lower halves.

The upper half acts as a reservoir and has no—or only slight—peristaltic contractions. The lower half has peristaltic contractions which increase in intensity towards the pylorus. It is in this part that the various constituents of the food are thoroughly mixed with each other and with the secretions of the glands of the stomach. Associated with this motor activity is an increase in musculature especially in the pyloric canal.

At the junction of the esophagus and stomach the epithelium changes abruptly from stratified squamous to simple columnar. The transition is seen strikingly in fresh specimens where the smooth, whitish mucosa of the esophagus gives way to the more irregular, pinkish mucosa of the stomach. The pink color of the stomach mucosa is due to the thinness of the epithelium and the proximity of the richly vascular connective tissue to the surface.

In the deeper structures the line of demarcation is not so clear, the muscularis mucosae of the esophagus being continu-

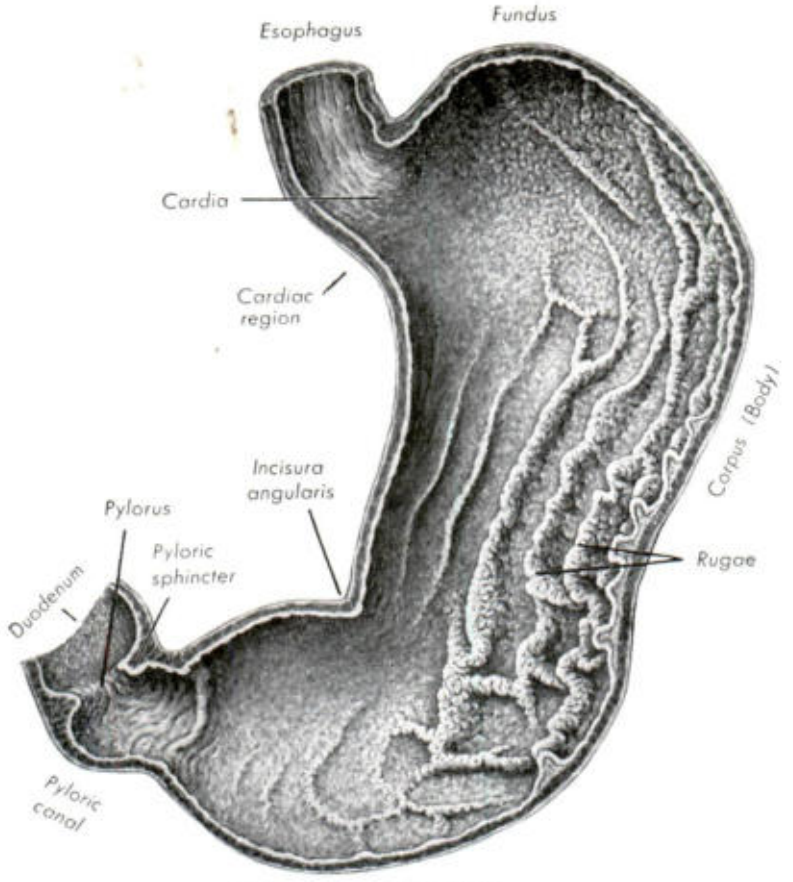


FIG. 254. Drawing of a cast of human stomach. The organ had been moderately distended *in situ* with formalin. Preparation and drawing made by Mr. Kellner. × 4/9.

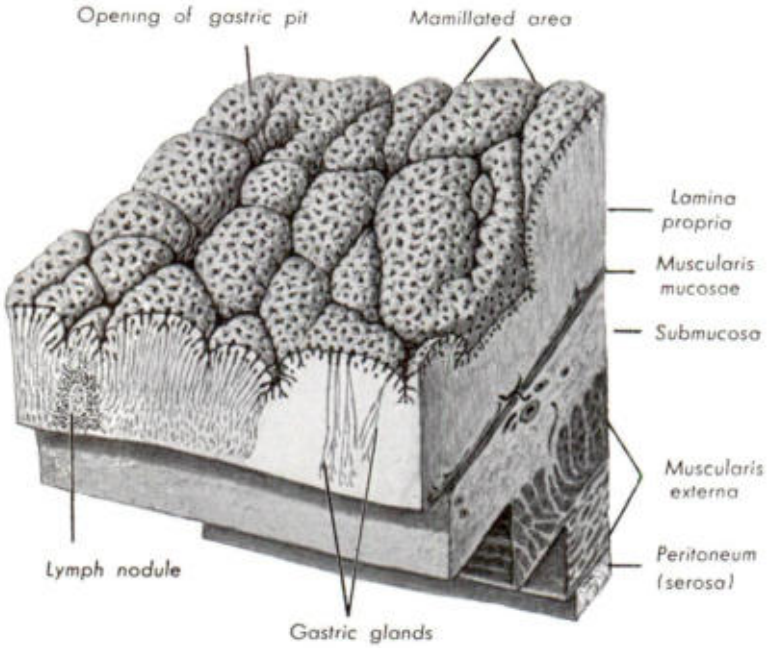


FIG. 255. Reconstruction of a portion of wall of stomach, semidiagrammatic. (Slightly modified after Braus.) × 17.

ous with that of the stomach, and glands of the stomach type extending up under the stratified epithelium of the esophagus (Fig. 252).

Mucosa. The *mucous membrane* of the stomach is folded into ridges or *rugae* (Fig. 254), the height and number of which depend upon the degree of distention of the organ. The rugae are most prominent in the empty stomach. When the stomach is fully distended they almost disappear. The epithelial surface is also divided by grooves into small irregular areas, 1–5 mm. in diameter, the *mamillated* or *gastric areas* (Fig. 255).

The entire surface of the mamillated or gastric areas is studded with minute depressions, the *gastric pits* or foveolae (Fig. 255). In the fundus they are comparatively shallow, extending through about one-fifth the thickness of the mucosa; in the pyloric region the pits are much deeper, extending through half or more of the thickness of the mucous membrane. The glands open into the bottoms of the gastric pits.

THE EPITHELIUM. The epithelium is simple columnar. It covers the entire surface of the gastric mucosa and lines the pits (Fig. 256). The cells are tall and cylindrical. The apical portion of each cell is filled with mucus, which is stained only by special methods. In ordinary histological preparations, the cells show a deep, clear distal zone characteristic of this cell type (Figs. 25, *A*; 256). The remaining cytoplasm and the nucleus are pushed to the base of the cell. The nucleus is oval, spheroidal or sometimes flattened, depending upon the shape of the cell and the amount of mucus it contains.

The mucous cells lining the stomach differ from goblet cells both in shape and in the way they accumulate and discharge their secretion. Whereas goblet cells expel their secretion intermittently,

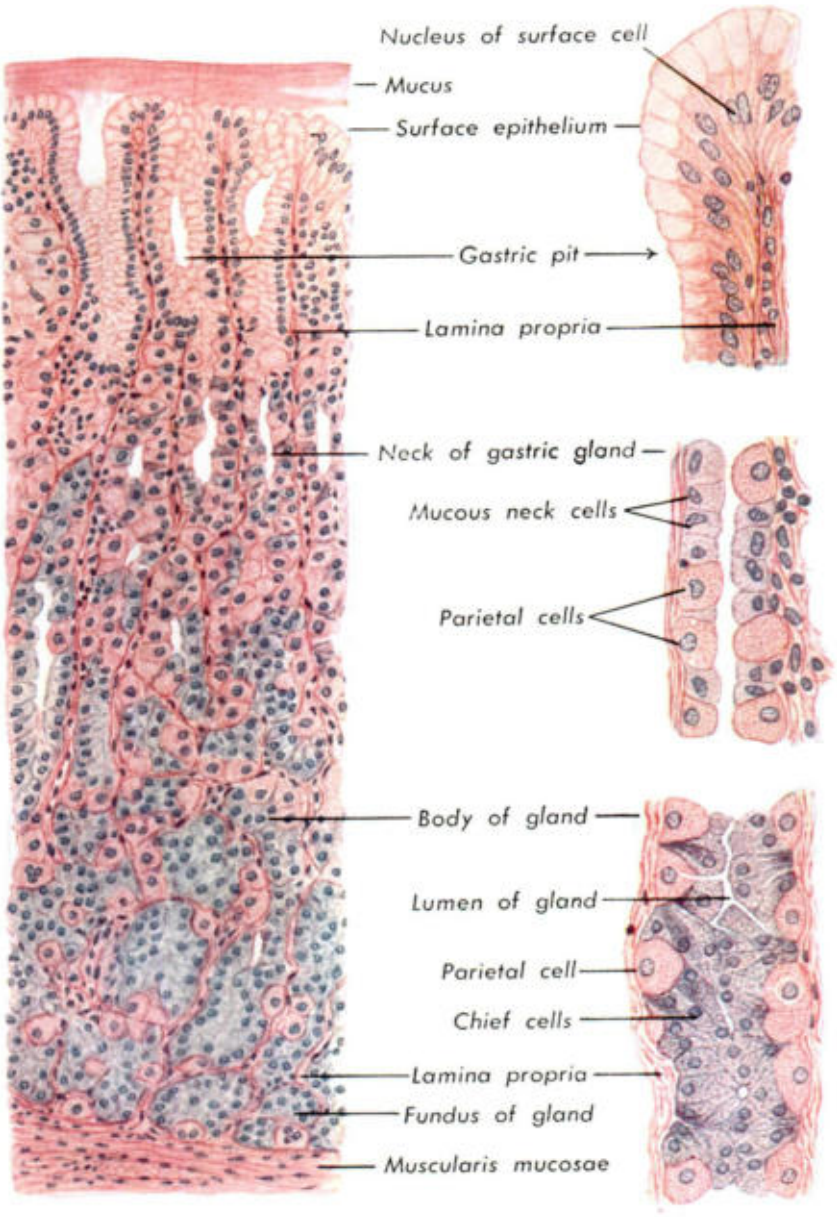


FIG. 256. Vertical section through mucous membrane of body of human stomach showing surface epithelium, gastric pits and gastric glands. Hematoxylin and eosin stain. Figure at left, × 250; figures at right, × 500.

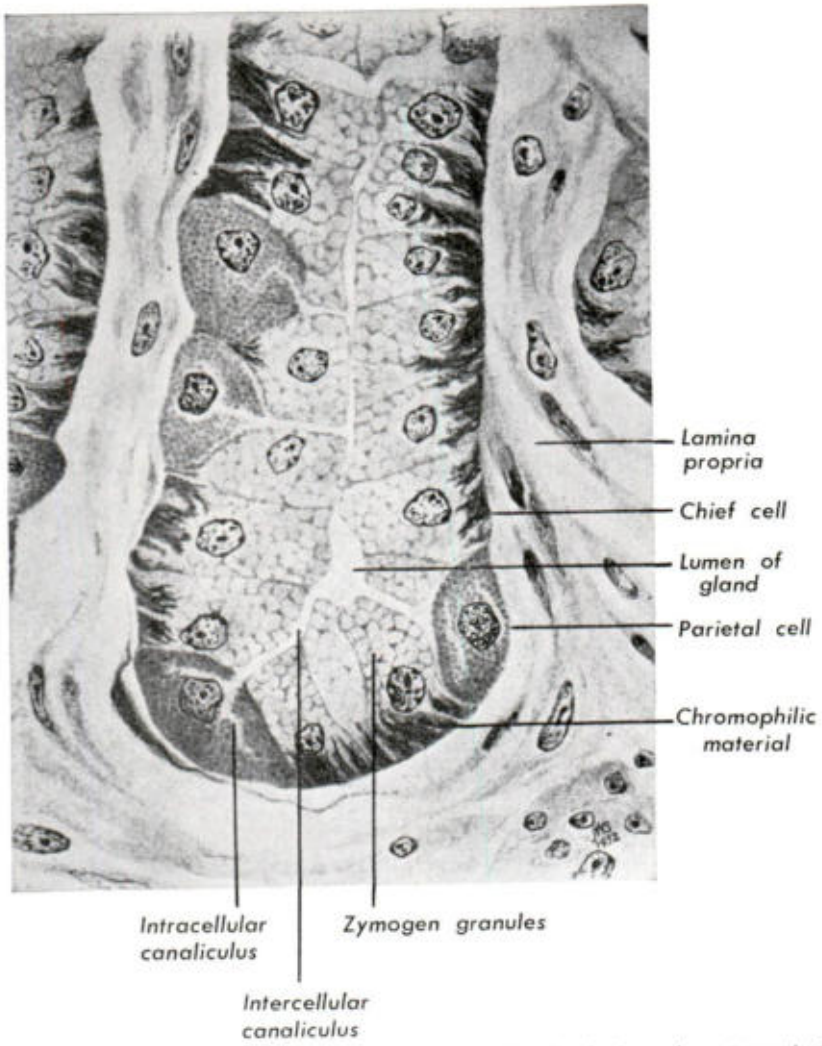


FIG. 257. Vertical section through the fundus of a gastric gland of monkey stomach. Regaud fixation; Harris' hematoxylin and eosin stain. × 950.

the mucous cells of the stomach secrete continuously. Thus, all the surface cells of the stomach have a similar appearance.

The epithelium lining the stomach, in contrast to that of the intestine, has no striated free border as seen with the light microscope. However, electron micrographs do show extremely fine cytoplasmic projections on the free surface of these cells. The absence of an obvious striated border aids in delimiting the epithelium of the stomach from that of the intestine at the stomach-duodenal junction (Fig. 260).

As the epithelium extends progressively deeper into the gastric pits, the cells become progressively shorter and have a narrower apical zone of mucus. This gradual transition, together with the occurrence of mitoses in the deeper lying cells

of the pits, has led to the conclusion that desquamated cells of the surface of the stomach are replaced by a migration of cells up the walls of the foveolae.

LAMINA PROPRIA. The lamina propria (Figs. 256, 257), in which the glands are located, consists of delicate interweaving connective tissue fibers, and fibroblasts and histiocytes. In most regions the glands are so numerous that connective tissue fibers are reduced to thin strands. There is a diffuse infiltration of lymphocytes throughout the lamina propria; in addition, there are scattered lymphoid nodules, or "solitary follicles," which occur most frequently in the pyloric region (Fig. 259).

MUSCULARIS MUCOSAE. The muscularis mucosae consists of a thin layer of smooth muscle in which the fibers usually course in both the circular and longitudinal directions. Strands of smooth muscle extend into the stroma between the glands.

Glands of the Stomach. These glands extend from the bottoms of the gastric pits and their epithelium is continuous with that of the pits (Fig. 256). There are three types of glands: (1) *gastric or fundic glands*, distributed through the greater part of the gastric mucosa, (2) *pyloric glands*, confined to the region immediately above the pylorus, and (3) *cardiac glands*, found in the cardiac region of the stomach near its junction with the esophagus.

The gastric glands produce the essential digestive elements of the gastric juice, and the pyloric and cardiac glands function largely as mucous glands.

The terminology is not entirely satisfactory. For example, the term *gastric* glands might be thought to indicate that these glands occur throughout the stomach, whereas they are absent from a narrow zone around the cardia and from the lower part of the pyloric region. A synonym, *fundic* glands, is even more misleading for it indicates that these glands are limited to the fundic region of the stomach whereas they are much more widely distributed.

THE GASTRIC GLANDS. The gastric glands (Fig. 256) are simple, sometimes branched, tubular glands, of which from three to seven open into each gastric pit. They extend downward through the entire thickness of the lamina propria, to the muscularis mucosae.

Each gland consists of (1) a *mouth* opening into the pit; (2) a constricted portion, the *neck*; (3) the *body* or main portion of the tubule and (4) a slightly dilated and bent blind extremity, the *fundus* (Fig. 257).

In the glands proper we may distinguish three types of glandular cells, the first two of which are easily recognized. These three types are (a) *chief cells*, (b) *parietal cells*, and (c) *neck chief cells* (mucous neck cell) (Figs. 256, 257 and 271).

Chief Cells. The chief cells (zymogenic cells), as the name indicates, are the most numerous cells of the gastric glands.

They are large squarish or pyramidal shaped cells (Fig. 257), whose bases lie against the basement membrane and whose apical borders form the lumen of the gland. The nucleus lies in the basal half of the cell. The apical region, in the usual histological preparations, appears as a delicate cytoplasmic meshwork enclosing clear vacuolated spaces, which represent the position of the unpreserved zymogen granules. With proper fixatives and stains these zymogen granules can be preserved and stained *in situ*.

In the base of the cell, below and lateral to the nucleus, is a substance which stains with basic dyes. It has been shown that this material (*chromophilic* or *chromidial substance*, *ergastoplasm*) is composed largely of ribose nucleic acid. In routine preparations, the chromophilic substance frequently appears striated or filamentous (Fig. 257). From electron

micrographs it can be seen that this appearance is due to the arrangement of the endoplasmic reticulum with its adherent RNA granules. In the basal portions of these cells, the endoplasmic reticulum is abundant and arranged in closely packed parallel lamellae, similar in appearance to that in pancreatic acinar cells. The high concentration of ribonucleoprotein in cells which are actively synthesizing protein (enzyme-producing cells, embryonal or dividing cells, etc.) has led investigators to believe that the substance is in some way essential in the production of protein products within the cell.

Mitochondria and a *Golgi apparatus* may be demonstrated by special methods. The mitochondria are arranged as parallel rods in the basal region of the cell. The Golgi complex occupies a position between the nucleus and the free border.

Various theories have been suggested for the function of both the mitochondria and the Golgi complex in the elaboration of secretory granules. The mitochondria are largest and most numerous when the cell is depleted of its granules and they decrease in size and number as new granules are formed. However, rather than providing the raw materials for the granule formation, as was once believed, it seems well established that the mitochondria contain many of the respiratory enzymes of the cell. Although the secretory granules are sometimes first seen in the region of the Golgi apparatus, it is unlikely that the Golgi material actually enters into the formation of the secretory products. It has been suggested that the Golgi material may act as a condensation membrane in the vicinity of which substances are segregated and recombined (see Chapter I). The endoplasmic reticulum has also been considered a possible site for granule formation in zymogenic cells, since in electron micrographs the secretory granules are sometimes seen, apparently forming, within its vesicles. Further support for this view is found in the localization of the RNA granules (essential for protein production) on the surface of the endoplasmic reticulum in these cells.

The zymogen granules of the chief cells are the antecedents of the enzyme *pepsin*. Various lines of evidence lead to this conclusion. First, it has been demonstrated that after vigorous secretory activity, the chief cells are depleted of their granules while the gastric juice is rich in pepsin. Secondly, in a fasting stomach, when the chief cells are filled with zymogen granules, the pepsin content of its mucosa is likewise at its maximum. And finally, the ingenious experiments of Linderstrøm-Lang demonstrate nicely the correlation between granule content and enzyme concentration at various levels within the glands. Small cylinders of tissue through the entire depth of the mucosa were punched out, frozen and sectioned, and alternate sections subjected to histological examination and chemical analysis. Those regions containing the greatest number of chief cells always yielded the highest concentration of the enzyme. In other words, the pepsin content of the stomach mucosa is directly proportional to the granular content of its chief cells.

Parietal Cells. The parietal cells were the earliest described cells of the gastric glands, and have retained their non-distinctive name. They are often spoken of as the HCl cells because they secrete the hydrochloric acid of the gastric juice. The cells are often larger than the chief cells and are oval or polygonal in shape (Figs. 256, 257). The nuclei are spherical and centrally located. Binucleate or multinucleate cells are occasionally seen. Unlike the chief cells, the parietal cells have frequently been reported to

divide. The cytoplasm of the parietal cells is finely granular throughout. It stains intensely with acid aniline dyes with the result that in stained specimens, these cells contrast sharply with the chief cells. In fresh, unstained preparations, the cytoplasm appears clearer than that of the chief cells.

The parietal cells are most numerous in the neck region of the gland where they are interspersed among the neck chief cells. Here their inner margins reach the glandular lumen. In the body and especially in the fundus of the gland the parietal cells are pushed away from the lumen by the crowding chief cells, so that they come to lie peripherally against the basement membrane. Although lying in this position, where they often bulge beyond the even line of the gland tubule, they always maintain a connection with the lumen. This is accomplished by means of *intercellular canaliculi* which extend between the chief cells to the parietal cells. In favorable preparations or by means of the silver impregnation method of Golgi not only the intercellular canaliculi may be demonstrated, but also their continuity with a network of minute canals within the cell, the *intracellular canaliculi* (Figs. 257, 258). The intracellular canaliculi are formed by an invagination of the free cell surface. The appearance of their walls as seen in electron micrographs is similar to that of the cell membrane, i.e., both have minute protoplasmic projections or microvilli constituting a striated border. This is undoubtedly the same structure which Bensley described in 1932 as a "thin film of exoplasmic condensation."

The mechanism of acid secretion in the stomach remains somewhat obscure. Since free acid is not found within the parietal cells it has been presumed that it must either be present in the form of bound acid or be formed in the vicinity of the cell membrane. The presence of intracellular canaliculi has been interpreted as a mechanism by which the extent of the cell membrane may be increased. In microdissection studies of living gastric mucosa using a variety of indicator dyes including neutral red, it has been found that, although the cytoplasm of the parietal cell gives a somewhat alkaline reaction, the canaliculi, both intra- and intercellular, and the lumen of the gland contain free acid in a nearly pure state. It appears, therefore, that the cell membrane of these cells is a highly selective and differentiated structure capable of segregating and secreting the constituents of the acid. The enzyme *carbonic anhydrase*, which is present in high concentration within the parietal cells, probably also plays an important rôle in the formation of the acid.

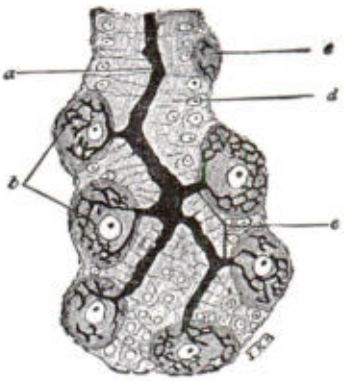


FIG. 258. Longitudinal section of fundus of gland from pyloric end of dog's stomach. (Golgi method.) *a*, Lumen of gland; *b*, intracellular canals in parietal cells; *c*, cut-off portion of parietal cell; *d*, chief cells; *e*, intercellular canals leading from lumen of gland to canals in parietal cells.

Mucous Neck Cells (Neck Chief Cells). The mucous neck cells were not identified until many years after the chief and parietal cells had been described. Certain cells which differ from the chief cells are found mainly in the neck region of the gland where they occur in groups inter-

spersed among the parietal cells. These cells, the mucous neck cells or neck chief cells, are cuboidal or low columnar in shape, with a finely granular cytoplasm which, in routine preparations, is paler than that of the chief cells but not as pale as the mucous surface cells. With special fixation and staining, it may be seen that the cells contain many small mucigen granules. The nucleus is situated basally and is frequently oval with its long axis perpendicular to the long axis of the cell. The upper surface of the nucleus is sometimes indented (Figs. 256, 271).

The mucous neck cells secrete the same type of mucus as do the cells of the cardiac and pyloric glands. The mucus produced by these cells, however, has a somewhat different composition and consistency (less viscous) than that produced by the surface cells of the gastric mucosa. There is a substance present in gastric juice and in the gastric mucosa, the *intrinsic factor* of Castle, which enhances the absorption of the extrinsic factor (vitamin B_{12}, the antipernicious anemia factor, p. 129) from the gut. There is good evidence indicating that the mucus (*glandular mucoprotein*) produced by the mucous neck cells contains the intrinsic factor.

A fourth type of cell, present in small numbers in the gastric glands, the *argentaffine cell*, will be discussed in the section dealing with the small intestine.

THE PYLORIC GLANDS. These are simple branched tubular glands, several of which open into each of the deep *pyloric pits*. These pits occupy a much greater proportion of the thickness of the mucous membrane than do the pits of the gastric glands (compare Figs. 256, 259) and the proportionate depth occupied by the glands themselves is correspondingly less. The pyloric glands, though short, are quite tortuous, so that in sections the tubules are seen cut mainly transversely or obliquely. In most of the pyloric glands but one type of cell is prominent. These cells are similar to the mucous neck cells of the gastric glands. Parietal cells are not always entirely absent, but occur infrequently here and there in the pyloric tubules, especially near the fundus of the gland.

The transition from the gastric to the pyloric type of stomach gland is not abrupt, but is marked by a "transitional border zone," in which gastric and pyloric glands are intermingled, and in which are also found single glands which combine the characteristics of both types.

CARDIAC GLANDS. In the transition zone between esophagus and stomach are found glands which resemble those in the lamina propria of the lower end of the esophagus (Figs. 252, 253). Because of their location they have been designated *cardiac glands*. Those nearest the esophagus are lined with clear cells which resemble closely the cells of the pyloric glands and the mucous neck cells of the neck in the gastric glands. As one passes further from the stomach-esophageal junction, parietal cells and chief cells make their appearance and become more and more numerous. The glands thus pass over by gradual transition into typical gastric glands.

Occasionally there are found in the adult stomach, chiefly in the pyloric and cardiac regions, patches of surface and glandular epithelium identical in structure with that of the intestine. Inasmuch as such inclusions are typically absent in children, and are present in so small a proportion of adult stomachs, they probably have a pathological significance.

Submucosa. The *submucosa* consists of coarse, loosely arranged connective tissue. It contains the larger blood vessels and nerves, including the plexus of Meissner.

Muscularis. The *muscular coat* of the

stomach is usually described as consisting of three layers, an inner oblique, a middle circular, and an outer longitudinal. In the fundus, however, the muscle bundles run in various directions, so that a separation of the muscular coat into layers having definite direction is made difficult. The pylorus has its inner and middle layers thickened to form the sphincter pylori. In the connective tissue which separates the longitudinal and circular muscles there are groups of parasympathetic nerve cells and fibers, which, while much less distinct, are homologous to Auerbach's plexus of the intestine.

Serosa. The *serous coat* consists of a layer of loosely arranged connective tissue which is covered by mesothelium.

Blood vessels, lymphatics and nerves are so similar throughout the stomach and intestines that they will be described together at the close of the sections on the intestines.

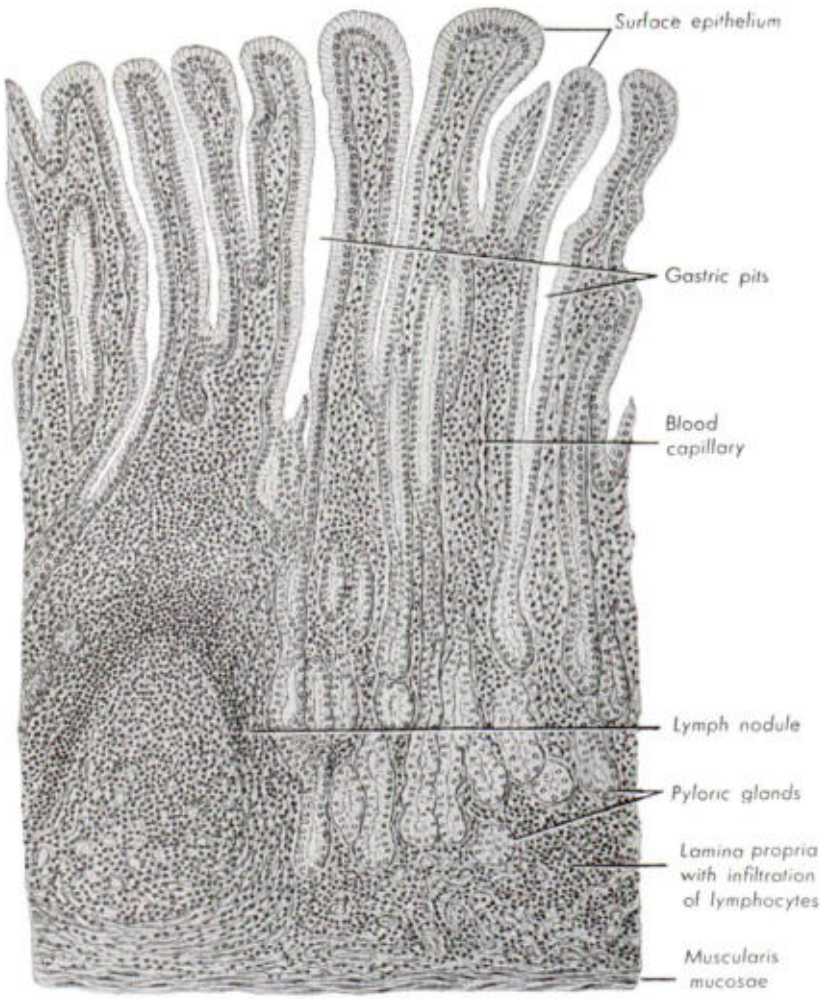


FIG. 259. Vertical section through mucous membrane of pyloric canal. × 110

The stomach of some mammals shows modifications for special diets. The cow's stomach, for example, has four distinct divisions: the rumen, reticulum, omasum and abomasum. The first three portions, which are often regarded as esophageal sacculations, are lined by stratified squamous epithelium; the fourth segment, sometimes called the "true stomach," has a glandular mucous membrane with the three types of gastric glands.

The rumen serves as a reservoir in which some digestion of food by bacterial action takes place; the food then moves into the reticulum, the inner wall of which is "honeycombed" by ridges. Here the cud is formed, to be regurgitated at will and rechewed. When the food is swallowed again, it passes directly into the omasum, with its high mucosal folds, and thence into the abomasum. In this final segment, enzymatic digestion is completed, and the food moves through the pylorus into the intestine.

THE SMALL INTESTINE

The small intestine, which extends from the pylorus of the stomach to the colon, is commonly divided into three regions, an upper, the *duodenum*; a middle, the *jejunum*; and a lower, the *ileum*. Although these divisions have certain distinctive features, to which attention will be directed, the similarity of their general structure makes it desirable to describe them together.

If the small intestine is opened by a longitudinal incision through its wall, a series of definite folds will be seen on its inner surface. They are in general parallel to one another and pass in a circular or oblique manner, partly around the lumen of the tube. These folds are variously

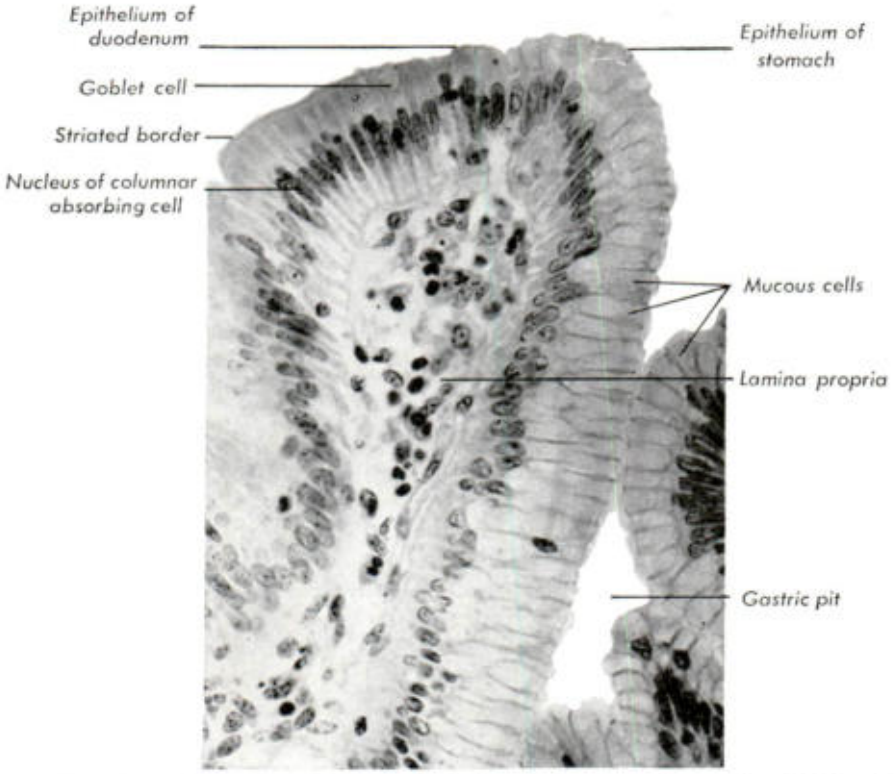


FIG. 260. Photomicrograph of stomach-duodenum juncture. Stomach epithelium is on the right, duodenum on the left. Monkey. Hematoxylin and eosin stain. Photograph by Mr. L. Koster. × 620.

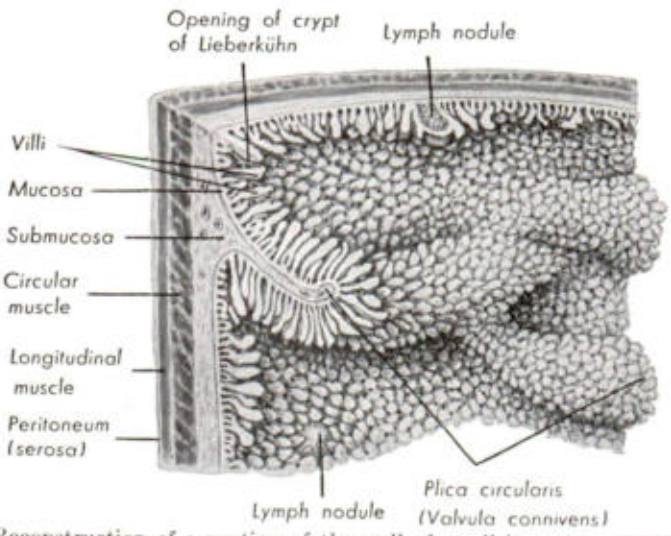


FIG. 261. Reconstruction of a portion of the wall of small intestine. (After Braus.) × 17.

known as *valvulae conniventes*, *plicae circulares*, *valves of Kerkring*. They are absent in the upper region of the duodenum, tallest in the jejunum and much less prominent in the ileum as it nears the colon. These folds involve the entire mucosa, and also carry into them a portion of the submucosa (Fig. 261). Unlike the folds of the stomach, the plicae cannot be completely flattened out by distention of the intestine.

The mucosa is further carried up into finger-like projections, the *villi*, which not only cover the surface of the valvulae but the entire surface of the small intestine (Fig. 261). The villi differ in shape in the different parts of the small intestine, being leaf-shaped in the duodenum, rounded in the jejunum, club-shaped in the ileum. The valvulae conniventes and the villi are characteristic of the small intestine. It is important to note that while the pits of the stomach are *depressions in* the mucous membrane, the intestinal villi are definite *projections above* its general surface. By means of its folds and projections the absorbing and secreting surface of the intestinal mucosa has been enormously increased. Opening between the villi and extending into the mucosa as far as the muscularis mucosae are simple glandular pits, the *crypts of Lieberkühn* (glandulae intestinales). All of the above modifications will be treated in detail in a discussion of the mucosa.

The wall of the intestine consists of the same four coats which constituted the wall of the stomach—*mucosa*, *submucosa*, *muscularis externa*, and *serosa*.

The Mucosa. The mucosa is composed of its lining *epithelium*, a *lamina propria* with its glands, and a limiting *muscularis mucosae* below. The most characteristic feature of the small intestinal mucosa is the *villus* (Figs. 261, 264).

The villi are mucosal projections barely visible to the naked eye. Situated close together and covering the entire mucosal surface, they give the interior of the intestine a soft, velvety appearance grossly.

Each villus consists of a core of delicate loose connective tissue and an epithelial covering. The connective tissue, the lamina propria of the mucous membrane, is infiltrated with lymphocytes to a

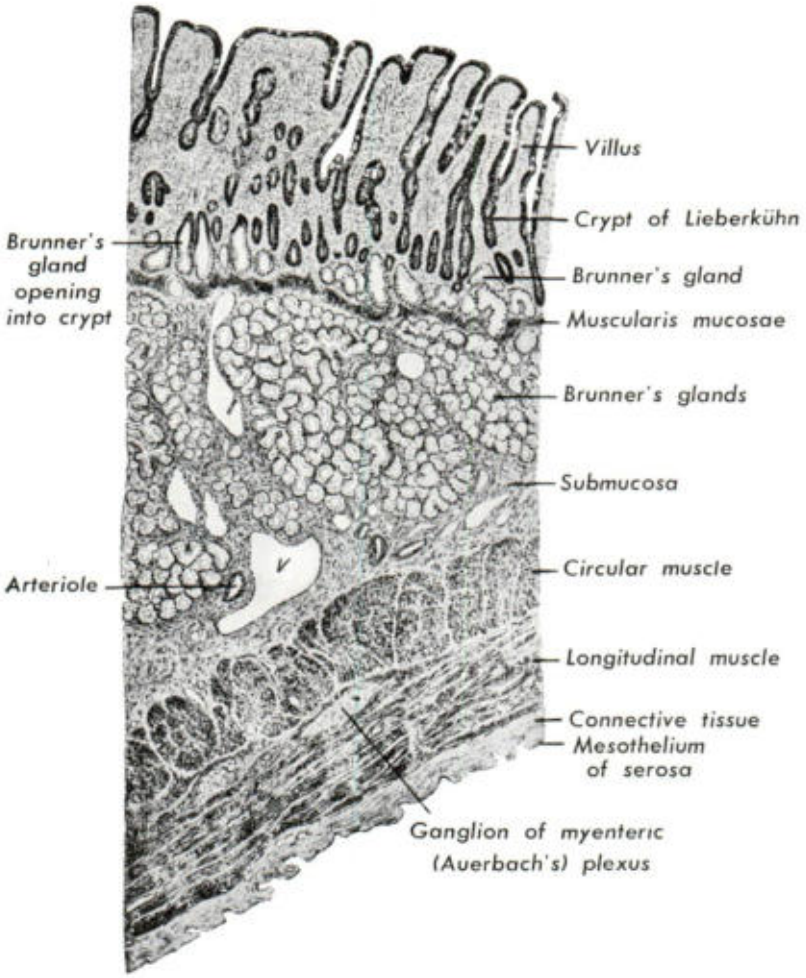


FIG. 262. Longitudinal section through upper duodenum of man. × 30. (After Schaffer.)

variable extent. In addition to these and the cells of the connective tissue, occasional isolated smooth muscle cells from the muscularis mucosae are present.

A single small lymph or chyle vessel with definite endothelial lining traverses the center of each villus, beginning at its tip in a slightly dilated blind extremity. Since this *central lacteal* is usually collapsed in fixed preparations, it is often difficult to see. It appears most frequently as two closely approximated rows of flat cells with bulging nuclei. During absorption of fat from the intestinal lumen, the lacteals become distended with fat droplets, and are then clearly visible (Fig. 266). The blood capillaries of the villus form a network which lies, for the most part, away from the lacteal, just beneath the basement membrane.

Observations of the villi in the living condition have revealed that they continually change in length and undergo waving motions. This is possible because

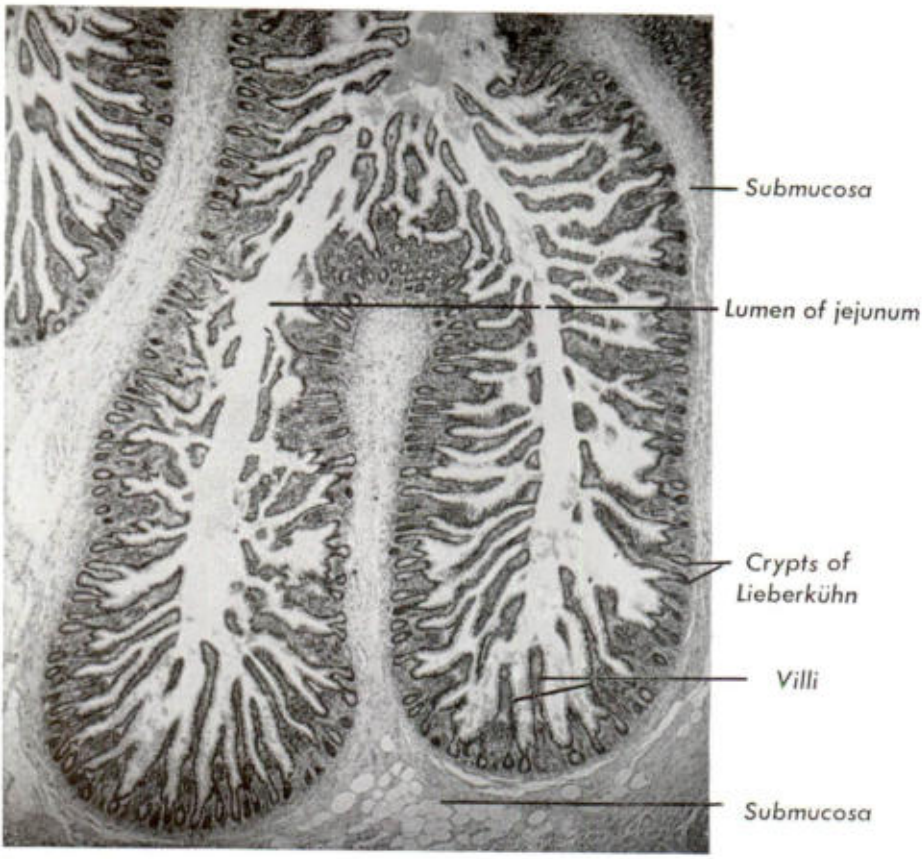


FIG. 263. Longitudinal section of mucosa of jejunum. The section shows a short plica circularis in the center of the field, and on either side of it part of a taller plica. The entire lumen surface is covered with villi; submucosal connective tissue forms the core of each plica. Human. Hematoxylin and eosin stain. Photomicrograph. × 32.

of the presence of smooth muscle in them. These movements serve to bring the villi into contact with new material to be absorbed and also aid in the circulation of the villus particularly in the movement of fluid in the lymph vessels (lacteals).

EPITHELIUM. The *epithelium* covering the villi is a single layer of columnar cells attached to a delicate basement membrane. The epithelium consists of two quite different kinds of cells, *columnar absorbing cells* and *goblet cells*. The columnar absorbing cells are quite plastic and, although generally long and narrow, they vary as to length and breadth as they adapt themselves to movements of the intestine. Their various shapes can best be studied in dissociated (macerated) epithelium. The cytoplasm is finely granular, its appearance changing somewhat in different phases of absorption. It frequently contains fat droplets. The nucleus is ovoid and is usually situated in the lower half of the cell (Fig. 260).

One of the most striking and distinguishing features of the columnar absorbing cells is their *striated free border* (cuticular border, brush border). With low magnification this is seen as a nearly homogeneous, refractile membrane

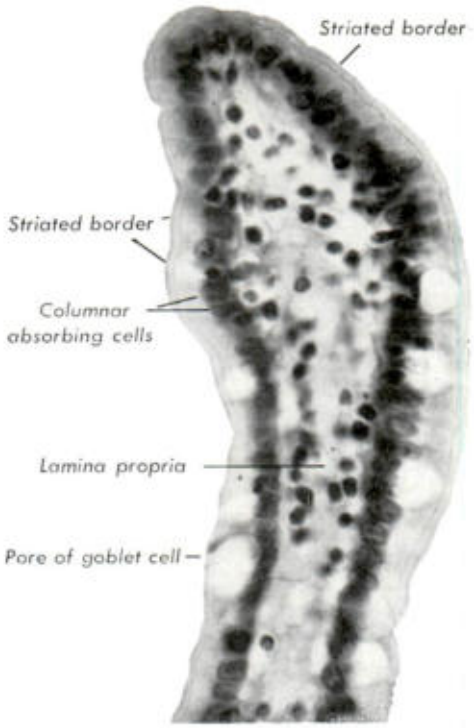


FIG. 264. Longitudinal section of a villus from the jejunum (same specimen as Figure 263). Human. Photomicrograph. × 850.

(Figs. 2, *C*; 264) covering the free surface of the cell, but with greater magnification it appears finely striated. By means of phase contrast and electron microscopy it is seen that the striated free border is actually composed of a great many very fine, closely packed filaments (Figs. 265, 267). The length of the filaments varies in cells of different regions, being greatest (1 to 2μ) on cells at the tips of the villi.

The striated border of these cells, like the brush border of the proximal tubule cells of the kidney, contains a high concentration of the enzyme *alkaline phosphatase*. Although the precise function of the enzyme in this location is not known, it is presumed to play a part in the absorption of foodstuffs, probably carbohydrates, from the intestine.

The *mitochondria* (Fig. 2, *C*) are distributed above and below the nucleus. In both regions they are rod-shaped or filamentous. Those in the basal portion of the cell are particularly numerous.

The *Golgi apparatus* lies between the nucleus and the free surface of the cell. Its possible significance in the absorption of foodstuffs is discussed below.

Scattered among the columnar cells are *mucous* or *goblet cells* (Figs. 264 and 2, *C*). These vary in appearance according to the amount of secretion which they contain. A cell at the beginning of secretion contains only a small amount of mucin near its free border. As secretion increases the mucus gradually displaces the cytoplasm until the latter is represented only by a basal crescentic mass containing a flattened nucleus. The cell discharges its mucus upon the free surface. The goblet cells possess no modified surface membrane, appearing, when seen from the surface, merely as openings surrounded on all sides by the thickened striated borders of the adjacent absorptive cells.

Although opinions differ as to the relationship of the goblet cell to the columnar absorbing cell, the majority of investigators look upon each one as a specialized cell type having its origin in a more primitive, undifferentiated columnar cell. Once differentiated, either type of cell probably does not revert back or transform into the other.

Goblet cells frequently appear to go through cycles of secretory activity alternating with periods of rest. The careful investigations of Clara (1926) disclosed the formation of a new cycle of small secretory granules within the fully distended goblet cell, indicating that at the time the cell discharges its mucous content, a second accumulation is already being elaborated. These observations show that at times this secretory

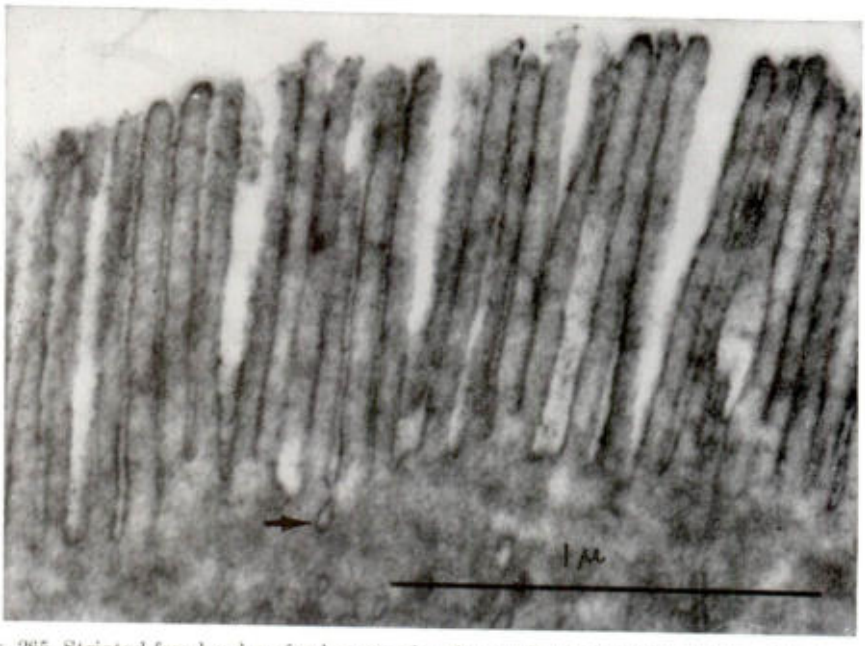


FIG. 265. Striated free border of columnar absorbing cell of mouse duodenum. Electron micrograph. Long, closely spaced cytoplasmic processes cover the entire free surface of the cell. Small vesicles, such as the one at the arrow, extending into the cytoplasm from between the processes have been interpreted by some investigators as absorption (pinocytotic) vacuoles. × 57,000. (Courtesy of Dr. A. J. Dalton.)

activity is a continuous process without any interpolated resting phase.

The number of goblet cells is small in the duodenum and becomes progressively greater in the jejunum, ileum and colon.

Crypts of Lieberkühn or *intestinal glands* occur throughout the small intestine. They are simple tubular glands located in the mucous membrane. They open between the villi, and extend down through the lamina propria as far as the muscularis mucosae. The epithelium of the crypt is continuous at its opening with the surface epithelium of the villi. In general, the columnar cells which lie near the fundus of the gland are less differentiated and somewhat shorter than the columnar absorbing cells of the villi. The striated border of the cells deep in the glands is poorly developed (with filaments less than 0.5μ in length). There is a gradual increase in the height of the filaments of the striated border on the columnar cells from the fundus to the mouth of the crypt. This fact, together with the presence of numerous mitoses, especially in the middle region of the gland, suggests that the columnar absorbing cells of the intestinal surface epithelium arise from the rather undifferentiated columnar cells of the crypt.

Goblet cells are also found in the crypt epithelium. They are more numerous in the upper portion, but may be found even in the region just above the base of the gland. Goblet cells, also, as stated above, most probably arise from the undifferentiated type of columnar cell.

In addition, there are also found in the depths of the crypts of Lieberkühn groups of coarsely granular cells, the *cells of Paneth* (Fig. 271). These cells are

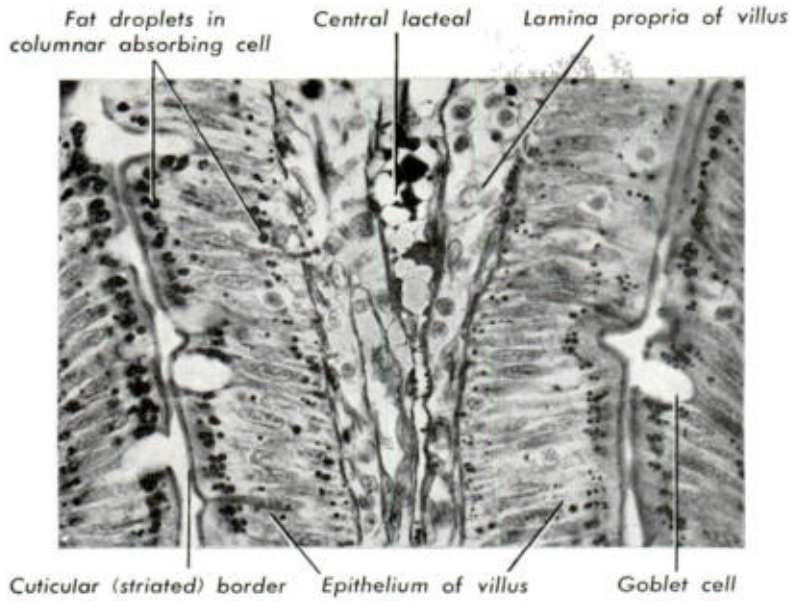


FIG. 266. Fat absorption in ileum of rhesus monkey given a meal with a high fat content by stomach tube four hours prior to autopsy. Parts of three villi and two intervillous spaces are shown. The fat droplets in the basal (infranuclear) regions of the columnar cells would appear from electron microscope studies to be between rather than within the cells. Osmic acid fixation; azocarmine stain. × 480.

serozymogenic, having the characteristic striated chromophilic material in the basal region and large, refractile acidophilic granules apically. The zymogenic nature of the cells is demonstrated by their cycle of activity during digestion. In fasting animals the secretory granules accumulate and the basal striations become prominent, whereas during active digestion the cell is depleted of both its granules and its chromophilic material. There are probably several digestive enzymes produced, but the exact number and identity are unknown. Paneth cells occur mainly in the crypts of Lieberkühn of the small intestine but may occasionally be present in the crypts of the large intestine as well.

Still another type of cell is present in small numbers among the crypt cells and occasionally among the surface epithelial cells. This is the *argentaffine* or chromaffin cell. Fine granules located in the basal portion of the cells are stained by silver and chromium salts. Similar cells are also found in the epithelium of the gastric mucosa. Their functional significance is entirely unknown.

Cytological Changes in Absorption. The epithelium of the gastro-intestinal tract must be considered as having two main functions: (1) The *secretion* of substances necessary in digestion; and (2) the *absorption* of the products of digestion. The first of these functions has been dealt with in connection with the description of the various secretory cells. The matter of absorption of digestive products deserves some further elaboration. The epithelium of the intestines must absorb, in addition to water, salts and mineral substances, the three classes of food—carbohydrates, proteins and fats.

Although we know that the columnar

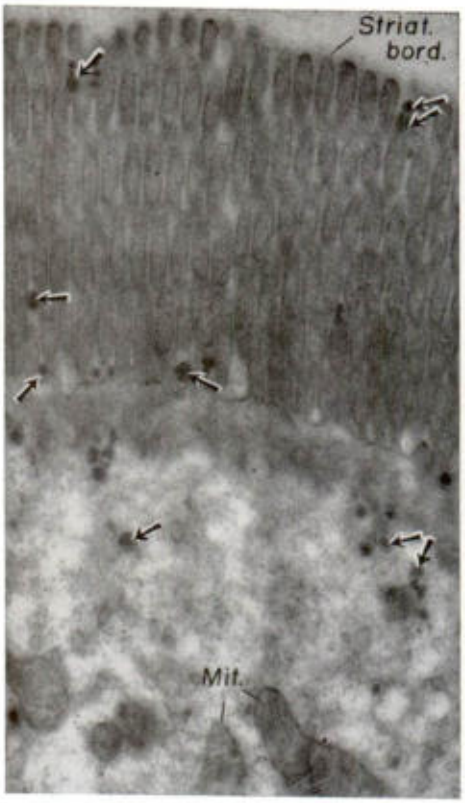


FIG. 267. Apical end of absorbing cell from the jejunum of a rat. Corn oil had been instilled into the stomach of the animal 22 minutes before autopsy. Fat droplets, blackened by the osmic acid, are seen between the microvilli of the striated border and within the apical cytoplasm of the cell. Arrows point to fat droplets. *Striat. bord.*, microvilli of striated border of absorbing cell; *Mit.*, mitochondria. Electron micrograph, × 36,525. (Courtesy of Dr. S. L. Palay.)

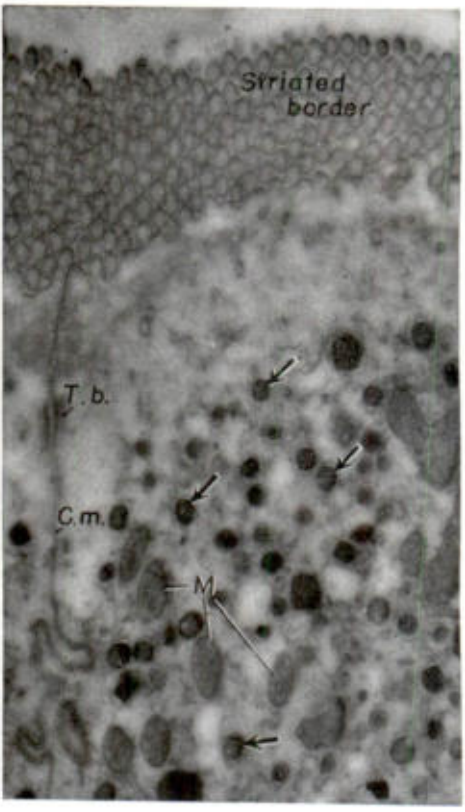


FIG. 268. Apical end of jejunal epithelial cell 70 minutes after corn oil had been instilled into the stomach of the rat. Microvilli of the striated border are cut obliquely. Fat droplets (arrows) are present within vacuoles in the cytoplasm, but no longer in the striated border nor in the immediately subjacent cytoplasm. Cell membranes (*C. m.*) and terminal bar (*T. b.*) are seen at the left in the figure. *M.*, mitochondria. Electron micrograph, × 24,225. (Courtesy of Dr. S. L. Palay.)

epithelium, especially in the large intestine, absorbs large amounts of water, cytological changes in the cell corresponding to this activity have not been established. We have some information regarding the absorption of a mineral substance such as iron. Macallum demonstrated that traces of iron in the form of granules (as demonstrated by the Prussian blue reaction) could be found in the columnar cells after heavy feeding of organic or inorganic iron compounds. He furthermore clearly demonstrated experimentally that the iron passes through the striated free border of the cells.

Proteins are absorbed as amino acids. As such their course through the epithelial cells cannot be traced visually. The cytological features such as the appearance of vacuoles and spaces within the cells or between them, which have fre

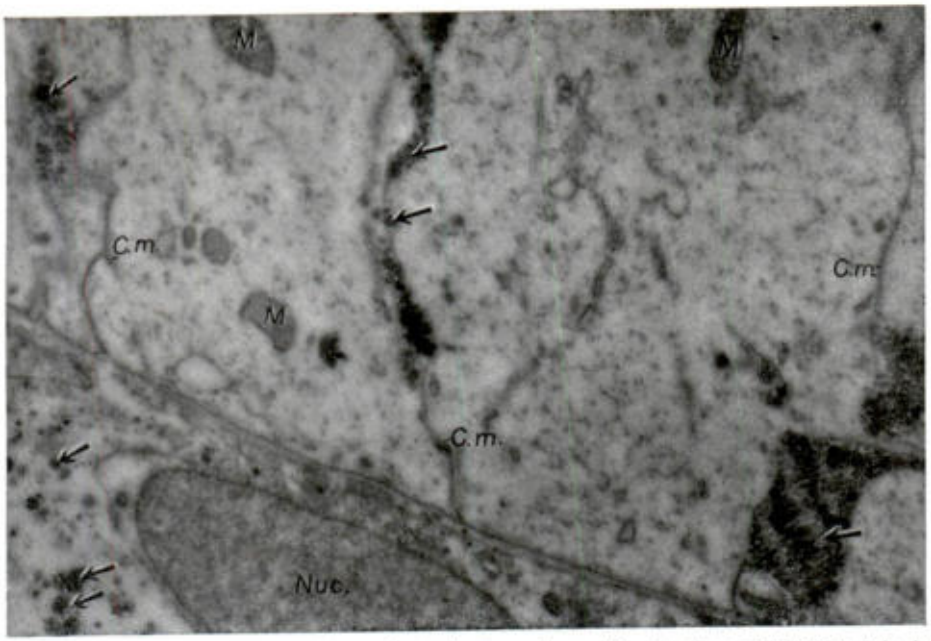


FIG. 269. Base of jejunal epithelium of rat about one hour after feeding with Purina lab chow. The fat droplets (arrows) lie between the epithelial cells and in the connective tissue spaces of the lamina propria. *C. m.*, cell membranes; *M.*, mitochondria; *Nuc.*, nucleus of connective tissue cell. Electron micrograph, × 14,775. (Courtesy of Dr. S. L. Palay.)

quently been reported as evidence of protein absorption are probably the result of post mortem changes and fixation shrinkage.

No changes in the epithelium associated with carbohydrate absorption have been described.

The absorption of fats has been studied extensively, largely because these substances are easily stained by a variety of dyes. Partial breakdown of dietary fats in the intestine is effected by the enzyme lipase with the aid of the bile salts, resulting in the formation of fatty acids and glycerol. The neutral fats which remain are emulsified by the action of the bile salts. Both the fatty acids, in the form of very fine droplets, and the neutral fats as larger droplets are absorbed by the intestinal epithelial cells.

The actual pathway taken by the fat droplets in their absorption and transport across the epithelial membrane has been demonstrated by electron microscopy. The fat, blackened by osmic acid, is first seen between the microvilli and in the cytoplasm just beneath the striated border (Fig. 267). Somewhat later, droplets of various sizes appear deeper in the apical cytoplasm. Each droplet is surrounded by a smooth membrane (Fig. 268). Some appear clustered or coalesced within the vesicles of the Golgi complex. At about the level of the nucleus, the fat droplets migrate out of the cells and appear in sizeable aggregations between the columnar cells; they are not seen in the cytoplasm below the nucleus (Fig. 269). From the intercellular clefts, the droplets make their way through the basement membrane and into the connective tissue spaces below (Fig. 269), finally entering a lymphatic capillary.

As indicated above, the Golgi complex of the columnar absorbing cells appears to play an important role in the transport of

fats. Various other factors also play a part. Bile from the liver is important in both the hydrolysis and the emulsification of fats in the intestine. One of the factors of the vitamin B complex appears to be essential for the transport of fats through the columnar absorbing cells. In the absence of this factor, huge droplets of fat accumulate and remain in the supranuclear region of the epithelial cells (perhaps due to faulty functioning of the Golgi complex). In the normal condition only fine droplets are seen streaming through the cytoplasm (Fig. 266).

The presence of both fatty acids and neutral fats within the epithelial cells has been adequately demonstrated. It was formerly believed that fats could not be absorbed unless they were hydrolyzed, i.e., in the form of fatty acids and glycerol, and that whatever neutral fats appeared within the cytoplasm of the cells had been resynthesized there. However, the work of Frazer has made it quite clear that some neutral fats in a finely emulsified state may be absorbed intact.

The pathway from the epithelial cells to the vascular system appears to differ for the two types of fat, however. The fatty acids find their way directly into the blood capillaries of the villi, and thence to the general circulation by way of the portal system and the liver where some of the fat is removed and stored by the hepatic cells. Much of the neutral fat, on the other hand, goes into the lymphatic capillaries (central lacteals) to reach the general circulation by way of the thoracic duct.

LAMINA PROPRIA. The *stroma*, besides forming the centers of the villi, fills in the spaces between the crypts of Lieberkühn and between the latter and the muscularis mucosae. It is composed of interweaving reticular and delicate collagenous fibers with a considerable number of elastic fibrils. Everywhere lymphocytes are abundant and in many places so numerous as to give the appearance of diffuse lymphoid tissue. Here and there the lymphoid cells are more closely packed together to form distinct lymph nodules. These are known as *solitary follicles* to distinguish them from groups of lymph nodules which occur in the lower sections of the small intestine and are known as Peyer's patches (see below). Solitary follicles are much more numerous in the

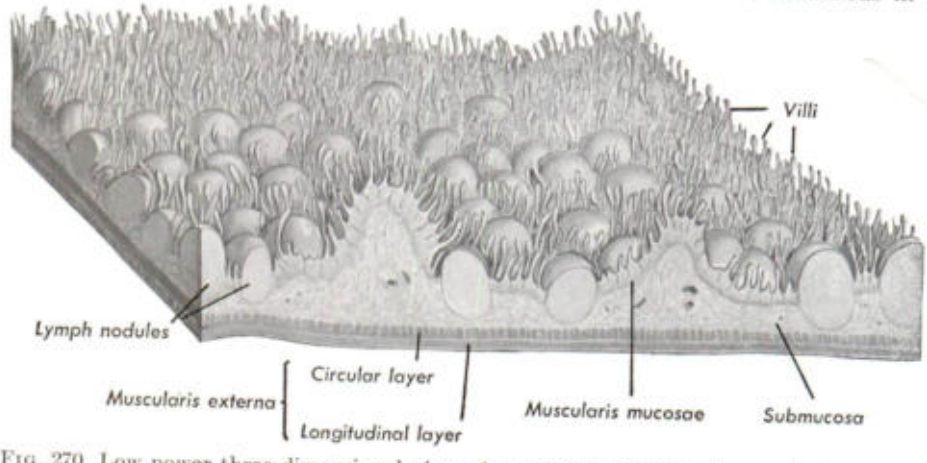


FIG. 270. Low power three dimensional view of a segment of human ileum showing part of a Peyer's patch. × 15.

small intestine than in the stomach. They are identical in structure with those of the stomach.

Peyer's Patches (agminated follicles). These are aggregations of solitary follicles or groups of lymph nodules found mainly in the ileum, especially near its junction with the colon. They always occur on the side of the gut opposite the attachment of the mesentery. Each patch consists of from ten to seventy nodules, which lie side by side and are so arranged that the entire patch has a generally oval shape, its long diameter lying lengthwise of the intestine. The pear-shaped apices of the follicles are directed toward the lumen and project almost through the mucosa. They are not covered by villi, a single layer of columnar epithelium alone separating them from the lumen of the gut, where their smooth surfaces can readily be seen in the gross dissection (Fig. 270).

The bases of the nodules are not confined to the lamina propria, but extend into the submucosa. The relation of the patch to the mucosa and submucosa can be best appreciated by following the course of the muscularis mucosae. This is seen to stop abruptly at the circumference of the patch, appearing throughout the patch as isolated groups of smooth muscle cells. Sometimes the individual nodules which make up a Peyer's patch are quite discrete and well defined. More frequently, however, the nodules tend to coalesce except at their apices, so that the individual nodules can be definitely outlined only at their apices and to some extent identified by their germinal centers. Peyer's patches are most prominent in children. In the adult and in old age the lymphoid tissue gradually undergoes regression and involution.

MUSCULARIS MUCOSAE. The muscularis mucosae is thin, consisting of an inner circular and an outer longitudinal layer of smooth muscle. Small groups of muscle fibers extend from the muscularis mucosae into the stroma of the villi.

Submucosa. The *submucosa* (Figs. 261, 270) consists, as in the stomach, of loosely arranged connective tissue and contains the larger blood vessels and lymphatics. The submucosa is free from glands except in the duodenum, where it contains the *glands of Brunner* (Fig. 262).

Brunner's glands are the most distinguishing characteristic of the upper duodenum and they are often spoken of as the duodenal glands. While their presence is an identifying feature of the duodenum, it must be remembered that these glands are absent in its inferior part. Brunner's glands are branched tubulo-alveolar glands lined with a columnar epithelium similar to that of the pyloric glands. The ducts are also lined with simple columnar epithelium. From the glands they pass up through the muscularis mucosae and lamina propria and empty usually into a crypt of Lieberkühn, although occasionally they empty on the surface between the villi. In the region of the juncture between the pylorus and duodenum there is a zone in which the glands of the two segments merge imperceptibly. Glands of similar structure lie partly in the lamina propria and partly in the submucosa, with strands of the muscularis mucosae present among the alveoli. The juncture of the stomach and duodenum thus is not sharply demarcated by the position of the glands. Brunner's glands, like the pyloric glands, secrete an alkaline mucoid substance.

Muscularis. The *muscular coat* (Figs. 261, 270) consists of two well defined layers of smooth muscle, an inner circular and an outer longitudinal. Connective tissue septa divide the muscle cells into groups or bundles, while between the two layers of muscle is a connective tissue

septum which varies greatly in thickness at different places and contains a plexus of nerve fibers and parasympathetic ganglion cells known as the plexus of Auerbach.

Serosa. The *serous coat*, as in the stomach, consists of loose connective tissue covered by a layer of mesothelium.

THE LARGE INTESTINE

The large intestine is divided topographically and to some extent structurally into three main segments: the colon, rectum and anal canal. Throughout the length of the large intestine, the wall consists of the same four coats which have been described for the stomach and small intestine, viz., mucosa, submucosa, muscularis and serosa (or fibrosa).

Colon

There is an abrupt change at the ileocecal juncture from the small intestine to the *colon*. The greater diameter of the colon is perhaps the most striking difference grossly. Other differences of structure will be noted in the description of the various coats.

Mucosa. The *mucous membrane* of the colon has a comparatively smooth surface, for *there are no plicae or villi* as in the small intestine. Long straight tubular glands extend from the surface down through the entire thickness of the mucosa. These glands are *crypts of Lieberkühn*.

The surface *epithelium* is tall columnar. The absorbing cells have a striated border which is thinner than that on the cells of the small intestine. Goblet cells are interspersed among the columnar absorbing cells. As the epithelium continues down into the tubular glands the columnar cells become lower, and goblet cells become exceedingly numerous. In fact, the walls of the glands frequently appear to be composed almost entirely of goblet cells. The cells at the base of the tubes are believed to be the more undifferentiated ones, which, by division, push new cells toward the surface to replace those which are being lost. Paneth and argentaffine cells are very rarely found.

The mucous membrane of the colon has two chief functions which are attributed to the columnar absorbing and goblet cells respectively: namely, the absorption of water and the copious

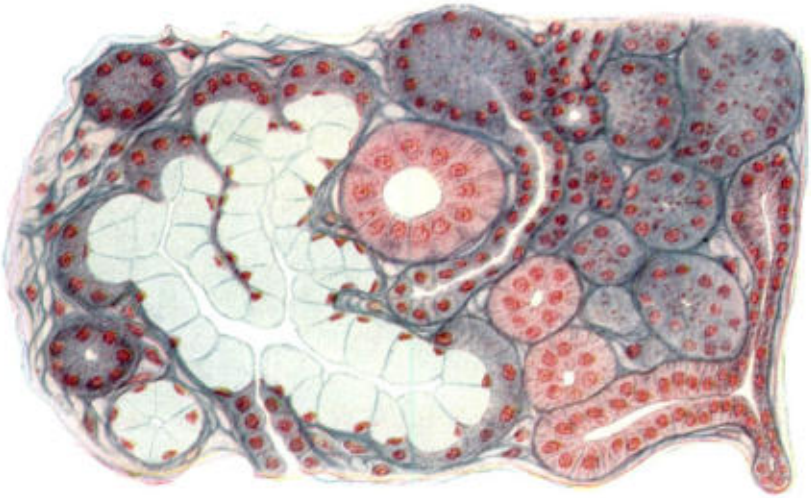
FIG. 271. Camera lucida drawings of human tissues (except epithelium of interlobar duct, which is from the parotid gland of a rhesus monkey). Stains: hematoxylin, Altmann's acid fuchsin and aniline blue.

Submaxillary gland. At the left, three mucous alveoli (light blue), all capped with serous demilunes, empty into the intercalated duct below. At lower left are cross sections through serous and mucous alveoli respectively. At lower right, two intercalated ducts join a secretory (salivary) duct, whose cells have a reddish-stained (fuchsin) granular cytoplasm with blue (aniline blue) basal striations. An intercalated duct from a serous alveolus curves around the right side of the large central salivary duct.

Epithelium of a parotid interlobar duct. Note that the blue goblet cell at the right, although greatly distorted, reaches the surface. The one to the left does so also, but it is cut off in this section. The large surface epithelial cell (second from left) stretches around the goblet cell to reach the basement membrane at separate places. Adjacent sections show that the four cells to upper right likewise reach the basement membrane. Thus the epithelium, stratified in appearance, is actually pseudostratified.

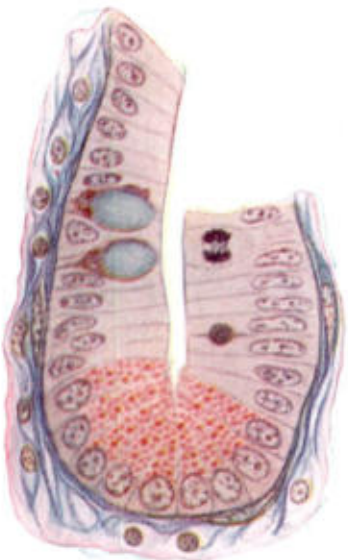
Crypt of Lieberkühn. The basal cells, full of large, red secretory granules, are Paneth cells. Above, at left, are two blue goblet cells. One of the columnar epithelial cells on the right is dividing by mitosis. The dark round nucleus of a lymphocyte, migrating between two columnar cells, is also shown.

Neck of gastric gland. This cross section shows two large red parietal (HCl) cells with their clear intracellular canals. The light blue cells with indented basal nuclei are mucous neck cells. The darker blue cells are chief cells.



Submaxillary gland

Epithelium of a parotid interlobar duct



Crypt of Lieberkühn

Neck of a gastric gland

FIGURE 271

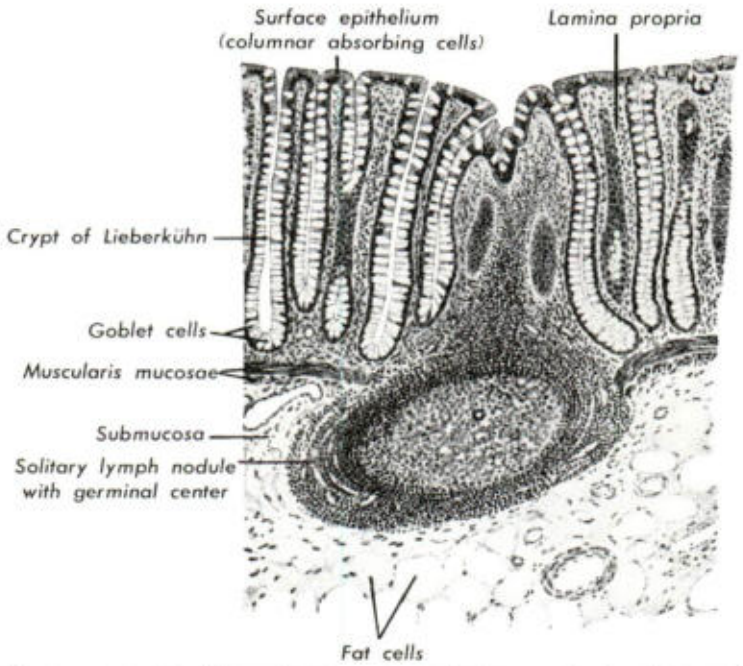


FIG. 272. Transverse section through mucosa and submucosa of colon of man. × 70. (After Braus.)

production of mucus. This mucous secretion lubricates the surface of the colon and facilitates the forwarding of the gradually dehydrated feces.

The connective tissue of the *lamina propria* extends up between the glands, but is reduced to a minimum because the tubules are close together. Solitary lymph nodules are present in the mucosa and push through the muscularis mucosae into the submucosa (Fig. 272).

The *muscularis mucosae* consists of an inner circular and an outer longitudinal layer of smooth muscle.

Submucosa. The *submucosa* is composed of loosely arranged connective tissue. It contains large blood vessels and the nerve plexus of Meissner. Solitary lymph follicles, while properly considered as structures of the lamina propria from which they originate, lie mainly in the submucosa.

Muscularis. The *muscularis externa* in the colon shows some variation from its usual structure. The inner circular layer is complete and prominent, but the longitudinal muscles become arranged into three strong, flat, equidistant, longitudinal bands, the *lineae coli* (taenia coli). Between these thick strands the longitudinal muscle coat becomes thinned but is rarely entirely absent. Nerve cell and fiber components of the plexus of Auerbach lie as usual in the connective tissue just external to the circular muscle layer.

Serosa. The *serous coat* is composed of a thin connective tissue layer covered by mesothelium. In certain regions, the mesothelial covering is absent. Here the connective tissue *fibrosa* binds the colon firmly to adjacent structures.

Vermiform Appendix

The vermiform appendix is a diverticulum from the cecum. Its walls are continuous with those of the latter, and

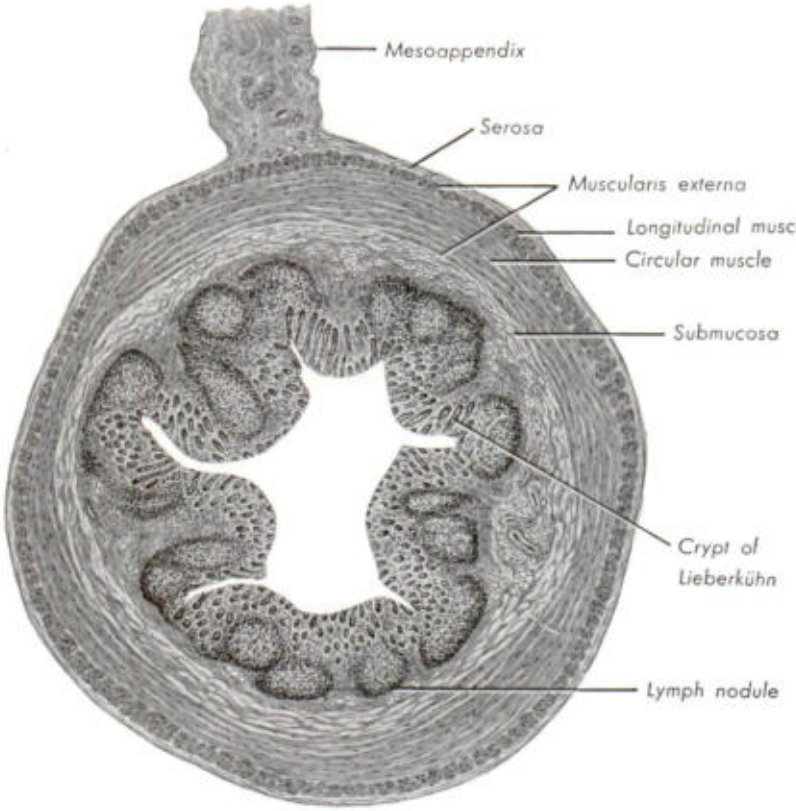


FIG. 273. Transverse section of human vermiform appendix. × 14.

closely resemble them in general structure. There are the same four coats: mucous, submucous, muscular and serous.

The *mucous membrane* has its usual characteristic structures: the epithelium, glands, lamina propria, and the muscularis mucosae. The epithelium is simple columnar with a striated free border. The surface epithelium continues into the deep tubular glands, where occasional goblet cells are found. These tubular glands are typical crypts of Lieberkühn. In the embryo the mucosal surface is lined with villi, but these disappear early in life. The lumen is often thrown into deep pocketed folds, and in many adults is nearly or completely obliterated.

The most characteristic histological feature of the appendix is the lymphoid tissue. Not only is the lamina propria infiltrated with lymphocytes, but it is often conspicuously occupied by a complete ring of solitary lymphoid nodules. These closely resemble the follicles which surround the crypts of the palatine tonsils. The follicles do not remain confined to the mucosa, but push through the muscularis mucosae and invade the submucosa. In fact, the large lymphoid development frequently makes it difficult to follow the muscularis mucosae and to separate the mucosa from the submucosa (Fig. 273). The *submucosa* contains numerous fat cells. It should be mentioned that in some instances, more commonly after middle age, the mucosa

and portions of the submucosa are largely replaced by fibrous connective tissue.

The *muscularis externa* varies greatly, both as to thickness and as to the amount of admixture of fibrous tissue. The inner circular layer is usually thick and well developed. The outer longitudinal layer differs from that of the large intestine in having no arrangement into lineae, the muscle tissue forming a continuous layer. The muscular layers are covered as usual by the *serosa*.

Rectum

The *rectum* is usually divided into two parts. The *upper part* (the rectum proper), measuring 5 to 7 inches in length, is structurally similar to the colon. The crypts of Lieberkühn are longer than in the colon, however, and are lined almost entirely by goblet cells. The longitudinal layer of the muscularis externa is thicker on the front and back than it is on the sides. This segment of the gut has no mesentery and the serous coat is incomplete.

The *lower part* (*anal canal*) is 1 to 1½ inches in length. It pierces the pelvic floor and is closed except during defecation. Its mucous membrane has a number of permanent longitudinal folds, the *rectal columns* (*anal columns*, *columns of Morgagni*) which terminate distally about half an inch from the anal orifice. These contain strands of smooth muscle and usually an artery and a vein. The bases (distal ends) of these columns are connected by transverse folds of the mucosa —the *anal valves* (Fig. 274).

Above the anal valves the mucosa is lined by simple columnar epithelium like that of the rectum proper, composed of columnar absorbing cells, interspersed among which are many goblet cells. Crypts of Lieberkühn are present.

At the level of the anal valves, the epithelium becomes a stratified squamous type, the surface cells of which are not

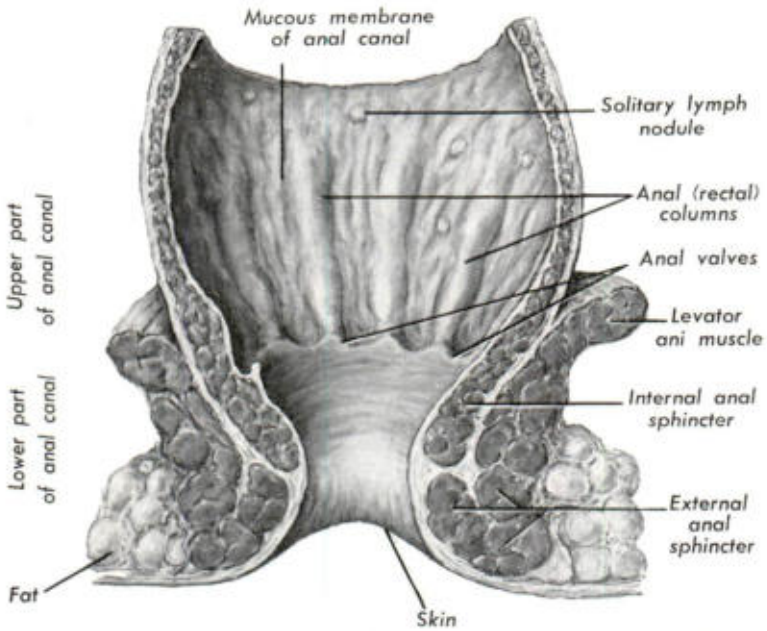


FIG. 274. Mucous surface of anal canal. Redrawn. (Courtesy of Wyeth, Philadelphia, Pa.)

cornified. No crypts of Lieberkühn are present below the juncture. The noncornified stratified squamous epithelium extends nearly to the anal orifice where it changes to epidermis. Hairs, sebaceous glands and sweat glands appear at the anal orifice.

The sweat glands are of two types. One type has the characteristic structure of the general body sweat glands (Figs. 208, 209, pp. 282 and 283). The other type (*circumanal glands*) are very large and are structurally similar to the axillary sweat glands. Their secretory cells contain granules, and inside the basement membrane are prominent myoepithelial cells (Figs. 210, 211).

At about the level of the anal valves, the muscularis mucosae becomes fragmented and soon disappears altogether.

The submucous coat of the anal canal has a rich plexus of blood vessels. The veins are often tortuous. Their size, arrangement, and the absence of valves are conducive to the formation of hemorrhoids.

The circular layer of smooth muscle of the anal canal is thick and forms the *internal anal sphincter*. The longitudinal layer of smooth muscle continues over the sphincter and attaches to connective tissue. The *external anal sphincter* is formed of skeletal muscle. It lies just inside the levator ani muscles which also act as a sphincter (Fig. 274).

THE PERITONEUM

The *peritoneum* (see p. 57) is a serous membrane which lines the walls of the abdomen (parietal peritoneum) and is reflected over the contained viscera (visceral peritoneum). It consists of two layers, a connective tissue stroma and mesothelium. The stroma consists of loosely arranged connective tissue bundles, which interlace in a plane

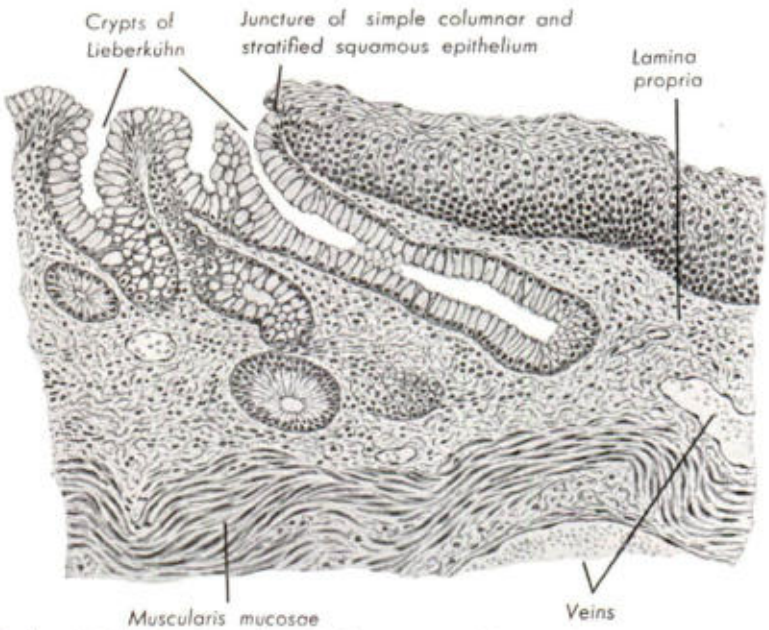


FIG. 275. Longitudinal section through the mucosa of the anal canal at the level of the anal valves. × 100.

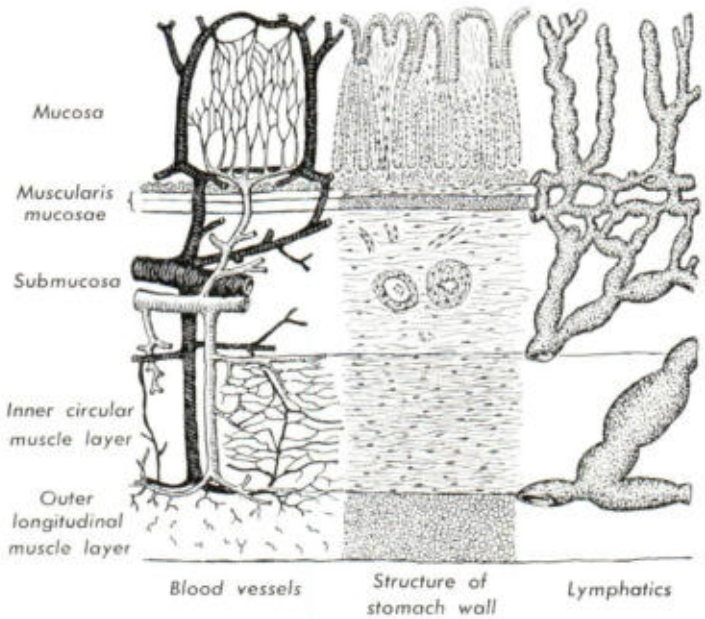


FIG. 276. Three sections of stomach wall placed side by side to show relationship of blood vessels and lymphatics to the different layers. (Redrawn after Mall.)

parallel to the surface. There are numerous elastic fibers, especially in the deeper layer of the parietal peritoneum. There are comparatively few connective tissue cells. The mesothelium consists of a single layer of flat polygonal cells with bulging nuclei. The cells have irregular wavy outlines, which are easily demonstrated with silver preparations. The shapes of the cells vary considerably according to the direction in which the tissues are stretched.

Over some parts, e.g., the liver and intestine, the peritoneum or serosa is thin and very closely attached. In places where the peritoneum is freely movable, a considerable amount of loose connective tissue, rich in elastic fibers and containing varying numbers of fat cells, connects the peritoneum with the underlying tissue. This is known as the "subserous tissue." The peritoneum is well supplied with blood vessels and lymphatics. The former give rise to a rich capillary network.

The *mesentery* is a sheet of loosely arranged connective tissue covered with peritoneum. It is reflected from the postabdominal wall to the viscera, and serves to carry to these organs their blood vessels, lymphatics, and nerves. Part of the mesentery is comparatively short, and certain organs are, therefore, quite firmly fixed to the abdominal wall. In other regions it is longer, allowing greater freedom of movement. The mesentery is richly supplied with lymph nodes and there is usually a considerable amount of fat. From the mesentery, the peritoneum continues onto and envelops the viscera.

The *omenta* and the visceral ligaments are similar in general structure to the mesentery. The great omentum usually contains large deposits of fat. Its connective tissue bundles are arranged in networks, the strands and meshes of which vary greatly in size and shape. The strands are covered by mesothelium.

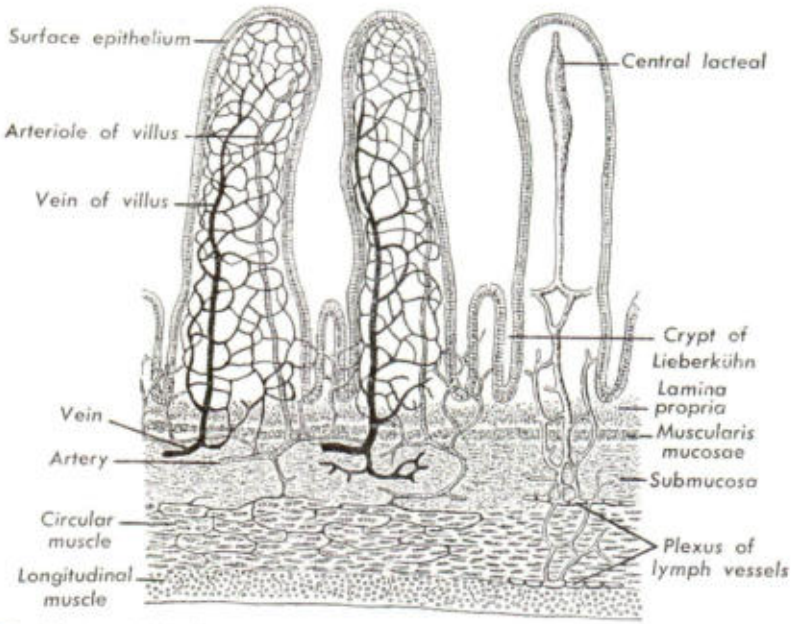


FIG. 277. Scheme of blood vessels and lymphatics of human small intestine. (Redrawn from Böhm and von Davidoff, after Mall.)

BLOOD VESSELS OF THE STOMACH AND INTESTINES

The arteries reach the gastro-intestinal canal through the mesentery, give off small branches to the serosa, and pass through the muscular coats to the submucosa, where they form an extensive plexus of large vessels (Heller's plexus) (Fig. 276). Within the muscular coats the main arteries give off small branches to the muscle tissue. From the plexus of the submucosa two main sets of vessels arise, one passing outward to form the main supply of the muscular coats, the other inward to supply the mucous membrane. Of the former, the larger vessels pass directly to the intermuscular septum, where they form a plexus from which branches are given off to the two muscular tunics. Of the branches of the submucous plexus which pass to the mucous membrane, the shorter supply the muscularis mucosae, while the longer branches[3] pierce the latter to form a capillary plexus among the glands of the stroma. These capillaries are most numerous around the bodies and necks of the glands. They pass over into a dense network of capillaries just beneath the surface epithelium. From the capillaries small veins take origin which pierce the muscularis mucosae and form a close-meshed venous plexus in the submucosa. These in turn give rise to larger veins, which accompany the arteries into the mesentery.

[3] Stöhr describes these small arterial branches to the stroma of the gastric mucosa as terminal or end-arteries each supplying a segment of mucosa from 1 to 2.5 mm. in diameter. As the capillaries of one end-artery do not have extensive anastomoses with those of other end-arteries, any obstruction or destruction of one of these arteries results in death of the dependent area of mucosa.

In the small intestine the distribution of the blood vessels is modified by the presence of villi (Fig. 277). Each villus receives one small artery, or, in the case of the larger villi, two or three small arteries. The artery passes through the long axis of the villus close under the epithelium to its summit, giving off a network of fine capillaries, which for the most part lie just beneath the epithelium. From these, one or two small veins arise which usually lie on the opposite side of the villus from the artery.

LYMPHATICS OF THE STOMACH AND INTESTINES

Small lymph or chyle capillaries begin as blind canals in the stroma of the mucous membrane among the tubular glands (Fig. 276). In the small intestine a lymph capillary (lacteal) occupies the center of the long axis of each villus, ending in a blind extremity beneath the epithelium of its summit (Fig. 277). The walls of the lymph capillaries are made up of a single layer of large endothelial cells. Although the lymph capillaries are considerably larger than the blood capillaries, they are usually collapsed and inconspicuous. These vessels unite to form a narrow-meshed plexus of lymph capillaries in the deeper part of the stroma, lying parallel to the muscularis mucosae. Vessels from this plexus pass through the muscularis mucosae and form a wider meshed plexus of larger lymph vessels in the submucosa. In this region they often expand into sinuses surrounding the lymphoid follicles. A third lymphatic plexus lies in the connective tissue which separates the two layers of muscle. From the plexus in the submucosa, branches pass through the inner muscular layer, receive vessels from the intermuscular plexus, and then pierce the outer muscular layer to pass into the mesentery in company with the arteries and veins. The larger lymph vessels are provided with definite valves, and their walls are also supported by a thin tunic of smooth muscle cells. In their course through the mesenteries they are associated with numerous mesenteric lymph nodes. These relations are well demonstrated in the cat by the injection of 1 per cent Berlin blue into the intestinal wall.

Although the primary function of the lymphatic system is the return of tissue fluids to the blood stream, the vessels of the small intestine form channels through which absorbed fats are drained. During digestion, the lymph fluid, carrying a rich emulsion of fat (chyle), gives to the vessels a white appearance, and renders them clearly visible.

NERVES OF THE STOMACH AND INTESTINES

The nerves which supply the stomach and intestines are mainly nonmedullated sympathetic fibers, and the medullated preganglionic fibers of the parasympathetic. They reach the intestinal walls through the mesentery. In the connective tissue between the two layers of muscle, these fibers are associated with groups of parasympathetic ganglion cells to form the *plexus myentericus* or *plexus of Auerbach*. The processes of the ganglion cells interlace, forming a large part of the plexus. The axons are grouped together in small bundles of nonmedullated fibers, which pass, together with the sympathetic fibers, into the muscular coats, where they form intricate plexuses, from which are given off club-shaped terminals to the smooth muscle cells. From Auerbach's plexus fibers pass to the submucosa, where they form a similar but finer-meshed and more delicate plexus, also associated with other groups of parasympathetic ganglion cells, the *plexus of Meissner*. Both fibers and cells are smaller than those of Auerbach's plexus. From Meiss-

ner's plexus delicate fibrils pass to their terminations in submucosa, muscularis mucosae, and mucous membrane.

THE SALIVARY GLANDS

The smaller tubular and tubulo-alveolar glands which form a part of the mucous membrane and submucosa of the alimentary tract have been described. There remain to be considered certain larger compound glandular structures, the development of which is similar to that of the smaller tubules but which come to lie wholly outside the alimentary tract and connected with it by their main excretory ducts. Functionally, they are an important part of the digestive system.

These structures are: (1) the *salivary glands*, (2) the *pancreas* and (3) the *liver*.

The salivary glands include a number of glandular structures which secrete a liquid, the *saliva*. The smaller of these glands are situated in the oral mucous membrane, which their secretions serve constantly to lubricate and moisten. The larger of the glands are some distance removed from the oral cavity, to which their products are conveyed through excretory ducts. These glands, which are paired structures and may be spoken of as the salivary glands proper, are the *parotid*, the *submaxillary* (or *mandibular*) and the *sublingual glands*. Their secretions contain enzymes which are important in preparing the food for the digestive processes which ensue in the stomach and intestines.

In the following account, only the salivary glands proper—the parotid, submaxillary, and sublingual—will be considered. These three are all compound tubulo-alveolar glands. In man, the parotid has only serous alveoli; the submaxillary and sublingual have both serous and mucous alveoli. Hence the latter are known as mixed glands, in distinction to the purely serous glands (parotid, von Ebner's glands) and the purely mucous (palatine).

Structure of the Salivary Glands. Each gland consists of gland tissue proper (*parenchyma*) and a supporting connective tissue framework (*interstitial tissue, stroma*). In the parotid and submaxillary, there is a definite connective tissue capsule which encloses the gland and blends externally with the areolar tissue of surrounding structures. The sublingual has no distinct capsule. Connective tissue septa divide each gland into *lobes* and *lobules*. In these septa are found the larger ducts, the blood vessels and occasional ganglion cells. For a description of the development of the duct system, see page 307.

The intralobular ducts show considerable variation in structure. There may be a few large, nonsecretory intralobular ducts lined by simple columnar epithelium with basally situated oval nuclei. Some segments of the intralobular ducts are lined with cells of a secretory nature and probably contribute to the secretion of the gland. They are known as *salivary ducts* (secretory or striated ducts). In the parotid and submaxillary glands these ducts continue as tubules which have a narrow lumen and are lined with low or flat epithelium. Since they lie between the salivary ducts and the terminal alveoli, they are known as *intercalated ducts* (intermediate tubules, necks, isthmuses). This is the only type of intralobular duct present in the pancreas.

The Serous Alveoli.[4] The serous alveoli are lined with pyramidal epithelial cells, resting upon a basement membrane and surrounding a narrow lumen. The

[4] The term "serous" is here used to refer to alveoli which secrete a watery albuminous fluid, without regard to whether or not the secretion is of an enzymatic nature. The term "serozymogenic" is usually employed in referring to cells, the serous secretions of which are known to contain an enzyme.

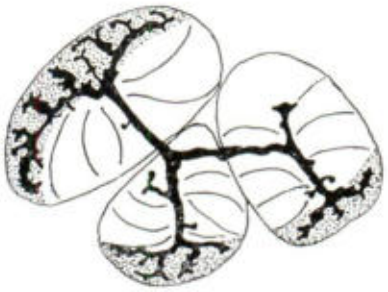


FIG. 278. Three mixed alveoli from the submaxillary gland of the dog, showing intercellular secretory canaliculi. Serous portions stippled, secretory canaliculi black. (Redrawn from Zimmerman, after G. Retzius.)

FIG. 279. Branching basal cells (basket cells) with dark-staining fibrils on a serous alveolus of the human submaxillary gland. (Redrawn after Zimmerman.)

cell boundaries are apt to be indistinct. The appearance of these cells varies according to their particular phase of activity (see Fig. 226). In a "resting" condition, the cytoplasm is filled with a number of small, highly refractile granules, which may be demonstrated clearly after formol fixation. These granules occupy the distal portion of the cell, toward the lumen, and, as they increase in number, the nucleus is pushed toward the base of the cell; the cell becomes slightly swollen and the lumen of the alveolus becomes decreased in diameter. Following active secretion, there is a diminution in the number of granules, so that they occupy only the more apical portion of the cell. These granules are the *zymogen granules* and must be looked upon as the intracellular representatives of the enzymes secreted by the cell.

As described previously (p. 300), these serous cells contain an abundance of *basophilic* or *chromophilic material* (RNA, ergastoplasm, chromidial substance, basal filaments, prozymogen), which is concentrated in the basal portions of the cells (Fig. 225). Its filamentous or striated appearance as seen with the light microscope can be correlated with the parallel arrangement of the rod-like mitochondria in this portion of the cell, and with the lamellar configuration of the elements of the endoplasmic reticulum as seen with the electron microscope (Fig. 288).

The mitochondria, Golgi apparatus and nucleus have all been described as being related to the process of elaboration of the secretory granules. The chromophilic substance (RNA) is believed to be essential for the synthesis of cellular proteins (e.g., zymogen granules in serozymogenic cells). The appearance of zymogen granules of various sizes within the vesicles of the Golgi complex and of the endoplasmic reticulum (Fig. 224) suggests either or both of these cytoplasmic components as the site of formation. It should be emphasized, however, that other cellular structures may also be involved either directly or indirectly in the synthesis of secretory granules.

By employing the technique of Golgi, fine *intercellular secretory canaliculi* may be seen in the serous alveoli (Fig. 278). These canaliculi open into the lumen and extend between the cells, perhaps even penetrating the cells themselves with delicate canals (*intracellular secretory canaliculi*). They are characteristic of serous alveoli.

Although not apparent in routine histological preparations, peculiar stellate-shaped cells may be demonstrated by special techniques in the region between the secreting cells and the basement membrane. They lie in close contact with the secreting cells and their processes form a sort of basket-work around the alveolus. These are the *basal* or *basket cells* (Fig. 279). They have been variously regarded as supportive cells and as "myo-epithelial" cells, which by their contraction assist in the discharge of the secretion products into the excretory ducts. They can be demonstrated only by special techniques.

The Mucous Alveoli. In the ordinary hematoxylin-eosin preparations, the purely mucous alveoli present a light bluish-purple appearance in contrast to the deep reddish-purple of the serous alveoli (see Fig. 280). Usually the lumen is fairly large and may have in it masses of mucin. The mucous cells rest on a basement membrane and their boundaries are usually quite distinct. In the resting condition, the nucleus stains deeply and appears flattened against the base of the cell, with a slight amount of cytoplasm around it. The remainder of the cell is occupied by a faint basophilic network, the trabeculae of which represent the cytoplasm and probably some precipitated material of the secretion granules. In the living condition, the mucigen droplets occupy the spaces of the network. Mitochondria may be demonstrated in the cytoplasm, but the basophilic material, so abundant in the serous cells, is present in such minute amounts as to be revealed only by microchemical methods or by electron microscopy. As the cell discharges its secretion, the mucigen droplets decrease in number, the nucleus becomes spherical or oval and its chromatin network becomes distinct. Intercellular secretory canaliculi are not seen in the mucous alveoli.

In the mixed glands, several types of terminal alveoli may be seen. Some alveoli may be composed entirely of serous cells; others, entirely of mucous cells. An alveolus may contain both kinds of cells, in which case the serous cells are more likely to occupy the blind end of the alveolus, with the mucous cells nearer the exit (see Fig. 280). In many instances, at the base of the mucous alveolus or along its sides, there occur crescent-shaped groups of serous cells, the *demilunes of Heidenhain* or *crescents of Gianuzzi* (Fig. 280). These may have direct access to the lumen of the alveolus, but more often communicate with it by means of intercellular secretory canaliculi which pass between the mucous cells and branch among the serous cells of the demilune (Fig. 278).

Consideration will now be given to the more important characteristics of the particular glands.

Parotid Gland. The *parotid gland* is the largest of the salivary glands and in man, dog, cat and rabbit is entirely *serous*. Its duct system is complex. The main excretory duct (Stenson's) opens into the oral cavity opposite the second upper molar tooth. It is lined with pseudostratified columnar epithelium. The main duct divides into interlobar ducts, which in turn divide to form interlobular ducts, the various branches following the connective tissue septa. Except in the larger of these ducts (Fig. 271), the epithelium becomes reduced to a simple columnar epithelium. From the interlobular ducts, branches are given off which penetrate the lobules.

Most of the large intralobular ducts are of the striated variety (*salivary ducts*). They are lined by a single layer of columnar cells in which the nucleus is spheroidal and centrally located. The cytoplasm is finely granular and acidophilic. In the basal portion of the cell it presents a characteristic striated appearance (Figs.

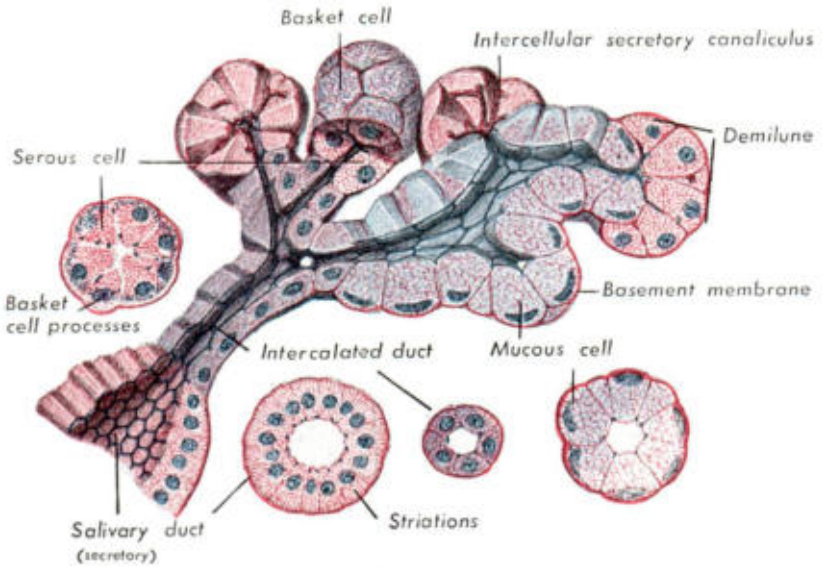


FIG. 280. Reconstruction of terminal ramification of human submaxillary gland, showing salivary and intercalated ducts and several alveoli. (From Braus, after a reconstruction by A. Vierling.)

280, 282). The rod-like mitochondria in this part of the cell are oriented perpendicular to the base of the cell. Also, electron micrographs show deep infoldings of the basal plasma membrane up into the cytoplasm. Both of these features probably contribute to the striated appearance of the cytoplasm of this region. These cells are believed to contribute water and certain salts to the secretion

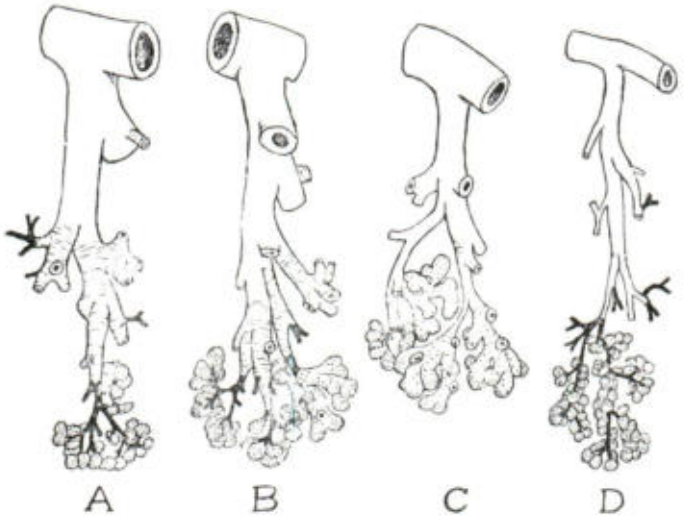


FIG. 281. Schematic drawing to illustrate the structure of the salivary glands and the pancreas. *A*, Parotid; *B*, submaxillary; *C*, sublingual; *D*, pancreas. Excretory ducts, white; salivary ducts, cross-striped; intercalated ducts, black; mucous alveoli, coarse stipple; serous alveoli, fine stipple. (Redrawn and slightly modified after Braus.)

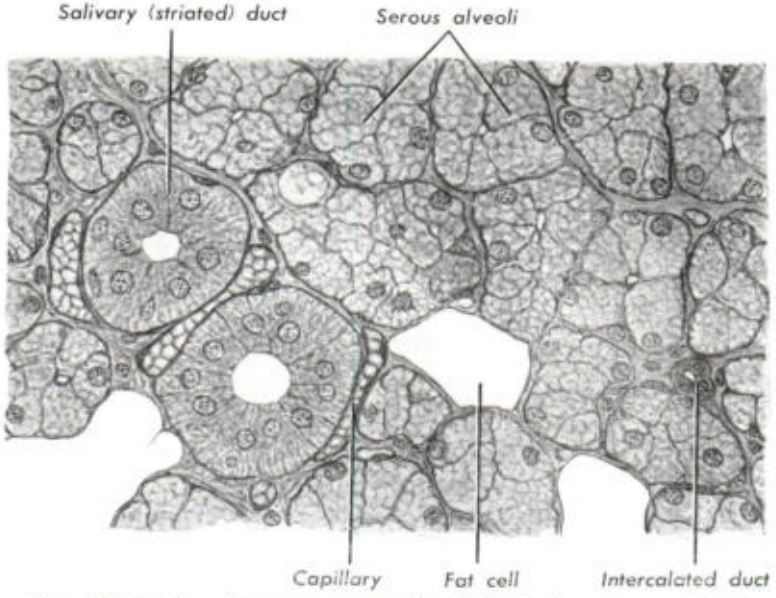


FIG. 282. Section of human parotid gland. Surgical specimen. × 465.

of the gland. The *intercalated ducts*, connecting the salivary ducts and the terminal alveoli, are of small diameter (Fig. 282), and are lined by low cuboidal epithelium. In the parotid they are fairly long elements.

The gland has a heavy connective tissue capsule, branches from which separate the lobes and lobules and envelop the individual alveoli. Fat cells are a frequent occurrence in the connective tissue of the parotid, and tend to increase in number with age.

Submaxillary Gland. The *submaxillary* (*mandibular*) *gland* in man and most other mammals is a *mixed gland*, but the proportion of serous and mucous alveoli is not the same in all species. In man, it is preponderantly serous (Fig. 283). The main duct (Wharton's) opens into the mouth beneath the tongue. It is lined by pseudostratified columnar epithelium and has, in addition, a richly cellular stroma and longitudinally disposed smooth muscle cells. The main duct branches in the manner described for the parotid, the pseudostratified epithelium continuing into the larger interlobular ducts. The intralobular ducts are of the same types as in the parotid gland. The salivary ducts are longer and more numerous than in the parotid; the intercalated ducts are short and narrow.

The serous alveoli greatly outnumber the mucous ones. The latter frequently are capped by serous demilunes. Some mucous alveoli have serous cells lining their terminal portions. The connective tissue stroma and capsule are well developed.

Sublingual Gland. The *sublingual gland* is also a *mixed gland* in man, dog, cat, rabbit and sheep. It is the smallest of the large salivary glands and in man

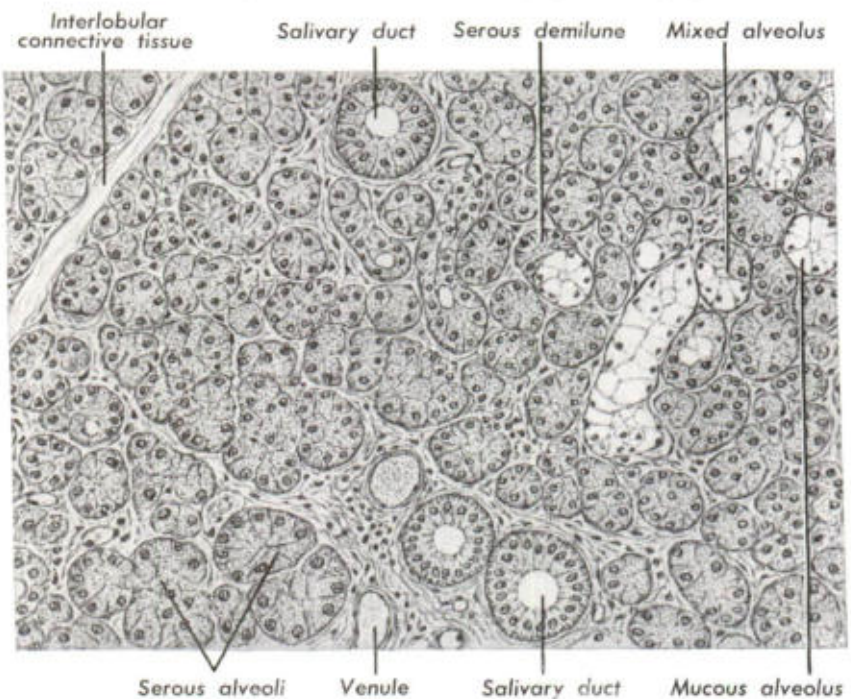


FIG. 283. Section of human submaxillary gland. × 216.

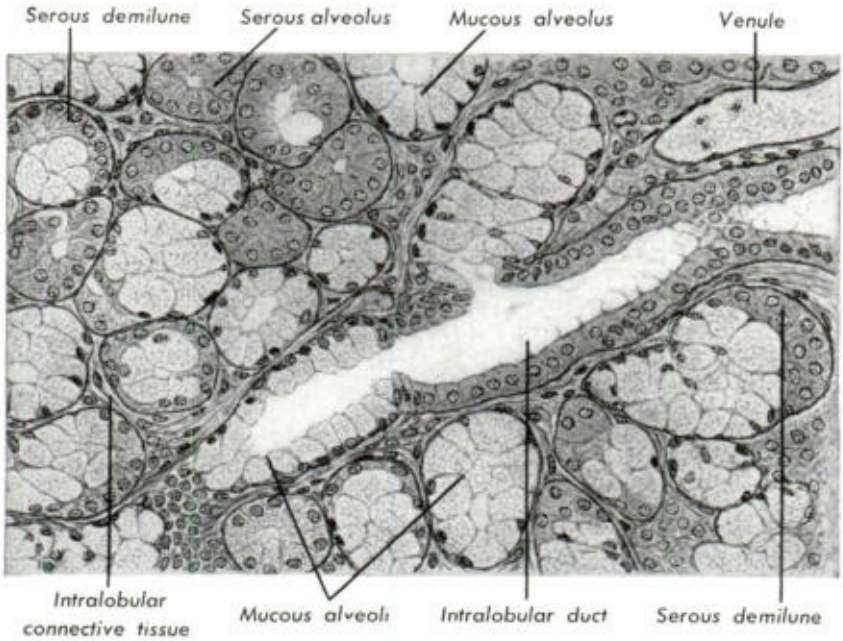


FIG. 284. Section of human sublingual gland. × 315.

is preponderantly mucous. A series of ducts open into the mouth at the side of the frenulum of the tongue, near the opening of Wharton's duct. The pseudostratified epithelium which lines the larger ducts is replaced by simple columnar in the smaller ones. There are relatively few intralobular ducts. Typical salivary ducts are rare. However, patches of striated cells may occur in the walls of intralobular ducts with an otherwise unmodified simple columnar epithelial lining. Intercalated ducts are likewise reduced in number so that most of the terminal alveoli, which are often quite elongated, either open directly into the larger intralobular ducts (Fig. 284) or into the interlobular ducts at the periphery of the lobule. Some authors consider that typical intercalated ducts are entirely lacking.

The terminal alveoli are mostly of the mucous type. Very few purely serous alveoli are seen. Serous cells occur in the form of demilunes around mucous alveoli. Such demilunes are numerous and large (Fig. 284).

The connective tissue septa are well developed in the sublingual but the gland does not have a distinct capsule, as do the parotid and submaxillary.

Blood Supply. The salivary glands have a relatively rich blood supply. The larger arteries run in the connective tissue septa with the ducts, giving off branches which accompany the divisions of the ducts to the lobules, where they break up into capillary networks surrounding the alveoli. These give rise to veins which follow the course of the arteries.

The lymphatics are relatively few in number. They begin as minute vessels in the smaller connective tissue septa and empty into lymph vessels which accompany the arteries.

Nerve Supply. The sensory innervation of the salivary glands is by the trigeminal nerve. The motor innervation is

derived both from the sympathetic and from the parasympathetic systems. Preganglionic sympathetic fibers run in the thoraco-cervical trunk to terminate in the superior cervical ganglion. Here they form synapses with postganglionic fibers which course in the walls of the branches of the carotid artery to the respective glands.

The parasympathetic supply to the parotid is by way of preganglionic fibers of the ninth cranial nerve to the otic ganglion, from which postganglionic fibers pass to the gland. The parasympathetic innervation of the submaxillary and sublingual glands is by way of preganglionic fibers in the chorda tympani nerve to the submaxillary ganglion, which usually lies within the glands. Short postganglionic fibers pass to the elements of the gland.

THE PANCREAS

The pancreas is a compound tubuloacinar gland, lying behind the stomach, and extending transversely from the spleen to the loop of the duodenum. The broader portion of it, the *head*, lies in the concavity of the latter organ. The head is joined to the *body* of the gland by a slightly constricted portion, the *neck*. The body tapers gradually into an extremity, the *tail*.

The pancreas is both a gland of *external secretion*, furnishing the pancreatic juice which is conveyed to the duodenum and which contains several digestive enzymes, and a gland of *internal secretion*, elaborating substances which, circulating in the blood, play an essential role in the regulation of the carbohydrate metabolism of the body. Serving the former function, it has a system of excretory ducts and terminal secreting acini, the arrangement of which somewhat resembles that of the parotid gland. For the performance of its endocrine function, there are highly vascular aggrega-

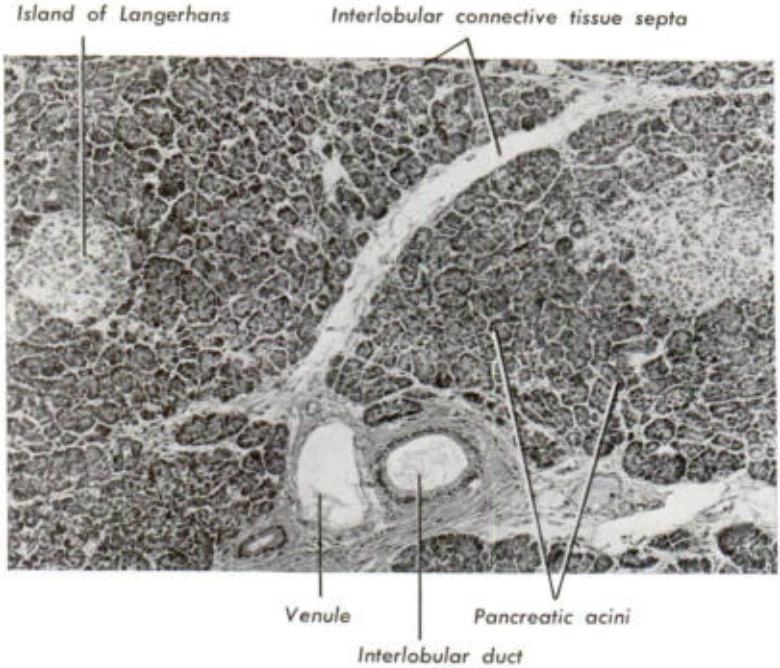


FIG. 285. Section of human pancreas. Surgical specimen. Photomicrograph. × 100.

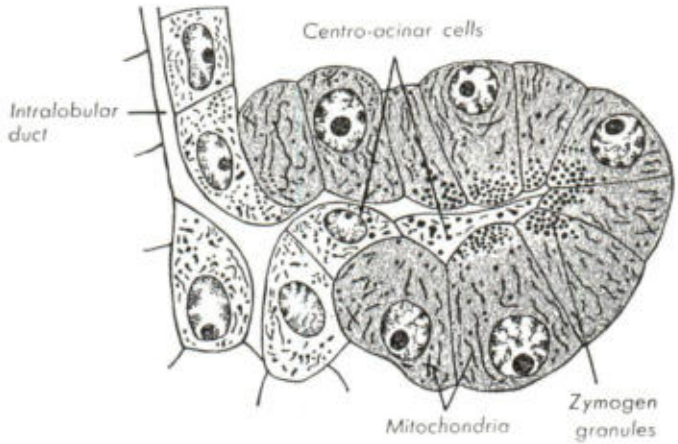


FIG. 286. Section of pancreatic acinus of the guinea pig, showing intercalated duct and centro-acinar cells. In the acinar cells may be seen zymogen granules and long mitochondrial filaments. (After Bensley.)

tions of secreting cells, the *islands of Langerhans*.

The pancreas has no distinct connective tissue capsule but is covered with a thin layer of areolar tissue from which septa pass into the gland, subdividing it into many small lobules. In some of the lower animals (as, for instance, the cat), these lobules are well defined, being completely separated from one another by connective tissue. A number of these *primary lobules* are grouped together and surrounded by connective tissue which is considerably broader and looser in structure than that separating the primary lobules. These constitute a *lobule group* or secondary lobule. The division into lobules and lobule groups is not distinct in the human pancreas, due to the incompleteness of the connective tissue septa, although it can usually be made out.

The main excretory duct of the pancreas, the *pancreatic duct* or *duct of Wirsung*, extends almost the entire length of the gland, giving off short lateral branches, one of which enters each lobule. These intralobular ducts are long and narrow, and they branch repeatedly before ending in the terminal secreting acini. All of the intralobular ducts in the pancreas have the structure of *intercalated ducts* (intermediate ducts); there are no striated ducts as are found in the salivary glands. The cells of the intercalated duct toward its termination become flattened and elongated in the direction of the long axis of the duct.

In addition to the main excretory duct, there is also a secondary excretory duct, the *accessory pancreatic duct* or *duct of Santorini*. It may have an independent opening into the duodenum; otherwise, it communicates with the duct of Wirsung.

The interlobular and main ducts are lined with a simple high columnar epithelium which rests upon a basement membrane. Outside of this is a connective tissue coat, the thickness of which is directly proportional to the size of the duct. Goblet cells are present in the lining epithelium; in the accompanying connective tissue of the main duct and its larger branches, there are small mucous glands. As the ducts decrease in size, the epithelium becomes lower. In the intercalated ducts, it is cuboidal.

The *acini* are all of the serous (serozymogenic) type. They are lined by an irregularly pyramidal epithelium resting on a basement membrane. Intercellular secretory canaliculi extend between the acinar cells. In each cell, there may be distinguished an inner zone, toward the lumen, and a more deeply staining basal zone, toward the basement membrane (Fig. 287). In the inner zone are numerous coarse *zymogen granules*, their number varying with the functional activity of the cell. During digestion, as the secretion is discharged, they diminish in number and may, after prolonged secretion, entirely disappear. In a resting condition (fasting), they again accumulate and may encroach upon the basal zone. Since the disappearance of the granules is coincident with the appearance of the fluid secretion of the gland, the zymogen granules are undoubtedly the intracellular antecedents of the enzymes.

The nucleus lies in the basal zone of the cell. It is spherical and characteristically contains one or more distinct nucleoli. Binucleate cells may occur but this condition is infrequently met with in man; in the white rat, it is said to occur in about half the acinar cells.

The basal zone also contains the *basophilic* or *chromophilic substance* (see p. 363) which, in routine preparations, usually appears as longitudinal striations or fine filaments (Fig. 287). Electron micrographs show the endoplasmic reticulum in this region to be highly developed and arranged in parallel or concentric lamellae (Fig. 288). The surfaces of its membranes are studded with RNA granules, which accounts for the basophilic staining. The striated appearance in the basal region of the cell is accentuated by the presence of numerous elongated mitochondria, which remain unstained except by special methods.

In sections of the pancreas, there are

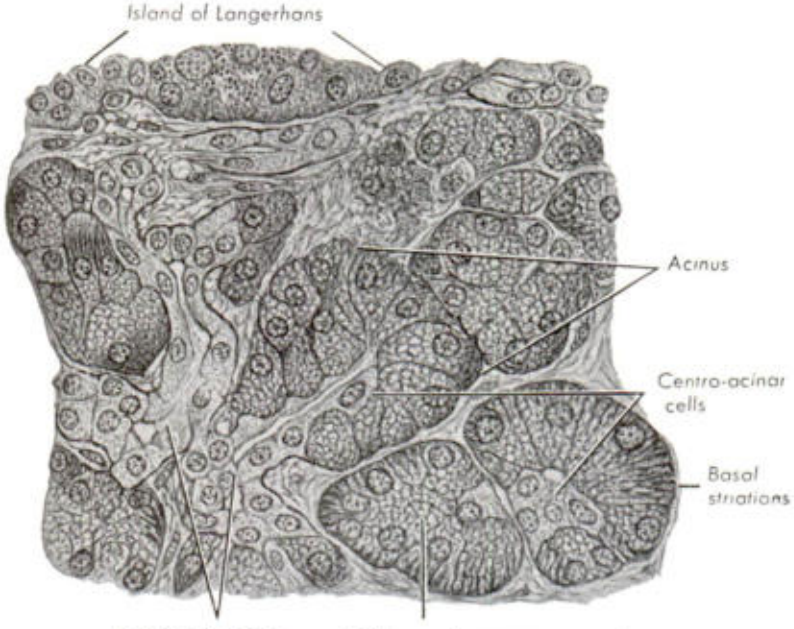


FIG. 287. Camera lucida drawing of a section of human pancreas removed surgically. Chrome hematoxylin and phloxine stain of 4 μ section. $\times$ 600.

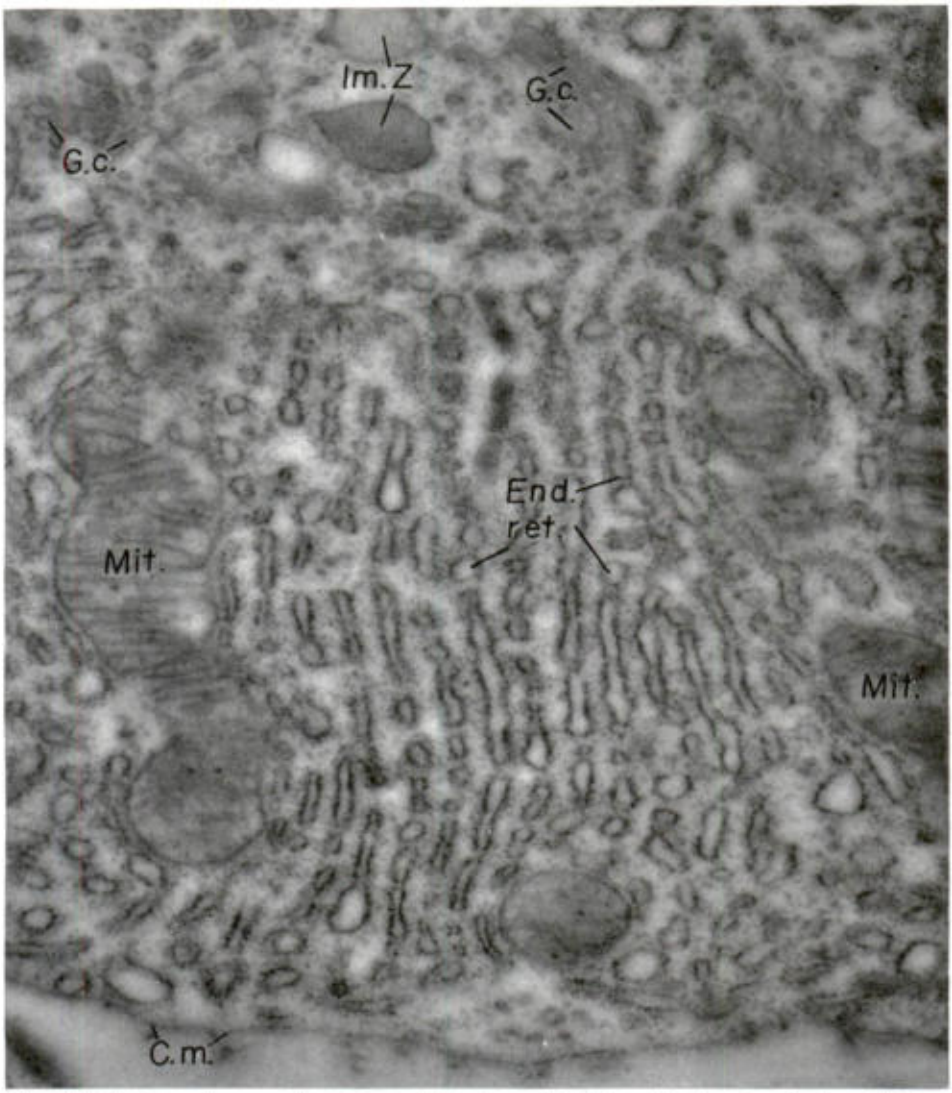


FIG. 288. Section of pancreatic acinar cell (guinea pig). The endoplasmic reticulum (*End. ret.*) in the basal portion of the cell is well developed. It consists of vesicles and cisternae the surfaces of which are studded with RNA granules. The orientation of both the elements of the endoplasmic reticulum and the mitochondria (*Mit.*) is largely perpendicular to the base of the cell. Toward the apical region can be seen parts of the Golgi complex (*G. c.*) and a few immature zymogen granules (*Im. Z.*). The nucleus was not included in this section. Basal cell membrane (*C. m.*) is at bottom of figure. Electron micrograph, × 26,000. (Courtesy of Dr. G. E. Palade.)

seen within the lumina of many of the acini one or more small epithelial cells, lying in contact with the apices of the secreting cells. These are the *centro-acinar cells* of Langerhans (Figs. 286, 287). They are a continuation of the epithelium of the intercalated duct, which instead of joining directly with the acinus, is carried over as a projection into the lumen of the latter. The centro-acinar

cell differs from the acinar cell in having a cytoplasm devoid of secretion granules or basophilic substance.

The Islands of Langerhans. As mentioned earlier in this discussion, the pancreas also has an *endocrine* function, which is carried on by cellular aggregations interspersed irregularly among the acini or along the ducts. These cell groups are the *islands of Langerhans;* their secretion is poured directly into the blood stream and they have no functional communication with the duct system of the gland.

In ordinary hematoxylin-eosin preparations, the islands appear as more or less spheroidal masses of pale staining cells, arranged in the form of irregular anastomosing cords (Fig. 289). Between the cords, closely applied to the epithelial cells, are numerous blood capillaries. Only a few fine connective tissue fibers are present within the islets. The islands may be more or less closely surrounded by pancreatic acini (Fig. 289), or they may lie in the interlobular connective tissue septa.

The size of the islands varies from those having only a few cells to islands which are macroscopically visible. The number of islands varies in different portions of the gland as well as in different individuals. They are more abundant in the tail of the pancreas than in the head.

In routine histological preparations all of the islet cells appear to be similar (Fig. 289); by special methods, however, three types of cells have been distinguished in the human pancreas, namely A or *alpha* cells, B or *beta* cells, and D cells. The A and B cells are much the

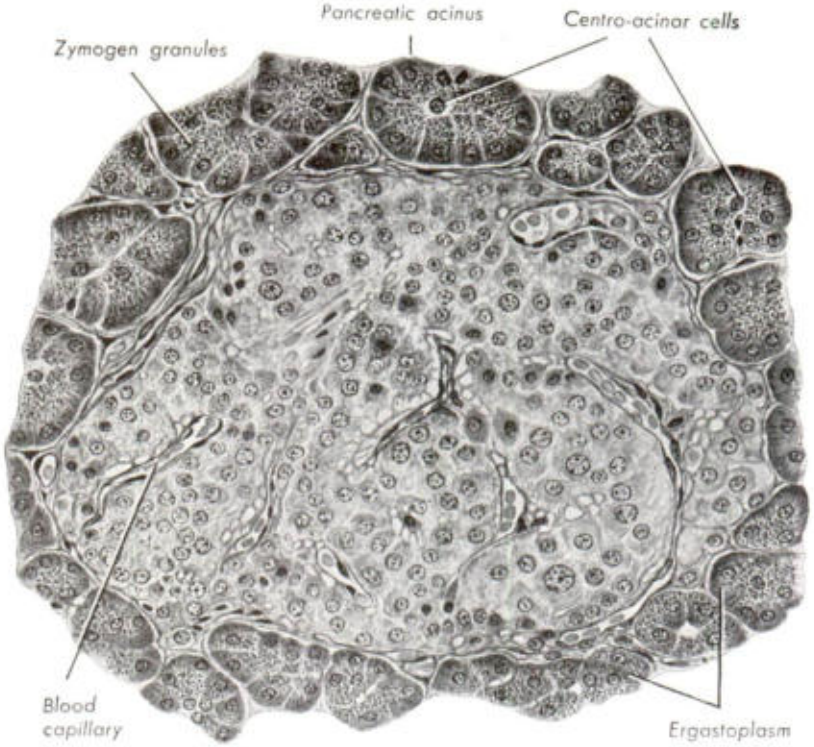


FIG. 289. A section through an island of Langerhans and surrounding acini. Human pancreas. Hematoxylin and eosin. × 450.

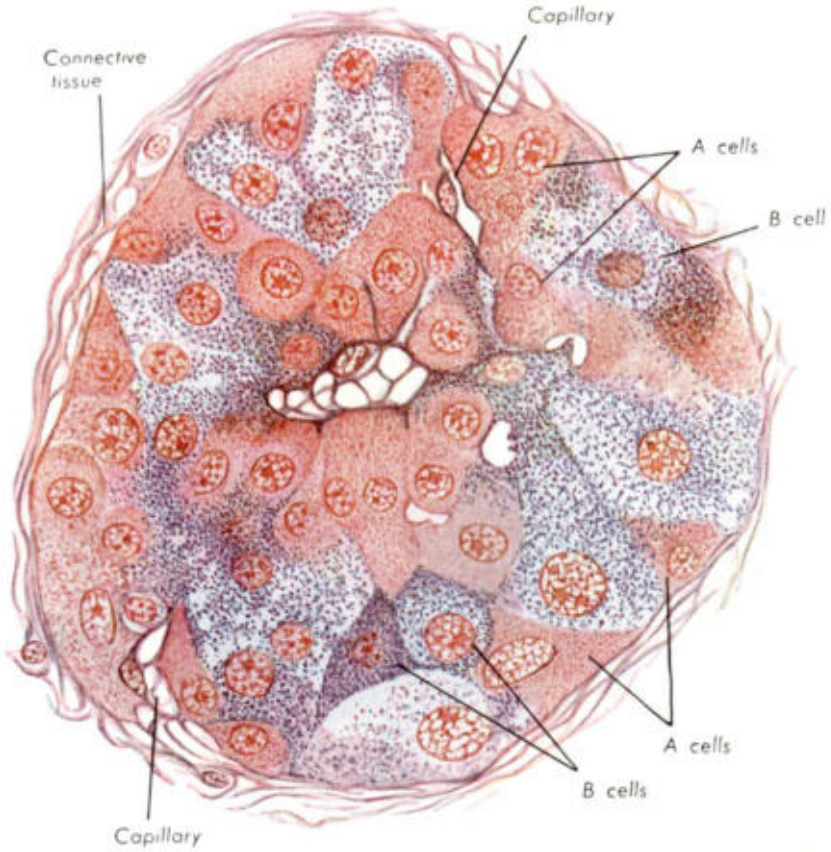


FIG. 290. A section of an island of Langerhans of human pancreas showing A and B cells. A 3 μ section of a surgical specimen. Chrome hematoxylin and phloxine stain (Gomori). $\times$ 1135.

most numerous, the D cells being relatively few in number.

Of the various staining techniques which may be used to differentiate the cell types of the islets (e.g., Masson's trichrome, chrome hematoxylin-phloxine, aldehyde fuchsin), the chrome hematoxylin and phloxine method is the one most commonly used. With this technique (Fig. 290), the A cells are seen to contain numerous fine, red-staining granules. The cell membranes are usually distinct. The B cells have coarse granules which frequently are rather sparse and are colored dark blue by the chrome hematoxylin. The membranes of the B cells are often indistinct; they may be indistinguishable in some cells.

Although the relative number of A and B cells varies in different islands, the B cells are generally more numerous. B cells may also occur outside the islets, either singly or as small groups, in association with fully developed ducts or with acini. Estimates of the percentages of the various cell types indicate that the B cells comprise 60 to 90 per cent of all the islet cells in the human pancreas (Gomori).

The D cells are differentiated by Masson's triple stain (Fig. 2, *B*), which also selectively colors the A cells. There are few D cells in the human, and their significance is unknown.

Another type of cell has also been described by Bensley in the pancreas of the guinea pig. Its cytoplasm contains no granules. It has been suggested that this type, the C cell, represents the progenitor of the A cells.

The question of the source of new islet cells in the adult, particularly whether acinar cells may be transformed into islet cells, has frequently been raised. In a study of the pancreas of the guinea pig, Bensley described a system of fine anastomosing tubules, which originate from the intralobular ducts and branch freely in the connective tissue surrounding these ducts. Smaller or larger islands of Langerhans are found attached to this system of tubules. Also attached to these tubules, but less frequently, are small pancreatic lobules, varying in size from a single acinus to a group of acini. Bensley regarded these branching tubules as tissue of a low order of differentiation, capable, under proper conditions, of producing by differentiation and mitotic division new islands of Langerhans or pancreatic acini. It must be remembered that this system of tubules does not comprise a duct system for the islands of Langerhans.

Secretions of the Pancreas. The *external secretion* of the pancreas is an alkaline liquid, the pancreatic juice, the important constituents of which are certain enzymes. Among these are *trypsin*, a powerful proteolytic enzyme which breaks down proteins into amino acids; *amylase*, which converts starches into maltose; and *lipase*, which splits fats into glycerin and fatty acids. It is interesting to note that although these several chemically dissimilar enzymes are elaborated by the pancreatic acini, the acinar cells are apparently cytologically similar.

The pancreas may be stimulated to activity either by nervous impulses from the vagus or by the action of a hormone, *secretin*, which is formed in the duodenal mucosa and carried to the pancreas in the blood stream. Secretin appears to be formed whenever acid substances, such as the acid contents of the stomach or acid bile come in contact with the duodenal mucosa.

The *internal secretion* of the pancreas includes two hormones. One of these, *insulin*, is known to play an important role in carbohydrate metabolism. Without this hormone, the cells of the body are unable to utilize the available glucose.

The clinical condition resulting from a deficiency of insulin, *diabetes mellitus*, is thus marked by hyperglycemia and glycosuria of variable severity. Although various theories have been proposed, the site (or sites) and mechanism of insulin action remain conjectural.

Insulin is produced in the islands of Langerhans, specifically by the B cells of the islets. Evidence for this is available from various sources, both clinical and experimental. Impaired sugar assimilation results from removal of the entire pancreas, but not from ligation of the pancreatic duct, which destroys the acinar tissue but leaves the islands intact. Furthermore, it has been amply demonstrated, as in the first successful extractions of insulin by Banting and Best, that insulin is present in such a duct-ligated pancreas. In man, functional tumors of the B cells produce hypoglycemia, the effect being comparable to that caused by the administration of insulin. Removal of the tumor restores the patient to a normal condition. Experimentally, diabetes can be produced in many animals by the selective destruction of the B cells by injection of the drug alloxan. Also, in certain species the repeated injection of crude extracts of the anterior pituitary gland results in B cell injury and diabetes.

The second hormone of the pancreas is *glucagon*, or the hyperglycemic-glycogenolytic factor. Experimentally, glucagon has an effect which is, in some respects, antagonistic to that of insulin. It causes an elevation of the blood sugar as a result of glycogenolysis in the liver.

Glucagon is presumed to be formed by the A cells of the pancreatic islets, although the evidence is not completely unequivocal. Like insulin, it is present in pancreatic tissue following duct ligation. Unlike insulin, it is present in the pancreas of a duct-ligated, alloxan-treated animal. It is said to be present as a contaminant in most commercial insulin preparations.

The physiological action of glucagon is not clearly understood.

Blood Supply. The pancreas receives its blood supply from the superior and inferior pancreatico-duodenal arteries and from pancreatic rami of the splenic artery. The vessels course in the interlobular connective tissue and give off branches which enter the lobules. These form capillary networks among the acini and in the islands. The islands have an extremely rich vascular network, a characteristic which they share with other endocrine tissues. The extensiveness of the capillary network within the islets is not usually appreciated in sections, but can be demonstrated in injected specimens. The blood leaves the pancreas by the pancreatico-duodenal veins to the superior mesenteric and portal veins and by several small pancreatic veins to the splenic vein.

Lymphatics. The lymph vessels lie mainly in the interlobular connective tissue. They drain chiefly into the celiac lymph nodes.

Nerve Supply. The nerves to the pancreas are from the splanchnic (sympathetic) and the vagus (parasympathetic). The former are nonmedullated. The latter are medullated preganglionic fibers; the cell bodies of their postganglionic fibers lie within the substance of the gland. The terminal fibers of the nerves are distributed about the secreting acini and on the walls of blood vessels. Pacinian corpuscles are occasionally found in the interlobular connective tissue of the gland.

THE LIVER

The liver is the largest gland of the body. It has an exocrine function, the secretion of bile, which is conveyed to

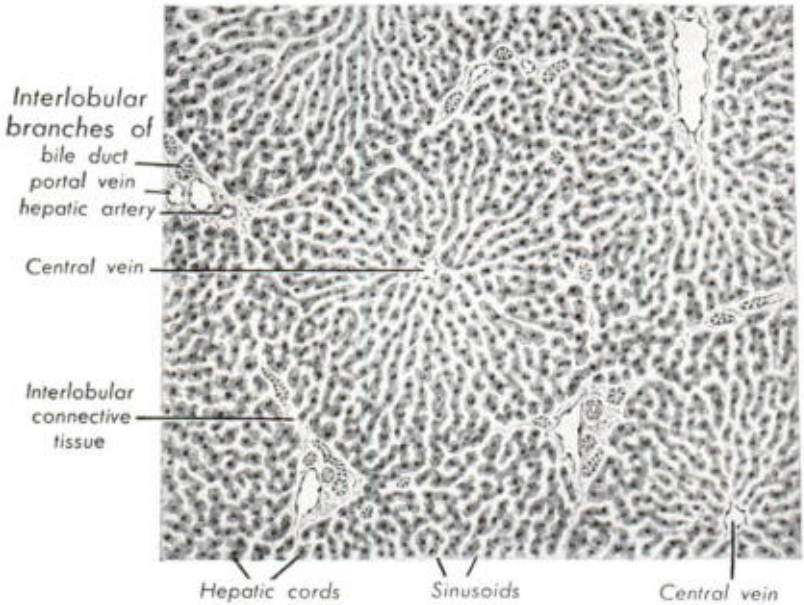


FIG. 291. Low power view of a portion of a section of human liver showing one complete lobule and portions of adjacent lobules. × 60.

the intestine by a system of ducts. It also performs the important function of storing carbohydrate foods and releasing them into the blood at such times as they are needed by the body—a function which was first described as one of internal secretion, although it is not now designated as such. Fats, proteins and vitamins are also stored in the liver cells. The liver is also an organ of excretion, essential in the removal of waste products from the blood. Despite the multiplicity and diversity of its functions, the liver has no groups of cells cytologically specialized for the performance of one function or the other, as is the case, for example, in the pancreas. It also presents several other structural features in which it differs from other glands.

The liver is situated in the upper and right part of the abdominal cavity, immediately below the diaphragm. Several fissures partially divide it into four *lobes*. It is incompletely invested by an outer *tunica serosa*, derived from the peritoneum, within which is a delicate connective tissue capsule, the *capsule of Glisson*. This capsule, which contains a fair abundance of elastic fibers, covers the entire surface of the organ. At the *porta hepatis* (transverse fissure, hilum), it surrounds the entering blood vessels and follows into the gland, forming a framework and dividing it into innumerable small *lobules*. In some animals, as, for example, the pig and camel, each lobule is completely invested by connective tissue. In man, the connective tissue is sparse and the lobular investment is incomplete. It is apparent mainly at points where three or more lobules meet (Fig. 291). The lobules are cylindrical or irregularly prismatic in shape and approximately 1 mm. in breadth and 2 mm. in length. Except just beneath the capsule, where they are frequently arranged with their apices directed toward

the surface, the lobules are irregularly arranged.

The *hepatic lobule*, which may be considered the anatomical unit of structure of the liver, has two main constituents: an epithelial parenchyma and a system of anastomosing blood channels. The parenchyma is made up of hepatic cells arranged in irregular, branching, interconnected plates. Since in sections the sheets of cells often give the appearance of cell cords (Fig. 291), they have frequently been called the *hepatic cords*. However, the groups are considerably more broad than they are thick. For this reason the terms *hepatic plates* or *hepatic laminae* are preferable. The plates tend to be arranged in a radiating manner around the central blood vessel of the lobule.

The hepatic plates or laminae form the secretory portions of the gland and are thus analogous to the secretory tubules of other glands. There are, however, no "terminal tubules," strictly speaking, in the liver, since the lumina and walls of neighboring units ramify and anastomose freely with each other. The hepatic plates are arranged in a definite manner relative to the blood channels, forming partitions between them. The blood vessels may therefore conveniently be considered first.

Blood Supply. The blood supply of the liver is peculiar in that in addition to the ordinary arterial supply and venous return which all organs possess, the liver receives venous blood in large quantities through the portal vein. There are thus *two afferent vessels*, the *hepatic artery* and the *portal vein*, the former carrying arterial blood, the latter venous blood from the intestines and spleen. Both vessels enter the liver at the porta and divide into large *interlobar branches*, which follow the connective tissue septa between the lobes. From these are given off *interlobular branches* which run in the smaller connective tissue septa between the lobules.

From the interlobular branches of the portal vein arise veins which are still interlobular and encircle the lobules. These send off short branches which pass to the surface of the lobule, where they break up into a rich intralobular capillary network (Fig. 292). These intralobular capillaries are sinusoidal in nature and are known as *hepatic sinusoids*. They all converge toward the center of the lobule, where they empty into the *central vein*. The central veins are the smallest radicles of the *hepatic veins*, which are the efferent vessels of the liver. As it passes through the center of the long axis of the lobule, the central vein constantly receives sinusoids from all sides, and, increasing in size, leaves the lobule at its base. Here it unites with the central veins of other lobules to form a *sublobular vein*, which is a branch of the hepatic vein.

The hepatic artery accompanies the portal vein, following the branchings of the latter through the interlobar and interlobular connective tissue. Some of the interlobular arterioles break up into capillary networks which supply the interlobular structures and then empty into the smaller branches of the portal veins. Other arterioles empty into the hepatic sinusoids, some at the periphery of the lobules, others traversing the lobule to enter the sinusoids near the central vein.

Duct System. The duct system of the liver serves to convey the external secretion, the *bile*, to the duodenum. The smallest branches of the duct system are the narrow intralobular *bile canaliculi* which form a ramifying network of channels between the cells of the hepatic plates (Figs. 295, 296, 297). Like the plates in which they lie, the canaliculi radiate outward from the central axis of the lobule. Most of the canaliculi drain from the network in the outer limiting

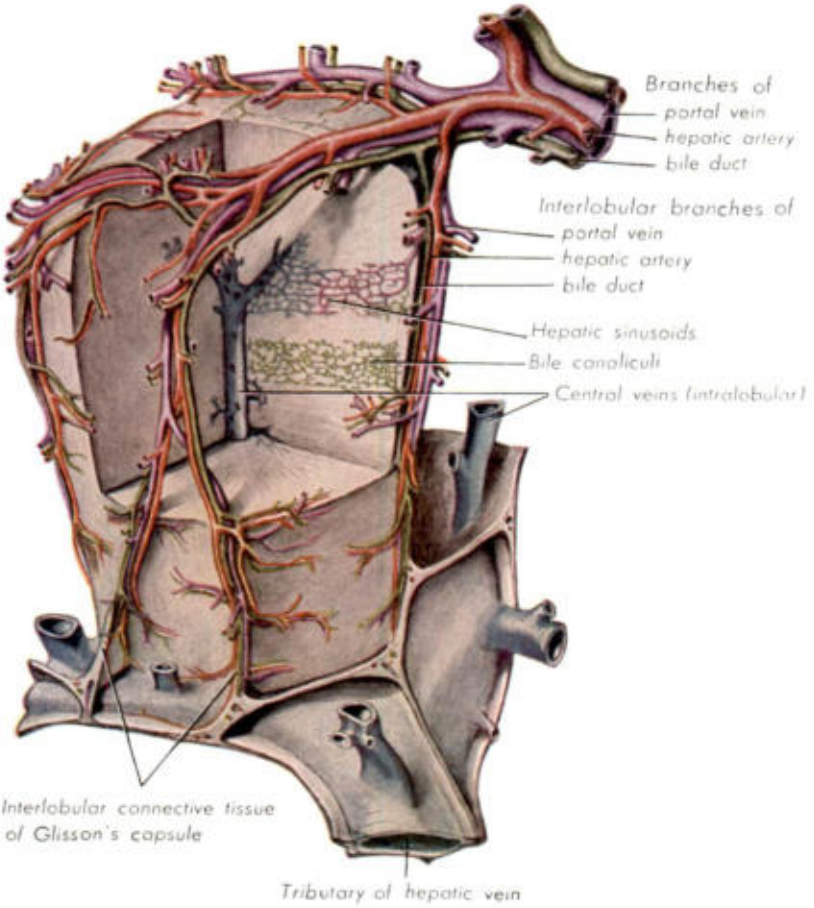


FIG. 292. Reconstruction of a lobule from the liver of a pig. A portion of the lobule is cut away to show the hepatic sinusoids and bile canaliculi. Redrawn and slightly modified from Braus, after a reconstruction by A. Vierling.

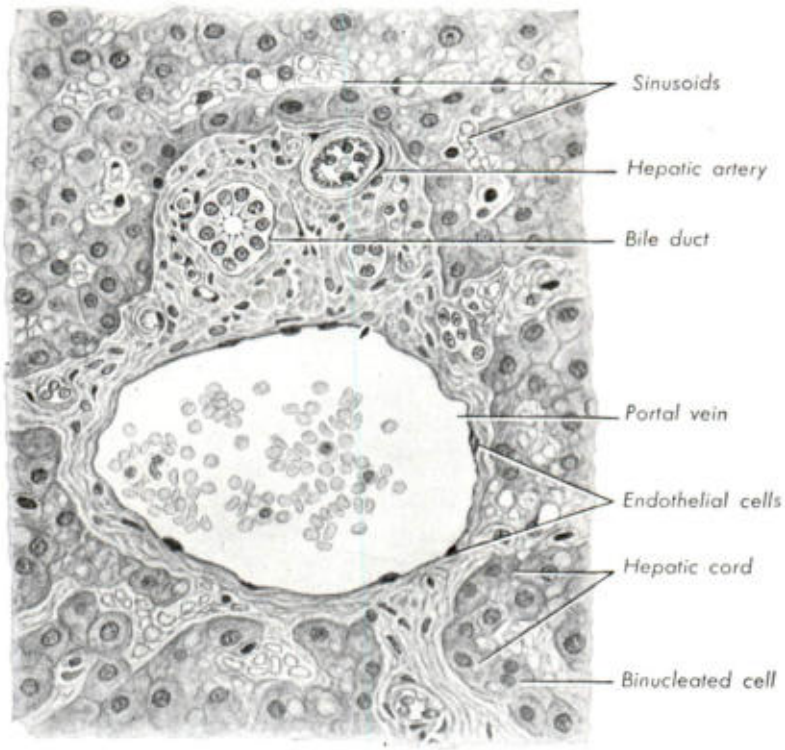


FIG. 293. The juncture of three lobules of the human liver showing interlobular branches of the portal vein, hepatic artery and bile duct. The region occupied by this aggregation of structures is frequently designated as a portal canal. Hematoxylin and eosin. × 455.

plate of the lobule into small *interlobular bile ducts* (*terminal bile ducts, cholangioles*) found at the periphery of a lobule. The short connections between the hepatic cells and the interlobular bile ducts are sometimes called the canals of Hering. They are lined by cuboidal cells which are smaller and paler staining than the hepatic cells. The lumen is little if any larger than that of a bile canaliculus (Fig. 297). A few of the canaliculi empty into small intralobular bile ducts which extend to variable depths within the lobule.

Each interlobular duct joins with others forming progressively larger ducts lined by cuboidal or columnar epithelium. As the ducts increase in size toward the porta of the liver, the epithelium becomes high columnar and the connective tissue layer becomes thicker, with many elastic fibers and a few scattered smooth muscle cells.

In their ramifications through the connective tissue septa, the interlobular bile ducts always accompany the branches of the portal vein and the hepatic artery. These structures, together with the interlobular connective tissue which marks the point of separation of three or more lobules occupy the *portal canal* or *portal area* (Fig. 293).

Extrahepatic Ducts. The right and left hepatic ducts join to form the *hepatic duct* which after its juncture with the cystic duct becomes the *common bile duct* (ductus choledochus) and conveys

the bile to the duodenum. The hepatic ducts, the cystic duct and the common bile duct may be referred to as the *extrahepatic ducts* of the liver, in contrast to the intrahepatic system of ducts within the gland.

The extrahepatic ducts are lined by high columnar epithelium resting on a connective tissue lamina propria containing smooth muscle and elastic fibers. The epithelium and underlying lamina propria are thrown into numerous folds. Goblet cells occur in the epithelium, more abundantly in the lower portion of the common bile duct. Small multicellular glands may be seen extending into the lamina propria. In the cystic duct, the smooth muscle fibers are disposed in transverse, longitudinal and diagonal directions. They are less abundant in the hepatic ducts and the common duct, except in the duodenal end of the latter, where they form a sphincter.

It is apparent that the hepatic lobule, the anatomical unit of structure of the liver, differs markedly from the lobules of glands such as the pancreas or salivary glands. In the latter, the lobule is surrounded by interlobular connective tissue and comprises a group of secreting tubules drained by the terminal branch of an interlobular duct. Thus, the unit of structure and the unit of function are identical.

In the liver, the parenchyma comprising one hepatic lobule is drained of its secretion by several interlobular bile ducts lying in the adjacent portal canals. As a result, a single interlobular bile duct carries off the secretions of portions of several adjacent hepatic lobules. This area of hepatic tissue, drained by a single interlobular bile duct lying in the axis of the area, has been designated a *portal lobule*. This classification is based solely on the external secretory function of the liver, without regard to its structure or its important endocrine-like functions. Aside from the function of bile secretion, the *hepatic lobule*, described as groups of cells arranged radially about an axial central vein, more logically defines the anatomical and physiological unit of the liver.

The Hepatic Plates or Laminae. The tissue comprising the hepatic lobule is quite unlike the secreting tubules of other glands. It is formed of plates or laminae which are but one cell in thickness. The plates are curved, branched and interconnected. They are arranged in an anastomosing fashion, radiating outward from the central vein. Between the plates of hepatic cells lie the anastomosing blood sinusoids (Fig. 291).

The liver lobule was for many years described as a system of hepatic cords radiating out from the central vein, each cord being formed of a double row of hepatic cells. The older, more conventional term, *hepatic cord*, therefore often appears in the literature. In some of the lower vertebrates the plates of liver cells are characteristically two cells in thickness and in sections give the appearance of double cords. This is not the case for man and other mammals, in which the plates are only one cell thick. Since the parenchyma is in the form of sheets, broader than they are thick, the terms *hepatic plate* or *hepatic lamina* are preferable. The number of cells seen in a section of any one plate will, of course, vary according to the angle at which the plate was cut.

Bile Canaliculi. Between the hepatic cells are minute channels, the *bile canaliculi*. These branch and have a very irregular course (Figs. 296, 297). Short side branches of the canaliculi frequently extend between the liver cells toward the surface of a plate. They ramify throughout the hepatic parenchyma, anastomosing with the canaliculi of adjacent anastomosing plates (Fig. 295), but seldom approach the surface of a plate.

Each canaliculus lies midway along the interface between adjacent hepatic cells, and is formed by the somewhat

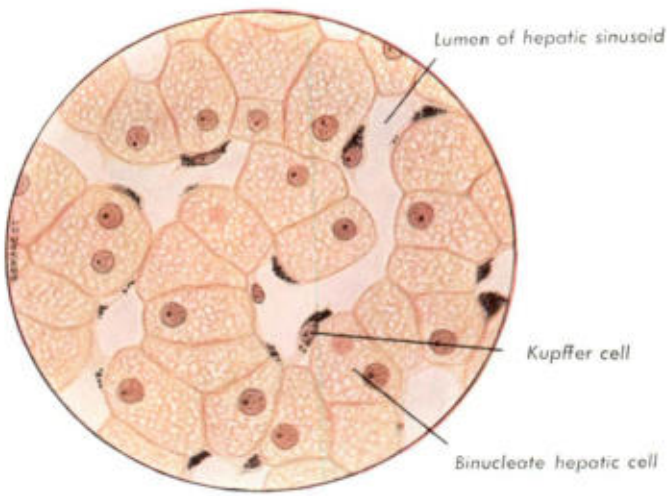


FIG. 294. Section of liver from a monkey which had received several intraperitoneal injections of trypan blue. Most of the sinusoidal lining (Kupffer) cells have taken in some of the dye by phagocytosis. Azocarmine and metanil yellow stain. × 720.

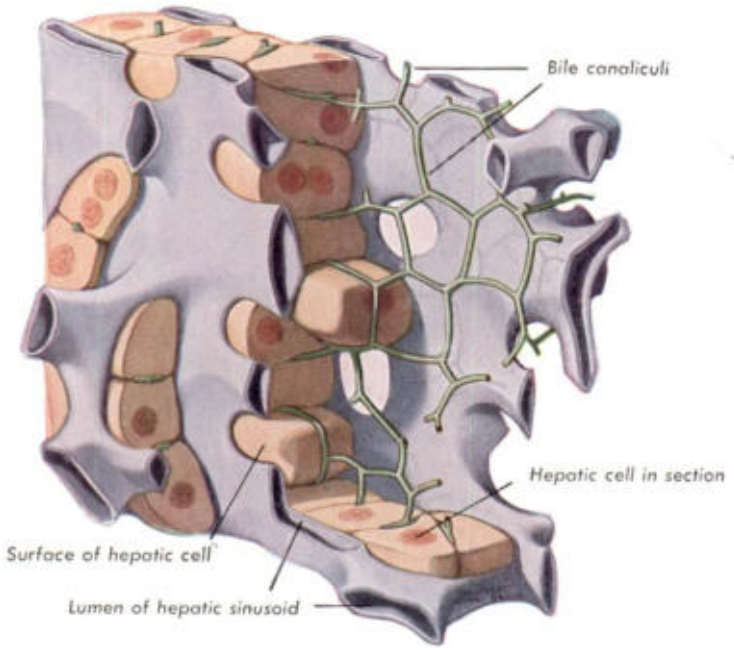


FIG. 295. Diagrammatic reconstruction of a small portion of human liver to show the relationship of the anastomosing plates of hepatic cells to the blood sinusoids and to the network of bile canaliculi. For the purposes of the diagram, both the sinusoids and the bile canaliculi are shown as independent structures, particularly at the right, where the hepatic cells have been "dissected away." It should be recalled, however that the bile canaliculi, being formed by the modified cell membranes of adjacent hepatic cells are always surrounded by hepatic cells, and that the lining of the sinusoids is not smooth and homogeneous as shown, but is made up of Kupffer cells and fine reticular fibers. (Redrawn and modified from Braus.)

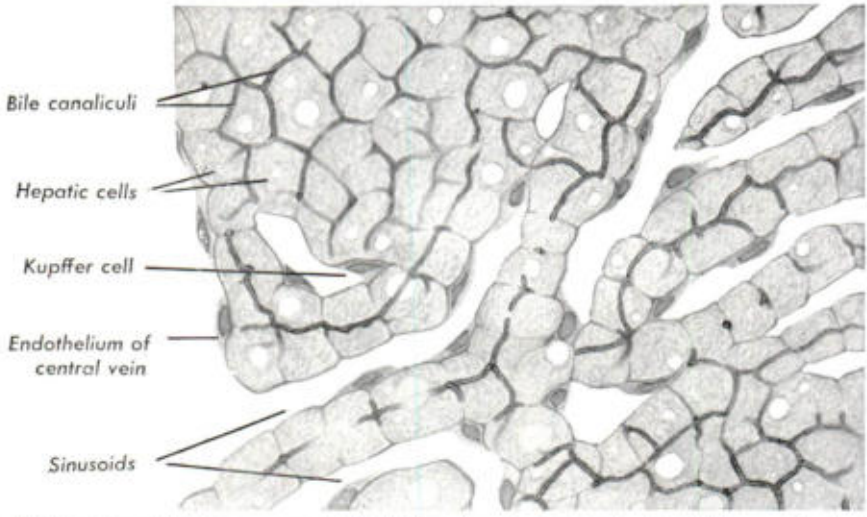


FIG. 296. Section of liver impregnated by Golgi silver technique to show bile canaliculi. Human. × 720.

modified cell membranes of the two opposing cells. In ordinary preparations the canaliculi appear to have firm but delicate membranes, and are often distinguished with difficulty. Electron micrographs show that the membranes of the hepatic cells which form the canaliculi have short microvilli projecting into the canaliculi (Fig. 298).

Hepatic Cells. The hepatic cells which make up the hepatic plates are polyhedral in form and, under normal conditions, their boundaries are quite sharply defined. Each cell has a central nucleus with a distinct nuclear membrane and one or more prominent nucleoli. Multinucleate hepatic cells are occasionally seen, the usual variation being the binucleate condition.

Binucleate cells result from mitotic division of the nucleus of a mononucleate cell without accompanying division of the cytoplasm (Beams and King, 1942). Each of the two nuclei is of approximately normal size and possesses the normal (diploid) number of chromosomes; the cytoplasmic portion of the cell may be large. Large hepatic cells with either one or two unusually large nuclei also occur. These result from mitotic division of the above-mentioned b nucleate cells in which all of the chromosomes merge on one spindle, with the formation of either (1) two large mononucleate cells, each with an unusually large nucleus containing a tetraploid number of chromosomes; or (2) one large cell with two large tetraploid nuclei. Further divisions of this type may produce mononucleate cells with still larger nuclei having a further increase in chromosome number.

The mitochondria of the hepatic cells are filamentous or rod-like, and are scattered throughout the cell. The Golgi apparatus lies either near the edge of the cell beneath the bile canaliculus (Fig. 11), or close to the nucleus. *Basophilic material* (RNA) is present in the form of small angular clumps dispersed throughout the cytoplasm, and is usually quite abundant. As in many other cells, the basophilic component appears in electron micrographs as small dense granules

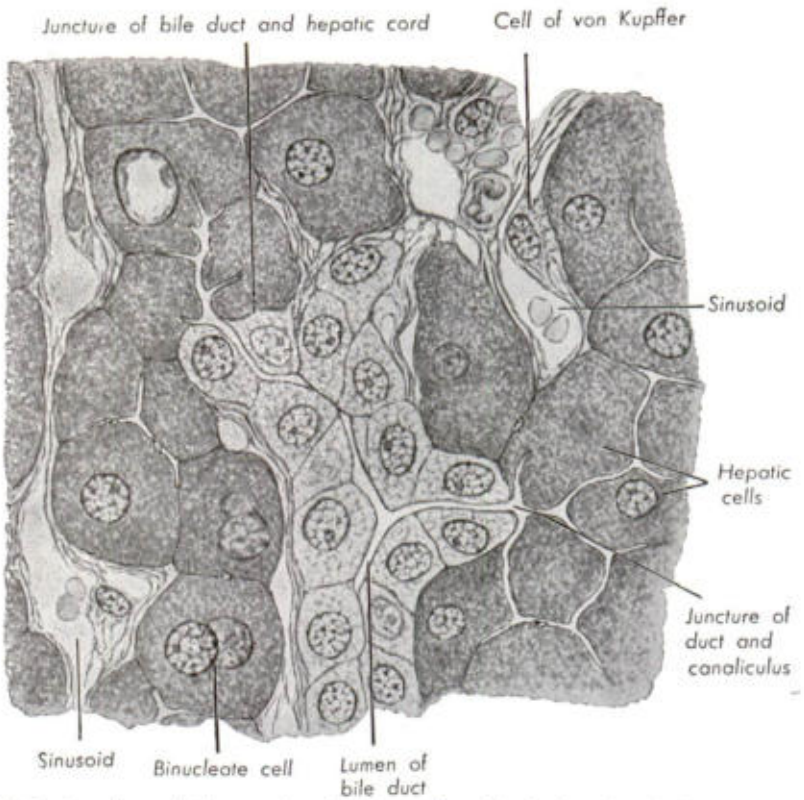


FIG. 297. Section through the margin of a human liver lobule showing the juncture of termina bile duct with hepatic cords. The lumens of two of the terminal branches of the duct are directly continuous with bile canaliculi. A 5 μ section stained with phosphotungstic acid hematoxylin and phloxine. × 1070.

either associated with the surfaces of the flattened vesicles of the endoplasmic reticulum or scattered in the cytoplasmic matrix (Fig. 299).

Scattered throughout the cytoplasm in ordinary preparations are small clear areas. These represent glycogen granules, or more correctly, *glycogen droplets*, which can be preserved and stained by appropriate special techniques. *Fat droplets* may also appear as inclusions in the cytoplasm, particularly after fasting or after ingestion of an excess of fats. The amount of fat which may be demonstrated in the hepatic cells varies inversely with the amount of glycogen. Pigment granules (bile pigments) are occasionally seen in the cytoplasm.

As in other gland cells, the appearance of the cytoplasm varies with the functional state of the cell. Both glycogen and the basophilic material are markedly reduced after a prolonged period of fasting, and they reaccumulate in the cytoplasm with refeeding. Glycogen is more abundant in the hepatic cells after digestion of a carbohydrate meal. It represents stored carbohydrate which is given back into the blood as glucose when the needs of the body demand it. When glycogen is released from the liver, it leaves the hepatic cells at the periphery

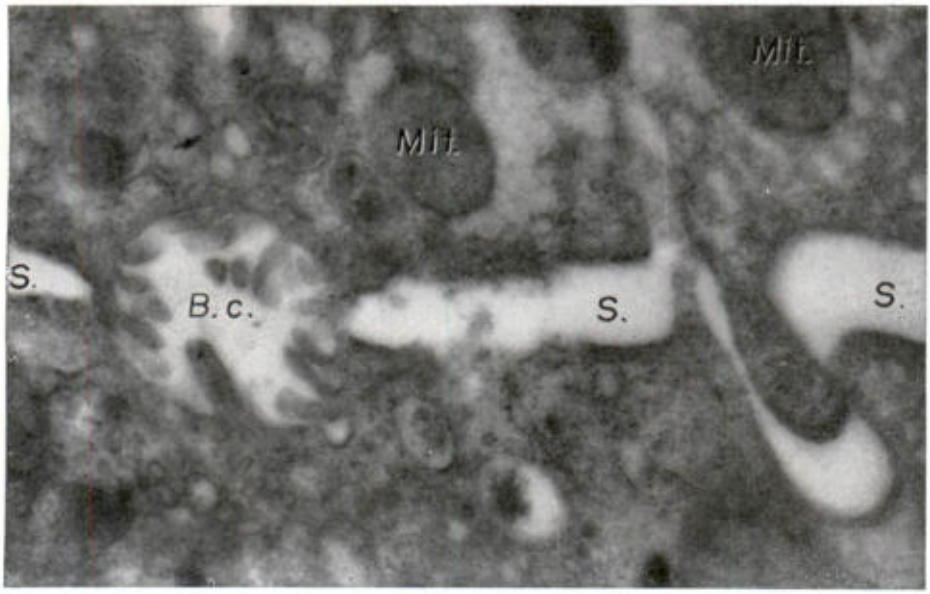


FIG. 298. Electron micrograph of portions of two adjacent hepatic cells to show the bile canaliculus. Bile canaliculus (*B.c.*), formed by opposing cell membranes, has microvilli projecting into it. At the right of the figure, the cell above shows a stud-like projection which fits into a concavity in the surface of the lower cell. This sort of attachment is not unique in liver cells. The space (*S.*) separating the two cells is an artifact. (Courtesy of Dr. D. W. Fawcett.)

of the lobule first. Regarding the region of the liver lobule where glycogen is first deposited, there has been some controversy. However, Eckman and Holmgren (1949) have reported that in rats and mice in which the liver is depleted of its glycogen by a prolonged fast, feeding results in the deposition of glycogen peripherally first, progressing centrally until the lobule is entirely filled again.

The membranes of the hepatic cells which border on the sinusoids show a surface modification similar to that on the free surfaces of cells in many other locations. Short, irregular microvilli which can be seen only with the electron microscope project from the cells (Fig. 301); in most areas the processes are covered by the lining cells of the sinusoids.

Hepatic Sinusoids. The hepatic sinusoids make up the intralobular system of blood capillaries which course centripetally through the lobule and convey the blood from the interlobular branches of the portal vein to the central vein. They have relatively wide lumina, anastomose irregularly and everywhere separate the hepatic plates one from another. It thus follows that the hepatic cells may have one or more surfaces abutting on bile canaliculi and one or more surfaces always adjacent to blood sinusoids (Fig. 295).

In the lining of the sinusoids there may ordinarily be distinguished two types of cells (or more likely two modifications of the same cell type). One is a cell with a dark nucleus and sparse cytoplasm lying flattened against the hepatic cells. The other is a larger cell with larger, more vesicular nucleus and branching cytoplasmic processes which protrude into the lumen of the sinusoid. Although the former resembles an endothelial cell,

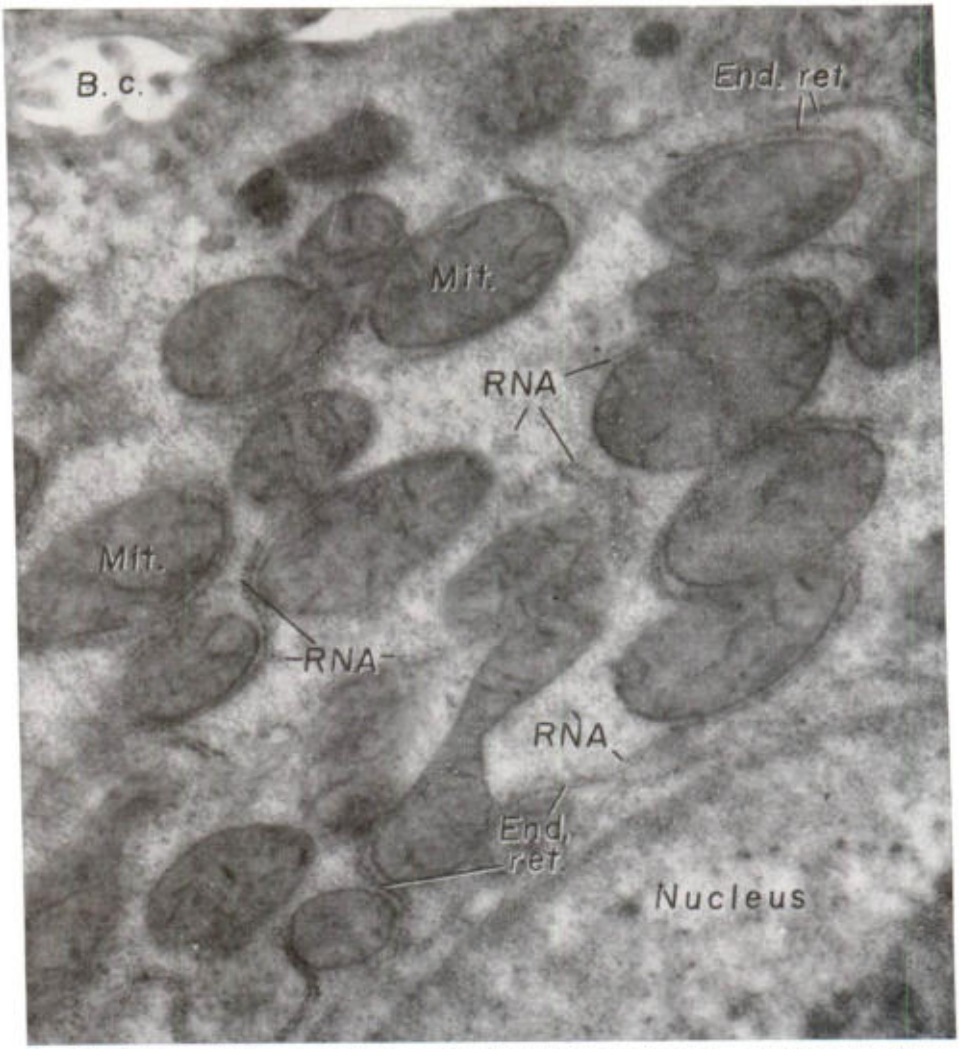


FIG. 299. Electron micrograph of hepatic cell. The field includes only a small portion of the cell. Segment of nucleus is at lower right, cell membrane and bile canaliculus (*B.c.*) at upper left. The intervening cytoplasm contains numerous mitochondria (*Mit.*), flattened vesicles of the endoplasmic reticulum (*End. ret.*), and dense RNA granules, some on the surfaces of the vesicles, but mostly scattered in the cytoplasmic matrix. Rat liver. × 20,000. (Courtesy of Dr. D. W. Fawcett.)

all gradations between it and the more branching form may be seen, and the two should probably be considered variations of the same type of cell, the stellate cells of von Kupffer, usually referred to as *Kupffer cells* (Figs. 294, 300). They are phagocytic cells and are a part of the reticulo-endothelial system (p. 75). Because of their intimate relation to the function of the liver, however, they must be considered an integral part of that organ.

If a substance such as trypan blue, or India ink is repeatedly injected into

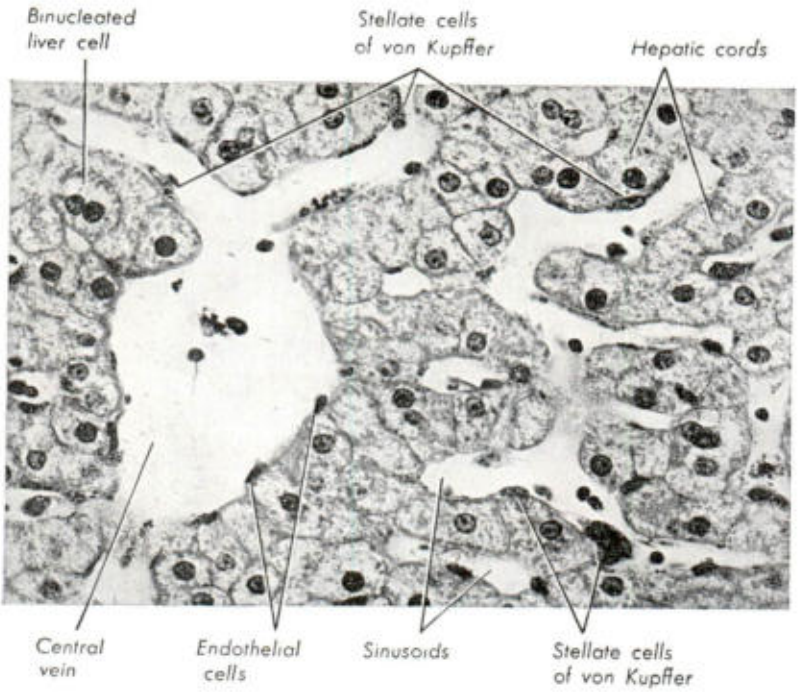


FIG. 300. Photomicrograph of a section of the liver of a monkey which had received several injections of trypan blue. All lining cells of the sinusoids contain some of the dye. They vary greatly, however, in the amount which has been ingested. At the lower right is a very heavily laden cell. The trypan blue granules photograph black as do also the nuclei of all of the cells. Azocarmine.

an animal, it will be found that the branching Kupffer cells readily ingest the foreign substance and store it in large amounts in their cytoplasm (Figs. 294, 300). Thus they act to free the blood stream of foreign particles. They are also said to be active in fat metabolism and in the formation of bile pigments. The flattened lining cells are somewhat less phagocytic. Although some investigators have felt that the flattened cells constituted a true endothelial lining of the sinusoids, this is probably not the case. They are more likely cells of the same type as the more phagocytic stellate cells, but in a less active state. The structure of the sinusoidal lining cells as seen in electron micrographs (except for the phagocytized material in those which were actively phagocytic at the time of fixation) does not differ sufficiently to distinguish two types.

There has also been some doubt in the past in regard to the completeness of the sinusoidal lining. In histological sections it often appears that there are spaces between the processes of adjacent Kupffer cells. Electron micrographs of normal liver tissue have confirmed this impression. Such an incomplete or fenestrated sinusoidal lining leaves the hepatic cells in some areas exposed to the blood stream. The mobile, non-endothelial character of the lining cells is further accentuated by their lack of firm attachment to the parenchymal cells of the hepatic plates.

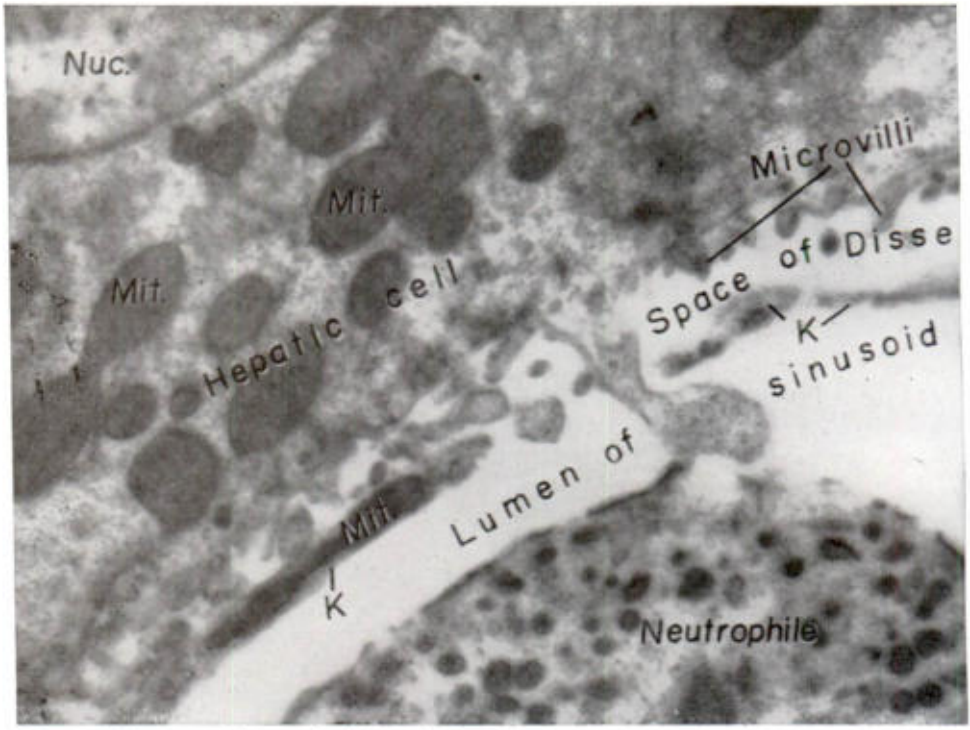


FIG. 301. Electron micrograph of hepatic sinusoid showing the surface of a liver cell facing the sinusoid, and parts of overlying Kupffer cells (*K*). The surface of the hepatic cell is studded with short, irregular microvilli. Part of one Kupffer cell included in the field contains a slender mitochondrion (*Mit.*). The Kupffer cells do not form a complete lining, but leave the hepatic cells exposed to the lumen of the sinusoid in some places. The edge of a neutrophilic leucocyte in the sinusoid is seen in the lower part of the figure. × 20,000. (Courtesy of Dr. D. W. Fawcett.)

Connective Tissue. Ordinary sections of the human liver reveal scant amounts of connective tissue. The interlobular connective tissue concentrated in the portal areas has already been described. Special methods show that there is, in addition, a framework of reticular (argyrophilic) fibers within each lobule (Fig. 302). This consists of extremely delicate fibrils lying between the Kupffer cells and the hepatic cells, forming a network which envelops each sinusoid, and a small number of coarser fibers which radiate outward from the region of the central vein.

Lymphatics. The lymph vessels of the liver form a rich plexus in the capsule of Glisson and in its connective tissue septa within the gland. Lymph vessels enmesh the larger blood vessels and ducts and there are anastomoses between lymphatics which surround the portal veins and those which surround hepatic veins. Anastomoses between lymphatics of neighboring portal canals enclose the hepatic lobules in a network of small lymph vessels, but so far as can be determined no lymphatic vessels penetrate the lobules themselves. Since the lobules comprise the bulk of the liver tissue it is puzzling to explain the fact that more lymph flows from the liver than from any other organ of the body.

There is a potential tissue space be-

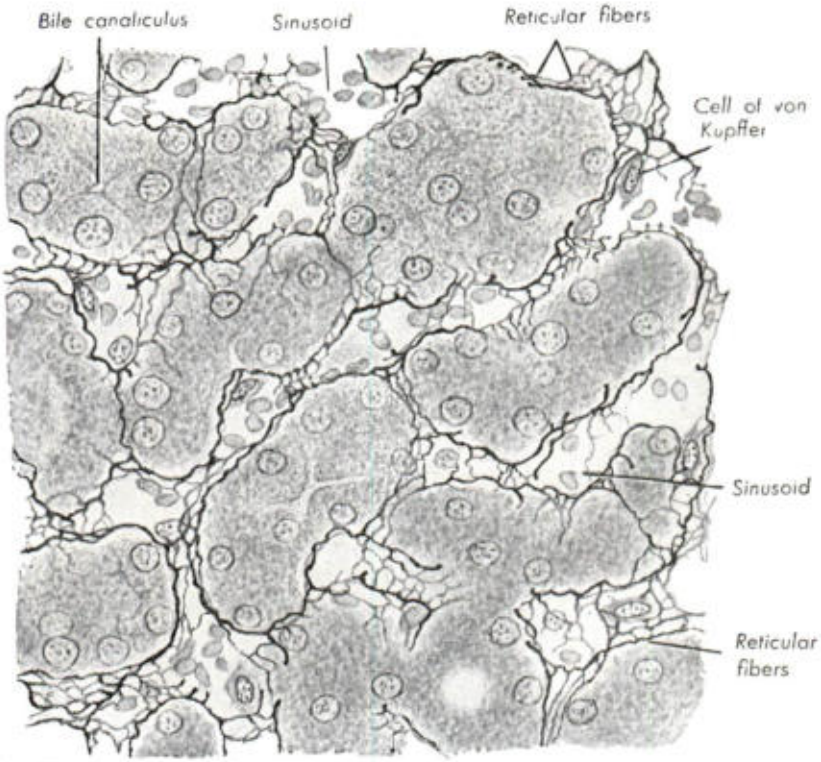


FIG. 302. Human liver showing reticular tissue in a lobule. Foot's silver technique. × 700.

tween the Kupffer cells lining the sinusoids and the hepatic cells. This is the so-called *space of Disse*, which is traversed by the reticular fiber network. In view of the large volume of lymph arising in the liver and the absence of any lymphatic capillaries within the liver lobule, some investigators have postulated that tissue fluid might collect in this space, seep into the portal connective tissue spaces, and thence into the smallest lymphatic vessels in the portal area. The space of Disse should not, however, be confused with a lymphatic vessel; its significance is still controversial.

Nerve Supply. The nerves of the liver are mainly nonmedullated fibers of the sympathetic system. They accompany the blood vessels and bile ducts, around which they form plexuses. These plexuses give off fibers which terminate on the blood vessels and bile ducts and among the hepatic cells.

Functions of the Liver. The exocrine function of the liver is concerned with the production of bile, which is carried by the system of bile ducts into the duodenum.

Bile is a product of the hepatic cells and is partly a secretion, important in the absorption of fats, and partly an excretion, carrying off waste products which are eliminated with the feces. Among the constituents of bile are the following: bile acids, bile pigments, cholesterol, lecithin, neutral fats and soaps, traces of urea, and water and bile salts. The bile salts, as emulsifying agents facilitate the absorption of fats in the intestine. They are themselves reab-

sorbed in part from the intestine and are again secreted by the hepatic cells. The bile pigments are derived from the breaking down of hemoglobin; this apparently takes place elsewhere in the body than in the liver, although the Kupffer cells may play a part. The bile pigments are removed from the blood by the liver and excreted in the bile as a waste product. Cholesterol is also excreted in the bile.

Claude Bernard was the first to show that the liver stored glycogen and gave it up as glucose. The liver not only stores reserve sugars but it is essential for the transformations which are necessary before some of the sugars can be utilized by the body. Conversion of fats and perhaps also of proteins to carbohydrates is accomplished in the liver (gluconeogenesis). Through its diversified chemical processes the liver is able to maintain the blood sugar level constant under widely different dietary conditions.

The liver has other important functions. It is involved in protein metabolism and is the chief site of deaminization of amino acids, with the production of urea as a by-product. Fats are likewise metabolized and stored in the liver. Some of the plasma proteins (fibrinogen, prothrombin, albumin) are synthesized in the liver. The liver is also an important place of storage for many vitamins, chiefly A, D, and B_2, B_3, B_4, B_{12} and K which are essential to the body.

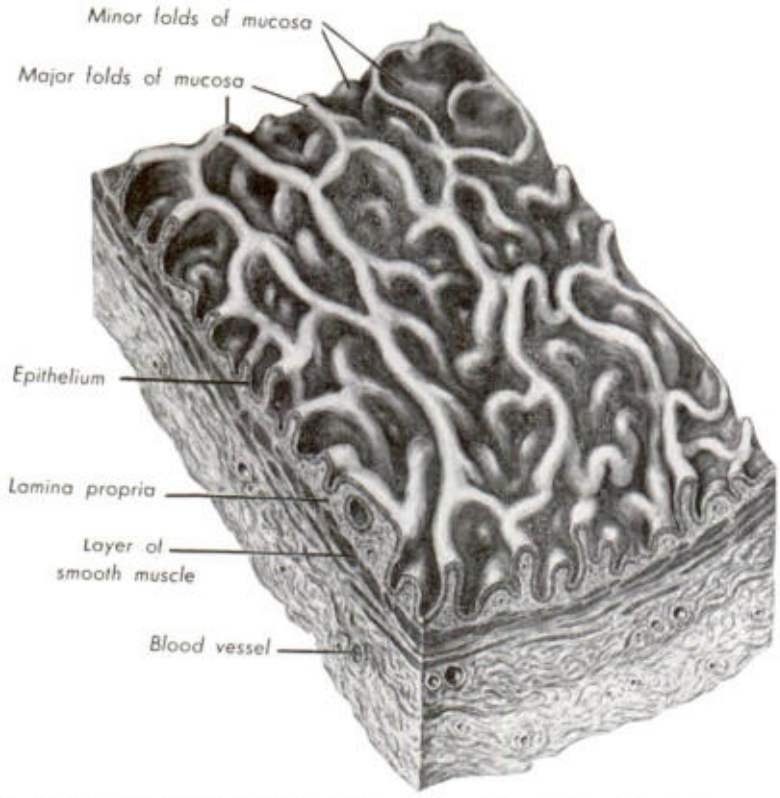


FIG. 303. Low power, three dimensional view of a portion of the wall of a human gall bladder. × 24.

Regeneration of Liver. When a part of the liver is removed by operation, or when there is partial destruction by toxic agents (chloroform, etc.), the organ regains its normal weight within a relatively short time. In the rat, surgical removal of as much as seventy-five per cent of the gland is followed by complete weight restitution within a month (Higgins and Anderson, 1931). The repair is accomplished by mitotic multiplication of parenchymal cells throughout the remaining portion of the organ and by cell enlargement. Differentiation of new liver cells from budding interlobular bile ducts may also play a part. In restored liver tissue, there is an increased number of the mononucleated cells which have large nuclei containing a multiple number of chromosomes (Sulkin, 1943). These are produced by division of binucleated cells. There is also a general increase in nuclear size of all the parenchyma cells.

THE GALL BLADDER

The gall bladder is a hollow pear-shaped organ lying obliquely on the inferior surface of the liver, and may be regarded as a diverticulum of the bile duct. It consists of a *fundus*, which is the blind end, a *body*, and a *neck* which passes into the cystic duct. In the neck, folds of the mucosa form the *spiral valve of Heister*.

The wall of the gall bladder is composed of three layers: *mucosa*, *muscularis* and an *adventitia* or *serosa*. The mucosa is thrown into numerous folds, which divide the surface roughly into irregular polygonal areas (Fig. 303). The epithelium is composed of tall columnar cells with oval nuclei situated in the basal zone (Figs. 304, 25, *B*). A thin striated border has been demonstrated on the free surface of the cells. It is less prominent than that of the intestinal cells, so that it is seen well only with phase contrast or electron microscopes (Fig. 305). The tunica propria consists of connective tissue containing extensive vascular plexuses and a few scattered smooth muscle cells derived from the muscularis. Near the neck of the gall bladder, small tubulo-alveolar glands occur in the mucosa.

Small diverticula of the mucosa which extend down into the muscular and perimuscular layers are known as the *Rokitansky-Aschoff sinuses*. Their epithelium is a continuation of the surface epithelium. They are interpreted as structures indicating a pathologic change, perhaps following prolonged over-distension of the gall bladder.

The muscular layer of the gall bladder is formed of interlacing bundles of smooth muscle fibers. Bundles of longitudinal fibers occur nearer the tunica propria, coursing the length of the bladder and curving over the fundus. The remainder of the muscle bundles, which form the greater part of the muscularis, are circularly disposed. Connective tissue, with an abundance of elastic fibers, occurs between the muscle bundles.

The external connective tissue layer is usually thick, but shows wide individual differences. Just external to the muscularis, it consists of fibrous connective tissue sufficiently distinct to be designated the *perimuscular layer*. Outside this is a subserous layer of connective tissue containing blood and lymph vessels and nerves. On its free surface, the gall bladder is covered by the peritoneum; the connective tissue of its attached surface merges with that of the liver.

Lymph nodules occur in the wall of the gall bladder. There may also be seen duct-like structures, lined with epithelium, which have no connection with the lumen of the bladder, although they have occasionally been described as having connections with the bile ducts.

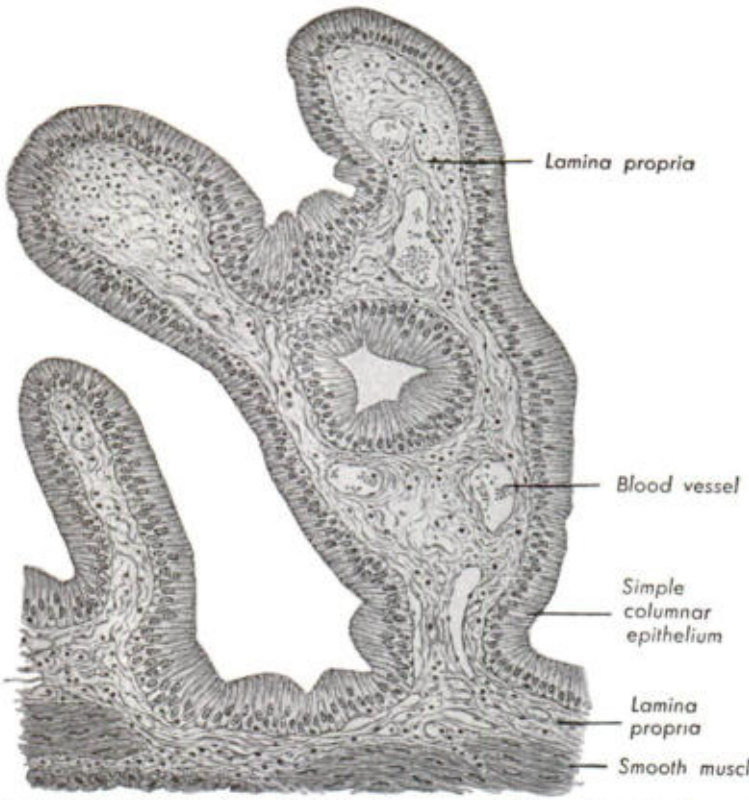


FIG. 304. A section through the wall of the human gall bladder which is shown in perspective in Figure 303. × 175.

These are known as true *Luschka ducts*. They are interpreted as aberrant embryonic bile ducts.

The gall bladder functions as a reservoir for the bile produced by the liver. By the reabsorption of large quantities of water and mineral salts through its mucosal layer, it also serves to concentrate the bile.

THE BILE DUCTS

There are three main ducts associated with the gall bladder. These are (1) the cystic duct, (2) the hepatic duct and (3) the common bile duct (*ductus choledochus*). The hepatic duct conducts bile from the liver to a point where the cystic duct from the gall bladder meets the common bile duct leading to the duodenum. The bile then passes through the cystic duct into the gall bladder for concentration and storage. Contraction of the gall bladder forces the bile back through the cystic duct and thence through the common bile duct to the intestine. As the ductus choledochus passes obliquely through the wall of the duodenum it lies side by side with the ductus pancreaticus. Where the ducts penetrate the muscularis mucosae, smooth muscle from this layer forms the sphincter of Oddi. A portion of its fibers which encircle only the bile duct are known as the *sphincter choledochus*. While this sphincter remains contracted (as for example during fasting), pressure is

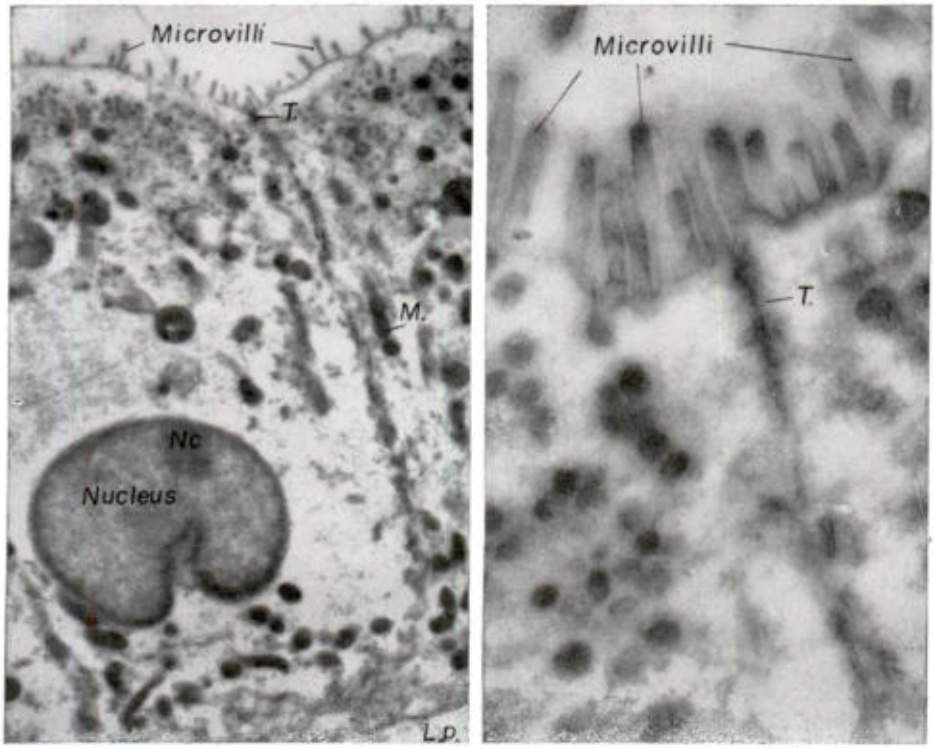


FIG. 305. Electron micrographs of the epithelium of the gall bladder (mouse) showing microvill on free surface. *Left*. Parts of three epithelial cells. Small granules in the apical cytoplasm may be absorbed material. *T.*, terminal bar; *M.*, mitochondrion; *Nc.*, nucleolus; *L.p.*, lamina propria. × 7,000. *Right*. Higher magnification of the apical region of parts of two epithelial cells. Extending into the cytoplasm between two of the microvilli in the cell at the left is an invagination (caveola intracellularis). This may be a stage in the formation of an absorption or pinocytotic vacuole. × 40,000. (Courtesy of Dr. E. Yamada.)

maintained in the duct system, and bile from the liver is forced into the gall bladder for storage. Upon the ingestion of food, especially fats or proteins, the sphincter of Oddi relaxes, the gall bladder is stimulated to contract, and bile traverses the cystic duct and common bile duct to the duodenum.

The ducts are lined with a columnar epithelium similar to that of the gall bladder. Near the neck of the gall bladder the mucosa of the cystic duct is thrown into a series of folds continuous with those of the spiral valve of Heister. The muscularis of the ducts is not as prominent as in the gall bladder. The arrangement of muscle fibers into layers is not definite, but they incline to be circularly arranged in the hepatic duct and longitudinally disposed in the cystic and in the common bile duct prior to its entry into the duodenum.

DEVELOPMENT OF THE DIGESTIVE SYSTEM

In the development of the digestive system all the layers of the blastoderm are involved. Mesoderm and entoderm are however the layers most concerned, as the ectoderm forms only a part of the mouth and a part of the anal canal. The *primitive alimentary canal* is formed by

two folds which grow out from the ventral surface of the embryo and unite to form a canal, in a manner that is quite similar to the formation of the neural canal. In this way the primitive gut is lined with cells which previously formed the ventral surface of the embryo, i.e., entoderm. A portion of the mesoderm accompanies the entoderm in the formation of the folds. This is known as the *visceral layer* of the mesoderm. The primary gut is thus a *closed sac* or *tube*. It is connected with the umbilical vesicle, but has no connection with the exterior. These connections are formed later by oral and anal invaginations of ectoderm which extend inward and open into the ends of the hitherto imperforate gut. The ends of the alimentary tract, including the oral cavity and all of the glands and other structures connected with it, are of ectodermal origin. The epithelial lining of the gut and the parenchyma of all glands connected with it are derived from entoderm. The muscle, the connective tissue and the mesothelium of the serosa are developed from mesoderm.

The mesodermal elements show little variation throughout the gut, the peculiarities of the several anatomical divisions being dependent mainly on special differentiation of the entoderm (epithelium). Beneath the entodermal cells is a narrow layer of loosely arranged tissue which later separates into lamina propria, muscularis mucosae, and submucosa. Outside this a broader mesodermal band of firmer structure represents the future muscularis.

The *stomach* first appears as a spindle-shaped dilatation about the end of the first month. Its entodermal cells, which had consisted of a single layer, increase in number and arrange themselves in short cylindrical groups. These are the first traces of tubular glands. They increase in length and extend downward into the mesodermal tissue. For a time the cells lining the gastric glands are all apparently alike, but at about the fourth month the differentiation into chief cells and parietal cells takes place.

In the *intestines* a proliferation of the epithelium and of the underlying stroma results in the formation of *villi*. These appear about the tenth week, in both small and large intestines. In the former they increase in size, while in the latter they atrophy and ultimately disappear. The simple tubular glands of the intestines develop in a manner similar to those of the stomach.

The mesothelium of the serosa is derived from the mesodermal cells of the primitive body cavity.

The *development of the larger glands* connected with the digestive tract takes place in a manner similar to the formation of the simple tubular glands. All originate as extensions of entodermal cords into the underlying mesodermal tissue. From the lower ends of these cords, branches extend in all directions to form the complex systems of tubules found in the compound glands.

The *salivary glands*, being developed from the oral cavity, originate as similar invaginations of ectodermal tissue.

The *pancreas* originates as three separate evaginations from the entoderm of the future duodenum. Of these, one atrophies, while the other two unite to form the adult pancreas. The two evaginations account for the two pancreatic ducts. The evaginations take place into the underlying mesenchyme, which develops the connective tissue framework of the organ. The growths are at first solid cords of cells which later become hollowed out to form tubules and differentiate acinar and centro-acinar cells, the former early showing zymogen granules. The proximal portions of the tubules remain as the duct system of the gland. The

islands of Langerhans develop as buds from a system of fine tubules which are derived from the ducts and which form a network around the ducts. Many of the islands become secondarily detached, but some of them retain their connection with the tubules in the adult.

The *liver* originates as an outgrowth of entoderm of the ventral wall of the future duodenum into the mesoderm of the transverse septum. At its cephalic end the outgrowth is solid and gives rise to the liver; at the caudal end it is hollow and gives rise to the gall bladder. As the evagination increases in size it becomes almost completely separated from the intestine, the slender connection which does remain becoming the ductus choledochus. Between the latter and the gall bladder anlage a slender connection also remains, the cystic duct. The mesenchyme surrounding these structures develops their connective tissue framework.

As the liver bud grows, it comes into intimate relation with the growing vitelline veins. The veins break up into a network of anastomosing vessels, the future hepatic sinusoids, which tunnel through the growing liver tissue, dividing it into irregular anastomosing trabeculae, the future hepatic plates. This relationship is maintained in the adult.

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XVII

THE RESPIRATORY SYSTEM

The respiratory system consists of the lungs and a series of passages leading to them—the nasal cavities, nasopharynx, larynx, trachea and bronchi. The oropharynx also serves for the conduction of air as well as of food.

NASAL CAVITY AND NASOPHARYNX

Nasal Cavity

The nasal cavity extends from the nares (nostrils) to the choanae through which it opens into the nasopharynx. The cavity of the nose is divided into lateral halves or *fossae* by a median cartilaginous and bony septum. The inferior region of each fossa is somewhat expanded and is named the *vestibule* (Fig. 309). This leads superiorly into the major cavity, designated usually as the respiratory region because of the type of its epithelial lining. In the uppermost part of each fossa is a relatively small area lined by an epithelium containing receptor cells for the sense of smell. This is the olfactory region.

The medial wall of each fossa (the septum) is smooth and flat, but the lateral wall, especially in the respiratory region, is very irregular in shape. This irregularity is due to horizontal scroll-shaped structures, the *conchae*, three in number (Fig. 309). Each concha, near its upper margin, has a relatively thin linear attachment to the lateral wall. Beneath each concha is a cavity or meatus. The lacrimal duct and certain of the accessory nasal sinuses open into the meatuses.

The main current of air in its passage through the respiratory portion comes in contact with the mucosa of the septum and the medial surfaces of the conchae. These are then more subject to cooling and drying by the air than are the more hidden surfaces of the meatuses, which in turn are more exposed than the lining of the nasal sinuses. There are differences in the mucous membrane which appear to be correlated with the degree of exposure.

The lining of the *anterior part* of the vestibule is quite similar to the epidermis of the wings (alae) of the nose. It has, however, long thick hairs or vibrissae with which are associated large sebaceous glands. Sweat glands also are present. The lamina propria is dense and becomes continuous with the corium of the skin in the distal region where cartilage is absent.

The zone of vibrissae is succeeded by the transitional zone of the vestibule. This in turn is continuous with the respiratory region.

The *transitional zone* is lined by a thick stratified squamous epithelium which is deeply indented by long highly vascular connective tissue papillae. Sweat and sebaceous glands and hairs are absent but there are a few mixed serous and

mucous glands characteristic of the respiratory region. The transitional zone is of variable width; it is also variable in the degree of its cornification.

The *respiratory region* is lined by a ciliated pseudostratified columnar epithelium which is the characteristic type of the conducting division of the respiratory system. In the more exposed areas, the epithelium is thick, goblet cells are numerous, and the basement membrane is very prominent (Fig. 307). In the meatuses, the epithelium is thinner, goblet cells are fewer and the basement membrane is not prominent. Intraepithelial glands (mucous crypts) are present in a few individuals. These glands are depressions in the epithelium and are lined by a continuous layer of goblet cells.

The *olfactory mucosa* occupies a small area in the upper part of the respiratory region. For details of the structure of the olfactory region, see Chapter XXII.

Experimental work shows that the nasal epithelium will change its character with increased ventilation. If all the air is made to pass through one side of the nose by occluding the other nostril, the epithelium of the side subjected to the increased ventilation at first hypertrophies and then changes to a stratified squamous type (metaplasia).

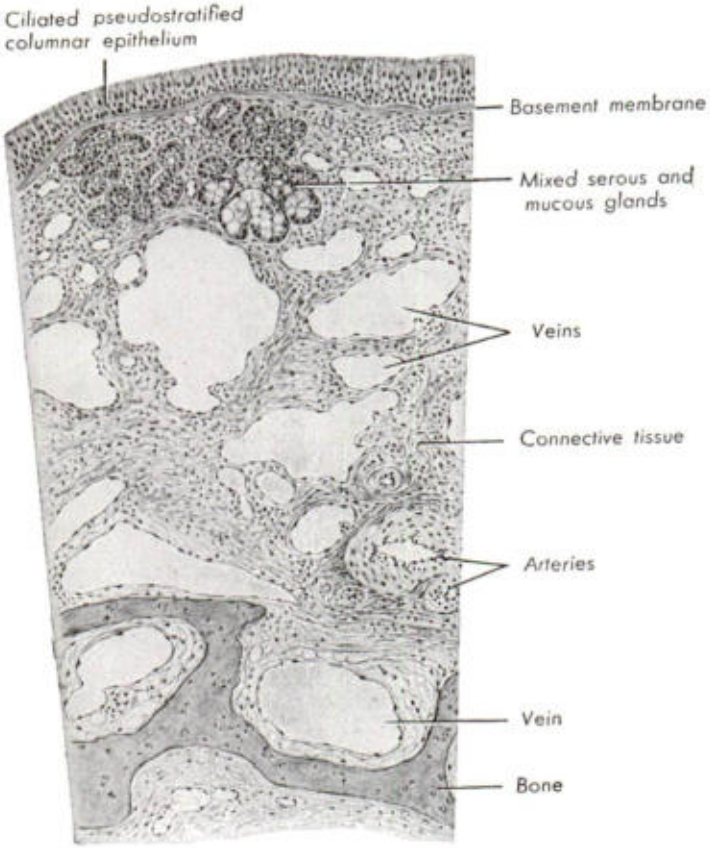

FIG. 306. Section through the medial portion of an inferior nasal concha of a woman forty-seven years of age. Blood which filled the veins is not shown. × 67.

The lamina propria contains numerous mucous and serous glands. They are more numerous in the exposed than in the more protected regions. Lymphocytes are usually present in both the epithelium and the lamina propria (Fig. 307).

The lamina propria of the nasal mucosa is quite vascular everywhere, but on the medial surfaces of the middle and inferior conchae, and to a degree on the apposed surface of the septum, it is so vascular as to be distinctive. There are many large veins (Fig. 306) which may become engorged with surprising rapidity. With their engorgement the mucosa becomes swollen and turgid obstructing the flow of air through the nasal cavities.

The *paranasal sinuses*—maxillary, ethmoid, frontal, sphenoid—are lined by an epithelium which is continuous with that of the nasal passages. It is a pseudostratified columnar ciliated epithelium (Fig. 308), but is only about half as thick as that which lines the nose, having two or three rows of nuclei instead of four or five as in the nasal epithelium. Goblet cells and glands are less numerous, also. In contrast to the nasal and tracheal epithelium, the basement membrane is very thin. The lamina propria is thin and is attached to the periosteum.

Nasopharynx

In the *nasopharynx* both stratified squamous and pseudostratified ciliated columnar epithelium are found. The distribution of these two types here and in the other respiratory passages is cor-

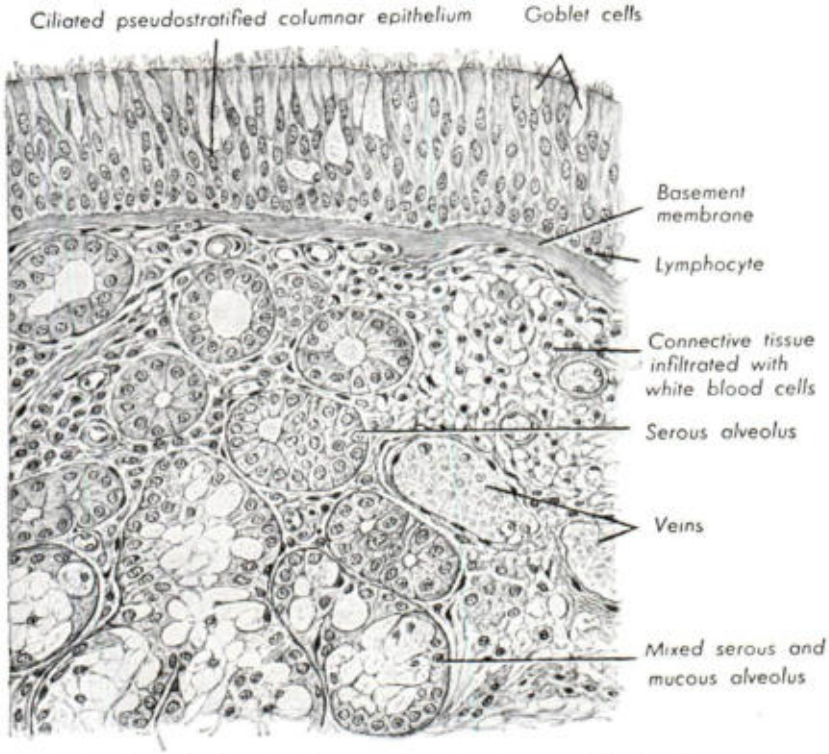

Fig. 307. Section through the epithelium and subjacent part of the lamina propria of inferior nasal concha. Human, 49 years old with no history of nasal inflammation. × 286.

related with the attrition to which the surface is subjected. When surfaces are frequently brought in contact with each other they are lined by stratified squamous epithelium. Those passages which usually remain open are lined by pseudostratified epithelium. Since, in the occlusion of the upper part of the naso- from the oropharynx, the soft palate and its appendage (the uvula) are brought in contact with the posterior wall of the nasopharynx, these surfaces are lined by stratified squamous epithelium. Other areas of the nasopharynx are lined by pseudostratified columnar epithelium.

In the deeper stratum of the subepithelial connective tissue in the posterior and lateral walls of the pharynx is a layer of elastic tissue whose fibers course largely in a longitudinal direction. It is closely applied to the subjacent muscle layer into which it sends fibers. Near the juncture with the esophagus the elastic layer disappears. In the superior lateral region, but not elsewhere in the pharynx a submucosa, which lies beneath the elastic stratum, can be distinguished.

Glands are present throughout the nasopharynx. In the regions covered by stratified squamous epithelium they are mucous and lie beneath the elastic layer. In the other areas they are mixed serous and mucous as in the other parts of the respiratory passages, and they lie more superficially.

Lymphoid tissue is especially abundant in the superior part of the nasopharynx. In addition to the general lymphoid infiltration of the connective tissue there are, in the posterior wall, aggregations of lymph nodules known as the pharyngeal tonsils. Lymphoid aggregations also occur behind the openings of the Eustachian tubes, forming the tubal tonsils.

The *muscle* of the pharynx is striated. It is deficient near the base of the skull. External to it there is a fibrosa.

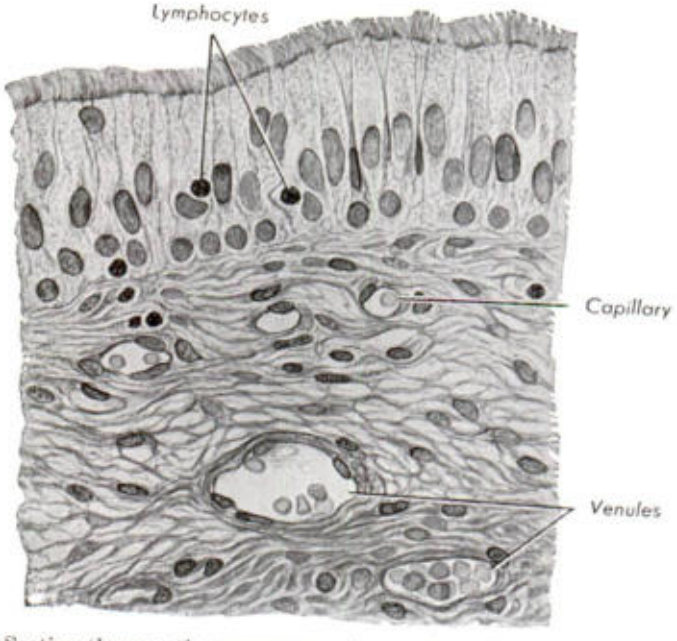

FIG. 308. Section through the mucous membrane of maxillary sinus of man. × 665.

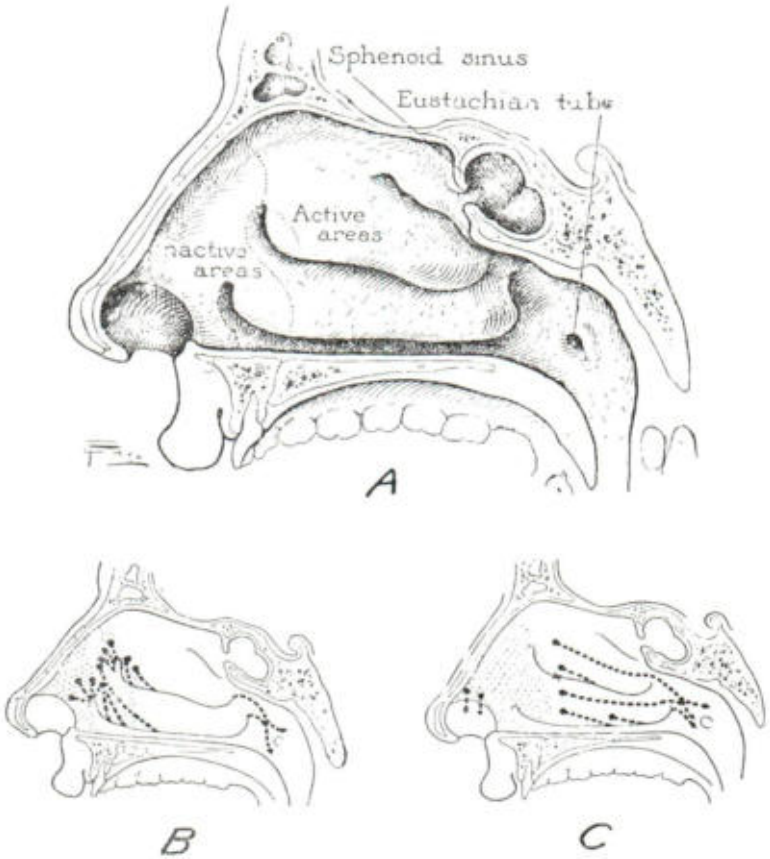

FIG. 309. *A*, The active and inactive areas of the lateral surface of the nasal cavity. *B*, The course of the mucous drainage of the inactive area. *C*, The course of the mucous drainage of the active area. (After Hilding.)

Ciliary Action. The epithelium lining the paranasal sinuses, nasal passages and nasopharynx is coated with a mucous film. Since the cilia of the paranasal sinuses beat toward the openings of these cavities into the nose, and since the cilia on the epithelium lining the nose and nasopharynx beat toward the oropharynx, there is a continuous movement of this mucous coat toward the oropharynx. The surfaces are thus freed of particulate matter which has impinged on and adhered to the mucous covering. In the posterior three-fourths of the nose, the movement is more rapid than it is in the anterior part, which has led to the characterization of these two portions as active and inactive parts (Fig. 309). The path of movement of the mucus is shown in Figure 309, taken from the work of Hilding.

THE LARYNX

The wall of the larynx consists essentially of a mucosa, a poorly defined submucosa, a series of irregular shaped cartilages connected by joints or fibroelastic membranes, and a group of intrinsic, skeletal muscles which act upon the cartilages.

Each lateral wall of the larynx has two prominent folds, and between these a

deep recess, or *ventricle*. The superior pair of folds, the *ventricular folds*, are also known as the false vocal cords; the inferior pair, or *vocal folds*, are the true vocal cords. The *epiglottis* is a broad, flat structure projecting upward from the anterior wall of the larynx. During swallowing, the epiglottis is depressed between the base of the tongue and the laryngeal orifice.

Two types of epithelium line the walls of the larynx, pseudostratified ciliated columnar and stratified squamous. These are distributed in accordance with the principle discussed in connection with the nasopharynx. At the juncture of these two types of epithelium, a third type—ciliated stratified columnar—is frequently present (Fig. 310). The wall of the aperture of the larynx, including the epiglottis down nearly as far as its tubercle or cushion, is lined by stratified squamous epithelium. This is succeeded by the ciliated pseudostratified type which lines the remainder of the laryngeal wall except for the vocal folds which have the stratified squamous variety.

The lamina propria is rich in elastic fibers. It is infiltrated with lymphocytes, and lymph nodules occur in the stroma of

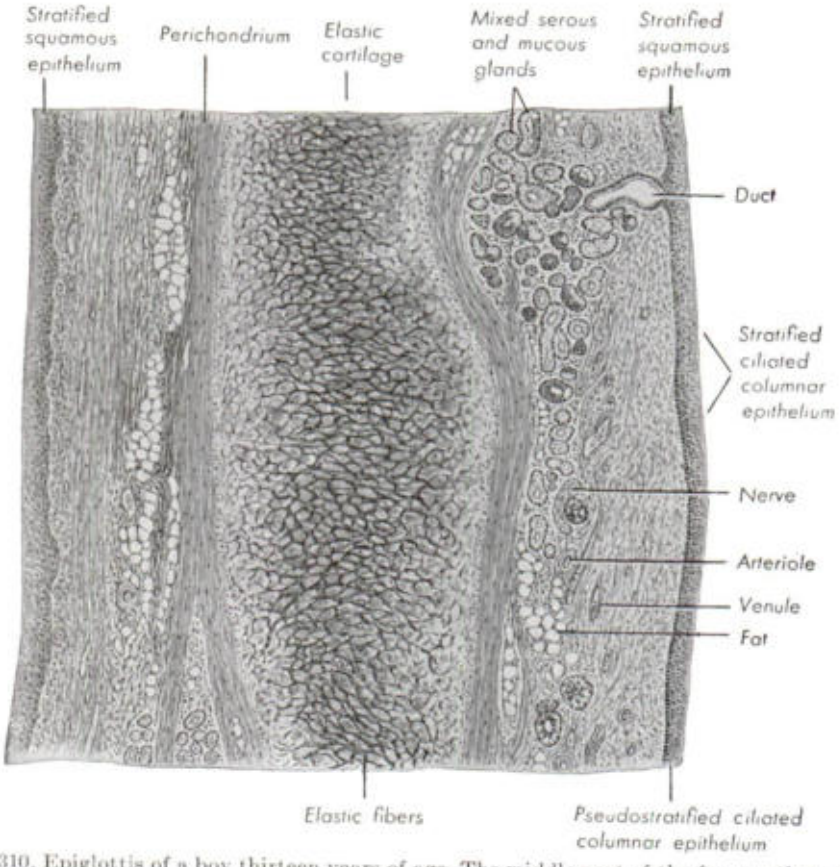

FIG. 310. Epiglottis of a boy thirteen years of age. The middle part of the free portion, cut in a sagittal plane, is illustrated. The lingual surface is at the left. Stained with elastin H, Delafield's hematoxylin and eosin. × 45.

the ventricles. There is no sharp demarcation between the mucosa and the submucosa. The latter is structurally less dense and more cellular than the lamina propria. Over the vocal folds and epiglottis the mucosa is more closely adherent to the underlying framework than in the other parts of the larynx. Mixed glands are present except in the vocal folds and are especially numerous in the ventricular folds (false vocal cords).

Most of the cartilages composing the framework of the larynx are hyaline. The epiglottic, the corniculate and the cuneiform cartilages are elastic and this variety of cartilage is said also to occur inconstantly in the apex and vocal process of each arytenoid cartilage. Calcification in the thyroid and cricoid cartilages begins in males during the second decade of life and at a somewhat later period in females.

THE TRACHEA AND CHIEF BRONCHI

The layers composing the larynx continue with certain modifications into the trachea and bronchi. The chief changes are in the cartilaginous framework and musculature.

The trachea and chief bronchi are lined by pseudostratified columnar ciliated epithelium which rests on a distinct basement membrane (Figs. 27 and 312). Goblet cells are numerous. The lamina propria contains many elastic fibers, which are especially numerous in the deeper zone of the lamina (membrana elastica interna). The submucosa consists of loose connective tissue, and contains fat and mixed glands, the latter often

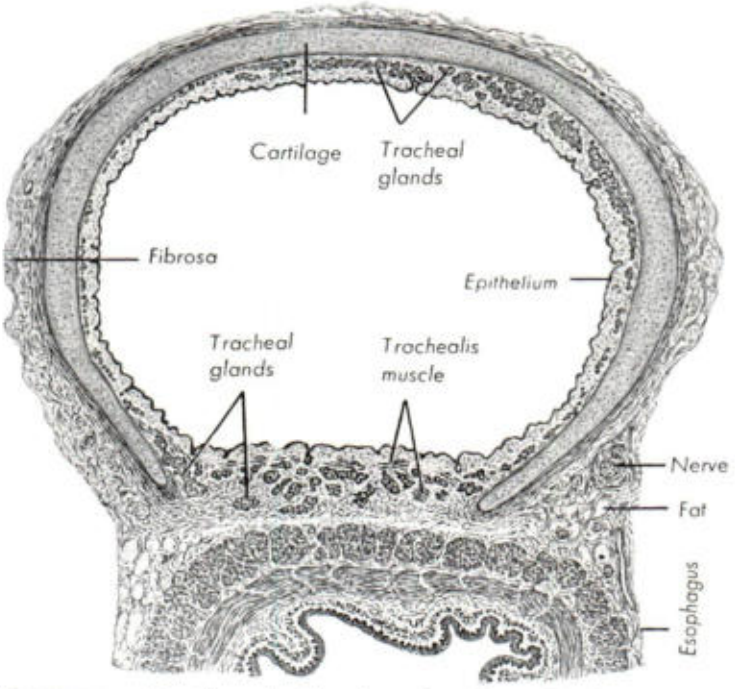

FIG. 311. Transverse section through mid-region of trachea and adjacent part of esophagus. The section is through a cartilage ring. Woman, 35 years of age. × 5.

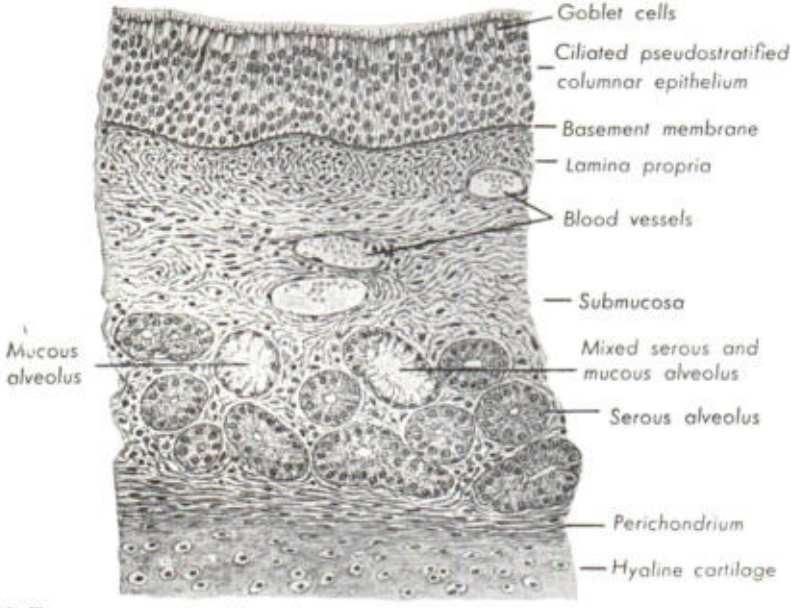

FIG. 312. Transverse section through anterior wall of trachea. Woman, 39 years of age. × 165 See also Figure 27.

penetrating into or even through the muscle layer (Fig. 311).

The framework of the trachea and chief bronchi consists of a series of regularly spaced, C-shaped hyaline cartilages. The open segment points dorsally. The cartilages vary in their width and thickness; their ends may bifurcate and they may have bars which fuse with an adjoining cartilage. The lowest tracheal cartilage (carinal cartilage) especially varies in shape. It has a ridge which runs dorsoventrally at the bottom of the trachea between the origins of the two bronchi.

A fibroelastic membrane which blends with the perichondrium of the cartilages extends across their open segments and connects adjacent cartilages to each other, thus forming a tube. Dorsally in the cartilage-free zone, smooth muscle is imbedded in the fibroelastic membrane. Most of these muscle fibers run transversely. Some, however, run longitudinally and obliquely. The transverse fibers attach largely to the inner surface of the ends of the cartilage rings and to a lesser extent to the intervening membrane. This is often called the "trachealis" muscle.

Localized areas of stratified squamous epithelium have been described in the trachea and bronchi of individuals suffering from chronic coughs. This change to a stratified squamous type of epithelium is possible because of the capacity of the pseudostratified epithelium of the respiratory passages to undergo metaplasia and change to the more resistant type when subjected to attrition, as has been pointed out in the description of the nasopharynx.

THE LUNGS

The lungs are paired structures which together with the mediastinum fill the thoracic cavity. On the right side, the lung is divided by two deep clefts into three lobes; on the left side, by a single deep cleft into two lobes. Entering the

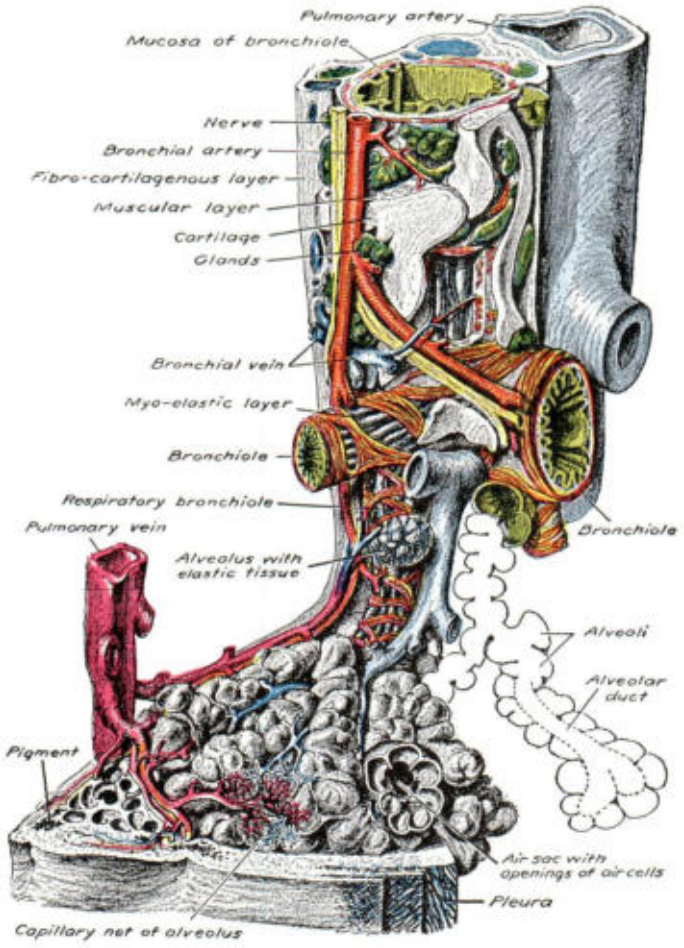

FIG. 313. A portion of a lung lobule. (After Braus from a reconstruction by A. Vierling.)

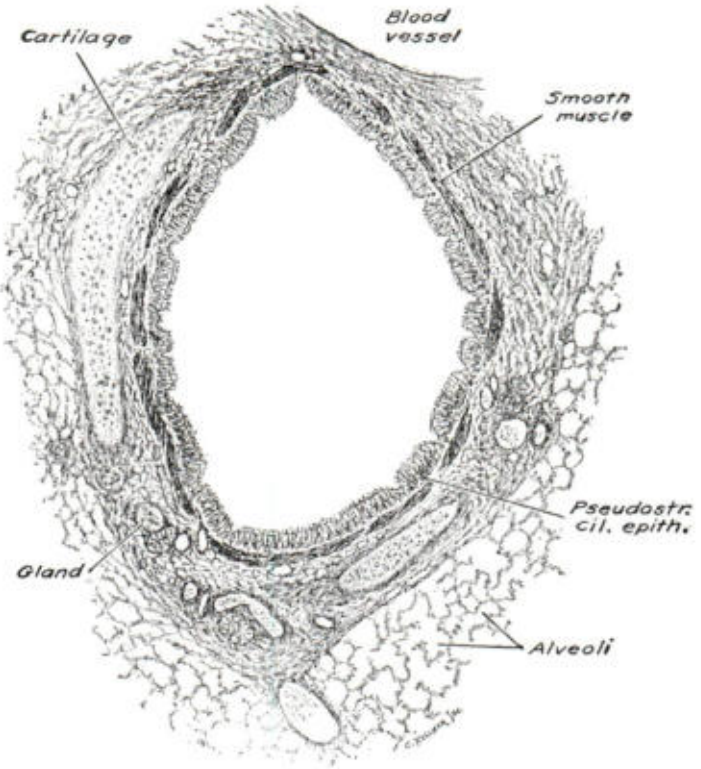

FIG. 314. Section through a bronchus approximately 2 mm. in diameter.

hilum, and forming the root of each lung is a bronchus, a pulmonary artery and vein, bronchial arteries and veins, lymphatics and nerves. These structures are imbedded in connective tissue.

The surface of the lungs is covered by a serous membrane, the visceral pleura which dips into the interlobar fissures, and covers the interlobar surfaces. At the hilum the visceral pleura becomes continuous with the parietal pleura.

The surface of the lungs is pinkish in early life but later becomes grayish in color due to the inspired particulate matter which is in the pulmonary tissue. On the surface of the lungs, small irregular shaped areas (1–2 cm.) are delineated by dark lines. Each of these areas is the base of a secondary lobule, the apex of which points toward the hilum of the lung. The dark lines are due to deposits of inspired particulate matter in the delicate interlobular connective tissue. Other more irregular and, usually, smaller secondary lobules lie deeper in the lungs. Each of the secondary lobules contains many primary lobules (pulmonary units).

PLAN OF THE LUNGS

A main bronchus, the primary division from the trachea, enters the root of each lung. There it divides, one secondary bronchus going to each lobe. Thus there are three branches to the right and two to the left lung. Each lobar bronchus divides, the number of branches varying from two to five in the different lobes. Each of these bronchi supplies a portion of a lobe known as a *segment*, or bronchopulmonary segment. There are ten segments in the right

lung and eight in the left lung. Although the segments are anatomical units somewhat separated by connective tissue planes, and are important from a surgical standpoint, they are not clearly demarcated on the surface of the lung.

The segmental bronchi then branch many times within the substance of the lungs, one small branch, a *bronchiole*, entering each secondary lobule. Within the secondary lobule, there are further subdivisions, the smallest being the *terminal bronchioles* (0.35–0.5 mm. in diameter). These are the terminal segments of the purely conducting division of the respiratory system. Each terminal bronchiole then divides into two or more *respiratory bronchioles*. Each respiratory bronchiole undergoes further divisions, each of which branches into *alveolar ducts* (ductuli alveolares). These in turn may further branch, terminating after a relatively long course in *alveolar sacs* (sacculi alveolares, air sacs). The smallest units or subdivisions are the *pulmonary alveoli* (air cells), small outpocketings which form the lining of the alveolar ducts and alveolar sacs, and in whose walls the interchange of gases between air and blood takes place.

Pulmonary alveoli are confined to the respiratory division of the lung. They first appear, in the branching system outlined above, in the respiratory bronchioles, which have characteristics of both the conducting and respiratory divisions. The alveolar ducts and alveolar sacs have continuous pulmonary alveoli forming their walls. The pulmonary alveoli of one system interdigitate with those of neighboring systems, so that two adjacent alveoli have a common capillary bed, an arrangement which gives a maximum surface area for the exchange of gases.

There appears to be considerable variation in the number of branches which each system of tubules may give off. The architecture, however, is such as to give but a relatively small increase in volume in the conducting division, with a very great increase in the volume of the respiratory division (the bellows portion of the lung).

The primary lobule (lung unit) has been variously defined. A respiratory bronchiole and its subdivisions is a commonly accepted unit. Miller considers that the lobule or lung unit is composed of an alveolar duct and its subdivisions, together with its blood and lymph vessels and nerves. This component is supplied solely by blood from the pulmonary artery.

Structure of the Lungs

The Conducting Division. Modifications in the structure of the bronchi appear with the first branching in the

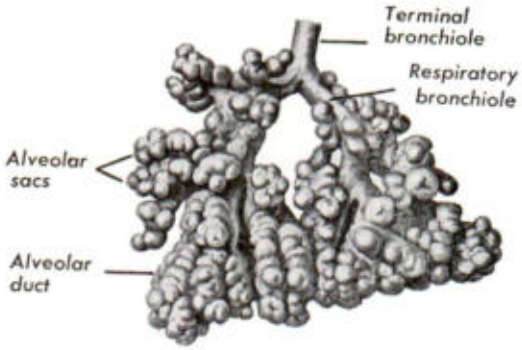

Fig. 315. Cast (Wood's metal) of a terminal bronchiole and its branches. (Braus after Loeschcke.)

root of the lungs and continue to take place with each subsequent branching. A comparison of a terminal segment (terminal bronchiole) with a main bronchus reveals how great these changes have been. The series of changes, however, is gradual and so the nature of the modifications which occur will be discussed rather than describing bronchi of different diameters.

The epithelium, although continuing as a pseudostratified columnar type through most of the conducting division, nevertheless decreases in height as the tubes become progressively smaller. In the terminal bronchiole it becomes a single layer of columnar or cuboidal cells which, however, still bear cilia (Fig. 317). The number both of the goblet cells and glands (sero-mucous) becomes fewer and both cease to be present before the terminal bronchioles are reached.

The lamina propria also decreases in thickness and elastic fibers become relatively more numerous. The layer of smooth muscle becomes a prominent

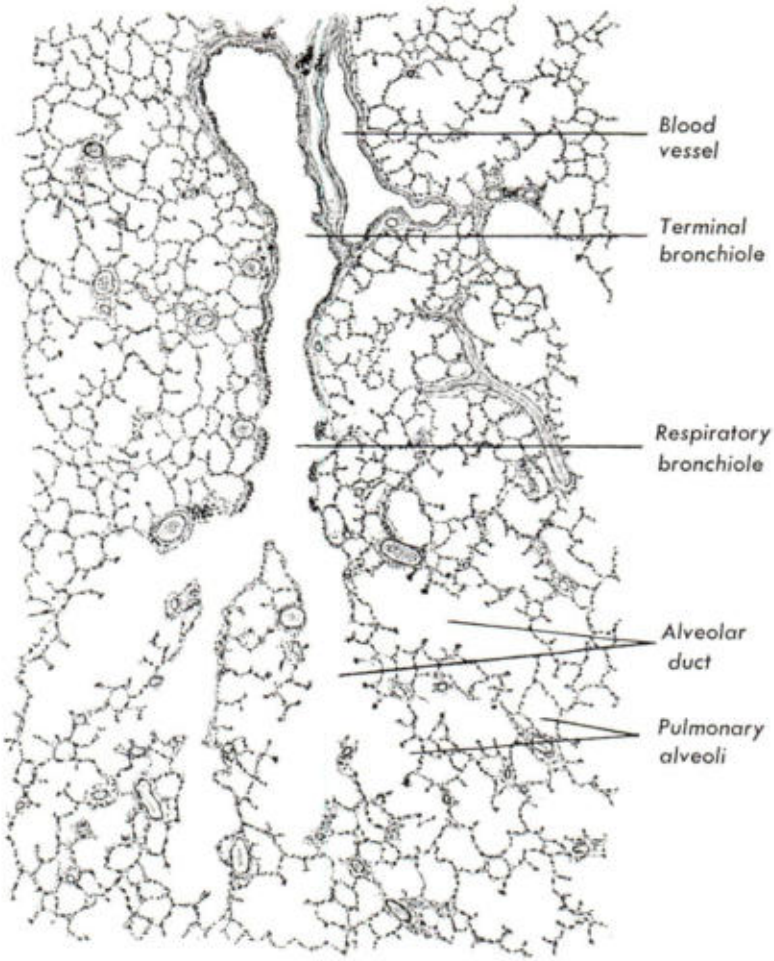

FIG. 316. Section through a terminal bronchiole together with part of the system arising from it. Camera lucida drawing. Human lung. × 19.

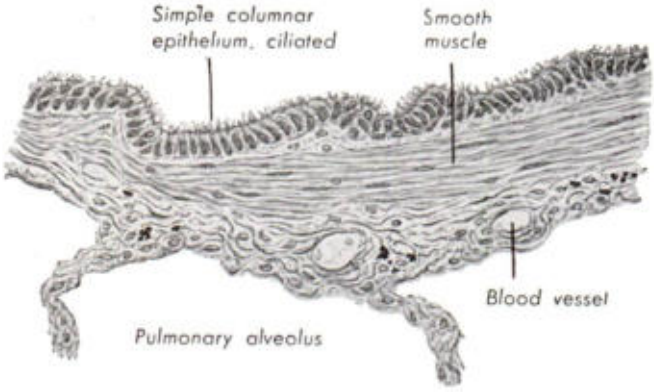

FIG. 317. Transverse section through a portion of the wall of a terminal bronchiole and adjacent pulmonary alveoli. Human lung. × 320.

component of the wall, reaching its greatest relative amount in the terminal bronchiole (Fig. 317). Its fibers, which pursue a spiral course, become closely associated with elastic fibers of the lamina propria thereby forming a myoelastic layer which is the most prominent layer of the terminal bronchiole.

The outer or fibroelastic, cartilaginous layer, also shows pronounced changes. With the first branching of the primary bronchi the cartilage ceases to occur as C-shaped rings and forms irregularly shaped plates, which have no regular arrangement. These plates decrease in size as the bronchi branch and become smaller and finally disappear in tubes of about one millimeter in diameter. Elastic fibers are said to be present in the periphery of the smaller plates.

Thus the terminal segment of the conducting division, the terminal bronchiole (about 0.5 mm. in diameter), is lined by a simple columnar or cuboidal ciliated epithelium free from goblet cells. No glands are present. The elastic tissue and muscle are closely associated and the amount of muscle is relatively increased. Cartilage is absent.

Due to the absence of cartilage and to the presence of elastic and muscular tissue, longitudinal folds form in the mucosa and are prominent in the terminal and somewhat larger bronchioles.

It is significant that the cilia extend farther down the tubes than do the mucous secreting elements. If the reverse were the case, accumulations of mucus might occlude the tubules.

The Respiratory Division. Each terminal bronchiole divides into two or more respiratory bronchioles (Fig. 315) which may further branch so that respiratory bronchioles of the first (I) and second (II) order are formed. The *respiratory bronchioles* bear pulmonary alveoli on those portions of their walls which are not in contact with the accompanying pulmonary artery (Fig. 318). The alveoli are few in number proximally but become more numerous distally. Between the alveoli, the wall of the respiratory bronchiole is lined in the proximal part by ciliated cuboidal epithelium. In the distal part cilia are absent. Smooth muscle and elastic fibers are very well developed in the respiratory bronchiole although they do not form as thick a layer as in the terminal bronchiole. Collagenous and reticular fibers are also present. The smooth muscle bundles and the elastic fibers course obliquely, i.e., in a spiral direction, but they branch and anastomose thus forming a loose elastic and con-

tractile network. In sections through the opening of an alveolus into a respiratory bronchiole the muscle fibers are seen to be cut obliquely or transversely (Fig. 318). The respiratory bronchioles branch into alveolar ducts.

Alveolar ducts are long, and branch repeatedly. Their walls are formed by pulmonary alveoli without intervening patches of epithelium. Alveolar sacs may also occur along them. Small smooth muscle bundles which branch and anastomose are present. They are concentrated around the openings of the alveoli, so that in a transverse section through an alveolar duct, the muscle bundles (Fig. 319) mark the circular extent of the lumen. Reticular, elastic and delicate collagenous fibers are also present. The alveolar ducts terminate in a variable number of alveolar sacs.

The *alveolar sacs* are extremely thin-walled structures which are closely beset with pulmonary alveoli. The walls of the alveolar sacs contain no smooth muscle but have elastic and reticular fibers. As in the alveolar ducts there is no epithelial lining other than that of the alveoli (see below under pulmonary alveoli).

The Pulmonary Alveoli

Pulmonary alveoli (air cells) are cup-shaped structures through whose thin walls the interchange of gas between the blood and air takes place. The open end or mouth of each alveolus opens into the lumen of a respiratory bronchiole, an alveolar duct or an alveolar sac (Fig. 313).

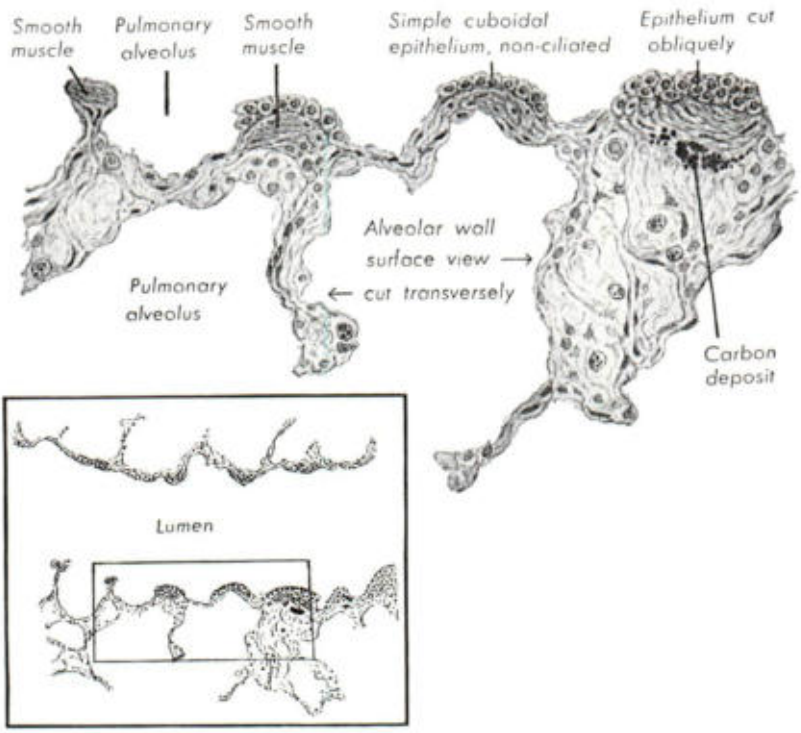

FIG. 318. Longitudinal section through wall of respiratory bronchiole near juncture with alveolar duct. Human lung. × 335. The insert at the lower left shows the orientation of that part of the wall which is illustrated.

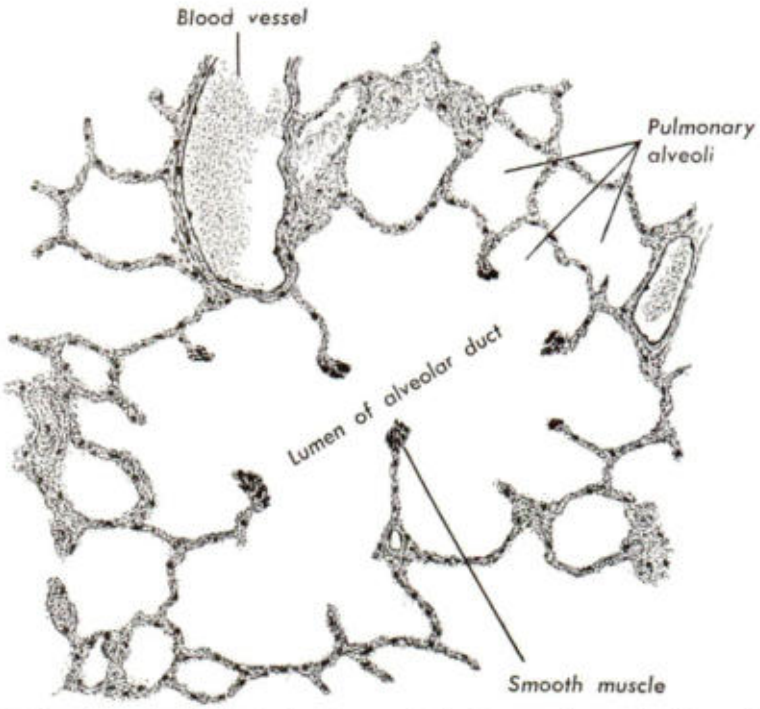

FIG. 319. Section of an alveolar duct showing especially the smooth muscle. Human lung. × 78.

Because of the interdigitating arrangement of the alveoli, a single wall, or *interalveolar septum*, is usually formed between adjacent alveoli.

A rich plexus of capillaries forms a large part of the alveolar wall. The capillaries have wide lumina, and they anastomose freely, thus forming a vascular network whose area exceeds that of the intervening spaces (Fig. 324). In the meshes of this network are interlacing fine elastic and reticular fibers, the arrangement of which is such as to permit an increase and decrease in the area of the wall.

Numerous nuclei are present in the alveolar walls. These can be identified as belonging to at least three types of cells. They are: endothelial cells of the capillaries; connective tissue cells, some of which have the potency of becoming phagocytic; and thin epithelial cells forming the lining of the alveoli.

The long-standing controversy concerning the nature of the alveolar lining, i.e., whether or not there is an epithelial lining, has been resolved satisfactorily by means of electron microscopy.

For many years there have been two views profoundly at variance with each other. Many investigators have held that the walls of the air cells have no epithelial lining, the connective tissue and other structures forming the wall being directly exposed to the alveolar gases. According to the other view, the air cells have a lining of squamous cells. Opinions differed, however, in regard to the presence or absence of nuclei in all of these cells. The lining has often been described as being composed of broad, extremely thin, nonnucleated plates interspersed among somewhat thicker nucleated cells. This concept of an alveolar lining arose through the use of methods by which cell boundaries are impregnated with silver, and it is probable that some of the cells outlined by the silver deposits were endothelial rather than epithelial cells. The fact that the eminent

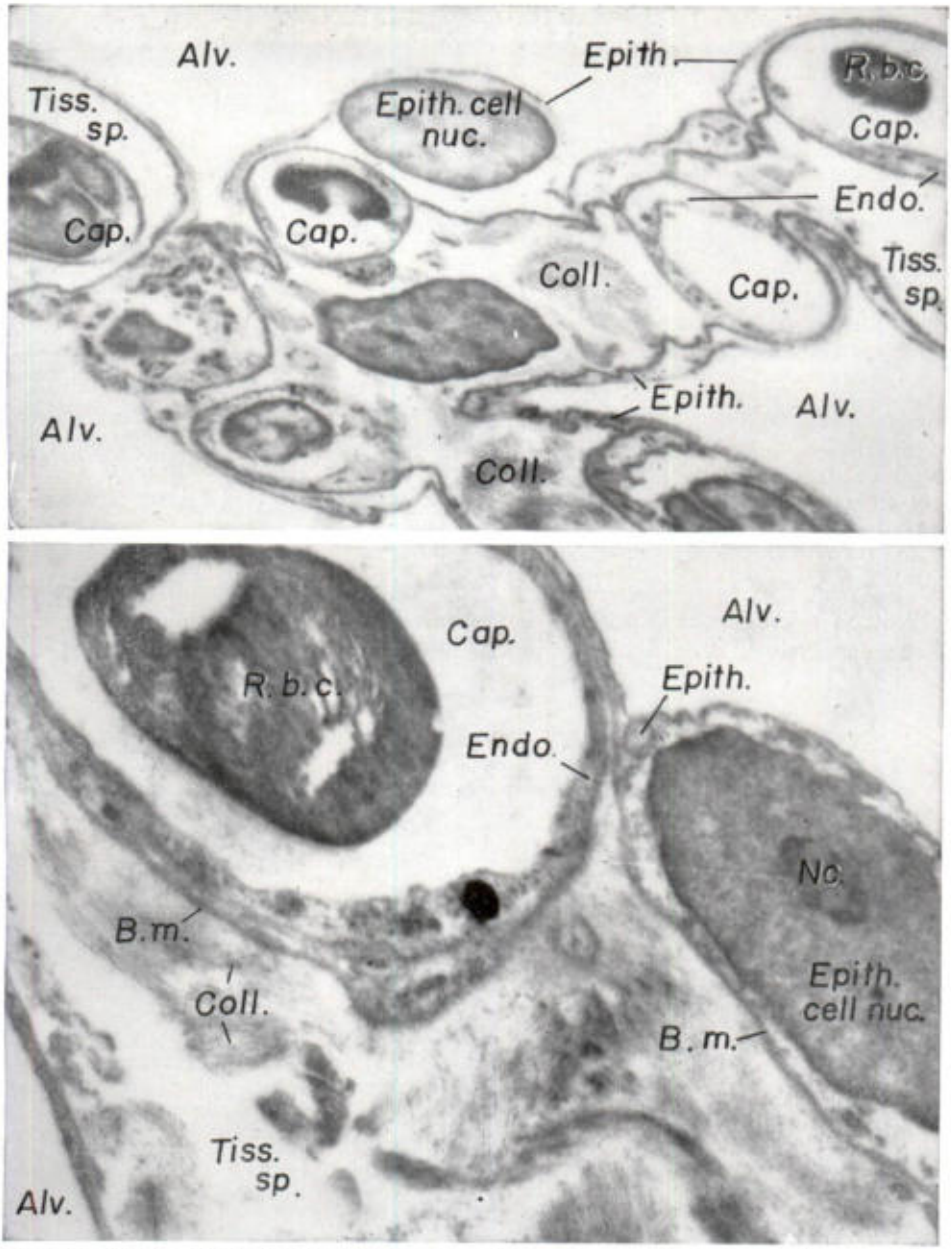

FIG. 320. Electron micrographs of lung. *Upper figure* shows parts of three pulmonary alveoli and their respective interalveolar septa. Rabbit lung. × 4,900. *Lower figure*, at higher magnification, shows the relationship of an alveolar epithelial cell and its underlying basement membrane to the capillary endothelium and its basement membrane. Note the thinness of the epithelial cell cytoplasm as it extends over the surface of the capillary, also, the thin layer of epithelial cell cytoplasm which lines the opposite wall. Human lung. × 14,300. (Courtesy of Dr. F. N. Low.)

Alv., alveolus; *Epith.*, alveolar epithelial cell cytoplasm; *Cap.*, capillary; *Endo.*, endothelial cell cytoplasm; *R.b.c.*, red blood corpuscle; *Coll.* collagen; *Tiss. sp.*, Tissue space; *B.m.*, basement membrane.

anatomist, Kolliker, sponsored the concept of an alveolar epithelial lining composed of interspersed nucleated cells and nonnucleated plates at least partially explains the heretofore general presentation of this concept in histology texts. More recently, the proponents of the epithelial lining theory have been inclined to believe that all the cells are nucleated. Support for this view is found in pathological material, where, in edematous conditions, a distinct layer of epithelium, separate from the connective tissue and capillary framework of the alveolar walls, can be seen. Also, from the standpoint of embryological development, it is difficult to conceive of air cells devoid of an epithelial layer. Failure to demonstrate the nature of the alveolar lining in the normal lung with the light microscope can be explained by the extreme thinness of the epithelial cells (at or beyond the resolving power of the light microscope).

Electron micrographs (Fig. 320) have shown conclusively that there is an alveolar lining composed of very thin, attenuated epithelial cells. Except in the region of the nuclei, the cells are so thin (about 0.2 μ in the human) as to be indistinguishable in ordinary sections. Underlying the epithelium is a thin, apparently homogeneous basement membrane. Wherever a capillary lies close to the surface of the alveolar wall, the basement membrane of the capillary and that of the alveolar epithelium become closely approximated (Fig. 320). This arrangement results in a minimum of tissue intervening between the air in the alveolus and the blood in the capillary, thus facilitating the gaseous exchange between the two media.

Small round or oval openings in the alveolar wall are sometimes seen, particularly in thick sections of the lung

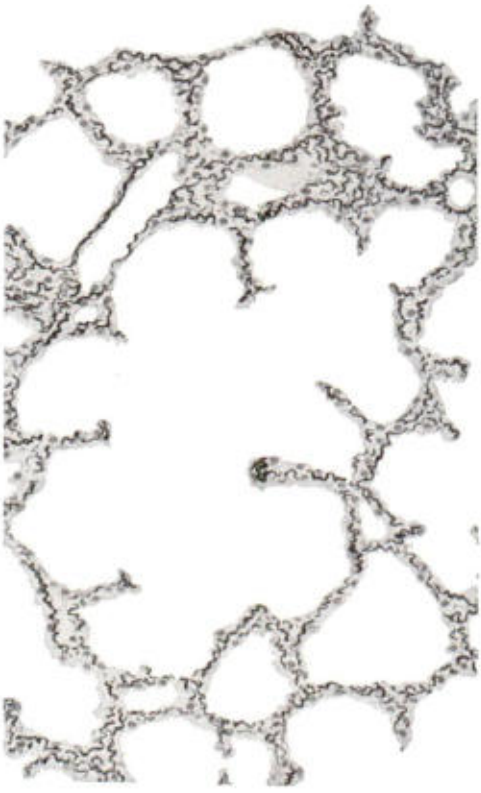

FIG. 321. Section through an alveolar duct showing the reticular fibers (black). Lung of rhesus monkey. Foot-Hortega silver carbonate method and Orth's carmine. × 310.

(Fig. 323). These are the so-called *alveolar pores*, which provide direct communication between adjacent air cells. In certain pathological conditions they are quite prominent, frequently containing strands of fibrin which extend from one alveolus into another. They occur between capillaries, and are often bordered by delicate elastic and reticular fibers. They are said to increase in number with age.

In spite of the fact that alveolar pores have been described repeatedly in all species studied, including man, their existence in the normal lung has been questioned by some investigators, who have interpreted their appearance as an artifact of fixation. They have not as yet been demonstrated in electron micrographs of lung tissue. The fact that they can always be found in histological sections of the most carefully prepared material, either with or without the injection of the bronchi with fixing fluids or gelatine, suggests that they are normal structures. As regards their functional significance, it has been suggested that they would tend to prevent overdistension or collapse of the alveoli if a small bronchiole becomes occluded.

INSPIRED PARTICULATE MATTER

Many particles of dust are present in the air which is inspired, and the number of particles is greatly increased in smoky regions and in industries or regions in which the air is laden with dust. Many of the inspired particles are removed by their adhering to the vibrissae in the nasal vestibule and by lodging on the mucous film of the respiratory portion of the nose, the nasopharynx, larynx and trachea. The mucous film and adherent particles are constantly being moved by ciliary action to the oropharynx from where

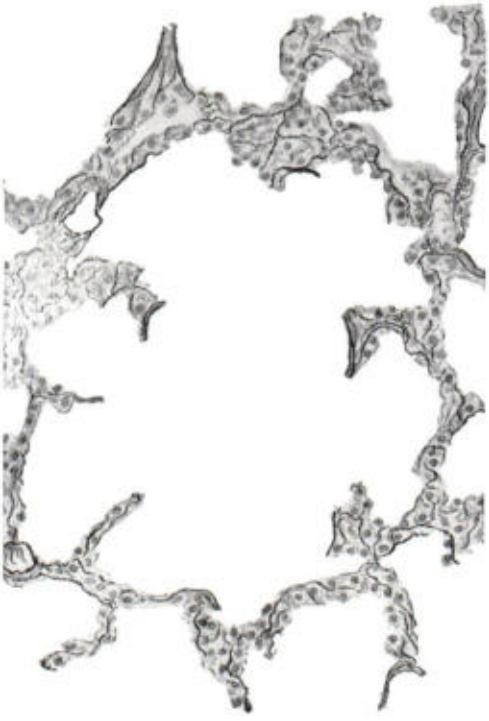

FIG. 322. Section through an alveolar duct showing elastic tissue (black). Lung of rhesus monkey. Weigert's elastic tissue stain and Orth's carmine. × 310.

they are either expectorated or swallowed. These structures thus serve not only to humidify and warm the air but, also, in the removal of particulate matter.

The bronchi and bronchioles in the lung also play a very important role in the removal of particulate matter. As repeated branchings take place, with only a slight increase in cross section area (volume), the surface of the walls is greatly increased. Due to the increased area, particles would be more likely to impinge on the mucous coat than in the upper larger passages. For this lower part of the conducting system Hilding has proposed the name "bronchiolar filter." As in the trachea and nasal passages, ciliary action carries the particles lodged on the mucous film to the oropharynx.

Many of the smaller particles, however, reach the alveoli of the lungs and their removal by ciliary action is not possible. A different mechanism operates to dispose of them. The particles are removed by alveolar phagocytes, called *dust cells*, because of the particulate matter which they contain (Fig. 323). These are phagocytic cells which appear to be identical to the macrophages of loose connective tissue elsewhere in the body. Thus, they are members of the reticulo-endothelial system. They may be found either within the alveolar wall or resting free on the alveolar surface of the wall.

The dust cells phagocytize the particles on the alveolar walls and deposit them, perhaps after passage through several cells, in the connective tissue of the lungs or in the lymphoid tissue. In this transport of the particles, the lymph vessels play an important role. The connective tissue may increase very greatly in amount (fibrosis) and encapsulate masses of the particles. The degree of the connective tissue overgrowth is influenced by the type of the inhaled dust. Siliceous dust and asbestos, for instance, cause a marked fibrosis, whereas coal dust even

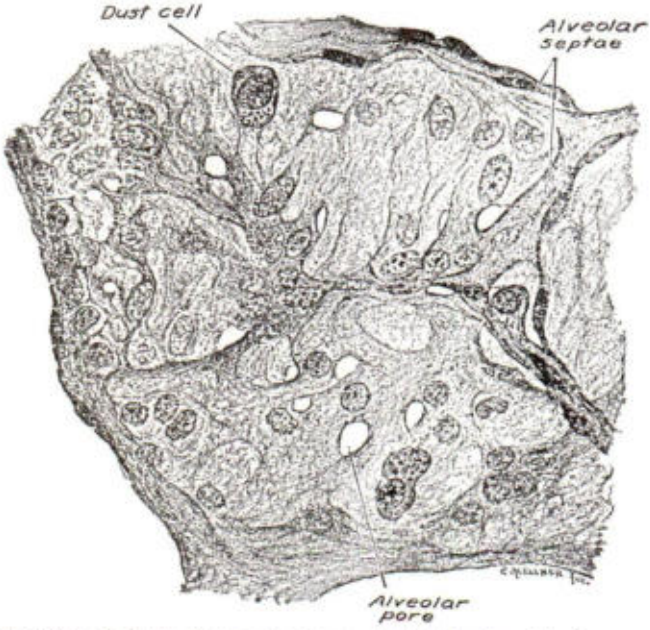

FIG. 323. Surface view of the walls of pulmonary alveoli showing alveolar pores. From a thick (50 μ) section of a lung of a rhesus monkey.

when present in large amounts induces a very slight or no reaction of the connective tissue. The condition which results from the dust is termed pneumoconiosis, special names (anthracosis, silicosis, asbestosis, etc.) being used to designate the reactions to the various types of dust.

THE PLEURA

The pleura is a serous membrane which completely lines the pleural cavity. The visceral portion invests and is closely adherent to the lungs. It is continuous with the parietal pleura at the root of the lungs. Beneath the mesothelial lining of the pleura is a fibroelastic stroma which contains smooth muscle. In the stroma are blood capillaries and a rich plexus of lymph vessels.

Continuous with the stroma of the pleura are delicate septa which extend between the secondary lobules, anastomosing with each other and with the peribronchial connective tissue. They are composed of fibrous and elastic connective tissue and contain smooth muscle, lymph and blood vessels and histiocytes.

THE BLOOD AND LYMPH CIRCULATION OF THE LUNGS

Blood Vessels. The lungs receive blood from two sources, venous blood, to be purified, through the *pulmonary arteries*, and arterial blood, for the nutrition of the walls of the conducting system and blood vessels, through the *bronchial arteries*. This blood is returned to the systemic circulation through the pulmonary and bronchial veins. The former, in contrast to the latter, are devoid of valves.

The pulmonary artery enters the lung with the corresponding chief bronchus. It branches with and follows the bronchial tree to the termination of the respiratory bronchioles. As the alveolar duct is reached the artery gives rise to the capillary plexus in the walls of the alveoli (Figs. 324 and 326). Veins arise from these capillaries. These do not immediately join the bronchioles but course in the septa. Later they join the bronchioles and course along them to the root of the lung.

The bronchial arteries likewise accompany the bronchi. Along their course

FIG. 324. Injected lung showing capillaries in walls of pulmonary alveoli. Dog. Preparation by Mr. Kellner. × 400.

they give origin to capillaries which supply the walls of the bronchi, arteries and veins and the peribronchial and septal connective tissue. The bronchial arteries do not extend beyond the respiratory bronchioles. The capillaries arising from them in that segment anastomose with the pulmonary capillary plexus. Part of the blood carried by the bronchial arteries passes into the pulmonary veins through this anastomosis. The remainder returns through the bronchial veins.

The pleura, in man, is supplied by the bronchial arteries. This blood returns through the pulmonary veins.

Lymphatics. There are two sets of lymph vessels in the lung, a superficial set which is in the pleura and a deep set which is in the substance of the lung. The superficial (pleural) lymph vessels are numerous. The larger vessels lie near the juncture of the interlobular septa with the pleura and thus outline the secondary lobules (Fig. 325). The pleural plexus receives afferent vessels from the deep plexus. Valves, which are numerous throughout the superficial plexus, prevent the passage of lymph from the superficial to the deep plexus. The efferent vessels of the superficial plexus course in the septa but later approach and course along the bronchi to the root of the lung. The deep plexus begins in the alveolar ducts and in the connective tissue accompanying the blood vessels and bronchioles. The lymphatics follow the bronchi and blood vessels to the hilum, where they anastomose with vessels from the superficial plexus. They are devoid of valves except for an occasional one in the larger trunks.

Lymphoid tissue in the form of lymph nodes or solitary nodules with germinal centers occurs along the larger bronchial branches. The amount of lymphoid tissue is said to increase with age. It becomes infiltrated with the inspired particulate matter. There is also a lymphocytic infiltration of the walls of the bronchi.

Nerves. The bronchi are accompanied by sympathetic and parasympathetic nerves. The latter come from the vagus. Ganglion cells are present in the walls of

FIG. 325. Lymphatics of pleura; the area outlined by the large (peripheral) vessels is the base of a secondary lung lobule. × 8. (Miller.)

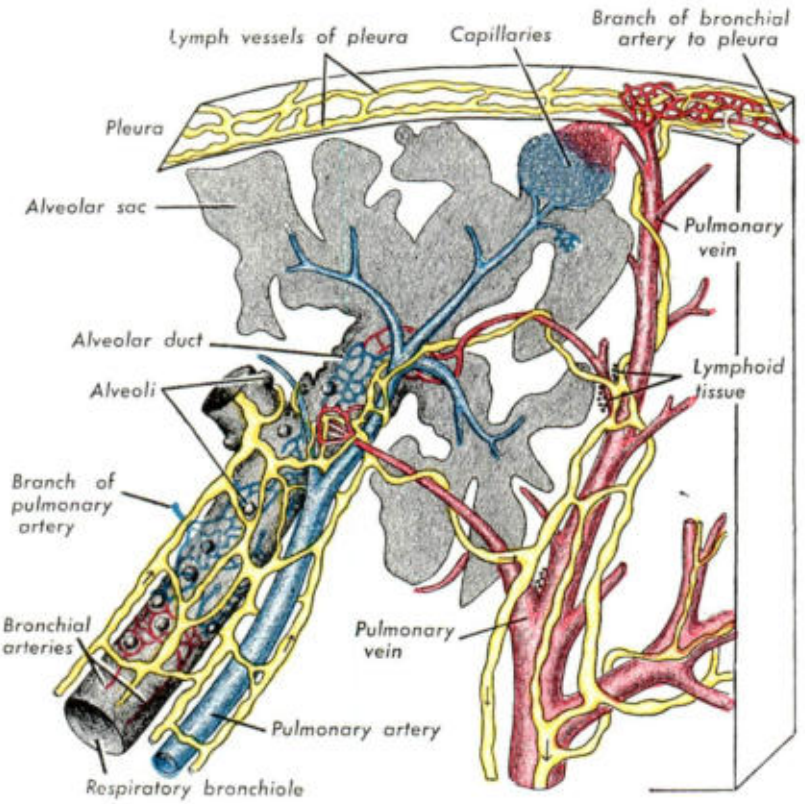

FIG. 326. The blood supply and lymph drainage of a primary lung lobule and the pleura. The primary lung lobule is defined by Miller as an alveolar duct and its branches. A respiratory bronchiole is also shown in this figure. (From Miller, The Lung. Charles C Thomas.)

the bronchi. The vagus fibers cause constriction of the air tubes; the sympathetic fibers, dilation.

CHANGES IN THE SIZE AND FORM OF THE PULMONARY STRUCTURES IN RESPIRATION

In inspiration the tubes of the conducting division increase in length and in diameter. The structure of the walls of the bronchi and bronchioles is such as to permit and aid in these changes. The external layer is elastic and the cartilages are separated from each other; the muscle fibers pursue a spiral course, and the elastic tissue forms a network. Progressive waves of contraction and relaxation (peristalsis) have been shown to take place in the larger bronchi and probably occur also in the smaller bronchi. This movement aids in the expulsion of mucus and foreign matter.

The changes which take place in the respiratory division during respiration are probably greater than those of the conducting division. In a functional consideration, the alveolar ducts and sacs can be grouped together. They have been termed the "bellows part" of the lungs. In inspiration the alveolar ducts and sacs are expanded. In this expansion, the entrance or base of the alveoli are enlarged and the alveoli become flattened. Their surface is probably not increased, since such an increase would attenuate the capillaries and consequently slow the blood flow.

When the pleural cavity is opened, the lung collapses. This is due both to the smooth muscle and to the elastic tissue. Since in the collapsed lung the bronchi and especially the bronchioles are greatly shortened, sections of them give the erroneous impression that their smooth muscle fibers are circularly instead of obliquely (spirally) arranged.

DEVELOPMENT OF THE RESPIRATORY SYSTEM

The epithelium of the respiratory system develops from entoderm. The rudiment of the larynx, trachea and lungs appears as a ventral, groove-like evagination in the floor of the primitive pharynx. This evagination grows caudally, becomes constricted off from the digestive tube except in its superior part, and its apex bifurcates. The portion which does not bifurcate together with the surrounding mesenchyme forms the larynx and trachea. The bifurcated part is the anlage of the epithelium and epithelial derivatives of the bronchial system and lungs, the mesenchyme forming the other portions of the walls. The right branch subdivides into three branches corresponding to the three lobes of the future right lung, and the left into two, corresponding to two lobes of the left lung. By repeated branching of these tubules, the entire bronchial system of the lungs is formed. This process is similar to the development of a compound gland. The last to develop are the respiratory bronchioles and the alveolar ducts and sacs, structures which are characteristic of the lung. During fetal life the alveoli are lined by a cuboidal epithelium. At the time of birth, these cells become extremely thin, so thin in fact that film-like peripheral portions of the cells are masked by the underlying denser tissue.

Following birth as well as during gestation there is a continued growth of the lung. The bronchioles increase in length and new respiratory units are formed. The increase in the length of the bronchioles, while due to a small degree to an interstitial growth of the existing bronchioles, is, in the main, due to the transformation of the respiratory bronchioles, alveolar ducts and alveolar sacs into bronchioles. The lengthening of the respiratory division takes place by a terminal budding and growth of the air sacs. Their growth is thus much like that which occurs in a tree.

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XVIII

THE URINARY SYSTEM

THE KIDNEY

The kidney is a compound tubular gland concerned with the excretion of urine. It is enclosed by a firm connective tissue capsule composed of collagenous fibers and a few elastic fibers. In many of the lower animals and in the human fetus, septa extend from the capsule into the gland, dividing it into a number of lobes or *renculi*. In human adults, the lobated character is obliterated by the great reduction of the interstitial connective tissue and the apparent blending of the peripheral parts of the different lobes. Rarely the fetal divisions persist in adult life, such a kidney being known as a "lobated kidney." In some animals, as the guinea pig and rabbit, the entire kidney consists of a single lobe.

On the mesially directed border of the kidney is a depression known as the *hilum*. This serves as the point of entrance for the *renal artery* and of exit for the *renal vein* and *ureter*.

On section, the kidney shows a parenchyma of *cortex* and *medulla* partially surrounding a cavity—the *renal sinus*—which opens at the hilum (Fig. 327). The renal sinus contains: (1) the upper, expanded portion of the ureter which is known as the *renal pelvis*; (2) subdivisions of the pelvis which form two or three *major calyces* and about eight *minor calyces*; (3) branches of the renal arteries, veins and nerves; (4) loose connective tissue and fat. In fresh, unfixed kidneys, the outer or cortical zone of the parenchyma is dark brown in color and granular in appearance. It contains many convoluted tubules and numerous round, reddish bodies, the *renal* or *Malpighian corpuscles*, which are barely visible to the unaided eye. The inner, medullary zone of the parenchyma is radially striated in appearance because its tubular and vascular elements run in parallel radial lines.

The cortex not only forms the outer zone of the kidney, but at intervals plugs of cortical tissue, the *columns of Bertini* or *renal columns*, penetrate the whole depth of the medulla. The main medullary mass consists of eight to eighteen *medullary* or *Malpighian pyramids*, the number corresponding to the number of lobes in the fetal kidney. The broad base of each pyramid is in contact with the cortex and the rounded apex projects into a minor calyx (Fig. 327). As a rule two or sometimes three pyramids unite to form a single papilla, hence the number of papillae is less than that of the pyramids. The tip of the papillary surface presents a sieve-like appearance, *area cribrosa*, due to the presence of 10–25 pores or *foramina papillaria* which are the openings of the uriniferous ducts. Even in gross preparations two zones may be distinguished in the medulla: an outer, more deeply colored zone in contact with the cortex, and an inner, somewhat paler papillary zone.

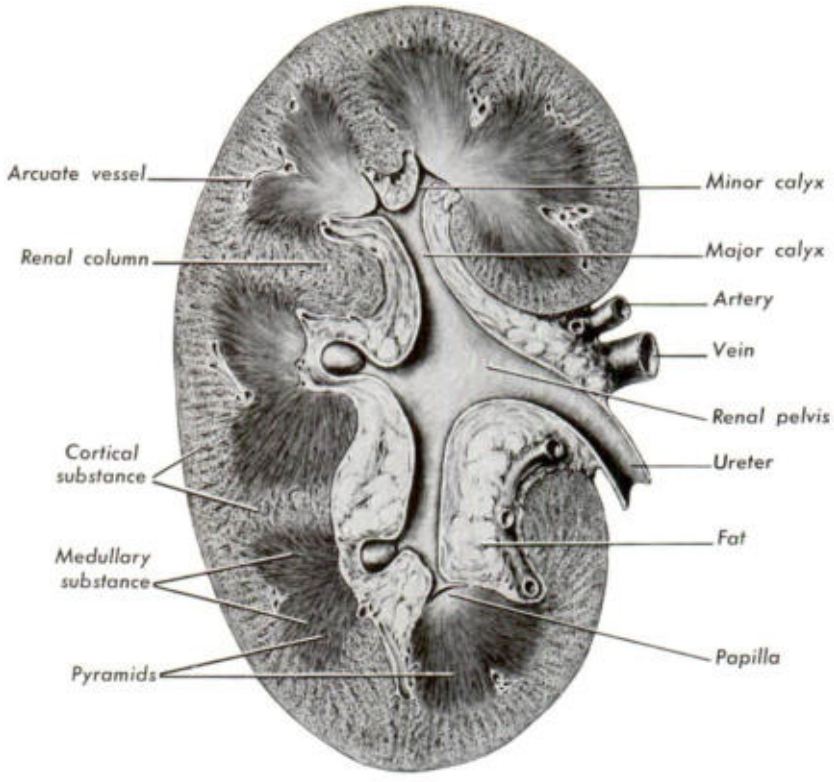

FIG. 327. Longitudinal section of a human kidney. Natural size

The cortical region is subdivided into many radiating slender columns composed of straight tubules alternating with regions containing glomeruli and convoluted tubules (Figs. 329, 330). The columns of straight tubules radiate outward from the medulla and hence they are named *pars radiata* or *medullary rays*. They are also known as the *processes of Ferrein*. The regions between the rays contain glomeruli and convoluted tubules, and are called *pars convoluta* or *cortical labyrinths*.

THE URINIFEROUS TUBULES

The parenchyma of the kidney consists of closely packed uriniferous tubules between which are blood vessels and a scanty amount of interstitial connective tissue. As in any other gland two types of tubules are distinguished: the terminal or "secretory" tubules concerned with the formation of the urine, and the collecting tubules which are the ducts conveying the urine to the pelvis and ureter. The terminal tubule constitutes a structural and functional unit to which Braus has given the name *nephron*. Each nephron is long (30 to 40 mm.), unbranched, and for the greater part of its course is highly tortuous forming compact convoluted masses (Fig. 328). Each nephron begins in a spherical expansion, known as *Bowman's capsule*, which encloses a tuft of capillaries, the *glomerulus*. Bowman's capsule and the glomerulus together form the *Malpighian* or *renal corpuscle* (Fig. 331).

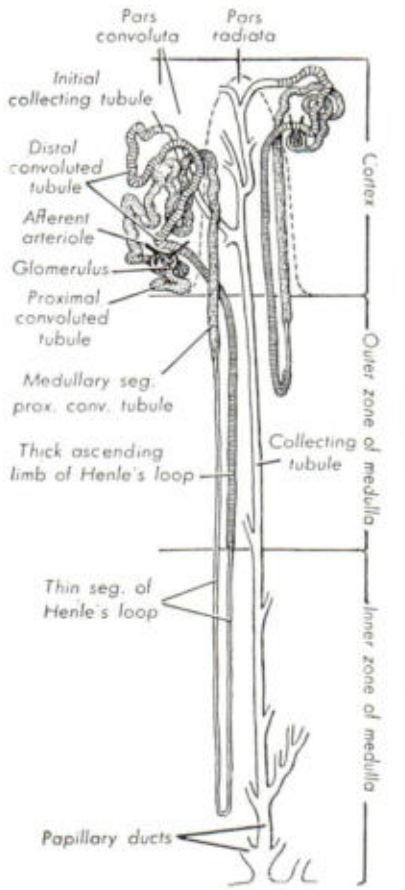

FIG. 328. Diagram of the subdivisions of the uriniferous tubules to show their relations and locations in a section extending from the capsule to the tip of a renal pyramid. (Redrawn and modified from Peter.)

The Malpighian Corpuscle. The renal corpuscles are spheroidal slightly flattened bodies which occur in large numbers in the cortical labyrinths (Fig. 330). Their diameter in the adult is close to 200 μ though there are considerable variations in size. As a rule the larger ones are found near the border of the medulla. Their structure is best understood by a brief reference to their development (Chapter 20 and Fig. 414). During development the rounded blind end of a growing uriniferous tubule becomes invaginated by the growing end of a blood vessel. By further invagination and differentiation, the expanded end of the tubule becomes transformed into a two-layered capsule, the capsule of Bowman, which encloses the glomerulus.

The glomerulus consists of a number of separate capillaries connecting an afferent arteriole with an efferent arteriole; in other words, *the entire vascular system of the glomerulus is arterial*. The afferent vessel is named the *afferent glomerular arteriole*; the efferent vessel is known as the *efferent glomerular arteriole*. These

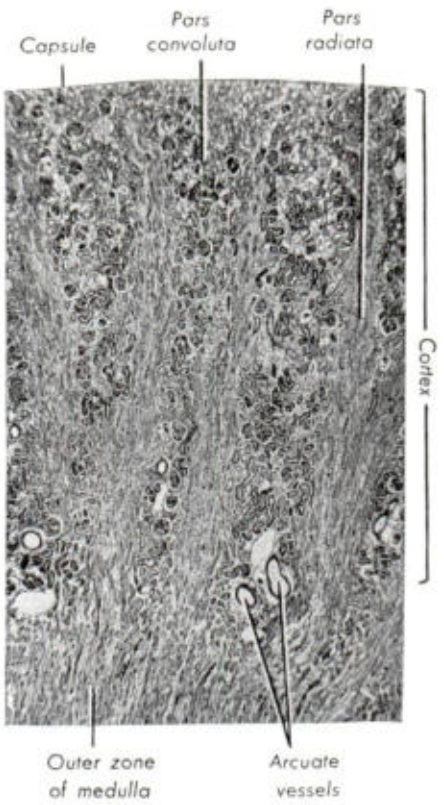

FIG. 329. Low power photomicrograph of a section of a kidney from an infant of eight months showing topography of the cortex and a part of the outer zone of the medulla. × 23.

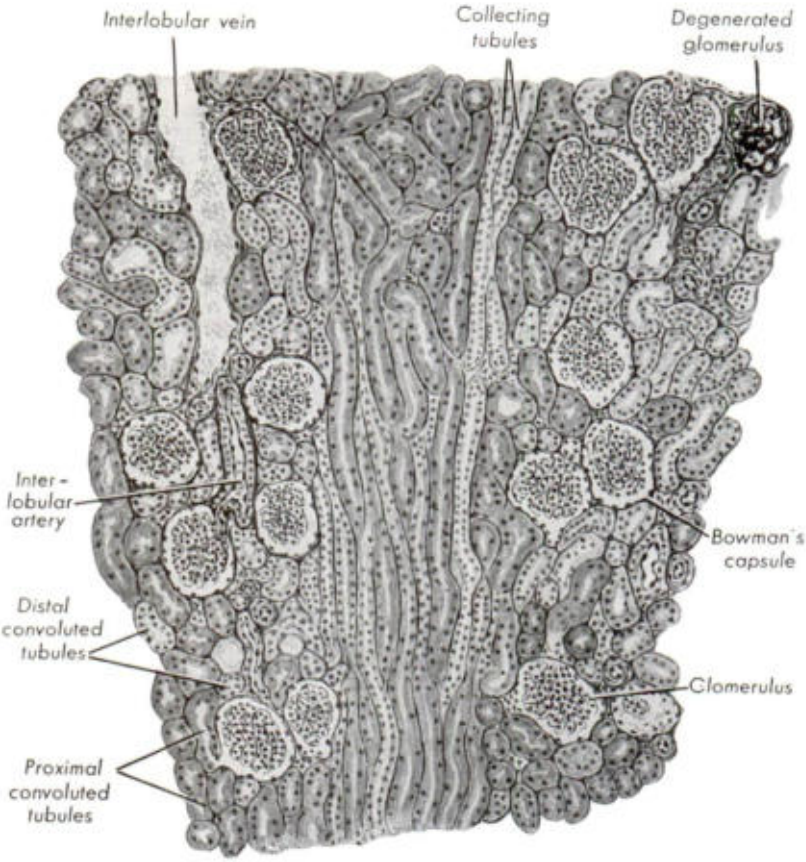

FIG. 330. Section through a part of the cortex of a human kidney showing the structure of a pars radiata (central part of figure) and of adjacent cortical labyrinths (lateral parts of figure). × 55.

vessels are usually close together where they enter and leave the glomerulus (Fig. 332), and the region where they enter and exit is spoken of as the vascular pole of the renal corpuscle.

As it enters the glomerulus, the afferent arteriole divides into four or five relatively large capillaries. Each of the latter vessels subdivides into a number of smaller capillaries which follow an irregularly-looped course in their pathway from the afferent to the efferent arteriole. The looped capillaries arising from each main branch of the afferent arteriole tend to be grouped together, giving the glomerulus a lobulated appearance. Carefully injected preparations show that there are numerous anastomoses between the capillaries within each lobule, and occasional anastomoses between those of different lobules. All of the capillaries of the different lobules eventually reunite to form the efferent arteriole which is always smaller in caliber than the afferent arteriole. The difference in size is correlated with a functional condition; the efferent vessel carries less fluid than the afferent vessel because a considerable

quantity of fluid is filtered from the blood while it flows through the glomerular capillaries. A small amount of connective tissue accompanies the arterioles into the renal corpuscle at the vascular pole but connective tissue elements do not follow the capillaries throughout their course as in most other parts of the body.

Bowman's capsule consists of an inner or "visceral" layer covering the glomerulus and an outer or "parietal" layer (Figs. 331 and 332). The visceral layer follows an irregular course as it closely invests the glomerulus and dips down between the lobules of glomerular capillaries. It is composed of simple squamous epithelium resting on a structureless basement membrane which is fused with the membrane beneath the endothelium of the capillaries, i.e., visceral epithelium and endothelium are separated merely by a single, thin basement membrane. The basement membrane is readily observable under the light microscope in sections stained with the periodic acid Schiff technique; it is less obvious in hematoxylin and eosin preparations.

Most of the controversial features of the structure of the basement membrane, endothelial cells, and Bowman's capsule have been resolved by electron micros-

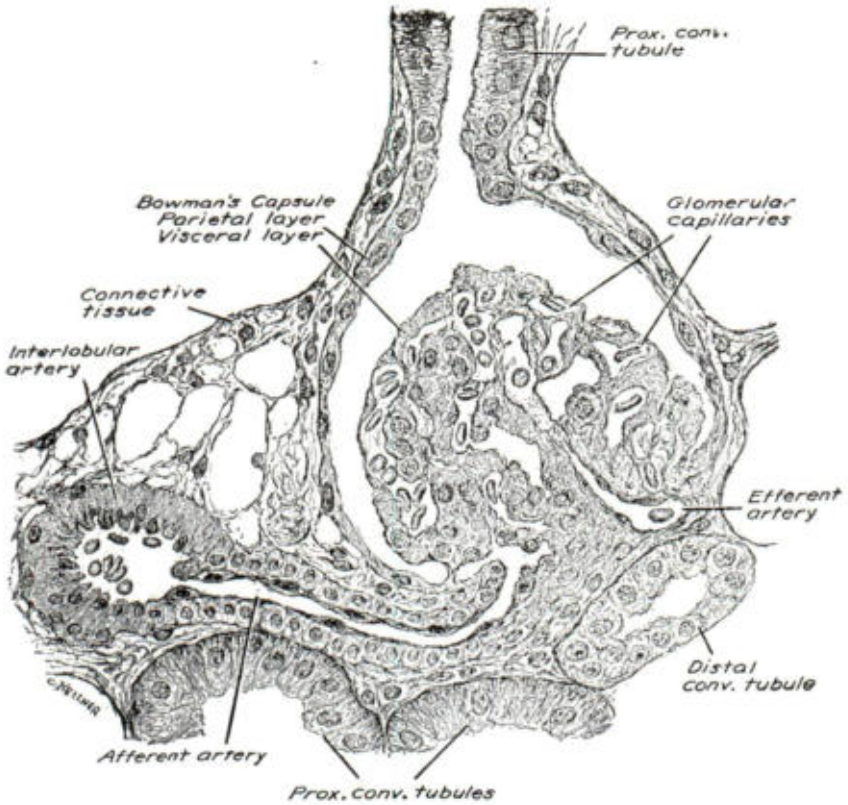

FIG. 331. Renal corpuscle from kidney of mouse. Drawing is from same preparation as colored Figure 2, A. × 1320.

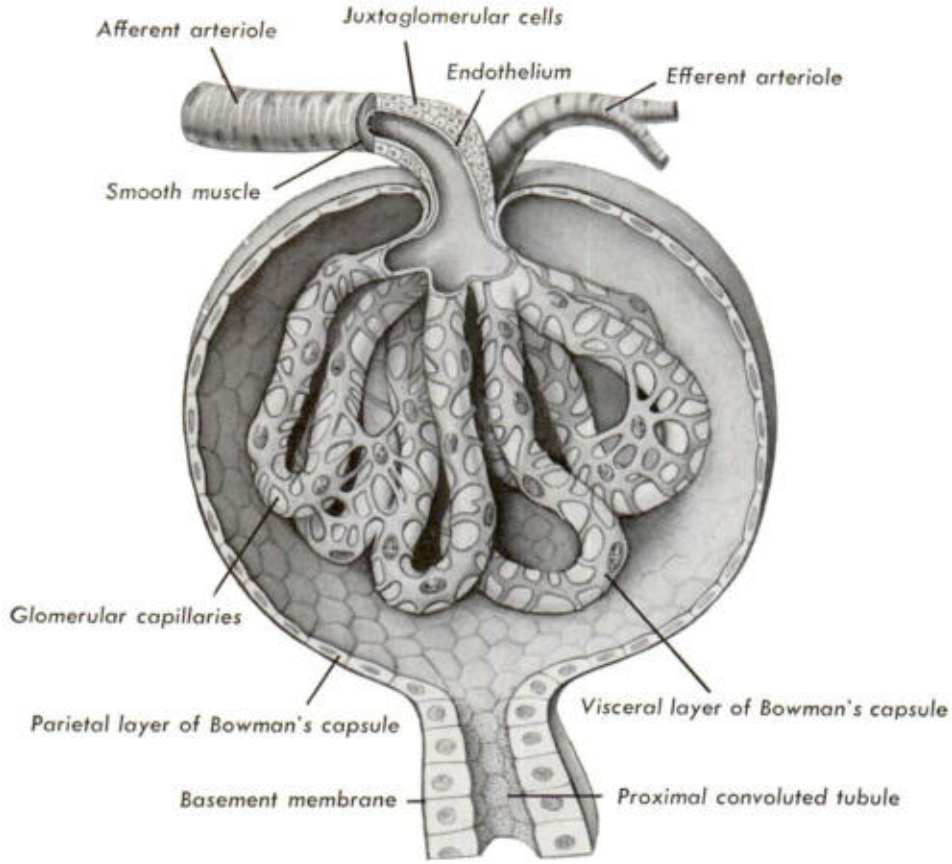

FIG. 332. Diagram of structure of renal corpuscle, showing relationship of Bowman's capsule to glomerular capillaries. (Redrawn and modified from Bargmann.)

copy. The basement membrane appears relatively dense in electron micrographs and is often referred to as the lamina densa. It varies in thickness from 0.08 to 0.12 microns. It occasionally forms a partial covering for an individual capillary in the manner of the visceral peritoneal covering of the intestine, but more often it forms an enclosure around groups of capillaries (Fig. 332).

The endothelial cells of the glomerular capillaries are extremely thin with the exception of the regions where the nuclei are located. Most of the endothelial cell cytoplasm extends along the basement membrane in the form of a cribriform plate (lamina fenestra), with pores which measure 0.04 to 0.08 μ in diameter (Fig. 333). From the functional standpoint, this means that any blood-borne material which is less than 0.04 μ in diameter can come into direct contact with the basement membrane. It should be noted also that the fenestrated layer of endothelial cytoplasm is only about 0.04 μ in thickness, i.e., it is too thin to be observed under the light microscope and this led to an erroneous conception that the glomerular endothelial cells are not in contact with each other.

The cells of the visceral layer of Bowman's capsule are relatively large and their nuclei bulge into the capsular space. Their cytoplasm is in the form of trabecular processes which subdivide into many small foot-like processes (pedicels) which are in contact with the basement membrane (Fig. 333). The entire glomerular epithelial cell with its little feet is sometimes referred to as a *podocyte*. The spaces between the pedicels vary in width from about 0.02 to 0.04 μ.

The visceral layer of Bowman's capsule is reflected at the vascular pole to become continuous with the parietal layer (Fig. 332). Opposite to the vascular pole, the parietal layer becomes continuous with the wall of the proximal segment of the renal tubule through a short transitional zone which is called the "neck." At this point, the cleft-like space between the visceral and parietal layers of the capsule becomes continuous with the lumen of the proximal tubule. The wall of the parietal layer is composed of simple squamous epithelium resting on a thin basement membrane but the epithelial cells are somewhat thicker than those of the visceral layer. The cells increase in height and become cuboidal in the neck region.

Terminal Uriniferous Tubule. The terminal tubule or nephron consists of an expanded portion, described above as Bowman's capsule, and an elongated tubular portion composed of a proximal thick segment, an intermediate thin segment, and a distal thick segment. The proximal thick and distal thick segments can both be subdivided into convoluted and straight portions. Hence, the tubular portion of the nephron is composed of the following segments: (1) the proximal convoluted tubule; (2) the straight or medullary segment of the proximal tubule (this part forms the thick proximal

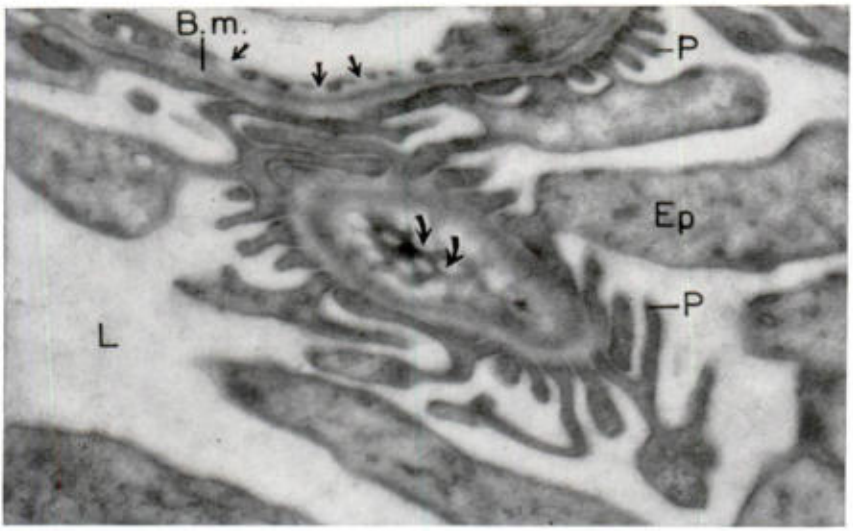

FIG. 333. Electron micrograph of a portion of a renal corpuscle showing pores (indicated by arrows) in the endothelium of the glomerular capillaries, a continuous basement membrane (*B. m.*), and fine processes or pedicels (*P*) of the epithelium of the visceral layer of Bowman's capsule (*Ep*). The endothelium is seen in transverse section in upper part of figure and in tangential section at center of figure. *L* indicates lumen of capsule. × 30,000. (Courtesy of Drs. G. D. Pappas, M. H. Ross, and L. Thomas.)

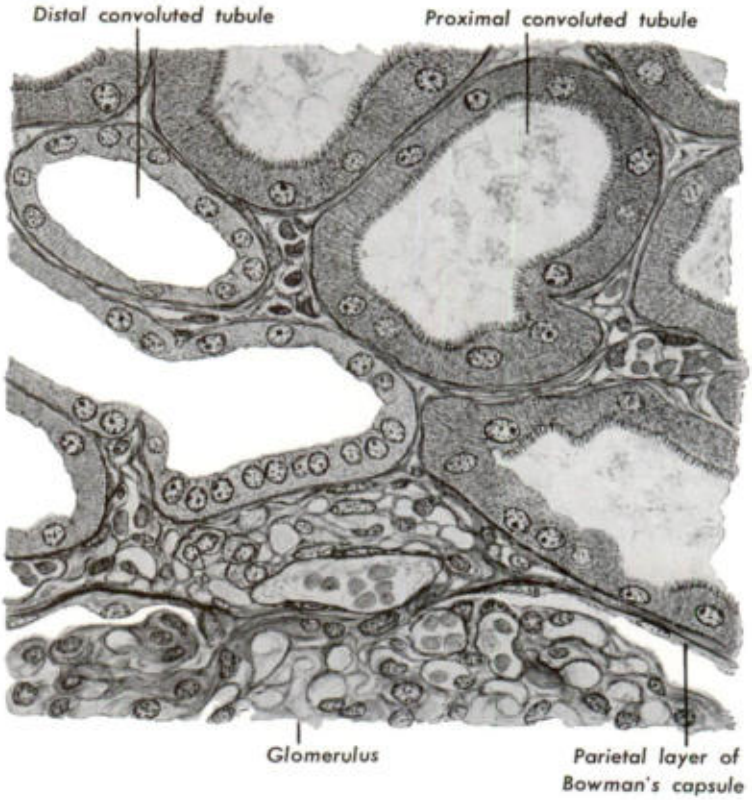

FIG. 334. Portion of a glomerulus and adjacent tubules from the cortex of a human kidney. One of the segments of a distal convoluted tubule shows a modification, known as the macula densa, where it borders on the vascular pole of the glomerulus. In this region, there is an increase in the height and number of cells with a concentration of nuclei. × 635. (The slide from which this drawing was made was kindly supplied by Dr. T. E. Hunt.)

segment of Henle's loop); (3) the thin segment of Henle's loop; (4) the thick distal (ascending) segment of Henle's loop; and (5) the distal convoluted tubule. The latter joins the arched (initial) collecting tubule which begins the system of ducts or collecting tubules.

(1) *The proximal convoluted tubule* is the longest and most convoluted segment of the nephron and it forms a major portion of the cortical substance. It has a length of about 14 mm. and a diameter of 57–60 μ. It takes a winding looped course in the vicinity of the glomerulus and eventually turns toward the nearest medullary ray (pars radiata of cortex) where it continues as the straight segment of the proximal tubule.

The proximal convoluted tubule is lined by a single layer of low columnar or pyramidal cells with round nuclei and granular cytoplasm which stains deeply with eosin (Fig. 334). The cell boundaries are as a rule difficult to make out in ordinary preparations. In silver impregnations they appear irregularly serrated, with processes which interdigitate with those of adjacent cells.

An important characteristic of the proximal convoluted tubule is the *brush*

border, a narrow finely striated zone at the free surface of the cells (Fig. 334). This region undergoes rapid post-mortem change and therefore the inner border of the tubule has a ragged appearance in most histological preparations. Electron micrographs of well-fixed material show that the brush border is composed of slender processes about 1.2 μ in length and about 0.03 μ in width (Fig. 335). The cell surface available for resorption is thus greatly increased. Histochemical studies show a concentration of alkaline phosphatase in the brush border. This enzyme is thought to play a rôle in the resorption of certain substances from the tubular lumen but the exact manner in which it functions in this location is not understood. The brush border also contains mucopolysaccharides as indicated by its intense staining with the periodic acid Schiff reagent.

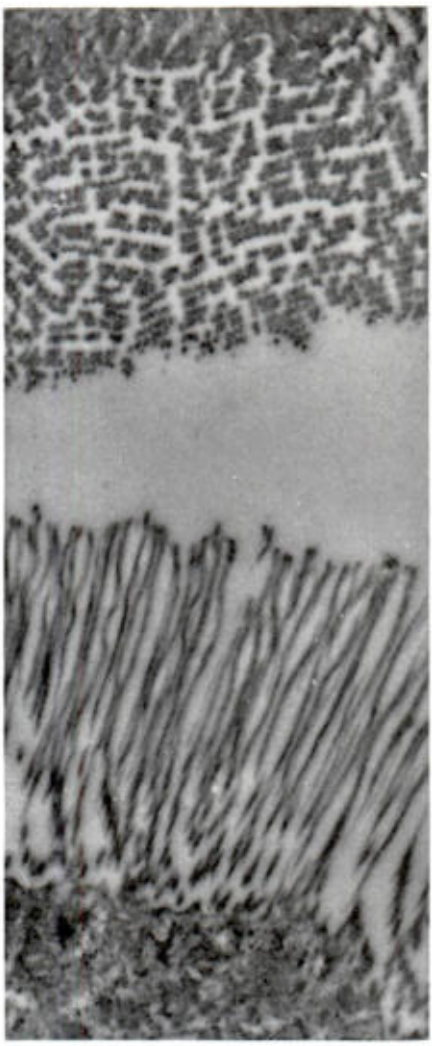

FIG. 335. Electron micrograph of proximal convoluted tubule of rat showing processes of brush border cut longitudinally in lower part of figure and transversely in upper portion. × 13,000. (Courtesy of Dr. Dan Moore.)

The basal portions of the cells of the proximal convoluted tubule have a striated appearance due to the arrangement of the cytoplasmic components. By special techniques, numerous mitochondria are found in the form of long rods and filaments arranged perpendicularly to the basement membrane (Fig. 2, *A*). By electron microscopy, numerous infoldings of the plasma membrane are found in the basal part of the cell (Fig. 336). The functional significance of the infoldings is not clear although they obviously increase the surface area of the basal side of the cell. Although neither the infoldings nor the mitochondria can be seen in ordinary preparations, they impart a characteristically striated pattern to the other components of the cytoplasm.

(2) *The straight or medullary portion of the proximal tubule* is a direct continuation of the convoluted portion of the proximal tubule and is not sharply delimited from it. As the proximal tubule leaves the cortical labyrinth and enters the medullary ray, it assumes first a spiral and then a straight course. This segment is known as the *straight portion* of the proximal tubule due to its course and it is also named the *medullary segment* of the proximal tubule because it is located in the medullary ray and in the

FIG. 336. Electron micrograph of proximal convoluted tubule showing numerous mitochondria (*Mit.*) and prominent infoldings (*In.*) from the basal portion of the cell membrane. × 29,700. (Courtesy of Dr. Dan Moore.)

outer, subcortical zone of the medulla (Fig. 328). It is lined by cells which appear structurally identical with those of the convoluted portion of the proximal tubule as seen in preparations stained with hematoxylin and eosin; that is, the cells have a brush border, an acidophilic granular cytoplasm and the other characteristics described for the convoluted segment. By means of special technique,

differences have been demonstrated for some laboratory mammals (see Foote and Grafflin, 1942).

The straight segment of the proximal tubule forms the proximal portion of the descending arm of Henle's loop (Fig. 328). It should be noted that this loop is composed of three parts: a proximal thick segment, a thin segment, and a distal thick segment.

(3) *The thin segment of Henle's loop* is about 15 μ in diameter and it is lined by a single layer of flattened epithelial cells with clear cytoplasm and nuclei which bulge into the lumen (Fig. 337). The change from the columnar cells of the proximal thick segment to the simple squamous epithelial cells of the thin segment is rather abrupt. The change usually occurs in the outer, subcortical zone of the medulla, but may occur in a higher level in some of the short loops.

The extent of the thin segment as well as the length of the entire loop varies greatly in different tubules. As a rule the tubules whose Malpighian corpuscles lie near the junction of cortex and medulla, have long loops and their thin segments

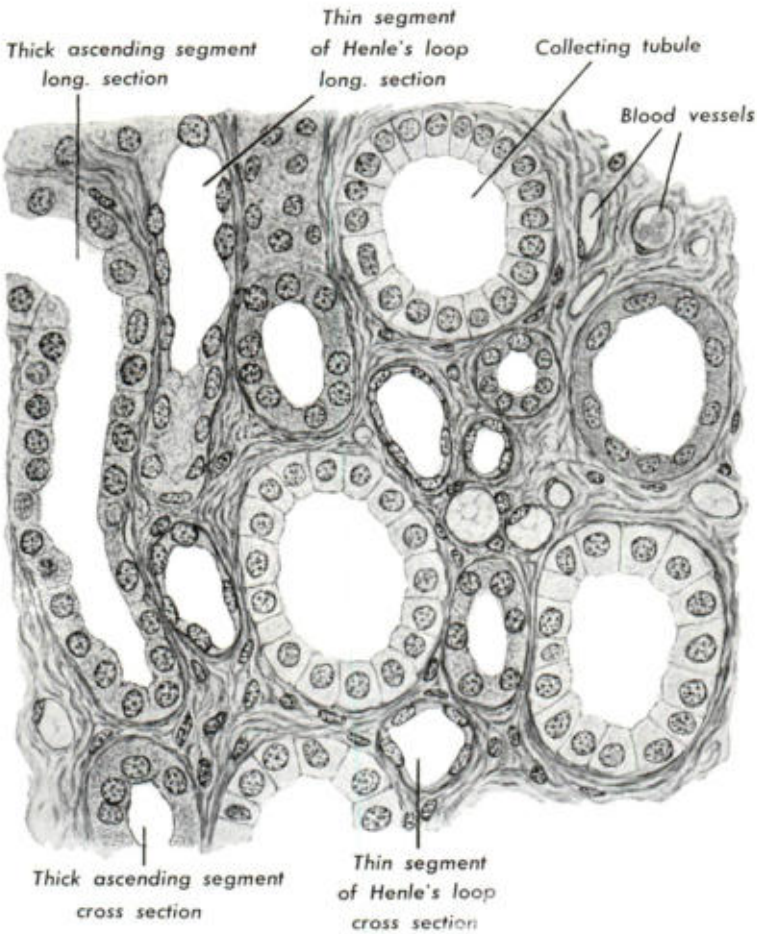

FIG. 337. Section from the medulla of a human kidney. × 575.

extend nearly to the apex of the medullary pyramid. In them, the thin segment forms the entire crest of the loop and continues a considerable distance up the ascending limb (Fig. 328). On the other hand, tubules associated with glomeruli of the outer part of the cortex have relatively short loops and their thin segments are limited to a small part of the descending arm (1–2 mm. or even less). There are many intermediate types between the two extremes described. The shorter forms are said to outnumber the longer ones by about seven to one.

(4) *The thick distal (ascending) segment of Henle's loop* is about 9 mm. long and 30 μ in diameter. In the short loops it may begin in the lower part of the proximal limb (Fig. 328). It ascends to the cortex and closely approaches the vascular pole of the glomerulus from which the nephron began. At this point, it becomes continuous with the distal convoluted tubule. It is lined by a layer of cuboidal cells which are very similar to those of the convoluted portion of the distal tubule.

(5) The second or *distal convoluted tubule* begins near the vascular pole of the glomerulus and terminates by becoming continuous with the arched collecting tubule (Fig. 328). At one point, it comes in direct contact with the afferent glomerular arteriole (Fig. 331). It is much less convoluted than the proximal tubule and is only 4½–5 mm. in length. It has an irregular outline and its diameter varies from 22–50 μ. It is lined by cuboidal cells containing a granular cytoplasm, which stains less intensely with acid dyes than the cytoplasm of the proximal convoluted tubules. The basal striations are faint and there is no brush border although a few short microvilli can be seen in electron micrographs. The cells are not as broad as those which line the proximal convoluted tubules and therefore the nuclei are closer together and more numerous in sections of distal tubules than they are in sections of proximal tubules (Fig. 334). The part of the distal tubule which is near the afferent glomerular vessel has the nuclei particularly close together and also has taller cells on the side which is adjacent to the arteriole. This zone of the distal tubule which consists of columnar, palisadic cells is named the *macula densa*. It is in contact with the juxtaglomerular epithelioid cells of the afferent arteriole.

The Collecting Tubules. The *arched* (initial) *collecting tubules* are still in the cortical labyrinth and empty into the *straight collecting tubules*. The straight tubules receive a number of arched tubules (7–10) as they pass down through the medullary rays of the cortex but receive no branches in the outer zone of the medulla (Fig. 328). In the inner zone of the medulla, they unite with other straight tubules and after a number of fusions the *papillary ducts* or *ducts of Bellini* are formed, which open on the area cribrosa of the papilla. According to Peter, seven successive fusions occur before the papillary ducts are formed.

The caliber of the collecting tubule while still in the medullary ray is about 40 μ. In the papillary ducts the diameter may exceed 200 μ. The lining of the collecting tubules consists of a single layer of cuboidal or columnar cells with round dark-staining nuclei and clear faintly staining cytoplasm (Fig. 337). The cells vary in height according to the caliber of the tubule and range from cuboidal in the arched tubules to tall columnar in the papillary ducts.

The length of the collecting tubule from its beginning in the medullary ray to its opening on the papilla is 20–22 mm.; that of the terminal tubule (nephron) 30–38 mm. The entire uriniferous tubule thus measures from 50–60 mm., the variations

TABLE 2

LOCATIONS OF PORTIONS OF URINIFEROUS TUBULES

Location in Kidney	Portion of Tubule
Cortex:	
Cortical labyrinth	Malpighian corpuscles
	Proximal convoluted tubules
	Distal convoluted tubules
	Arched collecting tubules
Medullary ray	Straight portions (medullary segments) of proximal tubules
	Thick segments of ascending arms of Henle's loops
	Straight collecting tubules
Medulla:	
Outer zone	Straight portions (medullary segments) of proximal tubules
	Thick segments of ascending arms of Henle's loops
	Thin segments of Henle's loops
	Crests of shorter loops
	Straight collecting tubules
Inner zone	Thin segments of Henle's loops
	Crests of long loops
	Straight collecting tubules
	Fusions of straight collecting tubules
	Papillary ducts

depending mainly on the length of Henle's loop.

A summary of the locations of the various portions of the uriniferous tubules is given in Table 2.

The epithelium of all portions of the uriniferous tubule rests on a clear, apparently structureless basement membrane (Figs. 333, 334). The interstitial connective tissue is very scanty and forms a fine network between the tubules. The tissue is more abundant in the papillary portion of the medulla.

THE EXCRETION OF URINE

The kidney has several important functions. It excretes urea and other nitrogenous waste substances, rids the body of undesirable salts, and, in conjunction with the skin and lungs, helps to maintain the constant volume of the blood by the elimination of excess water. Drugs and foreign substances which get into the blood are for the most part likewise eliminated by the kidney.

The kidney is not, properly speaking, a gland. The constituents of the urine, with the possible exception of ammonia and hippuric acid, are not synthesized in the kidney. They are already present in the blood circulating through the glomeruli and are merely eliminated by the uriniferous tubules.

Numerous experimental studies have been made to determine what part each portion of the nephron plays in the process of excretion. Although some of the details of the process are still controversial, it seems certain that the Malpighian corpuscles act as filtering mechanisms. As already pointed out, the barrier between the blood and the lumen of the upper, expanded end of the nephron is a thin membrane composed of a fenestrated endothelium, a basement membrane, and the visceral layer of

Bowman's capsule which is incomplete. Filtration through this membrane is a physical process and depends on the pressure within the glomerular capillaries, the condition of the filtering membrane, and the nature of the blood plasma. A large surface area for filtration is provided by the number of tortuous capillary loops in each glomerulus and by the total number of glomeruli (over 2,000,000 for the two kidneys). The filtration surface has been estimated as between 5200 and 5860 sq. mm. per gram of human kidney (Kirkman and Stowell, 1942). The total blood supply to the two kidneys also is unusually large. The average normal flow through the two kidneys is about 1200 cc. per minute, or about 1700 liters of whole blood or 900 liters of plasma in a 24-hour period. From this, about 170 liters of fluid are filtered daily through the walls of the glomeruli and reach the lumina of the nephrons.

According to the most widely accepted view, the *capsular* or *provisional* urine contains unchanged all of the constituents of plasma with the exception of the plasma proteins, fat droplets and blood cells which are unable to pass through the normal filtration membrane due to their molecular weight or size. Direct evidence for this view has been obtained from an analysis of urine withdrawn by micropipette from amphibian renal corpuscles (Richards, 1935). Although it is difficult to apply the micropipette method to the mammalian kidney, some studies of this type have been made on rats and guinea pigs with results essentially similar to those obtained on amphibians (Walker and Oliver, 1941).

The capsular or provisional urine becomes concentrated and altered in composition by the resorption of water and of certain other constituents as it passes through the tubular portion of the nephron. Some resorption occurs by diffusion of substances through the tubular epithelium into the surrounding capillaries. For example, some urea may reenter the circulation by this method, spoken of as passive resorption. A considerable amount of water may also be resorbed by this method. However, most of the tubular resorption is dependent upon the "vital activity" of the cells, a process by which various substances are resorbed selectively and not indiscriminately.

A number of factors influence resorption and determine the final composition of urine. For example, a hormone from the adrenal cortex facilitates the tubular resorption of sodium, and a hormone from the neurohypophysis (an antidiuretic hormone) facilitates the active resorption of water. Concentration within the blood plasma is an especially important factor in the resorption of substances which the body tends to conserve. Glucose, for example, is completely resorbed from the tubule under normal conditions and is maintained in the blood plasma at a definite level known as its threshold value. When the plasma glucose level exceeds its threshold value, as in diabetes mellitus, the amount of glucose filtered by the renal corpuscle exceeds the resorptive capacity of the tubule and glucose appears in the urine.

On the other hand, urea and other protein wastes are destined to be completely eliminated. They have no threshold values in the blood and are resorbed only passively or not at all. Foreign bodies which get into the blood stream are treated in the same way. This fact has been made the basis of the "clearance method" for studying renal function (H. W. Smith).

Different substances are resorbed at different rates by different parts of the tubule. For example, glucose is completely resorbed in the proximal tubule

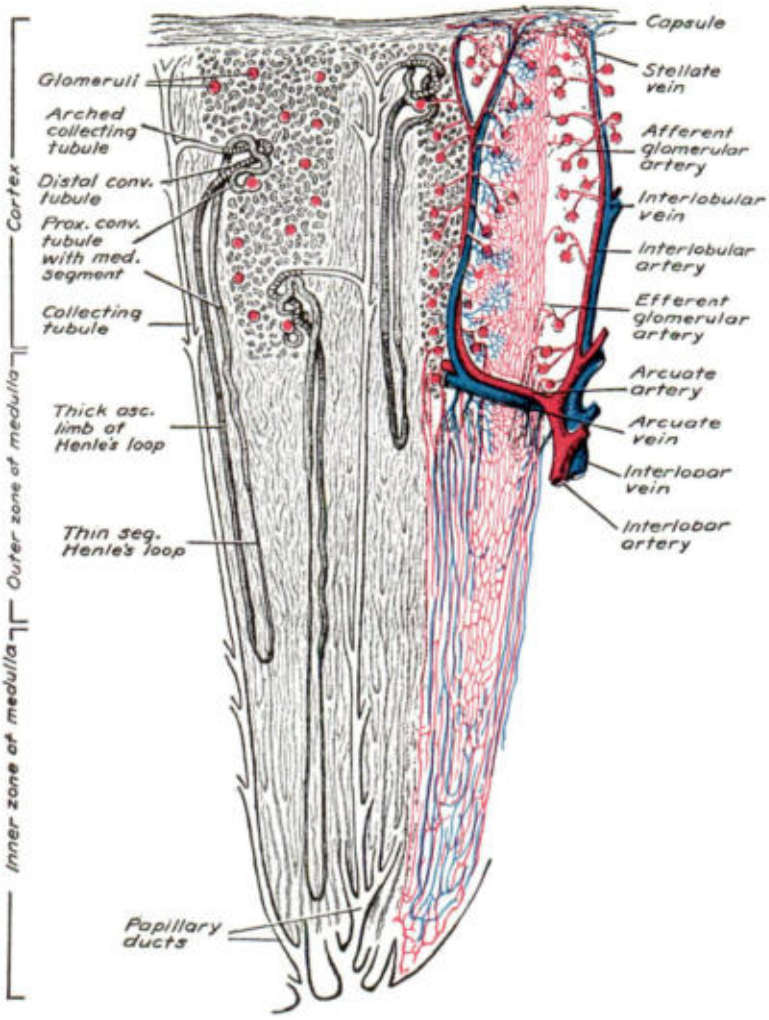

FIG. 338. Diagram of the structure of the human kidney. The cortical labyrinth is indicated by the presence of convoluted tubules and glomeruli, and the medullary ray is marked by the parallel arrangement of tubules. The medullary ray with adjacent regions of cortical labyrinths represents the lobule. Note that the straight medullary (*med.*) segment of the proximal tubule forms the upper part of Henle's loop and that these loops extend varying distances into the medulla. The thick ascending (*asc.*) limb of the loop is sometimes subdivided into a proximal opaque portion (stippled) and a distal more clear portion (closely cross striated). For clearer illustration, the convoluted tubules are simplified and all segments of the tubules are magnified more in width than in length. (Blood vessels are redrawn and modified from Braus and tubules are modified from Peter.)

whereas sodium may be resorbed in both the proximal and distal tubules but particularly in the latter. The resorption of water begins in the proximal convoluted tubule but is continued in the loop of Henle and in the distal convoluted tubule. The 170 liters of fluid which filter daily from the glomerular capillaries are concentrated in the tubular portion of the nephron to a liter or less of actual urine. The view that there are no changes in urinary composition in the collecting tubules has been questioned by recent studies which claim that water resorption may continue in this region.

There is considerable evidence that the cells of the proximal convoluted tubule function not only in resorption but also in secretion (excretion) of some substances, transferring them from the blood of the surrounding capillaries to the tubular lumen. This is a particularly important part of the tubular function in some species of marine fish which have an aglomerular type of kidney. In humans, there is tubular excretion of creatinine, hippuric acid and certain dyes. However, tubular secretion appears to be relatively unimportant as a normal physiological process in the human.

Blood Vessels (Fig. 338)

The *renal* artery enters the kidney at the hilum and splits into a number of large branches, the *interlobar arteries*. These give off fine twigs to the pelvis and to the capsule, then without further branching they pass between the pyramids to the boundary zone of medulla and cortex. Here they bend sharply and form short arches, the *arcuate* or *arciform arteries*, which run parallel to the surface. The arches do not communicate with one another as was formerly believed, each representing merely the curved terminal portion of an interlobar artery. The arcuate arteries break up into a large number of finer branches, the *interlobular arteries*, which ascend perpendicularly through the cortical labyrinths to the surface, about midway between adjacent medullary rays. From the interlobular arteries are given off numerous short lateral branches, each of which enters a Malpighian corpuscle as the afferent glomerular vessel and subdivides as already described (p. 418). Often the short branches subdivide and supply a cluster of glomeruli. The terminal portions of most interlobular arteries on reaching the periphery of the cortex, themselves become afferent glomerular vessels.

As the afferent glomerular arteriole approaches the glomerulus, some of its muscle cells are overlaid with or partly replaced by *myoepithelioid* cells which have very pale staining, afibrillar cytoplasm. Just before the arteriole enters the glomerulus, its wall contains a continuous cuff of this myoepithelioid tissue which has been called the "*juxtaglomerular apparatus*," or "polkissen," (polar cushion). A few isolated cells of this type have also been found on other parts of the arterial system of the kidney. They have also been seen in other parts of the body, particularly on the arterial vessels of the glomus caroticum and glomus aorticum. It has been shown by Goormaghtigh, that some of the cells of the juxtaglomerular apparatus of rabbits and dogs contain acidophil and basophil granules. It has been claimed that there is a hyperplasia of these cells in animals rendered hypertensive through experimentally produced renal ischemia. However, the suggestion that these cells secrete a hypertensive hormone remains unsubstantiated.

The efferent glomerular arteriole, immediately after leaving the corpuscle, divides into a *second* system of capillaries, *the peritubular plexus*, which form a

dense network around the tubules of the cortex (Fig. 338). So densely are the capillaries arranged that the tubules seem bathed in blood.

Nonglomerular arterioles may be found extending directly from an afferent arteriole to the peritubular plexus. They are known as Ludwig's arterioles. They are infrequent in normal kidneys and are said to arise from a continuity of afferent and efferent vessels following glomerular degeneration (MacCallum).

The arterial supply of the medulla is furnished by the efferent glomerular vessels of those Malpighian corpuscles which lie close to the medulla (Fig. 338). These vessels, the *arteriolae rectae spuriae*, pursue a straight course into the outer layer of the medulla and give rise to long-meshed capillary nets which extend to the apex of the pyramids. Numerous investigations have shown that the blood supply of the medulla comes mainly from the efferent glomerular vessels. The so-called arteriolae rectae verae which extend directly from the arcuate arteries into the medulla are few in number and probably arise following degeneration of glomeruli which previously existed along their course.

The blood from the peripheral portion of the cortex is collected into small venules which unite beneath the capsule to form the *stellate veins of Verheyen*. From these arise the *interlobular veins* which accompany the corresponding arteries and empty into the arcuate veins. The latter also receive short interlobular veins from the deeper portions of the cortex. Straight veins, the *venae rectae*, collect the blood from the capillary nets of the medulla and accompany the straight arteries to terminate in the *arcuate veins*. The venous arches unlike the arterial ones, combine with one another to form venous arcades. From the arcuate veins the large *interlobar veins* pass down between the medullary pyramids and unite to form the renal vein.

In addition to the distribution just described some interlobular arteries on reaching the periphery of the cortex break up into capillaries which supply the fibrous capsule of the kidney. These anastomose with capillaries from the suprarenal, lumbar and phrenic arteries and with those of the capsular twigs which arise directly from the renal artery or its interlobar branches.

Arteriovenous anastomoses have been described at various levels: between interlobar arteries and veins, between arcuate arteries and veins, and also between interlobular arteries and veins.

Lymphatics

The lymph capillaries are arranged in two systems: a superficial system which ramifies in the capsule, and a deeper system which lies in the glandular tissue. The capsular lymph is collected by superficial lymph vessels which communicate with the lymphatics of adjacent organs. The lymph from the parenchyma is collected by a number of lymphatic trunks which accompany the blood vessels. They leave the kidney at the hilum to enter lymph nodes which are situated on both sides of the aorta.

Nerves

The kidneys are richly supplied with nerves, the majority of which are unmyelinated. They are derived from the celiac plexus and from the 10th to the 12th thoracic nerves. It is probable that branches from the vagus also supply the kidney. The nonmyelinated fibers follow the blood vessels and terminate by numerous endings in the vascular wall, particularly in the glomerular arterioles. Delicate terminals have been described as ramifying in the basement membrane of the tubules or even between the epithelial

cells. Sensory myelinated fibers go to the capsule, the smooth muscle of the pelvis, and the adventitia of the renal vessels.

THE RENAL PELVIS AND URETER

The renal pelvis, with its subdivisions (the calyces) and the ureter, constitute the *main excretory duct* of the kidney. Their walls consist of three coats; an inner mucous, a middle muscular, and an outer fibrous.

The *mucosa* of the ureter is lined by transitional epithelium which varies in thickness depending on the state of distension of the ureter (Fig. 30). In the collapsed state the cells of the basal layers are cuboidal, almost columnar. The superficial layer consists of large cuboidal cells with lighter cytoplasm, often containing several nuclei. A basement membrane is not discernible. Beneath the epithelium is a stroma of delicate fibrous tissue containing few elastic fibrils and rich in cells (Fig. 339). Diffuse lymphatic tissue frequently occurs in the stroma, especially of the pelvis. Occasionally the lymphatic tissue takes the form of small nodules. There is no distinct submucosa, although the outer part of the stroma is sometimes referred to as such.

The *muscularis* consists of an inner longitudinal and an outer circular layer (Fig. 339). In the lower part of the ureter a discontinuous outer longitudinal layer is added.

The *fibrosa* consists of loosely arranged connective tissue and contains many large blood vessels. It is not sharply

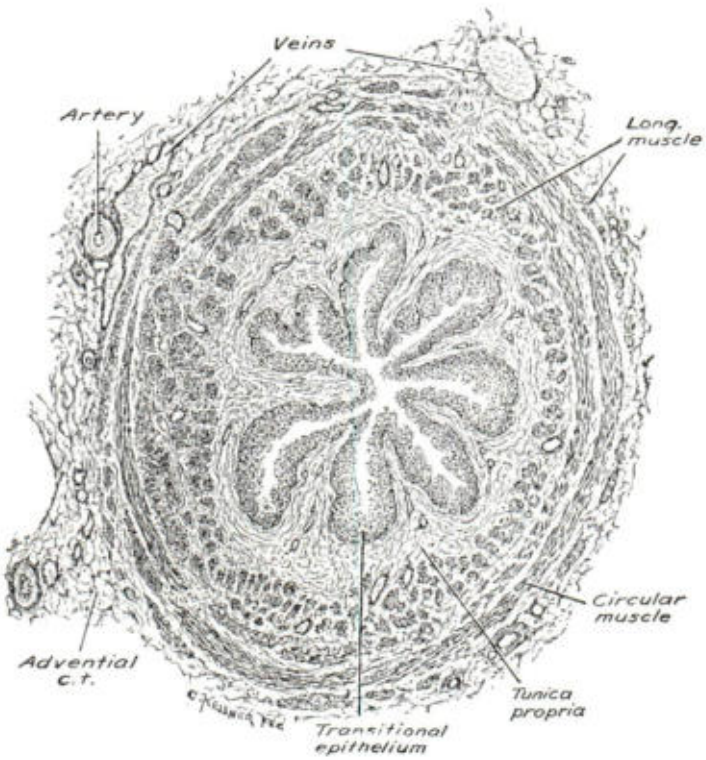

FIG. 339. Transverse section of contracted human ureter. Drawing is from lower part of ureter and shows the addition of a discontinuous layer of outer longitudinal (*long.*) muscle. × 30.

limited externally, but blends with the connective tissue of surrounding structures, and serves to attach the ureter to the latter.

The larger *blood vessels* run in the fibrous coat. From these, branches pierce the muscular layer, give rise to a capillary network among the muscle cells, and then pass to the mucosa, in the stroma of which they break up into a rich network of capillaries. The veins follow the arteries.

The *lymphatics* follow the blood vessels, being especially numerous in the stroma of the mucosa.

Plexuses of both medullated and nonmedullated *nerve fibers* occur in the walls of the ureter and pelvis. The nonmedullated fibers pass mainly to the cells of the muscularis. Medullated fibers enter the mucosa where they lose their medullary sheaths. Terminals of these fibers have been traced to the lining epithelium.

THE URINARY BLADDER

Except for the increased thickness of the muscular coat, the walls of the bladder are similar in structure to those of the ureter. The mucous membrane is thrown up into folds or is comparatively

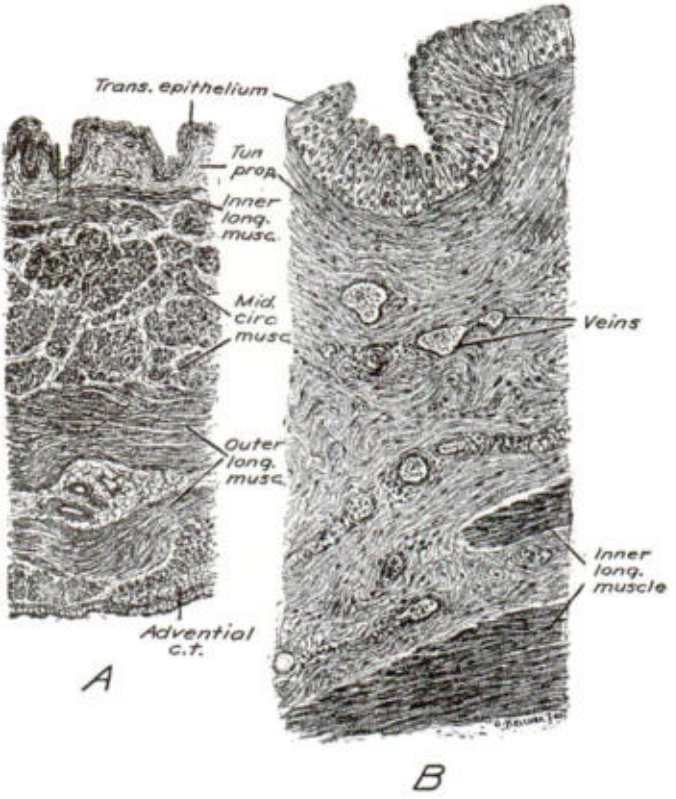

FIG. 340. *A*. Section through the wall of a contracted human bladder. × 12. *B*. Drawing of the region outlined by solid line in Figure *A*. × 100. *Inner long. musc*, inner longitudinal muscle; *Mid. circ. musc*, middle layer of circular muscle; *Outer long. musc*, outer layer of longitudinal muscle; *Tun. prop*, tunica propria.

smooth, according to the degree of distention of the organ. The epithelium is of the same general type—transitional epithelium—as that of the ureter and, as already described for the ureter, the surface cells may have two or more nuclei. The number of layers of cells and the shapes of the cells depend largely upon whether the bladder is full or empty. In the moderately distended bladder the superficial cells become flatter and the entire epithelium thinner than in the contracted organ. In the distended organ there is still further flattening of the superficial cells, and the entire epithelium may have a thickness of only two or three cells. The stroma consists of fine loosely arranged connective tissue containing many lymphoid cells and sometimes small lymph nodules. There is no distinct submucosa and, as in the ureter, there are no glands.

The three muscular layers of the lower part of the ureter are continued onto the bladder, where the muscle bundles of the different layers interlace and anastomose, but can still be indistinctly differentiated into an inner longitudinal, a middle circular, and an outer longitudinal layer (Fig. 340).

The fibrous layer is similar to that of the ureter, and attaches the organ to the surrounding structures.

The blood and lymph vessels have a distribution similar to those of the ureter.

Sensory myelinated nerve fibers pierce the muscularis, branch repeatedly in the stroma, lose their medullary sheaths, and terminate among the cells of the lining epithelium. Sympathetic fibers form plexuses in the fibrous coat, where they are interspersed with numerous small groups of ganglion cells. Axons of these sympathetic neurons penetrate the muscularis. Here they form plexuses, from which are given off terminals to the individual muscle cells.

THE URETHRA

The male and female urethra differ from each other in many respects. The short female urethra is merely the terminal urinary passage conducting urine from the bladder to the vestibule. The relatively long male urethra constitutes a urogenital duct conducting both urine and seminal fluid to the exterior.

The Male Urethra. The male urethra has a length of about 20 cm. and is divisible into three portions, prostatic, membranous, and cavernous. The *prostatic portion*, about 3–4 cm. in length, is surrounded by the prostate gland. From the dorsal wall of this portion a conical elevation, the *colliculus seminalis*, extends into the lumen. On the apex of the colliculus is the small opening of a blind tubule, the *utriculus prostaticus* or *uterus masculinus*, a remnant of the Müllerian duct. On either side of the utricle are the slit-like openings of the ejaculatory ducts, the terminal portions of the ductus deferens (see reproductive system). In this part of the urethra are also the numerous small openings of the ducts of the prostate gland.

The *membranous* portion is the narrowest and shortest, measuring about one centimeter in length.

The *cavernous* portion is about 15 cm. long and extends through the penis to open on the end of the glans. At its beginning the lumen is enlarged to form the *bulb* of the urethra. Then it continues with a uniform diameter to the glans penis where the lumen is again enlarged in a dorsoventral direction and is known as the *fossa navicularis*. Throughout its course this portion is surrounded by a cylindrical mass of erectile tissue, the *corpus spongiosum* or *corpus cavernosum urethrae* (Figs. 341, 371).

The structure of the mucous membrane varies in the different portions. The prostatic urethra is lined by a transitional

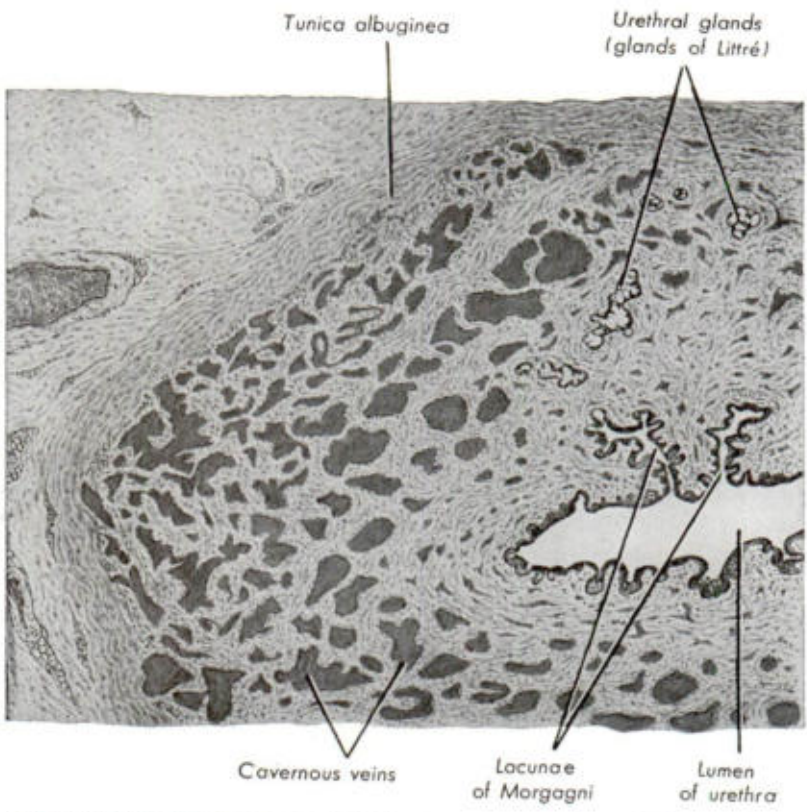

FIG. 341. Transverse section of a part of the cavernous portion of the urethra. See Figure 371 for lower magnification showing complete section of penis. × 19.

epithelium similar to that of the bladder. In the membranous and cavernous portions the epithelium is stratified columnar or pseudostratified up to the fossa navicularis. There it changes to stratified squamous which at the external urethral opening becomes continuous with the epidermis of the skin. More or less extensive areas of stratified squamous epithelium are often seen throughout the whole course of the urethra.

The epithelium rests on a thin basement membrane beneath which is a stroma of loose connective tissue rich in elastic fibers, and containing in its deeper portion a plexus of capillaries and thin-walled veins. Smooth muscle fibers, both longitudinally and circularly disposed, are found in the prostatic and membranous portions. A definite submucosa cannot be distinguished.

The prostatic urethra is surrounded by the fibromuscular tissue of the prostate, which under ordinary conditions keeps the urethral lumen closed. The membranous portion is encircled by a sphincter of skeletal muscle fibers from the deep transverse perineal muscle.

The mucosa of the cavernous portion contains very little muscle and is surrounded by a cylindrical mass of erectile tissue, the corpus spongiosum. The latter consists of a network of large irregular venous spaces, or lacunae, which are lined

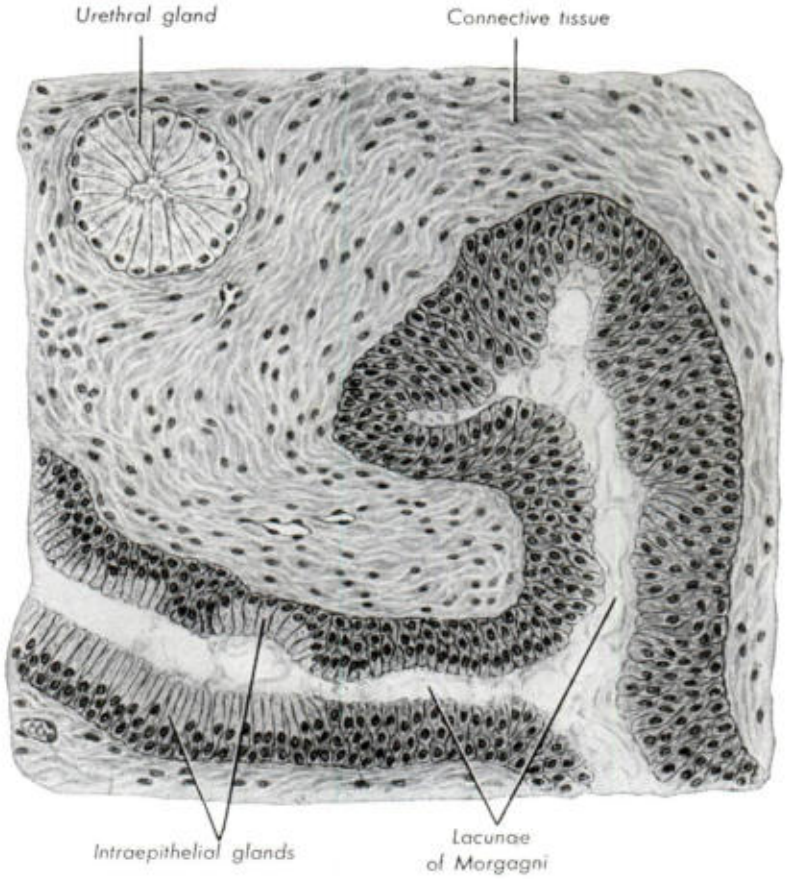

FIG. 342. Section through the dorsal portion of the corpus cavernosum urethrae showing clear, mucous secreting cells in a tubule of the urethral glands (glands of Littré) and groups of similar cells (intraepithelial glands) in the lacunae of Morgagni. × 294.

by endothelium and are separated from each other by trabeculae of fibroelastic tissue containing numerous smooth muscle fibers running both longitudinally and circularly. These lacunae connect with the plexus of veins in the mucosal stroma. The corpus spongiosum is enclosed in a connective tissue capsule containing numerous elastic fibers and on its inner surface smooth muscle cells.

The lumen of the urethra shows a number of deep, irregular outpocketings, the *lacunae of Morgagni*. The lacunae continue into branched tubular glands, the *glands of Littré*, which extend deep into the stroma and may even penetrate into the corpus spongiosum. They are most numerous in the dorsal part of the cavernous portion of the urethra (Figs. 341, 342). Most of the cells lining the gland tubules are clear staining, mucous secreting cells. Isolated mucous cells or groups of them (intraepithelial glands) are likewise found interspersed in the epithelium lining the lacunae of Morgagni.

The Female Urethra. The female urethra is a short tube 3–5 cm. long. The epithelium varies considerably in different

individuals. Near the bladder it is usually transitional. The remainder of the urethra is lined mainly by stratified squamous epithelium, with areas of stratified columnar or pseudostratified epithelium. The mucosa is thrown into longitudinal folds. Glands of Littré, though in fewer numbers than in the male, open into the lacunae between the folds.

The abundant stroma is rich in elastic fibers and contains a plexus of numerous thin-walled veins.

The rather indefinite muscularis contains both longitudinal and circular smooth muscle fibers, many of which penetrate into the stroma between the veins. An outer layer of skeletal muscle fibers forms a urethral sphincter.

A definite fibrosa is absent, the outer connective tissue fusing with that of the vagina.

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XIX

THE MALE REPRODUCTIVE SYSTEM

The reproductive system of the male consists of the testes, the various excretory ducts, the accessory reproductive glands—seminal vesicles, prostate and bulbo-urethral glands—and the penis (Fig. 343).

The essential constituents of the seminal fluid, the spermatozoa, are not products of cellular secretion, but are themselves cellular elements which are formed in the tubules of the testis and leave the body through the genital ducts. For this reason the testes, and ovaries as well, are known as *cytogenic* organs. The other constituents of the semen are not formed in the testis, but are secreted by the genital ducts, seminal vesicles, prostate, and bulbo-urethral glands. Besides forming the cellular sex elements, the testes and ovaries secrete physiologically important substances directly into the blood, and hence they are endocrine glands.

The *testes* are ovoid or walnut-shaped bodies which have the organization of compound tubular glands. Each testis is enclosed in a dense fibrous capsule, the *tunica albuginea*, underneath which there is a looser layer of connective tissue rich in blood vessels, the *tunica vasculosa*. A closed serous sac, the *tunica vaginalis*, surrounds the anterior and lateral surfaces of the testis. This cleft-like sac is a detached diverticulum from the peritoneal cavity. The testes have approximately the same relationship with this sac as they had with the peritoneal cavity before their descent into the scrotum. The visceral layer of the tunica vaginalis adheres as a smooth glistening membrane to the tunica albuginea; the parietal layer lines the inner surface of the scrotum. Both layers are lined by mesothelial cells. Posteriorly the serous sac is lacking, the testis lying behind and outside the tunica vaginalis.

The tunica albuginea of the posterior portion of the testis is greatly thickened to form the *mediastinum testis* or *corpus Highmori*, from which connective tissue septa, the *septula testis*, radiate into the organ and blend with the tunica albuginea at various points (Fig. 343). In this way the interior of the testis is subdivided into a number of pyramidal lobules, with bases directed toward the periphery and apices at the mediastinum. The septula do not form complete partitions, the lobules anastomosing with each other in numerous places.

Behind the testis and outside of its tunica albuginea is an elongated body, the *epididymis*. Three regions may be distinguished in it: (1) an upper expanded portion, the *head* or *globus major*, which projects above the upper pole of the testis; (2) a narrower middle portion or *body*; and (3) a somewhat thickened lower portion, the *tail* or *globus minor*. At the lower pole of the testis the tail of

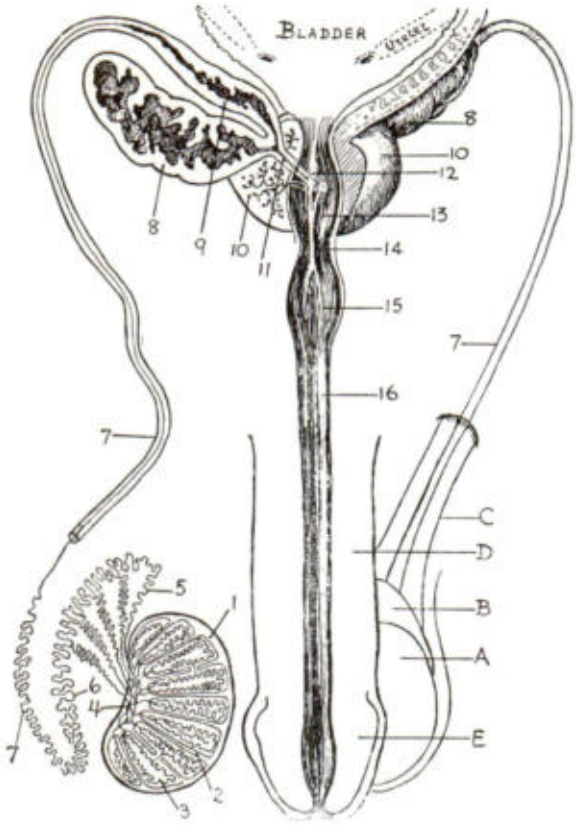

FIG. 343. Sketch of male genital organs. *A*, Testis; *B*, head of epididymis; *C*, spermatic cord; *D*, penis; *E*, glans penis; *1*, tunica albuginea; *2*, septum of testis; *3*, seminiferous tubule; *4*, mediastinum with rete testis; *5*, ductulus efferens; *6*, ductus epididymidis; *7*, ductus deferens; *8*, seminal vesicle; *9*, ampulla of ductus deferens; *10*, prostate gland; *11*, ejaculatory duct; *12*, colliculus seminalis with opening of utriculus prostaticus; *13*, *14*, *16*, prostatic, membranous and penile portions of urethra; *15*, bulb of urethra. (After Dickinson.)

the epididymis turns sharply upon itself and becomes continuous with the main excretory duct, the *ductus deferens*.

Each lobule of the testis contains several intricately coiled tubules, the *convoluted seminiferous tubules*, surrounded and supported by intertubular connective tissue. They have a length of 30–70 cm. and a caliber varying in different individuals between 150–300 μ. In the same individual the diameter is relatively constant.

The tubules do not end blindly but form single, double or even triple arches. Both limbs of an arch are not always in the same lobule. Communication between the tubules of adjacent lobules is established by lateral branches which pass through the incomplete interlobular septa. The course of the seminiferous tubules has been determined in laboratory mammals by teasing out the tubules after maceration and by reconstructions, and forms the basis of the description given here. Toward the apex of a lobule the convoluted tubules unite with the

narrow *straight tubules* which are about 30 μ in diameter. The straight tubules pass into the mediastinum and there empty into an irregular network of thin walled channels, the *rete testis* (Fig. 357). The straight tubules and rete testis form the beginning of the genital duct system.

From the rete testis arise eight to fifteen tubules, the *ductuli efferentes*, which pass into the head of the epididymis and there converge to form the *duct of the epididymis* (Fig. 343). The efferent ductules start as straight tubules, but soon after leaving the mediastinum pursue a tortuous spiral course, each tubule with its surrounding connective tissue forming a conical lobule or *conus vasculosus* of the head of the epididymis. The length of the efferent tubules is about 6–10 cm. The most anterior ductule becomes directly continuous with the duct of the epididmyis which then receives the remaining efferent ductules at shorter or longer intervals.

The duct of the epididymis is an enormously convoluted tubule having a length of about four meters. It begins in the head where it receives the ductuli efferentes and winds in a most intricate manner through the body and tail of the epididymis. At the caudal pole it turns sharply upon itself and passes without any definite demarcation into the *ductus deferens*.

THE TESTIS

The Convoluted Seminiferous Tubule. The wall of a convoluted tubule consists of (a) an outer capsule or tunica propria of fibroelastic connective tissue and flattened fibroblasts, which closely invests the tubule; (b) a thin homogeneous basement membrane; and (c) a lining of a complex stratified epithelium.

The epithelium consists of two kinds of cells, the supporting cells or cells of Sertoli and the spermatogenic cells.

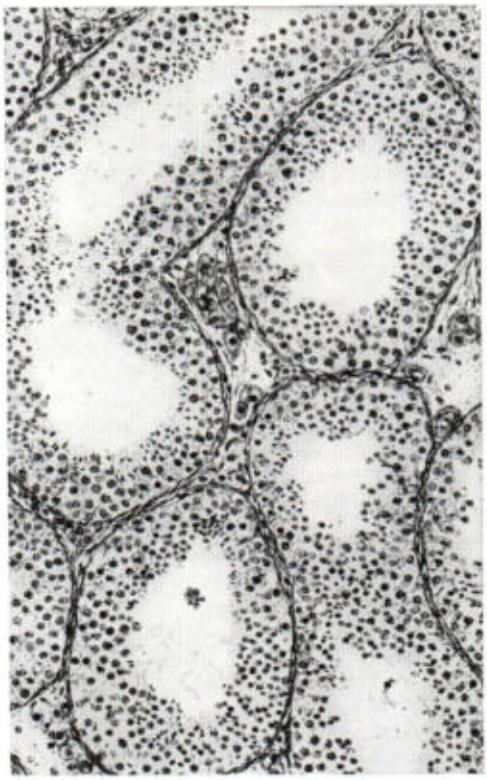

FIG. 344. Section through the right testis of a healthy man, 34 years of age. × 150. (After Stieve.)

1. The *cells of Sertoli* (Fig. 345) are relatively few in number. They are tall irregularly columnar cells which extend from the basement membrane to the lumen. Their sides are markedly uneven, showing pits and depressions into which fit the adjoining germ cells. The nucleus is ovoid, pale-staining, with finely dispersed chromatin and usually contains one or more prominent nucleoli. The nuclear membrane often shows a characteristic longitudinal or spiral groove or invagination. The position of the nucleus varies in different Sertoli cells from the basal position to a considerable distance from the basement membrane. The cytoplasm contains granular and fibrillar

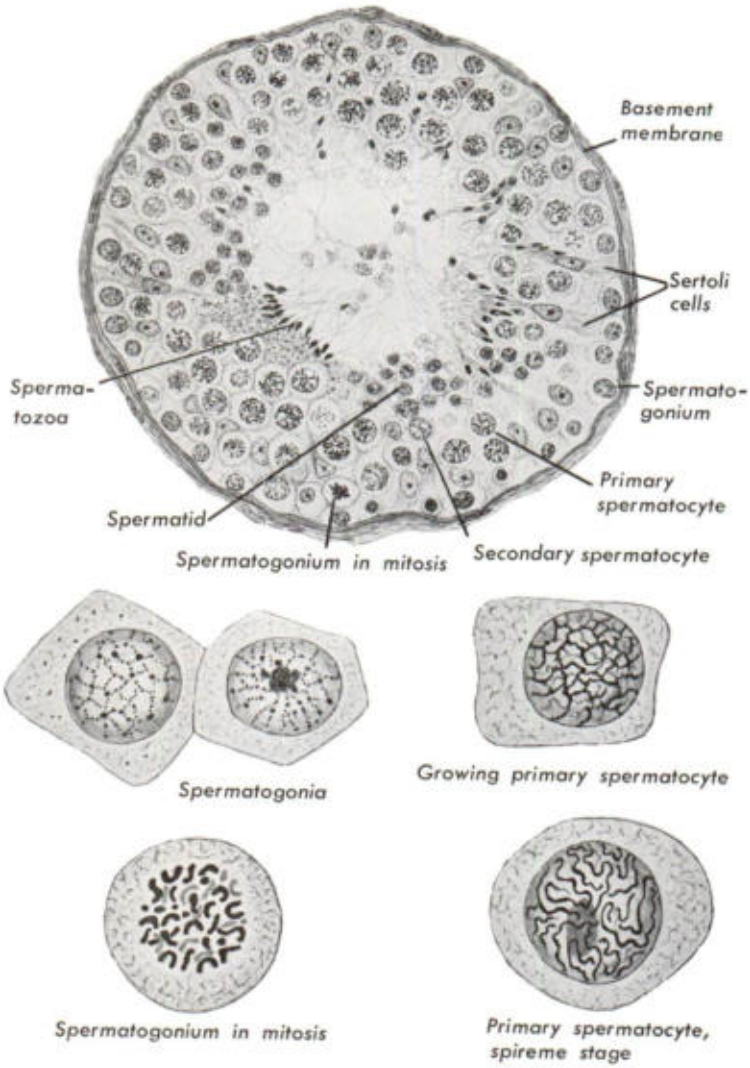

FIG. 345. The top figure illustrates a seminiferous tubule of a man 19 years old. × 360. (After Stieve.)

The lower figures illustrate cells from seminiferous tubules of a man 21 years old. × 1200. (After Stieve.) (See Fig. 346 for later stages.)

mitochondria, varying amounts of lipid droplets, and usually a peculiar elongated spindle shaped crystalloid placed near the nucleus. The cell borders are so indistinct that they can rarely be distinguished with the light microscope. Though of common origin with the germ cells, the Sertoli cells do not participate in sperm production but act as supporting and nutritive agents for the spermatogenic cells.

By special staining methods, it has been shown that the Sertoli cells have cytoplasmic processes which increase in num-

ber and size at the time when the spermatids are maturing. Thus, the Sertoli cells exhibit a cyclic activity which is correlated with the stage of spermatogenesis in any given region of the seminiferous tubule.

2. The *spermatogenic cells* lie between the cells of Sertoli, and from four to eight layers occupy the space between the basement membrane and the lumen. In the undeveloped testis only the primitive germ cells or spermatogonia are present. With the onset of sexual maturity the spermatogenic cells are represented in all stages of differentiation and are arranged in several more or less distinct layers (Fig. 345).

Directly inside the basement membrane

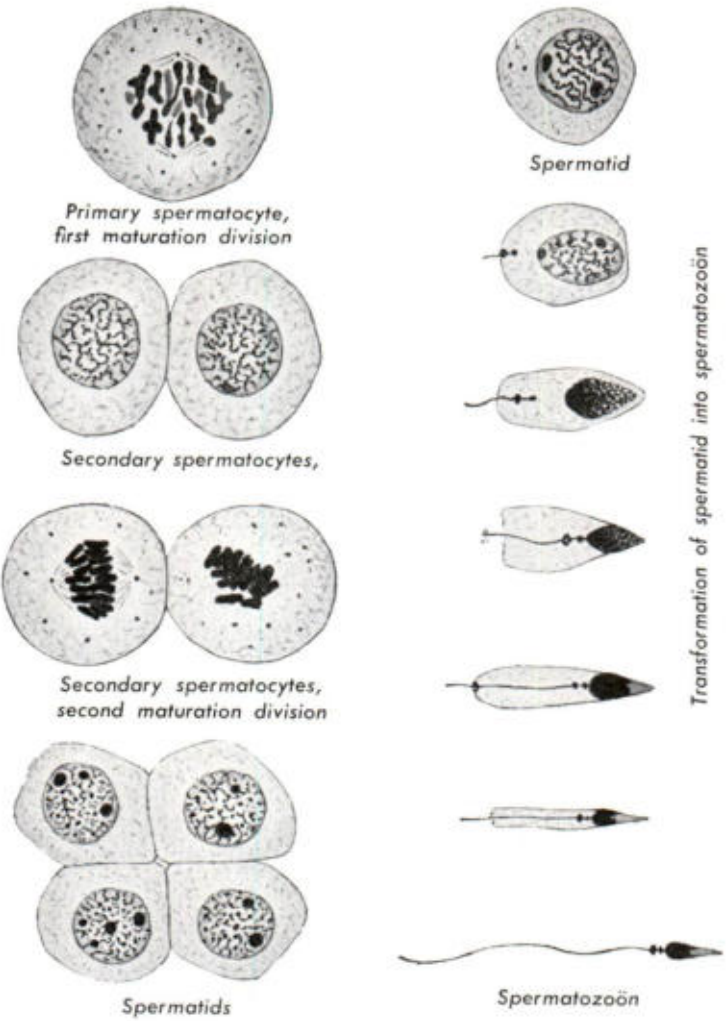

FIG. 346. Cells from seminiferous tubules of a man 21 years old. × 1200. (After Stieve.) (See Fig. 345 for earlier stages.)

are several layers of primitive germ cells or *spermatogonia* from which all the spermatozoa are ultimately derived. They are spherical or cuboidal cells with a diameter of about 12 μ. The nucleus is spherical and rich in chromatin. By mitotic activity, these cells maintain their own numbers and also provide the cells which differentiate into spermatocytes. In man, they usually undergo seven divisions before they differentiate into spermatocytes.

The *primary spermatocytes* lie next to the spermatogonia on their inner side. They are large cells with a diameter of 17–19 μ and are formed from the innermost layer of the spermatogonia. The chromatin of the large vesicular nuclei has a variable appearance depending on the functional state of the cells. It may be in the form of elongated spiremes, or in the form of condensed chromosomes preparatory to cell division (Figs. 345, 346).

Each primary spermatocyte gives rise by division to two *secondary spermatocytes* or *prespermatids*. They are smaller cells than the primary spermatocytes and lie internally to them (Fig. 345). Almost as soon as formed, each secondary spermatocyte divides to form two spermatids.

The *spermatids*, variable in number, adjoin the lumen of the tubule. They are easily recognized by their small size (about 9 μ) and location. These cells form the last generation in the spermatogenic process. They undergo no further division, but by profound changes in their structure become directly transformed into the mature *spermatozoa*. Groups of metamorphosing spermatids can often be seen in close attachment with the inner portions of Sertoli cells.

Thus in the mature testis the following cell types are distinguished passing from the periphery of the tubule toward the lumen: spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids, the latter often in process of transformation into spermatozoa.

Mature or nearly mature spermatozoa are frequently observed in the active tubule, with their heads in close attachment with the cytoplasmic processes of the Sertoli cells, and their tails extending out into the lumen. Free spermatozoa in the lumen of the tubule are relatively rare, for as soon as they are detached from the Sertoli cells they pass into the tubules of the epididymis where they are stored.

In animals with periodic rutting seasons, transverse sections through several seminiferous tubules will show clearly all stages of spermatogenesis. During the long intermissions between breeding seasons the tubules revert to a prepubertal condition and contain only spermatogonia and cells of Sertoli. In man and other animals where spermatogenesis is a continuous process the histological picture varies in different sections, since all the stages of sperm formation do not occur simultaneously in all portions of the seminiferous tubule. As a rule those stages which last a considerable time are easily seen in most sections, such as spermatogonia, primary spermatocytes and spermatids. Secondary spermatocytes which divide almost as soon as formed are usually few in number. For a similar reason the actual division of both primary and secondary spermatocytes is observed only in relatively rare instances.

In rodents, there is a definite wave of spermatogenetic activity along the seminiferous tubule and a clear correlation between the several developmental stages of the spermatogonia and the other generations of spermatogenic cells. In man, there is no distinct spermatogenic wave and no well-defined correlation between the developmental stages of spermatogonia and the other cell generations.

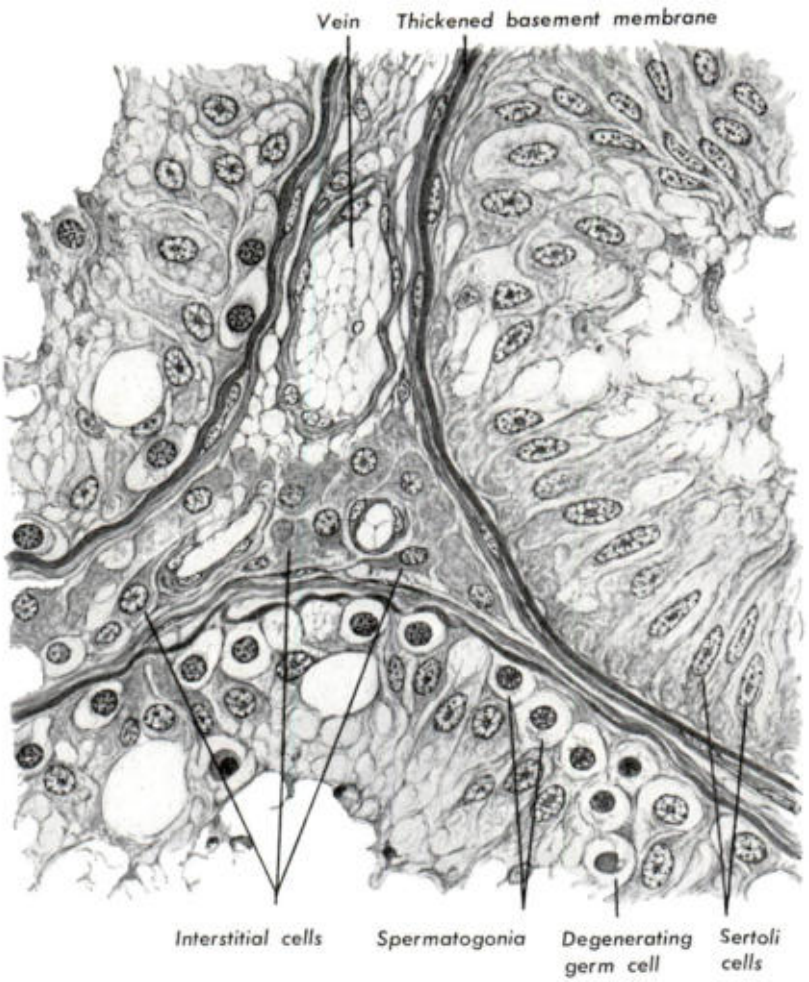

FIG. 347. Parts of three seminiferous tubules showing pronounced degeneration. The tissue was from a healthy middle-aged man. Camera lucida drawing. × 565.

Regions of spermatogenetic activity are intermingled in an irregular manner with regions of inactive germinal epithelium.

In many testes secured at autopsy there are regions in which spermatogenesis is greatly reduced or entirely absent, the seminiferous epithelium either reverting to a prepubertal condition or occasionally showing an entire absence of germ cells. After prolonged sickness and in senility the degenerative changes may be very pronounced. Although it has been known that there may be degenerative changes in the testes in medically normal men during the reproductive period of life, not many such cases have been reported until recently. Sand and Okkels have studied the testes from a large number of medically normal men, ranging in age from twenty to fifty years. They re-

port that only seventeen of their seventy-two cases showed a structural condition of the testes which is usually considered as normal. In the remainder, the basement membrane and capsule of the seminiferous tubules and often the intertubular connective tissue was thickened either diffusely or in localized areas. Hyalinization of the connective tissue was not infrequent. In about a third of their cases spermatogenesis was severely reduced. They emphasize that the figures of seminiferous tubules in textbooks of histology must be from carefully selected material. Figure 347 illustrates pronounced degenerative changes in the testes of a healthy middle-aged individual.

The mechanism causing the sperm to pass from the seminiferous tubules into the rete and hence into the efferent ductules, a very considerable journey, is of particular interest. The sperm do not become motile until they leave the testes and the duct system of the reproductive tract, and consequently their migration cannot be caused by an intrinsic movement. The seminiferous tubules have no muscle in their walls and so the sperm are not forwarded by peristalsis. Beginning with the efferent ductules, the remaining ducts have muscular walls which would move the sperm onward. Experimental studies on laboratory mammals have shown that a considerable quantity of fluid is "secreted" by the seminiferous tubules and resorbed by the rete tubules, the efferent tubules, and the duct of the epididymis. The current thus produced is probably an important factor in moving the sperm from the seminiferous tubules.

The testes of sexually mature mammals are rich in hyaluronidase. This enzyme is present elsewhere (p. 68) but the testes of slaughter-house animals is the main source of the hyaluronidase used experimentally and clinically. There is more than one hyaluronidase, that derived from testes being designated as "testicular hyaluronidase."

Spermatozoa are the source (or carriers) of testicular hyaluronidase. It is not present in the testes before sexual maturity or in those devoid of sperm. It is still present in sperm which have been washed several times. If sperm are autolyzed a large amount of the enzyme is found. Hyaluronidase is present in semen, being localized chiefly in the sperm.

Spermatogenesis. The cytological details and genetic significance of spermatogenesis are fully discussed in textbooks of genetics. Only a brief sketch can be given here of the various changes through which the primitive germ cells are transformed into mature sperm.

It is convenient to distinguish several successive periods or stages in spermatogenesis, though these merge into one another as parts of a continuous process. They may be called the period of proliferation, growth, maturation, and transformation (Figs. 345, 346).

All spermatozoa develop from spermatogonia. Those spermatogonia, which lie adjacent to the basement membrane, are relatively large cells with chromatic nuclei. Each one contains the somatic or diploid number of chromosomes characteristic of the species, as in any tissue cell. This number in man has usually been given as 48, consisting of 24 pairs of homologous chromosomes, one member of each pair being of maternal, the other of paternal origin. Recent studies indicate that the number may be 46 (or 23 pairs). During the period of proliferation the spermatogonia divide actively by ordinary mitosis, the daughter cells always containing the diploid number of chromosomes. The result of this proliferation is the production of several generations of smaller spermatogonia which are progressively pushed away from the basement membrane.

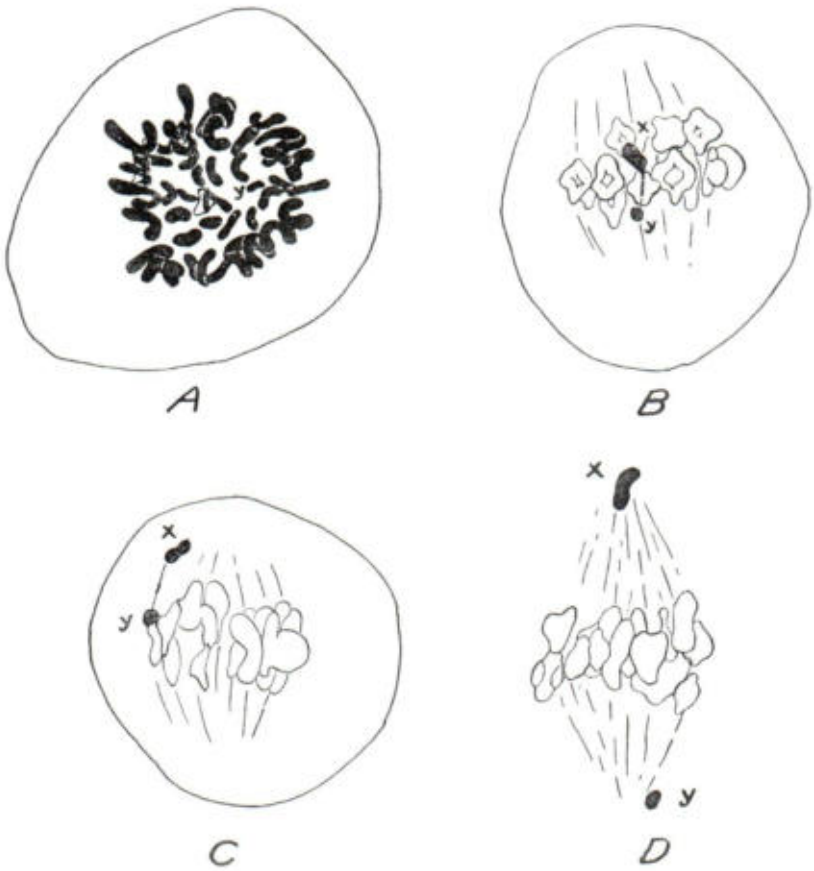

FIG. 348. Sex cells from human testis showing the number of chromosomes and the x and y chromosomes. *A*, Spermatogonium showing 48 chromosomes, including the small y chromosome. *B*, Primary spermatocyte showing the form of the x–y chromosome in the spindle. *C*, Primary spermatocyte in which the x–y chromosomes are dividing early. *D*, Primary spermatocyte showing the x and the y chromosome at the opposite poles of the dividing cell. (After Painter, 1923.)

In man, there are usually seven generations of spermatogonia. The last generation enters a lengthy intermitotic period characterized by growth and by important nuclear changes. The resultant large cells are known as primary spermatocytes.

After reaching its full size each primary spermatocyte quickly undergoes two successive divisions, the *maturation divisions*. The first division gives rise to two secondary spermatocytes, each of which in turn divides to form two spermatids. These are the last generation in the spermatogenic process and become directly transformed into spermatozoa. The important result of the two maturation divisions is that each spermatid and consequently each sperm received only the *haploid* or half the somatic number of chromosomes. A similar reduction takes place in oögenesis, the mature ovum likewise containing only the haploid number of chromosomes. When fertilization takes

place the somatic or diploid number is reestablished.

At the close of the growth period of the primary spermatocyte, the nucleus appears to contain convoluted spireme threads of chromatin granules, not unlike the spireme stage of the spermatogonial cells. The spiremes are not actually continuous with each other, but consist of a tangle of chromatin threads equal in number to the diploid number of chromosomes. As the threads thicken and shorten, their individuality becomes more apparent. Close inspection will reveal that the threads are not single but double, due to the fact that in the fine thread stage the members of each pair of similar chromosomes have come to lie side by side. This process is called *synapsis*. As the paired chromosome threads continue to thicken and shorten, they appear to fuse. Instead of 46 single thread-like chromosomes there are now 23 double threads. The fusion is not continuous, and between the points of fusion the threads may form a series of loops. Each chromosome thread now divides, giving loops in which four threads can be recognized. At this stage the pair of synaptic chromosomes, each of which will subsequently divide, is called a *tetrad*. These tetrads have consistent individual differences in size and form by which they may be easily identified. The tetrads now thicken and shorten and go on to the spindle as seen in Figure 348 *B*, *C*, *D*. Even in this greatly condensed stage the tetrads assume individual differences by which they may be recognized.

In the first maturation division the chromosome pairs which fused during synapsis become separated, one member from each pair going to each of the resulting two secondary spermatocytes. This division differs from ordinary mitosis in that no longitudinal splitting of chromosomes occurs with subsequent separation of the chromosome halves, but whole previously fused chromosomes are separated from each other, the daughter cells actually receiving only half the somatic number. It is a *reductional* or *heterotypic* division in contrast to the ordinary *equational* or *homeotypic* division. The process is known as *meiosis* in contrast to *mitosis*. Each tetrad separates in two dyads, each dyad representing an original single chromosome split precociously for a subsequent division. During the anaphase of the first maturation division one dyad from each tetrad passes to each daughter cell.

The two secondary spermatocytes, which are smaller than the primary spermatocyte, pass through a short intermitotic period and then divide to form four spermatids. The dyads are separated into single elements or monads, and one monad from each dyad goes to each daughter cell. Since the dyads are believed to be the split halves of single chromosomes, the second maturation division is of the ordinary equational or homeotypic kind. Each spermatid is about half the size of the secondary spermatocytes. Electron micrographs show that the spermatids have five protoplasmic connections for a temporary period, apparently due to a lack of complete division of the cytoplasm.

By spermatogenesis every primary spermatocyte, the tetrad spermatocyte, gives rise to four spermatids each containing the haploid number of chromosomes. Likewise, each spermatid nucleus contains only half as much desoxyribonucleic acid as that of the spermatogonia.

A similar reduction in number of chromosomes and in DNA content occurs during the maturation of the oocytes. Fertilization restores the diploid number of chromosomes with the characteristic amount of DNA.

The members of one pair of chromo-

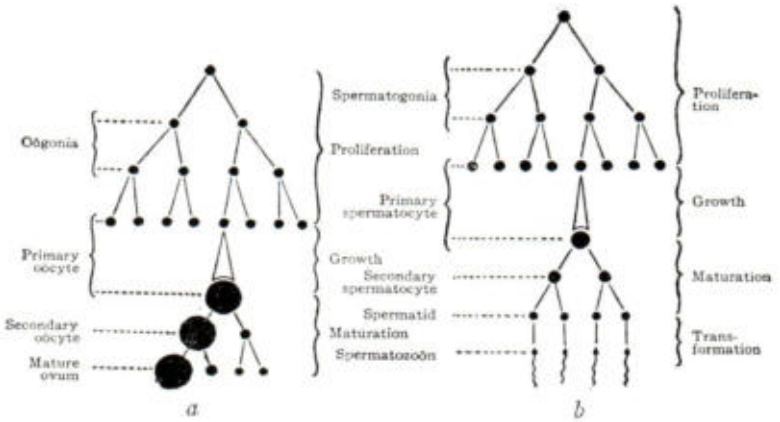

FIG. 349. Diagrams representing the comparative histogenesis of (*a*) the female sex cells and (*b*) the male sex cells. Modified from Boveri.

somes (the sex chromosomes) are dissimilar in the male. One is known as the X chromosome and is of maternal origin. The other is the Y chromosome, of paternal origin. When the members of homologous pairs of chromosomes separate during the meiotic division of the primary spermatocytes, one half of the secondary spermatocytes receives an X chromosome whereas the other half receives a Y chromosome. This differs from the condition in the female where each ovum contains an X chromosome. Consequently, when a sperm carrying an X chromosome fertilizes an ovum, an XX combination is established and a female develops. On the other hand, when a sperm bearing a Y chromosome fertilizes an ovum, an XY combination is produced and the embryo develops as a male.

Spermiogenesis. The spermatids do not divide but mature to form the spermatozoa by a process known as spermiogenesis. During most of their maturation, the spermatids are enclosed by the cytoplasmic process of the Sertoli cells from which they apparently receive nourishment.

The spermatids are small cells, about half the size of the secondary spermatocytes. They have round and rather dark staining nuclei. The stages of their transformation into mature sperm have been clarified by the use of electron microscopy.

One of the earliest changes occurs in the Golgi complex. A few of the vacuoles at the center of the complex enlarge and a relatively dense granule, the *acrosomal granule* of light microscopy, appears within one of the vacuoles. The granule apparently arises as a secretory product of the Golgi complex and it grows by accretion of new material to its surface. The vacuole also enlarges, perhaps through the incorporation of other vacuoles. In the meantime, the Golgi complex moves closer to the nucleus, with the acrosomal vacuole and granule making intimate contact with the nuclear envelope (Fig. 350). By this stage, the nucleus has become more ovoid and dense. Next, there is a change in the distribution of cytoplasm accompanied by an elongation of the cell. As the cytoplasm moves caudally, the remnant of the Golgi complex also shifts caudally, leaving the acrosomal granule and vacuole. The latter appears to collapse into a cleft-like space and forms a double-layered membrane

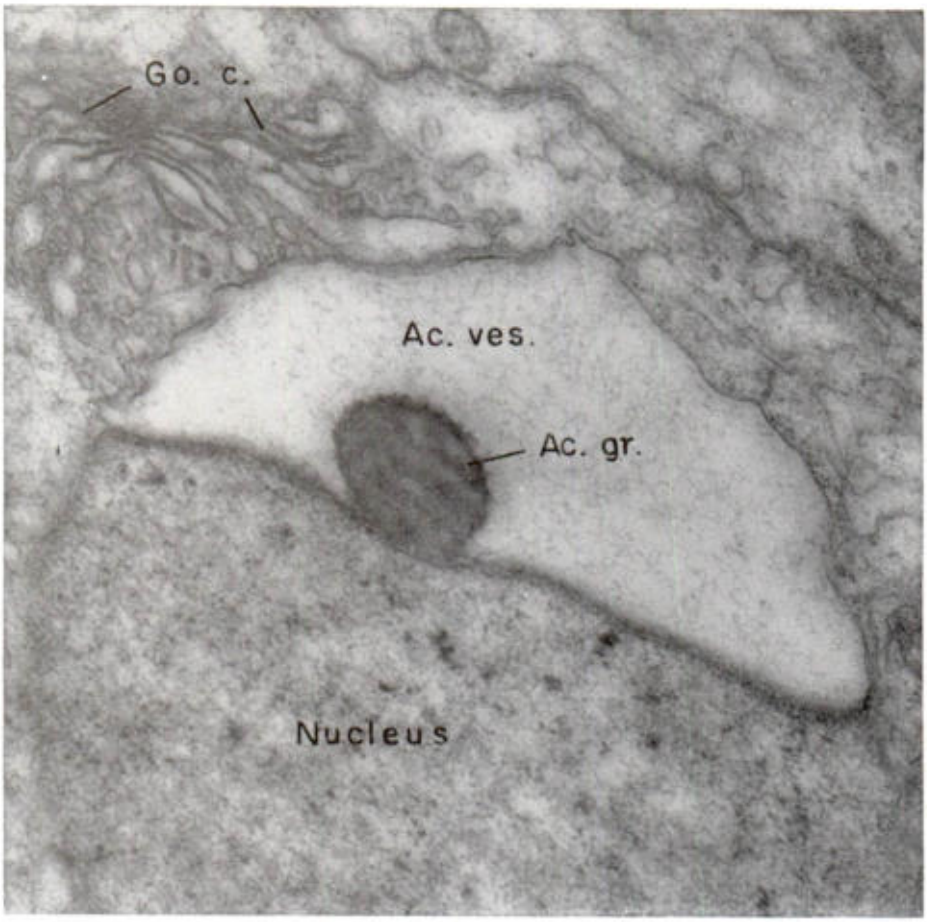

FIG. 350. Electron micrograph of cat spermatid showing nucleus, acrosomal granule (*Ac. Gr.*), acrosomal vesicle (*Ac. Ves.*), and the Golgi complex (*Go. C.*). (Courtesy of Dr. D. W. Fawcett.)

which becomes the *head cap* (Fig. 351). The acrosome eventually blends with the head cap and becomes indistinguishable in the mature human spermatozoon (Fig. 353).

Concurrently with the changes listed above, the centriole moves to a position in contact with the caudal pole of the nucleus. It is known as the proximal centriole. Longitudinal filaments comprising the intracellular part of the flagellum arise from its vicinity although they apparently are not continuous with it (Fig. 352). A dense ring known as the "ring centriole" encircles the filaments near their origin. The "ring centriole" differs in its fine structure from the proximal centriole and its origin is not clear. As differentiation proceeds, the "ring centriole" moves caudally to the posterior end of the middle piece (end ring of Fig. 353). The cytoplasm becomes reduced and is composed chiefly of mitochondria arranged in a spiral pattern in the middle piece.

The Mature Spermatozoa. The sper-

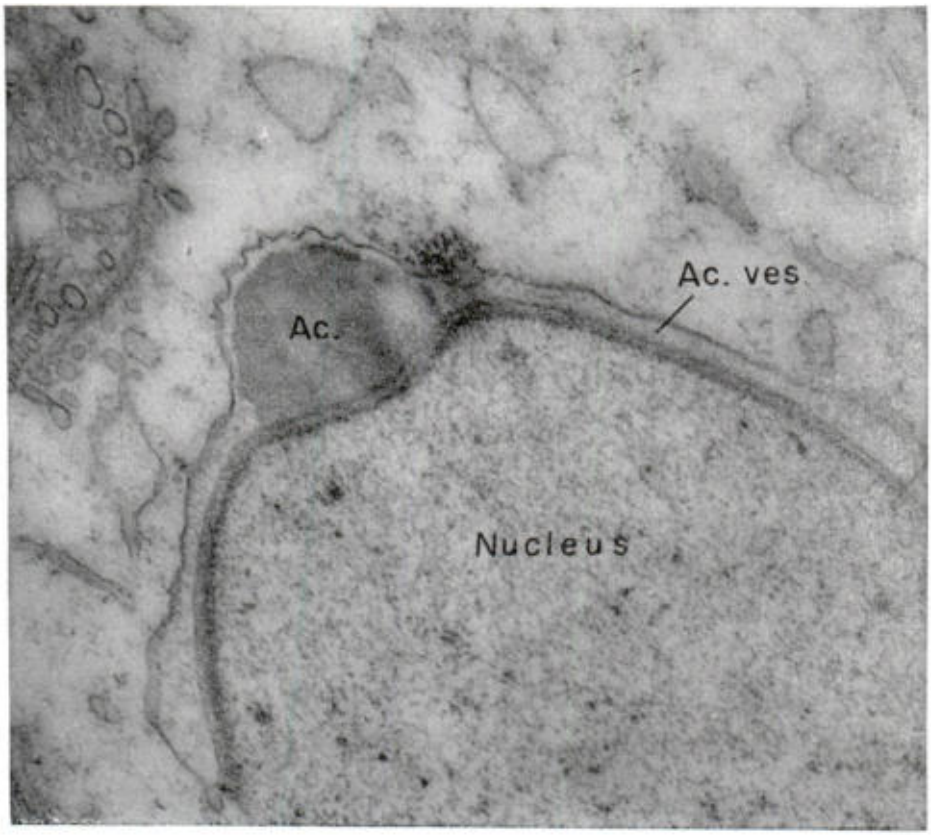

FIG. 351. Electron micrograph of human spermatid showing stage of maturation in which the acrosomal vesicle (*Ac. Ves.*) has collapsed to form a membrane-bounded narrow cavity, containing the acrosome (*Ac.*). (Courtesy of Dr. D. W. Fawcett.)

matozoa are slender, motile flagellate bodies having a length of 55–65 μ. They are formed in enormous numbers. It has been estimated that about sixty thousand spermatozoa are contained in a cubic millimeter of seminal fluid or 200–600 million in a single ejaculation. Through the undulatory motion of the tail they swim about freely, capable when fully active of covering a distance of 1–3 mm. per minute. In the seminiferous tubules and ducts of the testis they are sluggish or entirely quiescent. When expelled by the peristaltic action of the ductus deferens and duct of the epididymis they are activated into movement by the secretion of the accessory genital glands, especially the prostate. In a favorable environment the spermatozoa remain alive for some time after leaving the testis. Living spermatozoa have been found in the epididymis several weeks after the experimental ligation of the ductuli efferentes. In the female reproductive tract their life is very short.

The spermatozoön consists of (1) a head, (2) a middle piece or body, and (3) a tail or flagellum (Fig. 353).

The *head* is oval in shape with a length of 3–5 μ and a maximum width of about

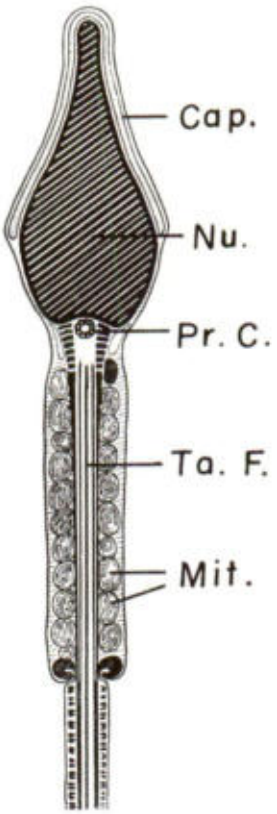

FIG. 352. Diagrammatic longitudinal section of human sperm showing the head cap (*Cap.*), the nucleus (*Nu.*), the proximal centriole (*Pr. C.*), the mitochondria (*Mit.*) of the middle piece, and the tail filaments (*Ta. F.*) within the middle piece and the proximal portion of the tail. (Courtesy of Dr. D. W. Fawcett.)

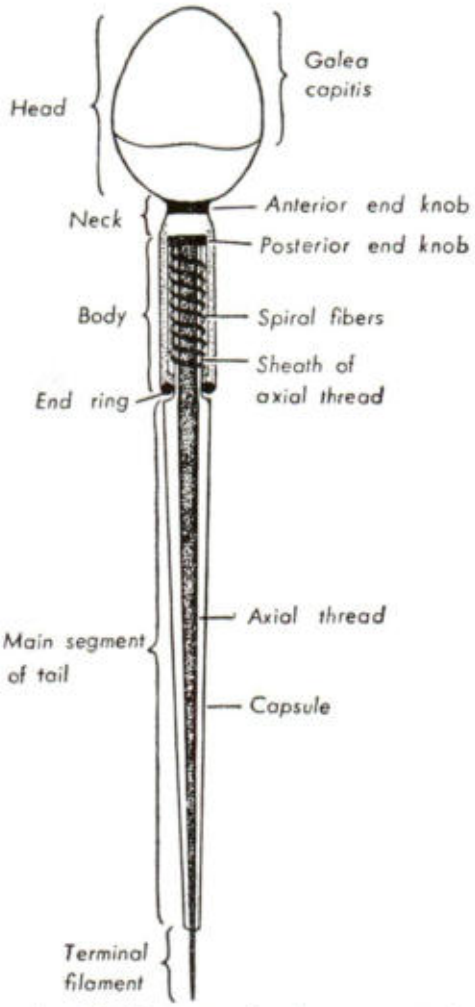

FIG. 353. Diagram of a human spermatozoön. (Meves, Bonnet.)

3 μ. The anterior portion is thinner than the posterior so that in profile it appears pear shaped. It consists mainly of a compact structureless mass of deeply staining chromatin derived from the nucleus of the spermatid. Enveloping the nucleus is a thin cytoplasmic membrane, the *galea capitis*.

The *body* is cylindrical, about 5–9 μ in length and 1 μ in diameter, and consists of a fibrillated central core, the *axial filament*, surrounded by a thin protoplasmic capsule. A short clear portion, the *neck*, connects the head and body. Its anterior end is formed by a disc shaped thickening, the *anterior end knob* (proximal centriole) which fits into a depression in the head. At the posterior end of the neck is a similar thickening, the *posterior end knob*, to which the axial filament is attached. The latter leaves the body through a perforated disc, the end ring or end disc ("ring centriole"). A closely wound *spiral*

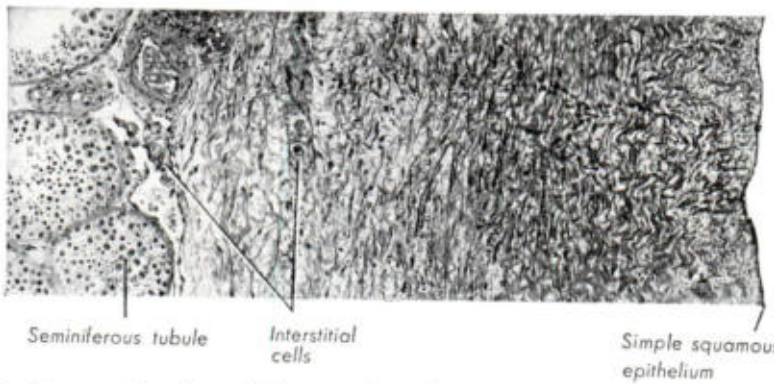

FIG. 354. Cross section through the anterior wall of a testis showing the tunics of the organ and subjacent seminiferous tubules. Masson's trichrome stain. Photomicrograph. × 104.

filament consisting of mitochondria, surrounds the body portion of the axial thread.

The *tail* consists of a *main segment* 40–50 μ in length, and a *terminal segment* having a length of 5–10 μ. In the main segment the fibrillated axial thread is enclosed in a thin cytoplasmic membrane. The terminal segment consists of the axial filament alone.

Of the above described parts of the spermatozoön only the head and tail can usually be distinguished, except by the use of special methods and very high-powered objectives.

The significance of the different parts of the spermatozoön has been brought out in describing its development. From this it is seen that the spermatozoön, like the mature ovum is a true sex cell containing half the somatic number of chromosomes. In spite of its small size and modified form it contains all the elements important for fertilization. The head furnishes the nuclear material and a part of the Golgi complex, the middle piece the centriole and mitochondria. The tail is a temporary accessory structure which enables the spermatozoön to reach the ovum by active movement.

Tunica Albuginea. The *tunica albuginea testis* is a tough fibrous membrane that encapsulates the testis (Fig. 354). It is about half a millimeter in thickness. Externally it is covered by mesothelium. During the early development of the testis this covering layer of epithelium is cuboidal and is the germinal epithelium.

Beneath the simple squamous epithelium is a layer of rather fine closely-woven collagenous fibers which blends with a deeper layer of interweaving coarse collagenous fibers. This deeper layer forms the main component of the tunica albuginea. The inner part of the tunic is composed of looser connective tissue, the innermost part of which is highly vascular and is called the *tunica vasculosa*. Just beneath the tunica vasculosa is the parenchyma of the testis. The delicate connective tissue forming the interlobular septa of the testis attaches to and is continuous with the tissue of the tunica vasculosa.

The surface epithelium of the testis and the subjacent connective tissue is often called the *visceral layer* of the *tunica vaginalis*. At the posterior part of the testis this layer is continuous with the parietal layer of the tunica vaginalis.

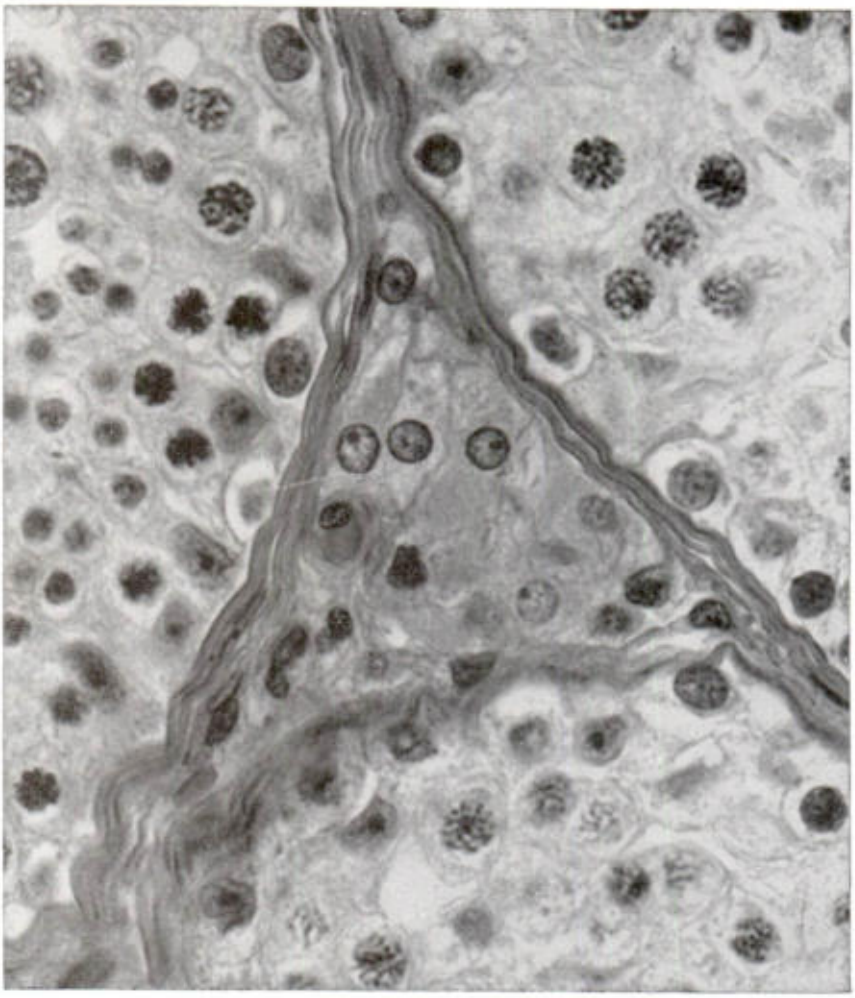

FIG. 355. Photomicrograph of a group of human interstitial (Leydig) cells between three contiguous seminiferous tubules. (Courtesy of Dr. D. W. Fawcett.)

Between the visceral and parietal layers is a cavity which is a remnant of the peritoneal cavity.

The tunica vasculosa contains numerous and quite large blood vessels. Branches of these penetrate the denser part of the tunica albuginea.

In the tunica vasculosa there are usually interstitial cells (cells of Leydig). These also occur in the denser tissue of the tunica albuginea (Fig. 354).

Interstitial Cells. Besides the usual connective tissue elements, the stroma contains characteristic cells known as the *interstitial cells* or *cells of Leydig*.

These cells occur in groups of various sizes and are quite distinct in the human testis (Figs. 355, 356). Small blood vessels are usually present in the groups. The interstitial cells are large and are ovoid or polygonal in shape. They have a large nucleus which is frequently eccentrically

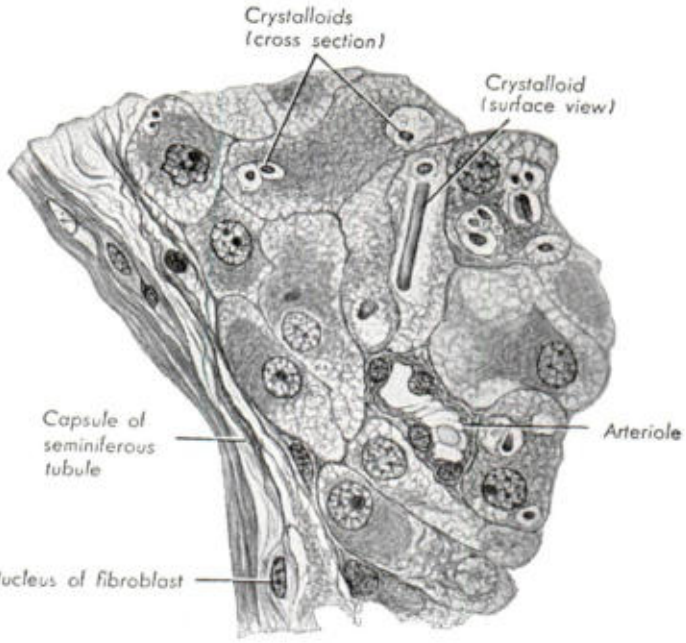

FIG. 356. A group of interstitial cells (cells of Leydig) of a normal adult man. Surgical specimen fixed in Worcester's fluid and stained with iron hematoxylin and erythrosin. × 990.

located. The cytoplasm of the interstitial cells is granular and fairly dense near the nucleus but peripherally it is vacuolated and in usual preparations stains quite lightly. This is largely due to the dissolving out of lipid granules and droplets. The interstitial cells also contain pigment and crystalloids. The crystalloids are rod-shaped structures and in cross section are oval or round. Their number and size vary greatly. They are formed of an albuminous substance and are quite resistant to solvents. Fixation soon after death is necessary to preserve them.

The interstitial cells arise from fibroblasts and may revert to cells indistinguishable from fibroblasts.

The internal secretion of the testes (testosterone) is elaborated by the interstitial cells (see page 468).

Blood Vessels. Branches of the spermatic artery ramify in the mediastinum and tunica vasculosa. These send branches into the septa of the testis, which give rise to a capillary network around the convoluted tubules. The blood is collected by veins which accompany the arteries.

Lymphatics. In the intertubular tissue are numerous lymphatics, appearing as clefts lined by endothelium. These connect with lymph vessels in the mediastinum. Relatively few lymphatics are found in the tunica albuginea.

Nerves. Nerve fibers accompany the blood vessels and enter the interior of the testes through the mediastinum. Some fibers go to blood vessels and the termination of other fibers is not known.

THE GENITAL DUCTS

Straight Tubules and Rete Testis. At the juncture of a seminiferous tubule with a straight tubule there is an abrupt change in structure. The junctures occur at varying distances from the rete, the

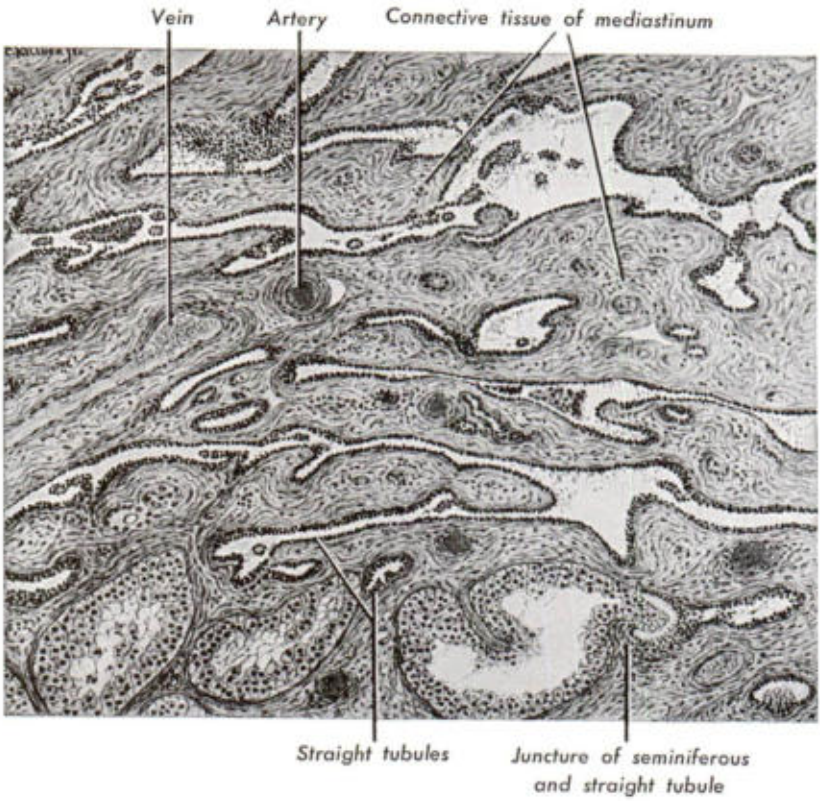

FIG. 357. From a longitudinal section through a testis showing a part of the mediastinum and rete testis and the adjacent seminiferous tubules. The juncture of a seminiferous with a straight tubule is shown in the lower right quadrant of the figure. The separation between the rete and the straight tubules is topographical, not structural. The upper pole of the testis is to the right. Human, accident case. Fifty-four years old. × 65.

straight tubules thus varying in length. Close to a juncture, the developing sex cells of a seminiferous tubule decrease in number and then finally disappear. The Sertoli cells change somewhat in structure, their cytoplasm becoming more vacuolated and their nuclei more dense. They increase in number and finally form a continuous epithelial layer. This protrudes into the enlarged end of the straight tubule (Fig. 358). The epithelial lining then changes abruptly into the cuboidal or columnar type characteristic of the straight tubules and rete.

The rete testis is composed of wide anastomosing channels, the general course of which is upwards toward the ductuli efferentes. The spaces of the rete are lined by a simple epithelium which varies in height from a low cuboidal to a columnar (Fig. 357). Some of the cells have a single cilium (flagellum) which is connected to the distal centriole. No secretion droplets are present. In contrast to Sertoli cells, the nuclei stain deeply and the cell wall is well defined. The tubules of the rete have no definite lamina propria that is distinct from the connective tissue composing the

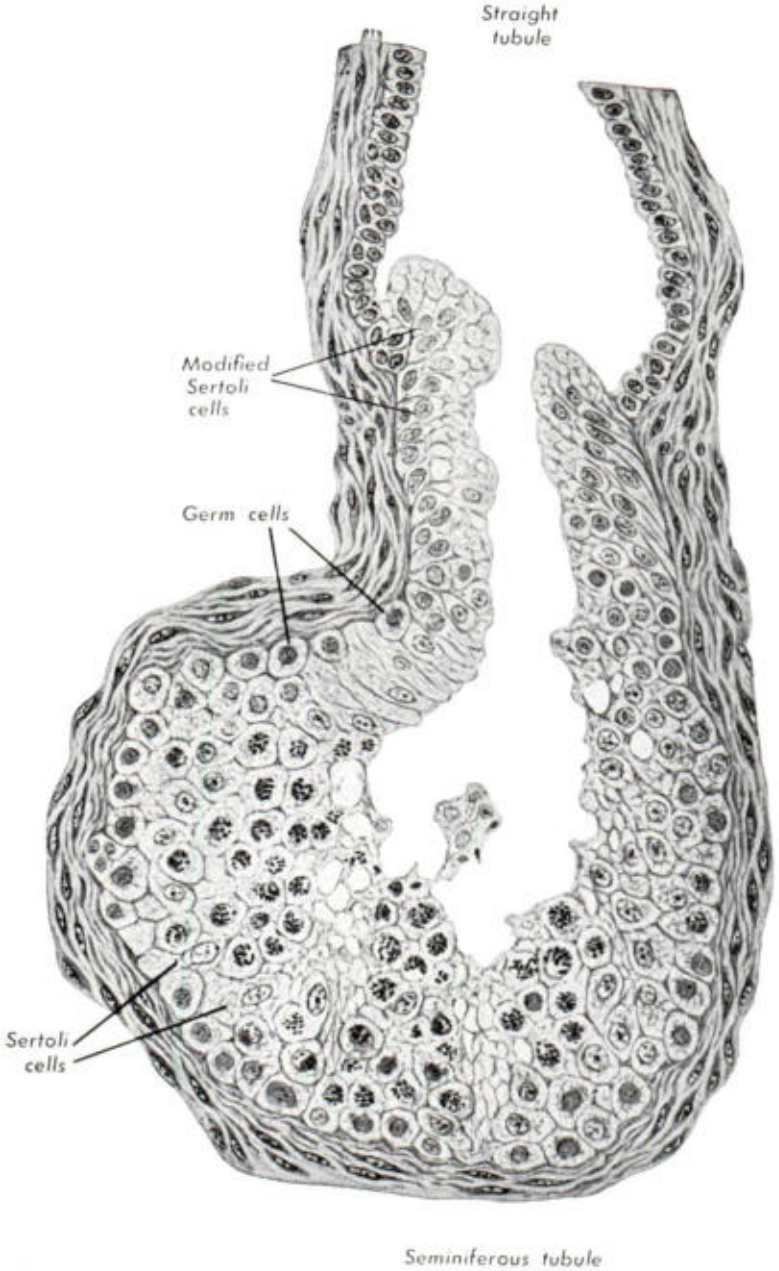

FIG. 358. A juncture of a seminiferous tubule and a straight tubule. The seminiferous tubule is cut somewhat obliquely. From the same testis shown in Figure 357. × 445.

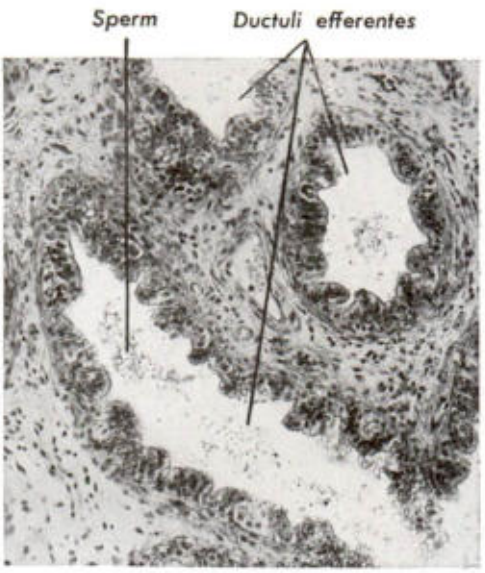

FIG. 359. Sections of ductuli efferentes from the head of the epididymis. Adult man. Photomicrograph. × 130.

mediastinum. No smooth muscle is present around the straight tubules or rete testis.

Ductuli Efferentes. The epithelium of the efferent ductules is simple and consists, mainly, of two kinds of cells, groups of high columnar ciliated cells alternating with nonciliated cuboidal cells. The distribution of the cilia is, however, quite variable. Often they are missing on the tall cells and present on the cuboidal cells (Fig. 359).

On account of the varying height of the cells the inner surface of the tubules appears folded or wavy, the tall cells forming conical crests, the cuboidal cells lining crypt-like depressions or pockets. The basement membrane is not affected, and externally the tubules have a smooth contour.

Both the tall and low cells are secretory in function and have a granular cytoplasm. Bleb-like droplets of secretion are occasionally seen adhering to the free surface of the nonciliated cells. Many of the cells contain pigment granules. As the tubules approach the epididymis the pockets become fewer and the epithelium consists mainly of tall cells.

In addition to the columnar and cuboidal cells, rounded, basally-placed cells which do not reach the surface of the epithelium also occasionally occur. In thin serial sections it can be determined that the columnar and cuboidal cells extend around these basal cells, and reach the basement membrane. The epithelium is thus a modified simple type or is more accurately designated, due to the basal cells, as pseudostratified. The basal cells in the ductuli efferentes and in the other ducts have a cytoplasm which is rich in lipids so that in ordinary preparations their cytoplasm stains very lightly. Cells which are intermediate in size and shape between the basal and columnar cells are not present. The function of the basal cells is unknown.

The epithelium rests on a distinct basement membrane, surrounded by a lamina propria of lamellar connective tissue containing many capillaries and some circular smooth muscle fibers.

Ductus Epididymidis (Duct of the Epididymis). This duct has an even contour, not only externally, but internally as well, for the epithelial cells all end at the same level and the smooth muscle in fixed preparations has not undergone a contraction sufficient to cause a folding of the mucosa as is the case with the ductus deferens. The lining epithelium of the epididymis is composed of two types of cells: very narrow, tall columnar cells, and rounded or angularly-shaped basal cells (Fig. 361). Although it cannot be determined with certainty, even in very thin sections, that all of the columnar cells overlying the basal ones reach the basement membrane, nevertheless many of them certainly do by bending around the basal cells. Presumably all of

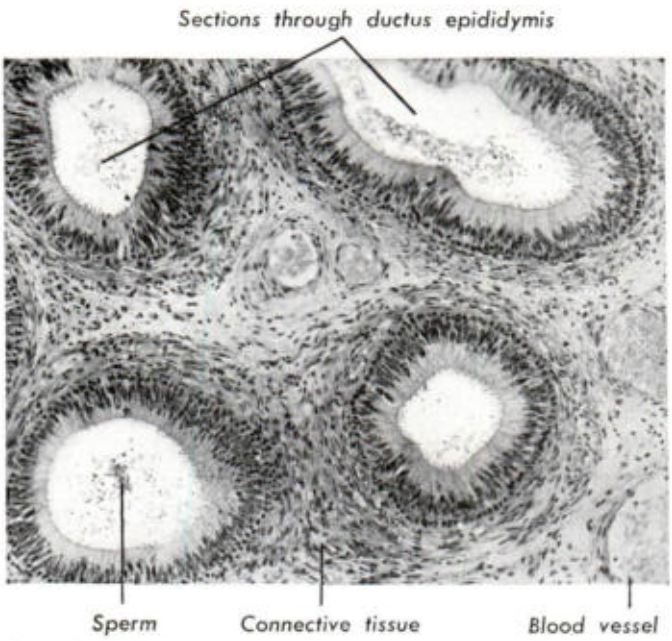

FIG. 360. Sections through the duct of the epididymis. Adult man. Photomicrograph. × 105.

them reach the basement membrane. The epithelium is thus pseudostratified.

The columnar cells bear non-motile processes (stereocilia), and the cytoplasm of most of the columnar cells contains distinct droplets and granules of various sizes (Fig. 361). Some of the droplets are undoubtedly secretory materials and others may represent the products of resorption from the lumen. Pigment granules also occur but are not as numerous as they are in the epithelium of the efferent ductules. The stereocilia are supposed to assist in forming a pathway for the secretion of the epithelium into the lumen of the tubule. The nuclei of the columnar cells are elongate and lie at somewhat different levels. The basal cells are similar to those already described in the efferent tubules, but are much more numerous (Fig. 361).

There is a basement membrane and lamina propria. The smooth muscle is circular but is small in amount. As the juncture with the ductus deferens is approached, the muscle increases in amount and longitudinal bundles appear.

Ductus Deferens. The main genital duct is a direct continuation of the duct of the epididymis. Its proximal portion which runs along the epididymis is coiled. Then it straightens out and, as part of the spermatic cord, passes into the abdominal cavity to terminate in the prostatic portion of the urethra. Shortly before reaching the prostate the ductus deferens shows a spindle-shaped dilation, the *ampulla*, which gradually narrows to form the thin *ejaculatory duct*. The two ejaculatory ducts penetrate the prostate gland and empty into the urethra on either side of the prostatic utricle. When fully straightened the duct is about half a meter in length.

The wall of the ductus deferens consists of three coats—mucosa, muscularis, and fibrosa (Fig. 362).

The *mucosa* is lined by a pseudostrati-

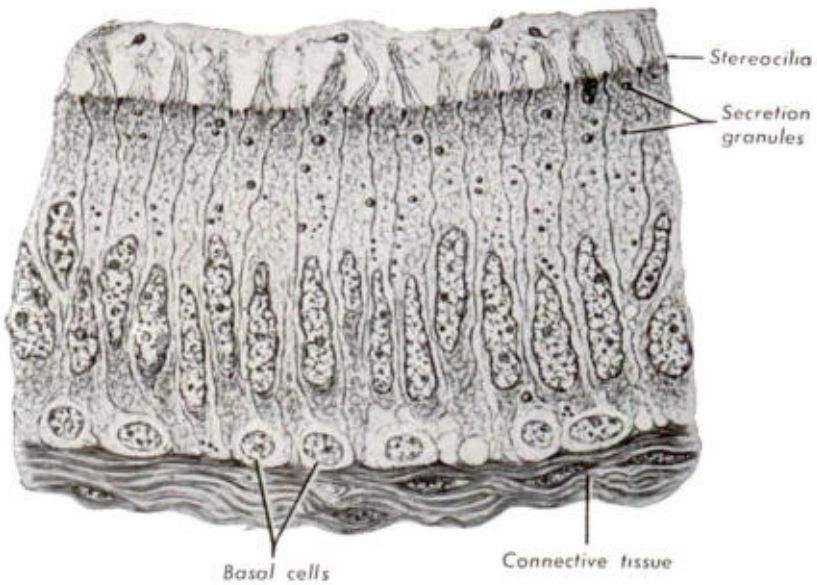

FIG. 361. A section through the ductus epididymidis showing the epithelium and subjacent connective tissue. Numerous secretion granules are present. Human, accident case. Fifty-four years old. Mallory-azan stain, 3μ section. × 945.

fied columnar epithelium somewhat similar to that of the duct of the epididymis. However, the surface cells are lower and the stereocilia show a variable distribution, being absent on some cells and present on others. Secretion granules are not as numerous as they are in the epithelium of the epididymis. The epithelium is surrounded by a stroma exceedingly rich in elastic fibers which form a compact lamina propria around the delicate basement membrane. In the deeper portion of the stroma are numerous blood vessels. Owing to the abundant elastic tissue and strong muscularis the mucosa is thrown into four or five longitudinal folds, so that in transverse section the lumen appears star shaped.

The *muscularis* is by far the thickest coat (1–1½ mm.) and consists of three smooth muscular layers, an inner longitudinal, a middle circular, and outer longitudinal. The middle and outer coats are strongly developed layers. The inner longitudinal layer is relatively thin.

The *fibrosa* consists of fibroelastic tissue containing numerous blood vessels, nerves, and often scattered bundles of smooth muscle fibers. It blends without definite demarcation with the surrounding connective tissue.

In the *ampulla* the mucosa shows numerous folds forming crypts or recesses, many of which extend as tubular structures into the stroma (Fig. 363). These are glandular structures lined by a cuboidal or columnar epithelium of a secretory character, the cells frequently containing yellow pigment granules.

The *ejaculatory ducts* have a thin mucous membrane thrown into numerous fine folds, with glandular recesses like those of the ampulla. The epithelium is simple columnar or pseudostratified and becomes transitional near the urethral opening. Beneath the epithelium is a

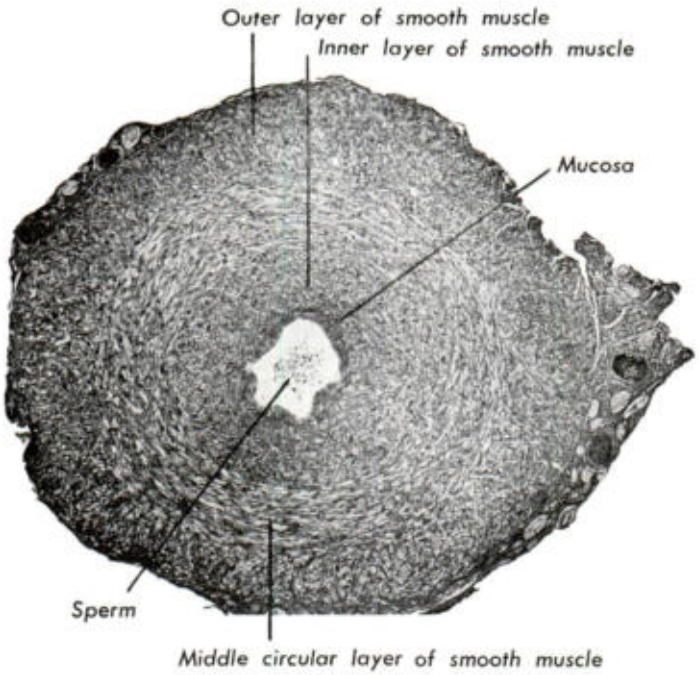

FIG. 362. Transverse section through ductus deferens. Adult man. Photomicrograph. × 38.

rich network of elastic fibers. A distinct muscularis is present only at the beginning. In the prostatic portion it disappears and its place is taken by the fibromuscular tissue of the prostate gland.

STORAGE OF SPERM

The ductuli efferentes, epididymis and the first part of the ductus deferens are the storehouse for the sperm. The passage of the sperm through the straight tubules and rete testis must be relatively rapid for they are rarely seen in these ducts, although numerous in the ductuli efferentes and ductus epididymidis. The storage place of the sperm is thus correlated with the secretory capacity of the epithelial cells of the different segments of the duct system. It has been pointed out that in the straight tubules and rete, the epithelial cells show no evidence of secretion, whereas in the efferent ductules and epididymis secretory granules (glycogen, et cetera) are numerous. Ligation experiments in animals have shown that when the testes are left intact, the sperm retain their capacity of becoming motile for 40 to 60 days, although the period in which they retain the capacity to fertilize the egg is somewhat shorter. During at least a part of their sojourn in these ducts, they are probably undergoing a further maturing. It is certain that their survival is aided by a secretion from the epithelium. If the testes are removed, thus depriving the animal of the internal secretion of these organs, and the ducts left intact the epithelium involutes and the life of the sperm is reduced about one half.

VESTIGIAL STRUCTURES IN TESTIS AND EPIDIDYMIS

Connected with the testis and its ducts are remains of certain fetal structures

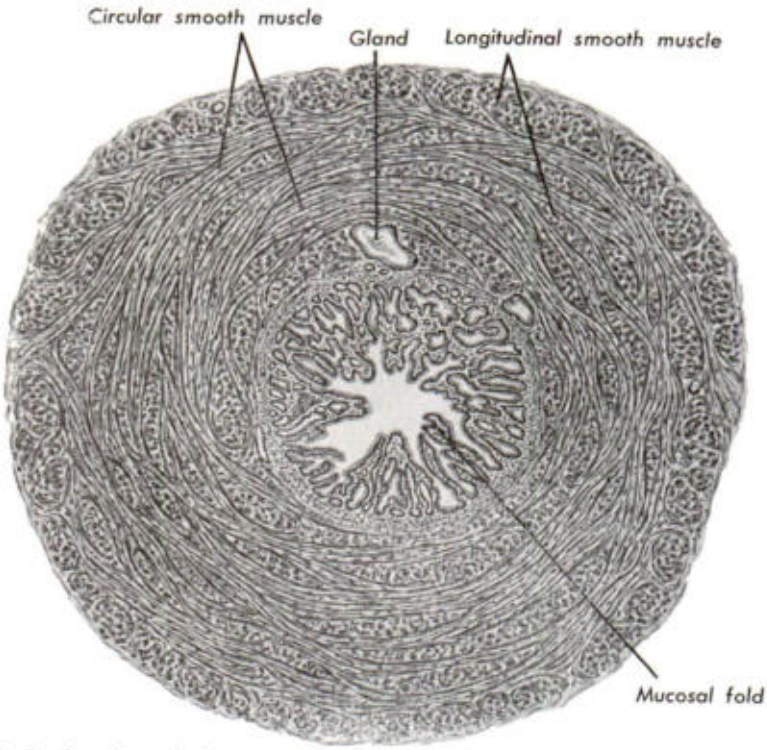

FIG. 363. Section through the ampulla of the ductus deferens. Man, 21 years of age. × 30. (After Stieve.)

associated with the development of the genital system.

(1) The *paradidymis* or *organ of Giraldés,* is situated between the vessels of the spermatic cord near the testis. It consists of several blind tubules lined with a simple columnar epithelium, part of which is ciliated. The cells may vary in height and the tubule have the appearance of an efferent duct.

(2) The *ductus aberrans Halleri* or inferior aberrant duct arises from the lower portion of the ductus epididymidis and extends toward the head where it ends blindly. It is lined with simple columnar ciliated epithelium.

A smaller *superior aberrant duct* is often present. It arises from the rete testis and ends blindly in the epididymis.

(3) The *appendix testis* (hydatid of Morgagni) is situated on the cranial pole of the testis near the epididymis. It is a vesicular structure lined by simple columnar epithelium surrounded by vascular connective tissue.

(4) The *appendix epididymidis* (stalked hydatid) is found occasionally on the head of the epididymis near the appendix testis. Its lumen is lined with a single layer of cuboidal or columnar cells.

The paradidymis, aberrant tubules and appendix epididymidis represent vestiges of the fetal mesonephros, while the appendix testis is derived from the Müllerian duct.

ACCESSORY GENITAL GLANDS

The Seminal Vesicles. The seminal vesicles are elongated convoluted sacs which lie closely apposed to the ampullae

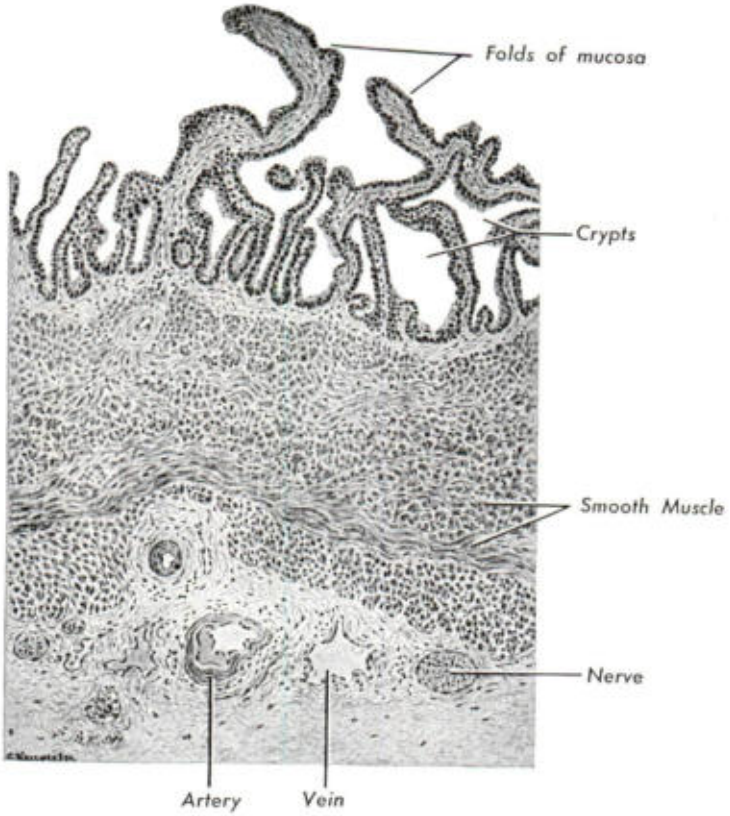

FIG. 364. From a section through wall of seminal vesicle. Human, thirty-four years old. Mallory-azan stain. × 65.

and open into the ductus deferens at the junction of ampulla and ejaculatory duct. The mucosa is folded in a complicated manner, forming numerous irregular chambers or crypts many of which are continued as glandular tubules similar to those of the ampulla (Fig. 364). The epithelium varies somewhat but is usually pseudostratified, being composed of cuboidal or columnar cells which reach the surface, and irregularly-shaped basal cells similar to those described in the seminal ducts. The walls of the surface cells are very distinct. The cytoplasm of these cells contains secretion granules and a yellowish lipochrome pigment (Fig. 365). The pigment makes its appearance at sexual maturity and increases with age.

The lamina propria is rich in elastic fibers and forms a continuous layer around the vesicle. The stroma of the folds is likewise rich in elastic fibers and contains some smooth muscle cells. Outside of the lamina propria is smooth muscle, which can be rather indefinitely divided into inner circular and outer longitudinal layers, both layers being thinner than in the ductus deferens.

Spermatozoa in varying numbers are often seen in the seminal vesicles. Their presence there is accidental, however. The seminal vesicles are not storehouses for sperm, but are glandular structures contributing a slightly alkaline viscid secretion to the seminal fluid. The secretion is rich in fructose.

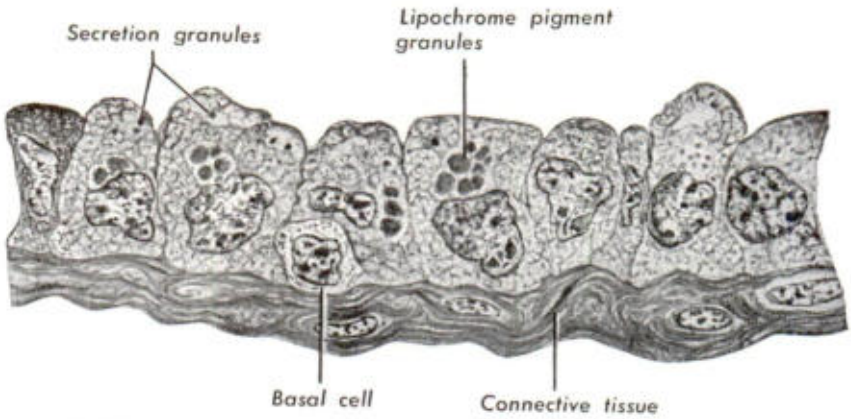

FIG. 365. Epithelium of seminal vesicle. Human, thirty-four years old. Mallory-azan stain, 3μ section. × 1660.

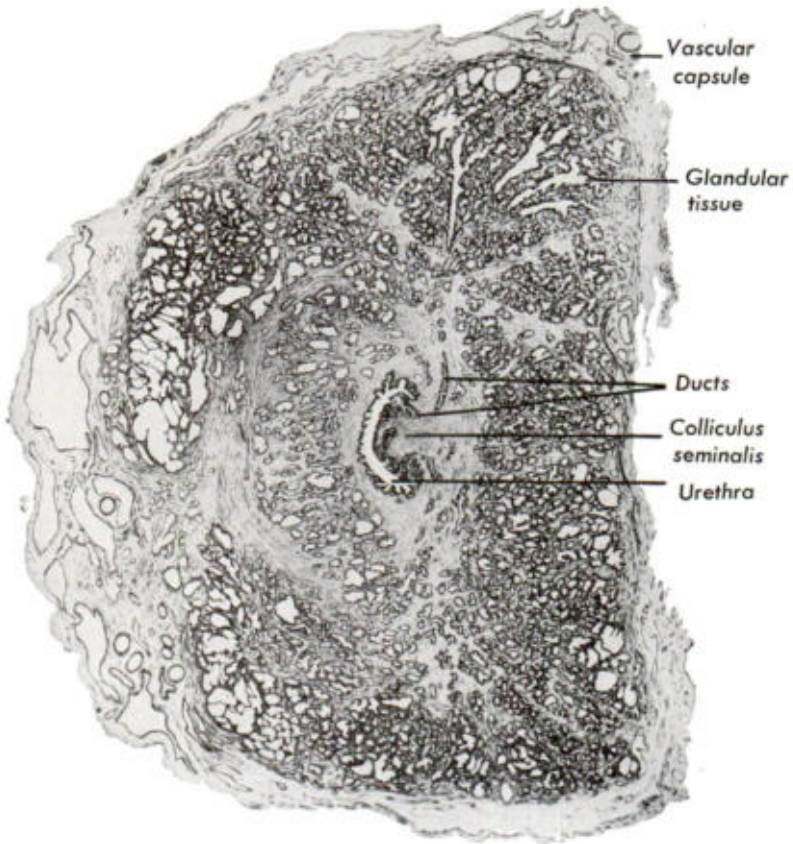

FIG. 366. Transverse section through the prostate at the level of the colliculus seminalis. Man, 19 years old. × 4. (After Stieve.)

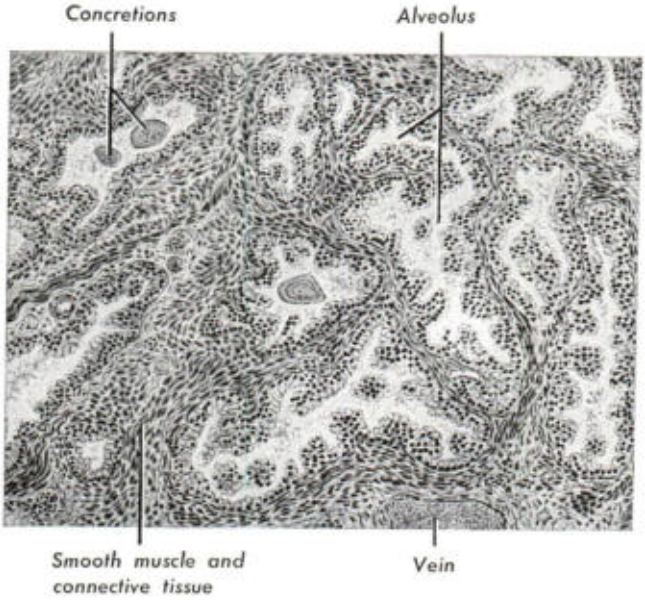

FIG. 367. Section of prostate gland. Human, fifty-four years old. Accident case. H & E. × 65.

The Prostate Gland. (Figs. 366, 367). The prostate is in reality an aggregation of many branched tubulo-alveolar glands with wide ducts and terminal tubules. The glands number from 30–50, and their ducts converge to form twenty or more terminal ducts which open into the urethra.

The gland is surrounded by a vascular capsule of fibroelastic tissue containing numerous smooth muscle fibers in its inner layer. From the capsule broad septa penetrate into the interior to form the unusually abundant stroma which separates the scattered tubules or alveoli. As in the capsule, the stroma is rich in elastic fibers and contains numerous smooth muscle fibers which course in various directions (Fig. 369). This fibromuscular tissue may constitute a third or even more of the whole mass of the prostate.

The epithelium shows a great variation in different glands and alveoli and even in a single alveolus. It is usually a simple cuboidal or columnar type. Basal cells may be present. The walls of the epithelial cells are usually distinct. Certain cells show bleb-like protrusions. The cytoplasm contains secretion granules and lipid droplets. The epithelium and subjacent stroma form folds which project into the cavities of the glands. The ducts are lined by a simple columnar epithelium which near their termination changes to the transitional epithelium of the urethra.

Corpora amylacea occur normally in some of the alveoli of most prostate glands (Fig. 367). Typically, they are spherical bodies about 250 microns in diameter but there is considerable variation in size. In the fresh condition they are fairly soft and light yellowish brown in color. In sections, concentric layers which stain with different intensities are evident. They are composed of protein and nucleic acid. Corpora increase in number with age. They may become calcified, and then are known as *calculi*, some of which reach a very large size.

Prostatic secretion is weakly acid (*p*H

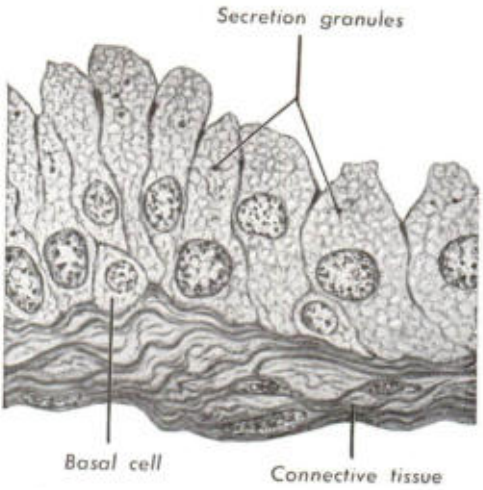

FIG. 368. Epithelium of prostate gland. Human, fifty-four years old. Accident case. Mallory-azan stain, 3μ section. × 1660.

FIG. 369. Prostate of a man thirty-four years old. The large number of smooth muscle fibers in the stroma is shown. × 190. (After Stieve.)

6.3–6.45), is rich in citric acid and contains lipids. "Lipid bodies" which stain with eosin are frequent. Prostatic secretion also contains large amounts of acid phosphatase which due to a normal daily discharge of prostatic secretion (0.5–2 cc. a day) is present also in urine. Normal blood serum also contains acid phosphatase in small amounts. However, in prostatic carcinoma there is frequently a pronounced discharge of the enzyme from the prostate into the blood stream. The acid phosphatase content of the blood may be increased sufficiently to make its determination of clinical significance in judging if metastasis has occurred. It may also be useful in indicating the response to therapy.

Within the prostate is found the *vesicula prostatica* (*utriculus prostaticus*, *uterus masculinus*), the remains of the fetal *Müllerian duct*. It consists of a blind tubule with folded mucous membrane lined by a simple or pseudostratified columnar epithelium which dips down to form short tubular glands.

The *blood vessels* of the prostate ramify in the capsule and trabeculae. The small arteries give rise to a capillary network which surrounds the tubules. From these arise small veins which accompany the arteries in the septa and form venous plexuses in the capsule.

The *lymphatics* begin as clefts in the trabeculae and follow the general course of the blood vessels.

The *nerves* of the prostate are both motor and sensory, the majority of which are unmyelinated. Many of them come from groups of autonomic ganglion cells which are found underneath the capsule and in the larger trabeculae. Axons of these cells pass to the smooth muscle of the trabeculae and blood vessels, and probably also to the epithelium of the tubules.

The Bulbo-urethral Glands (Fig. 370). The bulbo-urethral glands or *glands of Cowper* are two small glandular structures placed close to the bulb of the urethra. They are compound tubulo-alveolar

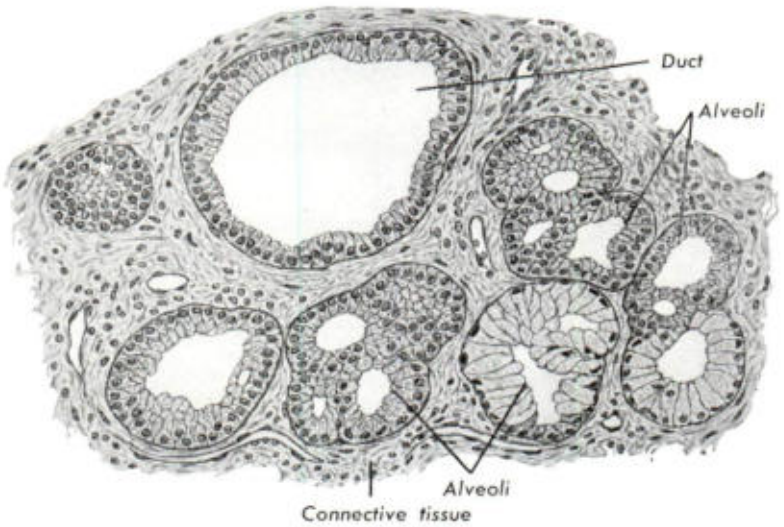

FIG. 370. Section through a lobule of the bulbo-urethral (Cowper's) gland. Human, 23 years of age. × 200.

glands whose tubules and ducts have a very irregular diameter. The terminal portions may be tubular or alveolar, or in the form of cyst-like dilatations. They are lined by a simple epithelium whose height varies from columnar to low cuboidal, and may even be flat in distended alveoli. Most of the columnar cells are of the mucous type, the nuclei basally placed, the cytoplasm containing mucin or pre-mucin droplets. Other cells stain darker with eosin and have a granular appearance, often containing fibrillar or spindle-shaped inclusions. The secretion is a glairy substance resembling mucus.

The smaller ducts are lined by a simple epithelium and seem to be secretory in character. They unite to form two main ducts which run parallel to the urethra for a variable distance and then open into the latter. The main ducts have a stratified columnar epithelium.

The stroma between the tubules consists of fibroelastic tissue with few muscle fibers. Smooth and skeletal muscle fibers are however quite numerous in the septa between the lobules. Externally the glands are enclosed by a layer of skeletal muscle fibers from the deep perineal and bulbocavernosus muscles.

THE PENIS

The penis (Fig. 371) consists largely of three cylindrical masses of erectile tissue, the paired dorsally placed *corpora cavernosa* and lying underneath them the unpaired *corpus spongiosum* (*corpus cavernosum urethrae*). The latter surrounds the urethra and terminates distally in a conical enlargement, the *glans penis*. The three cylindrical bodies are enclosed in a common fascia of areolar tissue rich in elastic fibers, to which the covering skin is loosely attached. In the glans the areolar tissue is lacking so that the skin adheres firmly to the underlying erectile tissue.

Each corpus cavernosum is surrounded by a dense capsule or *tunica albuginea* composed of collagenous fibers, the inner ones running circularly, the outer longitudinally. Elastic fibers are quite numerous. Between the cavernosa the capsules

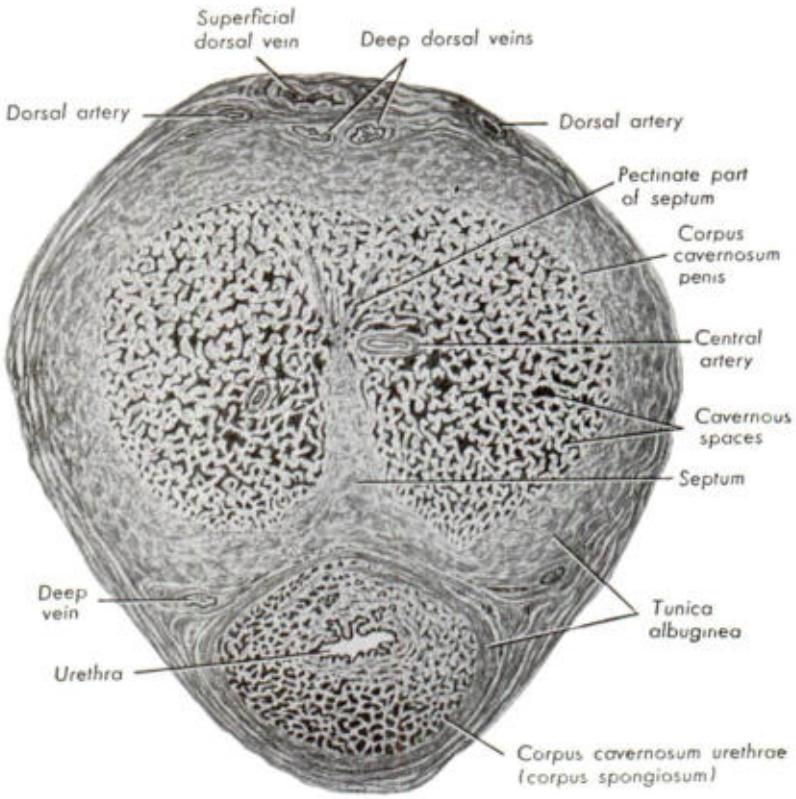

FIG. 371. Cross section of penis of adult man. The section is at the juncture of the proximal two-thirds and the distal one-third of the organ. The skin has been removed. × 4.5.

fuse to form a median septum which is thickest and most complete near the root of the penis. Further forward it becomes thinner and contains numerous slit-like spaces which permit a communication between the two bodies (Fig. 371). Directly underneath the albuginea is an irregular plexus of small veins.

The interior of each body consists of a network of large spaces or lacunae lined by endothelium (cavernous veins). These are separated by fibrous trabeculae rich in smooth muscle fibers which are disposed both circularly and longitudinally. The lacunae are large and trabeculae thin in the central portion. At the periphery is a layer of narrower spaces which communicate with the venous plexus on the inner surface of the albuginea. In the flaccid condition the lacunae are kept closed by the tonus of the trabecular muscle, and appear as mere slits.

The corpus spongiosum has a similar structure, but the albuginea is thin and contains many elastic fibers, so that the organ is not highly resistant to expansion. The trabeculae are thin, the lacunae quite uniform in size, and a peripheral layer of smaller lacunae is absent. Towards the urethra the lacunae become continuous with the mucosal plexus of veins; at the periphery they communicate with the venous network of the albuginea.

In the glans, the erectile tissue has the character of a dense venous plexus. An albuginea is lacking, the skin being firmly attached to the erectile tissue.

The skin of the penis is characterized by

tall dermal papillae and a thin epidermis containing considerable pigment in the cylindrical layer. Coarse hairs are found only at the root, but fine lanugo hairs are distributed over all of the shaft. Only the glans and inner surface of the prepuce are entirely hairless.

The *prepuce* is a fold of skin which overlies the glans. It consists of fibroelastic tissue containing bundles of muscle fibers and is covered by a very thin epidermis. On its inner surface, and on the glans as well, are found a number of modified sebaceous glands, the *glands of Tyson*.

Blood Vessels. The penis has a complicated blood supply which can respond to its varying functional states. The organ is chiefly supplied by the *arteria dorsalis* and the *arteria profunda*, which are branches of the *arteria penis*. The dorsal artery sends twigs to the albuginea and the larger cavernous trabeculae, where they break up into capillaries. Leaving the capillaries the blood enters the lacunae and is drained by the plexus of albugineal veins. This is the course of most of the blood during the flaccid state.

The principal vessels for filling the lacunae during erection are the deep arteries (*arteriae profundae*), one of which runs lengthwise in each corpus cavernosum. They give off numerous branches which are supported by the trabecular tissue and end in minute arteries which open directly into the cavernous spaces. In the flaccid organ many of the arterial branches project into the lacunae as looped or spiral vessels, the *helicine arteries*. Similar vessels are found in the corpus spongiosum.

The helicine arteries have an unusually thick media of circular muscle fibers, and in many of them the intima shows valve-like structures in the form of marked longitudinal thickenings or cushions. When the muscle of the arteries contracts these thickenings plug up the lumen and shut off the blood supply.

During erection the muscle of the helicine arteries and of the cavernous trabeculae relaxes, and the lacunae are flooded with arterial blood from the helicine vessels which empty mainly in the central spaces of the cavernous bodies. The sudden filling of the large central spaces tends to compress the narrower peripheral ones which communicate with the venous plexus of the albuginea. Egress of blood is blocked or at least greatly reduced, and the organ becomes swollen and rigid. At the end of the erectile state the muscle of the helicine arteries contracts and the inflow of blood is shut off. The trabecular muscle regains its tonus and the blood is slowly driven out into the venous plexus. According to Kiss there are special provisions for emptying the corpora cavernosa. One or perhaps several veins originate directly from the central lacunae. These are equipped with funnel shaped valves whose small opening permits the passage of only a small stream of blood. When the blood supply of the helicine arteries is cut off, these veins begin to empty the central lacunae. The internal pressure is reduced, the peripheral lacunae open, and then the blood is pressed out more rapidly into the peripheral veins, the organ gradually returning to the flaccid condition. Most of the blood from the corpora cavernosa is drained by the *vena profundis penis*.

The corpus spongiosum is filled in a similar manner. However, the albuginea is more elastic, the lacunae more uniform in size, and the outflow of blood is not blocked to the same extent. The spongiosum naturally swells during erection, owing to the increased blood supply, but it always remains compressible and does not assume the rigidity of the paired cavernous bodies.

Lymphatics. Numerous lymphatics are found in the skin of the shaft, prepuce and glans (superficial plexus) and in the mucosal stroma of the urethra. A deeper lymphatic network in the erectile tissue has also been described. The lymphatics drain chiefly into the inguinal lymph nodes.

Nerves. The penis is abundantly supplied with spinal, sympathetic and parasympathetic nerve fibers. The sensory spinal fibers terminate in a variety of end organs—free sensory endings, Meissner's corpuscles in the papillae, Pacinian corpuscles and end-bulbs of Krause in the superficial connective tissue, and the more deeply placed genital corpuscles of Dogiel.

Sympathetic and parasympathetic motor fibers form extensive networks in the walls of the blood vessels and the smooth muscle of the cavernous trabeculae.

INTERNAL SECRETION OF THE TESTIS

It has been known for a long time that the testes are in some way responsible for the appearance of the secondary sex characters. When the testes are removed before puberty, these characters remain in an infantile condition or are entirely suppressed. The penis and prostate gland are small; the male type of chest and pelvis fails to develop; the face, chest and limbs are hairless; the larynx remains small and as a result the voice maintains its infantile pitch. The bones grow beyond their normal length but are not robust. Sometimes there is considerable deposition of fat, its distribution characteristic of the feminine type.

When castration is performed after puberty, the effects are less pronounced for the sex characters are already established and the changes are retrogressive.

The most profound and constant effects of castration in mammals, as shown experimentally, are upon the genital ducts and accessory glands. The epithelium of these structures fails to develop to the normal height and does not show secretory activity if the testes are removed before puberty, or if removed after maturity the epithelium of these structures will involute. The effect of the testes on these accessory reproductive structures appears to be solely due to a hormone secreted by the testes called *testosterone*. There are other male sex hormones which are found in the urine, but presumably they are not secreted by the testes but are transformation products of the testis hormone or are secreted by other organs (adrenals) although not in sufficient amounts to substitute for the testis hormone. The response of the various accessory reproductive organs and of the comb of the capon have been used in assays of the male hormone content of extracts.

The evidence is quite conclusive that the interstitial cells of the testes secrete the male sex hormone. In undescended or partially descended testes (cryptorchid) the epithelium of the seminiferous tubules, but not the interstitial tissue, atrophies. Extensive degeneration of the spermatogenic cells is likewise produced by treatment with x-rays, the interstitial cells being uninjured when the correct dosage is used. In both cases the secondary sex characters remain normal. Histochemical studies have shown that compounds with the chemical properties of testosterone are present in the interstitial cells but not elsewhere in the testis (Pollock). There is a correlation between the androgen content and amount of interstitial tissue (Hooker).

Estrogen, the female sex hormone, also occurs in the male. It has been shown that about 80% of the estrogen arises from the Leydig cells and about 20% from the adrenal gland.

The endocrine and spermatogenic activity of the testes is dependent on internal

secretions of the anterior hypophysis. This gland only indirectly influences the accessory reproductive structures through its action on the testes.

SEMEN

Semen consists of seminal plasma, spermatozoa and usually some cells cast off from the lining of the reproductive ducts and glands. Seminal plasma consists of the secretion of the prostate, seminal vesicles, bulbo-urethral glands and epididymis, the chief contribution being from the prostate and seminal vesicles. The seminal plasma serves as a food source and vehicle for the spermatozoa.

The volume of semen from a normal ejaculation varies greatly among different individuals and in the same individual, as does, also, the number of sperm. The usual range in volume is from 2 cc. to 5 or 6 cc. The total number of sperm ranges from a high of 500,000,000, or even more, to a low of a few million or a complete azoospermia. A variable percentage of sperm are malformed or inactive.

Testicular or epididymal sperm are inactive but quickly become active in seminal plasma (or saline). They carry little of the foodstuff for metabolism but acquire this from the carbohydrate, chiefly fructose, in the seminal plasma. The sugar is reduced to lactic acid, glycolysis being best carried out under nearly anaerobic conditions.

The rôle of the high content of hyaluronidase in sperm has not, as yet, been definitely established. One of the postulates which has been proposed is that the enzyme aids in some way in the penetration of a sperm into an ovum.

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XX

THE FEMALE REPRODUCTIVE SYSTEM

The female genital organs comprise the ovaries in which the egg cells are formed; a system of genital ducts—the Fallopian tubes, uterus and vagina; and the external genitalia including the labia majora, labia minora and clitoris. The mammary glands, although not one of the genital organs, are important glands of the female reproductive system. The ovaries, like the testes, are also important glands of internal secretion.

THE OVARY

The ovaries are somewhat flattened ovoid bodies, measuring about 4 cm. in length, 2 cm. in width and 1 cm. in thickness. One lies on each side of the uterus in relation to the lateral wall of the pelvis. Each is attached at its hilum to the back of the broad ligament by a peritoneal fold, the mesovarium, and by the ligament of the ovary. The ovary is pinkish gray and does not have the glistening or shiny appearance characteristic of the peritoneum. Its surface is uneven and becomes puckered to an increasing degree with ageing.

At the hilum the connective tissue of the mesovarium and ovarian ligament passes into the ovary and becomes continuous with the ovarian stroma. At the hilum also the mesothelium of the mesovarium abruptly ceases and is replaced by a layer of cuboidal cells, the *germinal epithelium*, which covers the ovary.

In sections of the ovary two zones may be distinguished, a central deeper portion, the *medulla* or *zona vasculosa*, and a broad outer layer, *the cortex* (Fig. 373). The two zones blend into each other without any distinct demarcation.

The medulla is composed of a stroma of loose connective tissue rich in elastic fibers and containing numerous large blood vessels, lymphatics and nerves. Bundles of smooth muscle fibers are found near the hilum. In some individuals vestiges of certain fetal structures, the *rete ovarii*, occur as epithelial strands or tubules in the region of the hilum.

The cortex is a broad peripheral layer interrupted at the hilum where the medulla becomes continuous with the tissues of the mesovarium. It consists of a more compact, richly cellular stroma in which are scattered the peculiar glandular structures of the ovary, the *ovarian follicles* (Fig. 373). The connective tissue cells are fusiform or spindle-shaped, with elongated vesicular nuclei, and resemble smooth muscle fibers. They are placed in a feltwork of delicate interlacing collagenous fibrils. Elastic tissue, except in the walls of the blood vessels, is practically absent. Directly underneath the germinal epithelium the stroma forms a denser fibrous layer, the *tunica albuginea*, composed of fewer cells and more closely packed fibers.

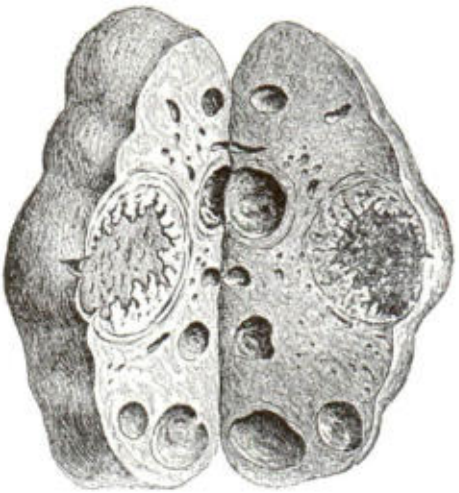

FIG. 372. Ovary opened by longitudinal incision. Ovum has escaped through tear in surface. Cavity of follicle has irregular projections composed of lutein cells. (Kollmann's Atlas.)

The Follicles. Ovarian follicles are formed during fetal life from the germinal epithelium. At that time the latter proliferates actively and forms a many-layered epithelial mass composed of two kinds of cells—a large number of small cuboidal cells which stain intensely, and scattered among them larger spherical cells with vesicular nuclei and pale staining cytoplasm, the primitive ova or *oögonia*. The underlying embryonal connective tissue penetrates into this mass and subdivides it into a number of cell clusters or cords in each of which are the two types of cells. At first each cluster contains a number of ova scattered among the smaller (follicular) cells. With further invasion of the connective tissue the clusters are ultimately subdivided into smaller bodies, the *primary follicles*, each composed of one large ovum surrounded by a single layer of now flattened follicular cells. Rarely a follicle may contain several egg cells, of which, however, only one develops normally while the others degenerate. Near the time of birth the tunica albuginea is formed. The germinal epithelium becomes reduced to a single layer of cells which are columnar in early life and cuboidal in the adult.

The follicles in the ovaries of the newborn are all of the primordial type. The new formation of egg cells from the germinal epithelium during postnatal life, which has been reported in some of the common laboratory mammals, apparently does not occur to any extent in the human. Estimates regarding the total number of eggs in the ovary vary within wide limits. As many as 400,000 primitive follicles have been reported in the ovaries of a newborn infant.

Of this large number relatively few are destined to reach full maturity. The active sexual life of a woman, from puberty to menopause, is about 30 to 35 years. During this period, even if no disturbing factors appear, one ovum matures normally each month, so that altogether some 400 eggs may actually reach maturity. All of the others ultimately degenerate, and from birth on, the follicles progressively diminish in number. Follicular degeneration occurs most intensely in early life but continues actively throughout the period of sexual maturity. After the menopause the remaining follicles degenerate within a few years.

In the mature ovary, the follicles are found in all stages of growth. Most numerous are the primary follicles found mainly in the peripheral layer of the cortex. As a follicle grows it occupies progressively deeper positions.

The primary follicles (Fig. 374) are spheroidal bodies measuring 30–40 μ. The central ovum, about 20 μ in diameter, has a large vesicular nucleus with a deeply staining chromatin reticulum and a rather indistinct nucleolus. The cytoplasm is finely granular. A connective tissue capsule is lacking, the follicle being placed directly in the cortical stroma.

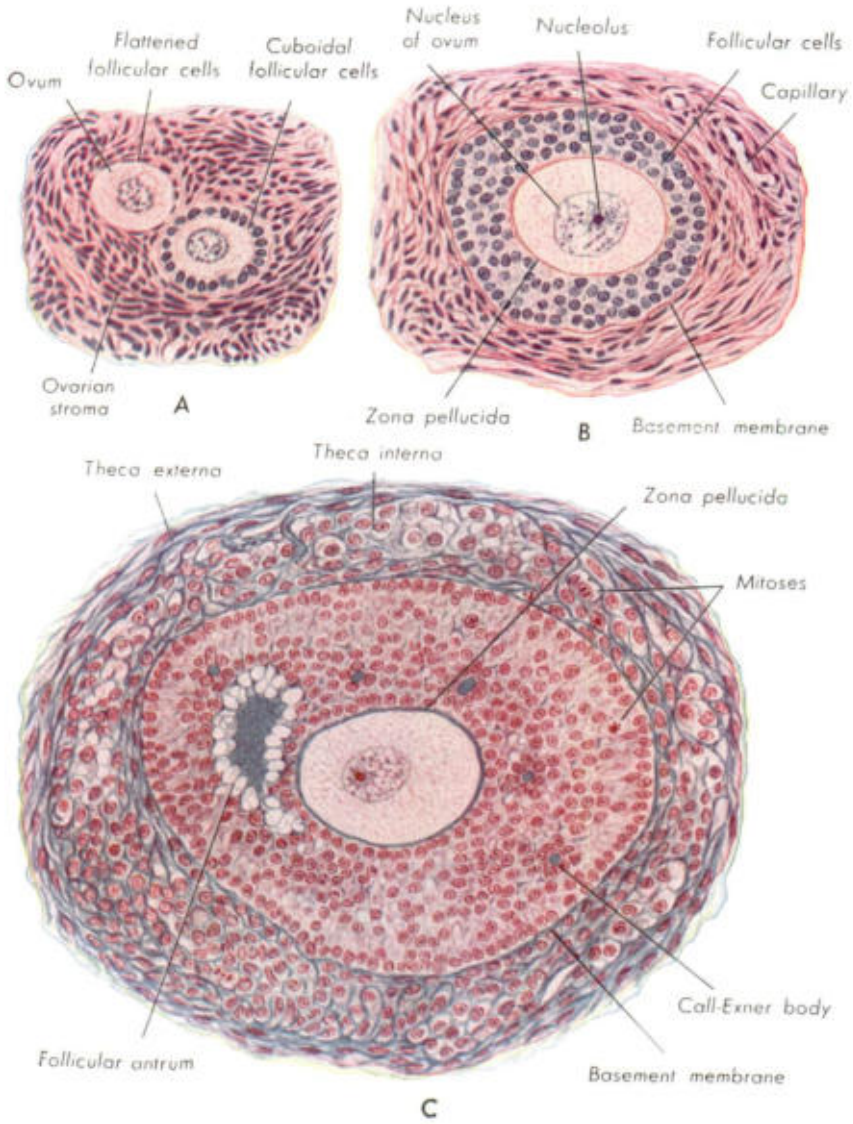

FIG. 374. Follicles in various stages of development. These were selected from a normal human ovary removed surgically on the fourteenth day of the cycle, and fixed by injection with Bouin's fluid. The patient was thirty-six years of age. The illustrations are drawn at a uniform magnification, × 289. Sections illustrated in *A* and *B* were stained in hematoxylin and eosin, *C* in Masson's trichrome stain without a preliminary application of hematoxylin.

A. Two primordial follicles in slightly different stages of development.

B. An early stage of a growing follicle.

C. A follicle showing the beginning of the formation of the antrum.

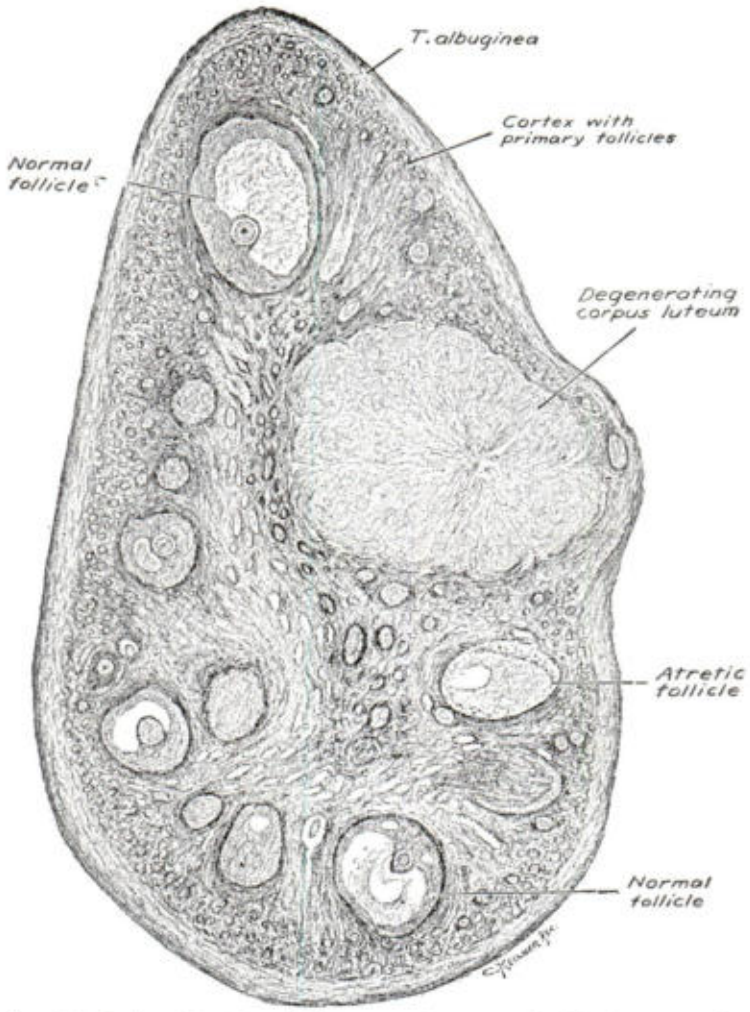

FIG. 373. Longitudinal section through the ovary of a normal adult rhesus monkey. The corpus luteum is probably degenerating.

Maturation of the Follicles. The further growth of the primary follicles is characterized by the proliferation of the follicular cells, the increase in size of the ovum, and the formation of a connective tissue capsule. The flattened follicular cells become cuboidal, divide actively and soon form a stratified layer around the ovum (Fig. 374). Irregular small spaces appear in the follicular mass and fuse to form a crescent-shaped cavity, the *antrum* or follicular cavity, filled with a serous fluid, the *liquor folliculi*.

As the follicle increases in size it assumes an ovoid shape and moves to a deeper position in the cortical stroma. The follicular cells continue to divide, the follicular cavity with its contained fluid increases in size, and the ovum is pressed to one side, where, surrounded by an accumulation of follicular cells, it forms a definite projection into the cavity (Fig.

375, *A*). This is known as the *germ hill* or *cumulus oöphorus* (*discus proligerus*). Elsewhere the follicular cavity has a fairly regular epithelial lining, the *membrana granulosa*, composed of several layers of cuboidal or polyhedral follicular cells. The cells of the germ hill nearest the ovum become columnar and arrange themselves radially into a regular layer around the ovum, the *corona radiata*.

On the surface of the follicle, which is now known as a *vesicular follicle*, the stroma has become modified to form a follicular sheath, the *theca folliculi* (Figs. 375, *A*, *B*) which soon differentiates into two layers, an inner vascular layer, the *theca interna*, and an outer fibrous layer, the *theca externa*. The theca interna consists of loose connective tissue containing numerous capillaries in close contact with the membrana granulosa. Between the capillaries are delicate collagenous fibrils and peculiar epithelioid connective tissue cells containing fatty granules in their cytoplasm. The theca externa consists of more closely packed, circularly disposed fibers and spindle-shaped cells. Between the theca and membrana granulosa is a homogeneous or finely fibrillar basement membrane, the *glassy membrane*, to which the follicular cells adhere closely.

While these changes are going on in the follicle, the ovum rapidly increases in size and reaches a diameter of 100–150 μ. The nucleus becomes large and vesicular (germinal vesicle) and has a definite nuclear membrane, a sparse chromatin reticulum and a large deeply staining nucleolus (germinal spot). The cytoplasm (vitellus) shows an inner coarsely granular zone containing yolk granules (deutoplasm) and a peripheral layer of clearer or finely granular protoplasm. Surrounding the ovum is a thick membrane, the *oölemma* or *zona pellucida*, probably formed by the follicular cells, but believed by some to be a product of the ovum (Figs. 375, *A*, *C*). This is a tough resistant membrane which, in atresia, persists much longer than does the ovum. In the fresh condition it appears to be homogeneous, although after fixation fine radial markings may be seen. Studies with the phase microscope show that processes from the cells of the corona radiata extend through the zona pellucida where they terminate in close contact with the plasma or vitelline membrane of the ovum (Fig. 376). It is thought that the corona radiata cells supply nutritive substances to the ovum by way of the processes. A narrow cleft, the perivitelline space, is frequently observed between the ovum and the zona pellucida.

The *mature* or *Graafian follicle* attains a size of 10–12 mm. It extends through the whole thickness of the cortex, and at one place encroaches upon the albuginea and actually bulges on the surface of the ovary. At this place, the *stigma*, the albuginea becomes thin and the equally attenuated theca of the follicle becomes practically devoid of blood vessels. The large follicular cavity, distended with fluid, is lined by a granulosa consisting of several layers of cuboidal follicular cells. In the cumulus oöphorus the ovum has grown to its full size and is surrounded by a thick zona pellucida and a well formed corona radiata. Outside the corona, small irregular spaces filled with fluid have appeared between the cells of the germ hill, weakening considerably the attachment of the ovum to the follicular wall. The prominent theca interna, which doubles in thickness during the period of maturation, contains a rich network of capillaries and numerous large epithelioid cells which become secretory, with fine lipid droplets in their cytoplasm. The glassy membrane (basement membrane) appears as a homogeneous line between theca and granulosa.

Ovulation. When the follicle has reached maturity, rupture at the stigma

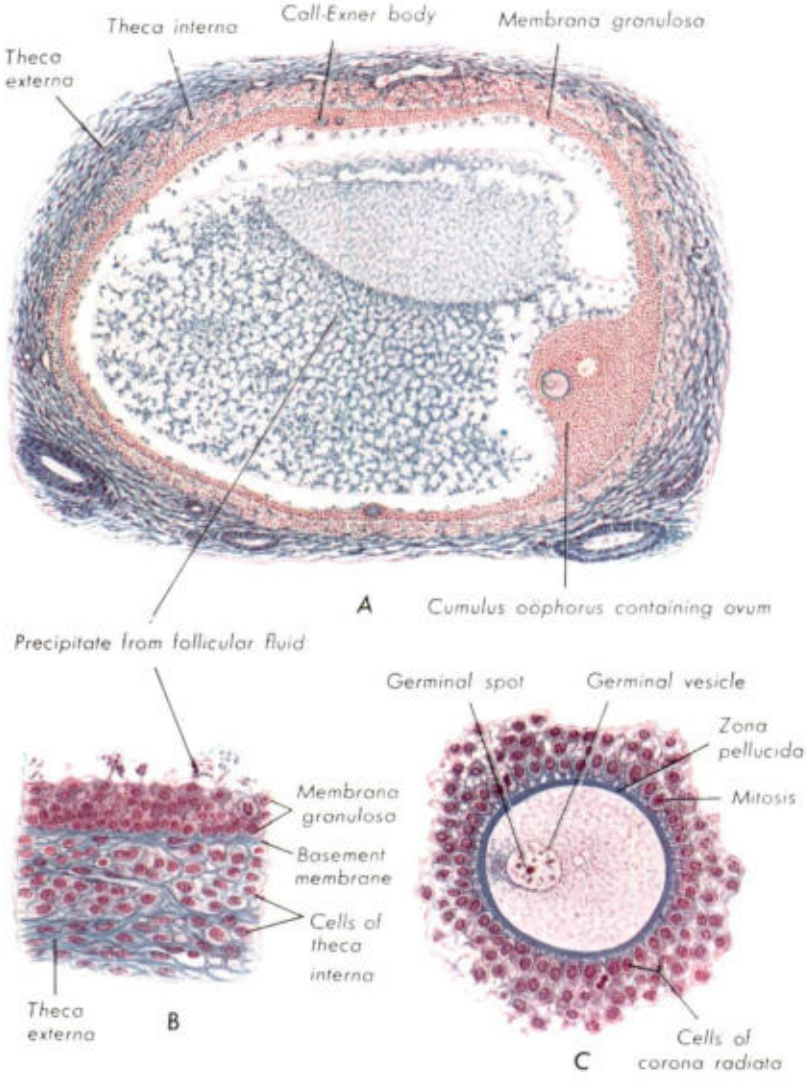

FIG. 375. On this plate is illustrated a follicle with a maximum dimension of 4.5 mm. From the same ovary as Figure 374. Masson's trichrome stain.

A. A low power view, × 53.

B. A portion of the wall of this follicle, × 287.

C. The ovum of this follicle drawn at the same magnification as the illustrations in Figure 374, × 287.

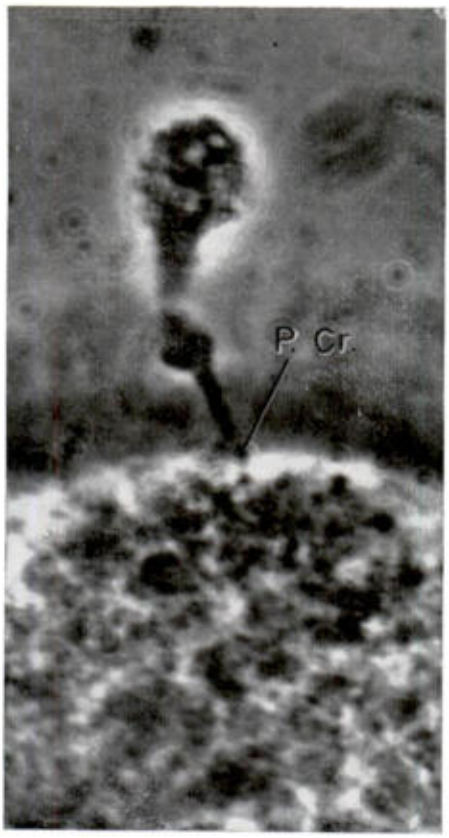

FIG. 376. Photomicrograph of a corona radiata cell with a process extending through the zona pellucida. The end of the process (*P. Cr.*) is seen in the perivitelline space around the ovum. It is thought that nutritive material is supplied to the ovum in this manner. (Courtesy of Dr. L. B. Shettles.)

takes place but the mechanism causing this rupture is not well known. Observations on animals have shown that the follicular fluid oozes out. It is a gradual and not cataclysmic process. The germ hillock is freed from the wall, and the ovum, surrounded by the radially-arranged follicular cells, passes out with the follicular fluid and enters the abdominal cavity and from there passes into the fimbriated funnel of the Fallopian tube. There it is ultimately freed of the adhering follicular cells. The rupture of the follicle with consequent discharge of the ovum is termed *ovulation*.

The ovum must be fertilized soon after ovulation or it will degenerate, fragment and disappear. Although no data are available in the human, studies on animals show that the ovum does not retain the capacity to be fertilized much longer than 24 hours. In some forms fertilization must occur within 4 to 6 hours after ovulation. The union of ovum and sperm takes place either before or immediately after the ovum enters the fimbriated extremity of the tube. Following fertilization the journey of the egg down the tube to the uterus is a leisurely one. Data from early human embryos secured by Hertig and Rock show that the ovum reaches the uterus about 3 days after ovulation. It is implanted in the endometrium about 6 days after ovulation.

Atresia. Of the numerous follicles only a few reach full maturity and discharge the ovum. The vast majority undergo degeneration either as primary follicles or after a varying period of growth. The degeneration of follicles is known as *atresia*.

In atresia of the *primary follicles* the ovum undergoes degeneration accompanied by chromatolysis and fragmentation of the nucleus, and this is followed by similar changes in the follicular cells. The follicle is resorbed and disappears and the space which it occupied is filled with stroma, leaving as a rule no trace of the destroyed follicle.

Atresia of the *growing follicles* is a more complicated process which varies in details depending on the size of the follicle and the behavior of the theca interna. Here too the disintegrative changes start in the ovum and spread to the follicular cells. These changes are in the nature of fatty degeneration, evidenced by the accumulation of fatty granules in the cytoplasm, chromatolysis of the nuclei and ultimate liquefaction. The zona pellucida

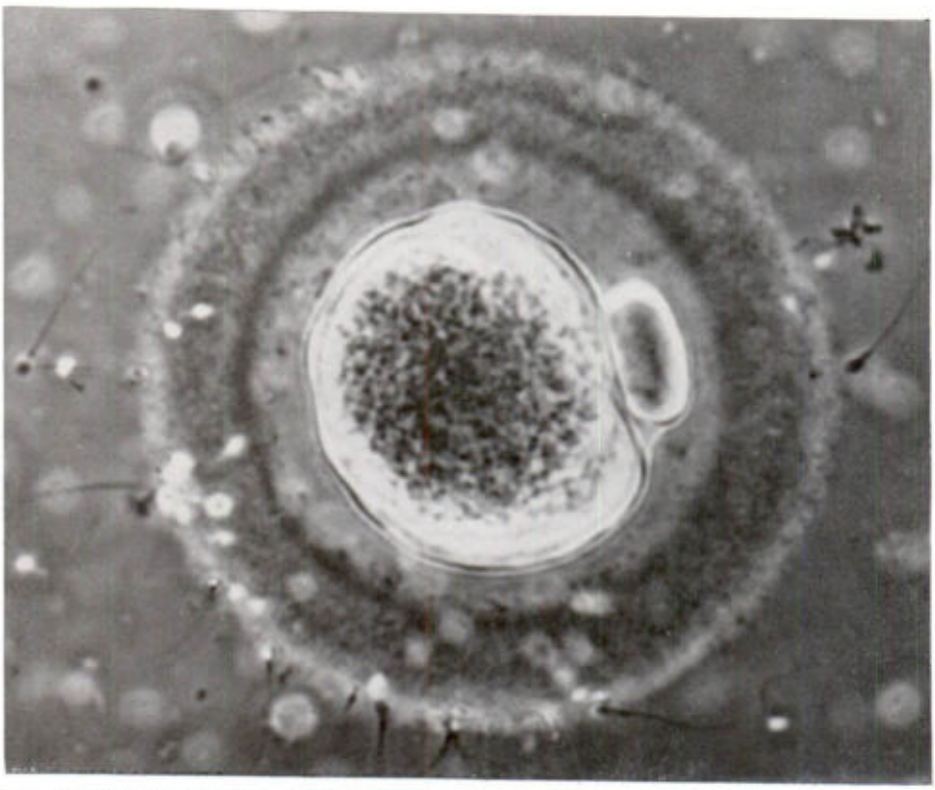

FIG. 377. Photomicrograph of living human ovum and its first polar body surrounded by zona pellucida and by cumulus cells. Sperm, which are attempting to reach the ovum, are seen at the periphery of the cumulus cells. (Courtesy of Dr. L. B. Shettles.)

swells and becomes folded and may persist for some time after the disappearance of the ovum and follicular cells.

In the larger degenerating follicles the cells of the theca interna enlarge, and their cytoplasm shows an increase in fatty granules. They form a compact layer around the follicle, separated from the latter by the glassy basement membrane which in some cases hypertrophies and appears as a folded hyaline band (Fig. 378). The granulosa cells disintegrate and are resorbed with a consequent shrinkage in the size of the follicle. After resorption of the follicular cells, the theca cells also degenerate and are replaced by connective tissue, leaving only the corrugated glassy membrane which persists for a time and then likewise disappears (Figs. 379, 380). When a thickened glassy membrane is absent, invasion of the theca cells takes place along the whole periphery of the follicle, and the degenerative processes occur more rapidly.

In the mature ovary there are always several antrum-containing follicles of various sizes. Most of these are destined to undergo atresia at some stage in their growth. As a rule only one follicle matures each month, and the process of transformation from a growing to a mature follicle is accomplished in about two

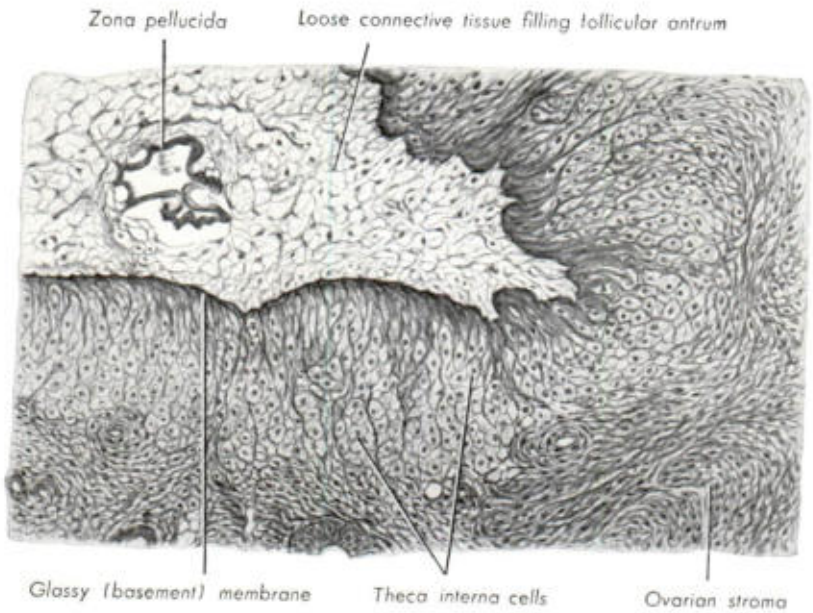

FIG. 378. A moderately early stage in follicular atresia. The ovum and the granulosa cells have been resorbed. The glassy (basement) membrane is much thickened and the theca interna cells have reached their maximum development. Normal ovary of a woman 36 years old. Surgical specimen removed at the mid-cycle. Masson's trichrome stain. × 193.

weeks. In rare instances two or even several follicles may ripen at the same time.

Oögenesis. In preparation for the union with the spermatozoön the ovum passes through a series of changes similar to that described for the sperm cells (see spermatogenesis, p. 444) and with the same end result, namely, the reduction of its chromosomes to one half the somatic number. The changes differ however in one important respect. In the male each primary spermatocyte gives rise to four functioning spermatozoa. In the female, one primary oöcyte gives rise to but one mature ovum, the other three being discarded as abortive minute bodies, the *polar bodies*.

The *oögonia* or primitive ova which contain the somatic (*diploid*) number of chromosomes, divide mitotically as do the spermatogonia, the daughter cells likewise containing the full number of chromosomes. After an indefinite number of generations the oögonia of the follicles enter a period of rest and growth at the end of which they are known as *primary oöcytes*. During the growth period synapsis of chromosomes occurs. The primary oöcyte then passes through the two *maturation divisions*, as a result of which the chromosomes are reduced to the *haploid* number. In these divisions the chromatin is divided equally between the daughter cells, but the division of the cytoplasm is extremely unequal. The spindle of the first maturation division forms near the periphery of the cell (Fig. 381), and when cleavage occurs, one of the *secondary oöcytes* receives most of the cytoplasm, while the other, the *first polar*

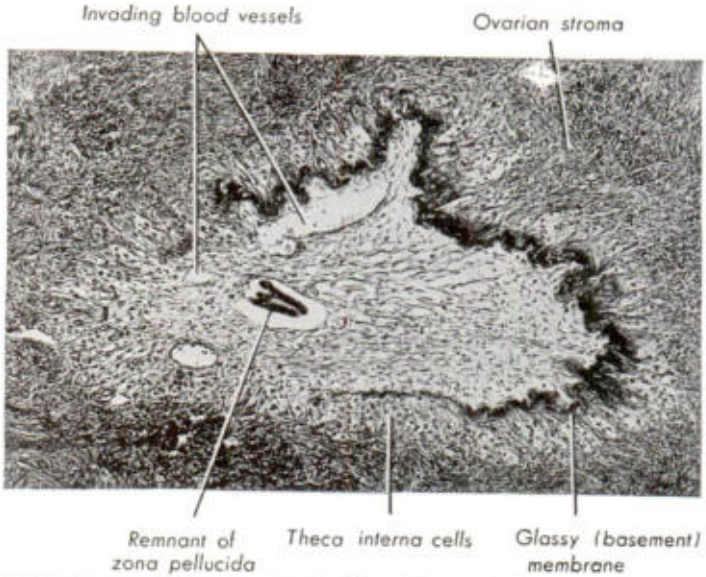

FIG. 379. Medium stage of follicular atresia. Normal ovary of a woman 36 years old. The ovary was surgically removed at the mid-cycle. Masson's trichrome stain. Photomicrograph. × 95.

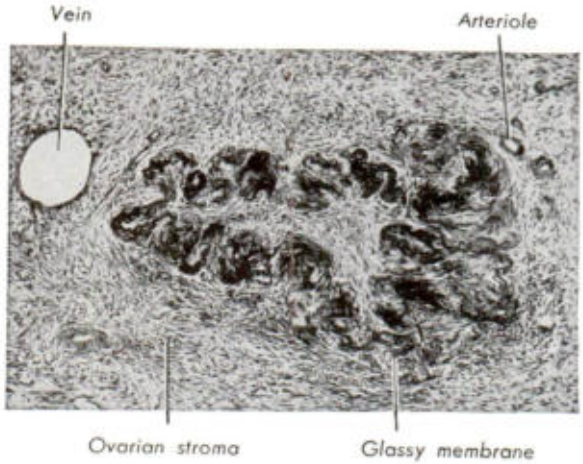

FIG. 380. Late stage of follicular atresia. Normal ovary of a woman 36 years old. The ovary was surgically removed at the mid-cycle. Masson's trichrome stain. Photomicrograph. × 95.

body, receives practically none and soon degenerates.

The second maturation division is similar to the first. Once more the spindle forms at the periphery and again two cells of unequal size are formed. One, the *mature ovum*, retains most of the cytoplasm; the other is cast off as the *second polar body*. In some lower forms the first polar body may also divide, so that alto-

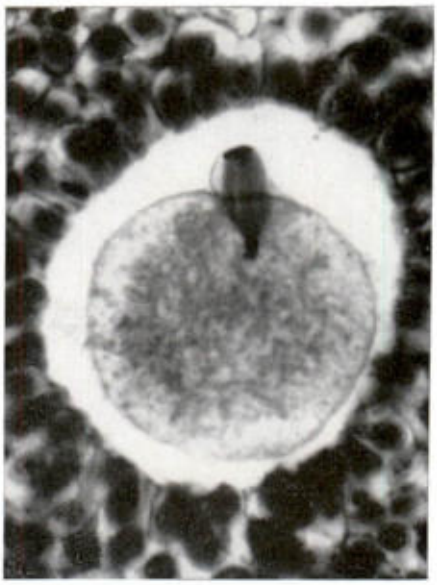

FIG. 381. Photomicrograph of ovum during first maturation division. Corona radiata cells are seen encircling the ovum. × 700. (From a preparation by Dr. T. H. Hunt.)

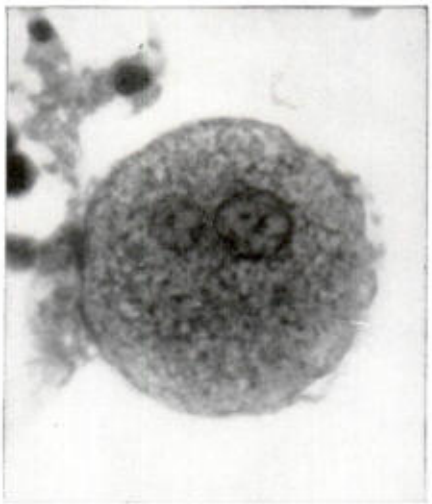

FIG. 382. Photomicrograph showing male and female pronuclei in an ovum shortly after sperm penetration. Ovum was located by sectioning the Fallopian tube. × 700. (From a preparation by Dr. T. H. Hunt.)

gether three polar bodies are formed, all of which ultimately degenerate. Thus of the four cells formed from the primary oöcyte only one reaches functional maturity, retaining practically all of the cytoplasm with its nutritive contents. The volume of the mature ovum is about 250,000 times greater than that of the spermatozoön.

The egg cells of the primary follicles represent the last generation of oögonia which are entering the period of rest and growth. The majority of these undergo degeneration before much growth has taken place but degeneration may occur at any stage of development of the follicle. Fully grown primary oöcytes are found only in the mature Graafian follicles. The exact time of occurrence of the maturation divisions is not definitely known for the human ovum. In most mammals the first polar body is formed in the ovary, while the second is cast off after ovulation and after fertilization.

The Corpus Luteum (Figs. 383, 384). After ovulation, the ruptured follicle does not degenerate at once but is transformed temporarily into a glandular structure, the *yellow* body or *corpus luteum*. The follicular cavity closes over by healing of the wound and becomes filled with a serous fibrin-containing fluid which usually contains some blood. The granulosa cells of the follicle do not proliferate but increase greatly in size. The nuclei become vesicular and stain more lightly. The abundant cytoplasm, at first finely granular shows a progressively greater accumulation of fine lipid droplets and yellowish pigment granules which give the gland its characteristic color. These cells, now known as *granulosa-lutein* cells, are arranged as a thick stratified layer around the remains of the central cavity. In the corpus luteum of the human, this layer has large folds (Fig. 383).

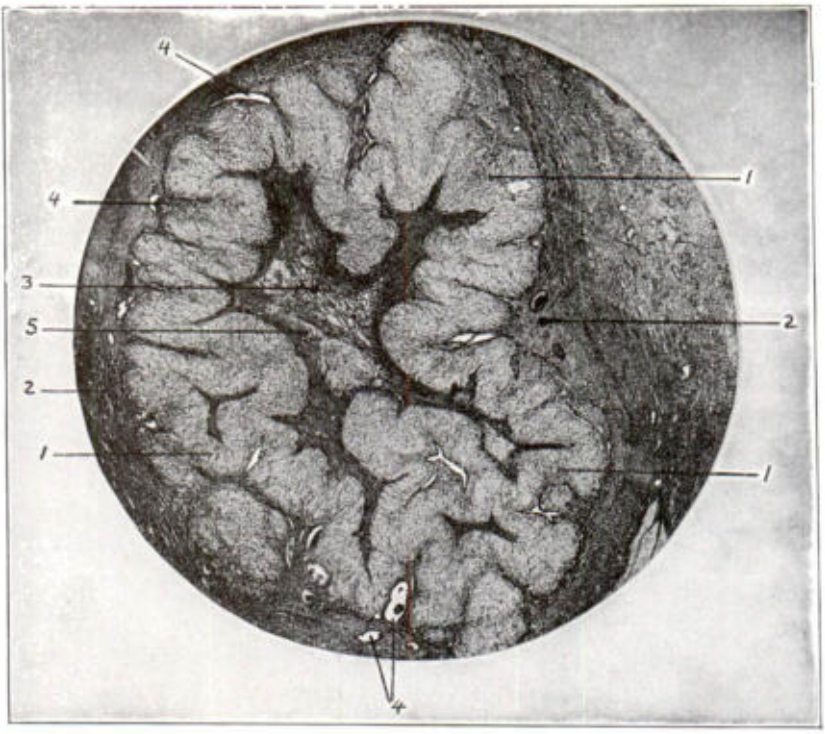

FIG. 383. Section through corpus luteum from human ovary. Photograph. *1*, Layer of lutein cells; *2*, theca folliculi; *3*, remnant of follicular cavity containing fibrin; *4*, blood vessels; *5*, loose connective tissue covering inner surface of lutein layer.

The large epithelioid cells of the theca interna are scattered in groups at the periphery of the lutein layer, especially in the recesses between the folds. As they resemble the lutein cells in shape and in content of fatty droplets, they are known as *theca-lutein* cells in contrast to the *granulosa-lutein* cells of follicular origin. In the well-formed corpus luteum they may be distinguished by their peripheral location, their smaller size and more compact, darker staining nuclei (Fig. 384).

Connective tissue from the theca externa penetrates into the lutein mass and forms delicate interlacing septa in which are numerous capillaries. The connective tissue finally penetrates the entire layer and spreads to form a continuous covering on the inner surface of the lutein cells. In the center the follicular cavity remains as a greatly reduced space of irregular outline, still filled with a serous fluid or more rarely with the disintegrating remains of the blood clot.

If the discharged ovum is not fertilized and dies on its way to the uterus, the corpus luteum reaches its greatest development about one week after ovulation and then begins to degenerate. This is the *corpus luteum menstruationis* (*spurium*). The cells of such a corpus luteum gradually decrease in size, show increasing vacuolization, and finally are resorbed. The connective tissue between the lutein cells increases in amount and a loose

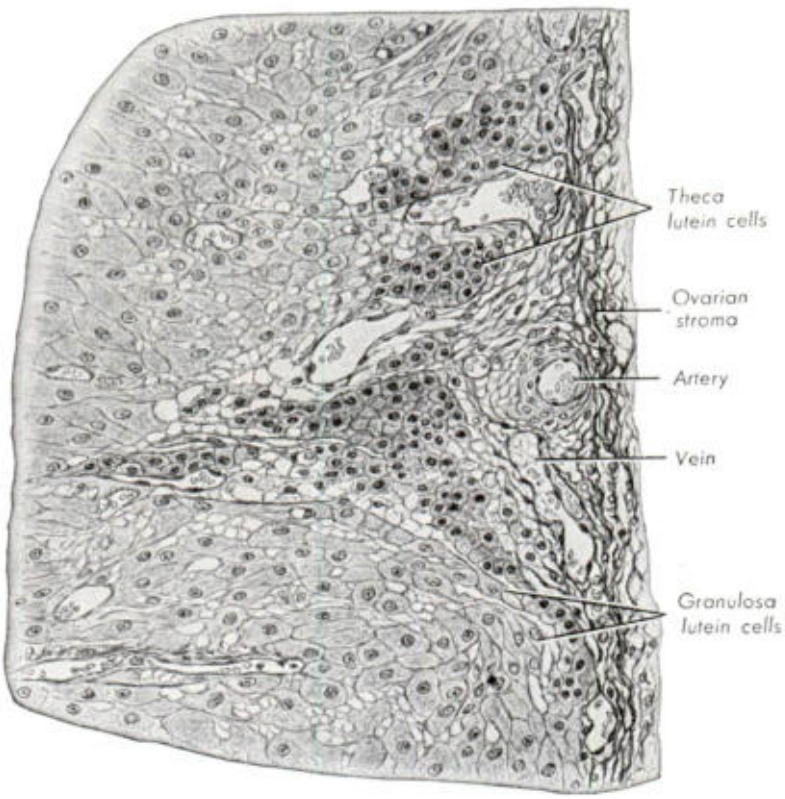

FIG. 384. Section through the peripheral part of corpus luteum removed during the progravid stage of the sexual cycle. Masson's trichrome stain. × 175.

gelatinous type of connective tissue, often containing brownish pigment of the extravasated blood, fills the central cavity. The gland becomes progressively smaller, and several weeks after beginning of involution is transformed into a whitish scar of microscopic size, the *corpus albicans*.

If the ovum is fertilized, the corpus increases in size for a time and is known as the *corpus luteum graviditatis (verum)*. It attains a size of 20–30 mm., and persists until the later months of pregnancy when it likewise undergoes a slow involution.

Although human corpora lutea are bright yellow, this is not true for all mammals. The corpora lutea of cows have an orange hue. In some animals (dog, cat and some rodents) they contain no pigment and consequently are pale in color. The composition of the pigment has not been extensively studied although carotene has been identified as one of the constituents in certain animals.

Interstitial Cells. In many mammals the cortical stroma contains clusters or strands of epithelioid connective tissue cells whose cytoplasm contains fine lipid granules. These form from the theca interna and are known as *interstitial* cells. In the mature human ovary the theca interna cells of degenerating follicles form radiating cords which persist for only a short time (Figs. 378, 379).

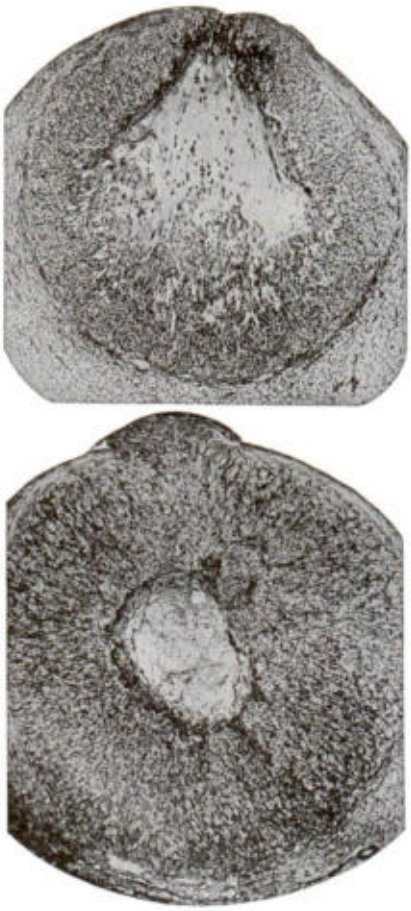

FIG. 385. Stages in the formation of the corpus luteum in the rabbit. *A*, Corpus luteum from an ovary removed 48 hours after mating (about 36 hours after ovulation). *B*, Corpus luteum from the other ovary of the same animal. This ovary was removed four days after mating (about 84 hours after ovulation).

Hormones of the Ovary. The ovary is under the direct influence of hormones of the anterior hypophysis. These hormones, the gonadotropins, control the maturation of follicles and the formation of corpora lutea. The ovary in turn produces hormones of its own, which affect the accessory reproductive organs such as the uterus, Fallopian tubes, mammary glands, and, in addition, exert a regulatory effect upon the anterior hypophysis.

The ovarian hormones are steroids. One of them, *estrogen* (principally estradiol), is secreted by the growing follicles, and to a lesser degree by the corpus luteum. The other, *progesterone*, is produced mainly by the corpus luteum, but also to some extent by the mature follicle just prior to ovulation. Since there is a wave of follicular maturation during the first half of each month, followed by formation of a corpus luteum immediately after ovulation, the levels of the two hormones normally show regular cyclic fluctuations. Estrogen secretion is high during the preovulatory period and reaches a peak at about the time of ovulation; progesterone secretion increases rapidly as the ruptured follicle becomes luteinized and remains at a high level until regression of the corpus luteum. The fluctuations in ovarian hormone levels are reflected, as will be discussed later, in cyclic variations in other structures, notably in the mucous membrane of the uterus.

Blood Vessels. Branches of the ovarian and uterine arteries enter the medulla at the hilum and divide into a number of spirally coursing vessels which run to the boundary zone of cortex and medulla where they ramify and anastomose into plexuses. From these are given off branches which enter the cortex radially and break up into extensive capillary networks in the thecae of the growing and mature follicles. From the capillaries veins arise which accompany the arteries, form an extensive plexus in the medulla and leave the ovary at the hilum.

Lymphatics. Lymph capillaries begin in the theca externa of the follicles, and unite into somewhat larger vessels which pass radially through the medulla and leave at the hilum. There they are collected in a number of lymphatic trunks which drain into the lumbar lymph nodes.

Nerves. Nerve fibers, mostly unmyelinated, enter at the hilum and follow the

course of the blood vessels. Many terminate in the muscle fibers of the medullary blood vessels. Others enter the cortex and form delicate plexuses in the thecae of the follicles, but apparently do not penetrate into the granulosa. According to some, groups of sympathetic ganglion cells are found in the medulla.

Vestigial Structures. As is the case with the testis, certain rudimentary organs, the remains of fetal structures, are found connected with the ovary.

The *epoöphoron* consists of a number of blind tubules situated in the folds of the broad ligament between the ovary and the oviduct. The tubules open into a longitudinal duct, the canal of Gärtner, which passes along the lateral wall of the uterus and reaches the vagina. The duct is often interrupted and may be altogether absent in some cases.

The *paroöphoron* is situated in the connective tissue of the hilum and consists of a few blind tubules or cords. It is rarely found in the adult.

Both epoöphoron and paroöphoron are remains of the embryonal mesonephros, while Gärtner's canal represents a vestige of the Wolffian (mesonephric) duct.

THE FALLOPIAN TUBES

The Fallopian (uterine) tubes are paired structures each of which is about 15 cm. long and 6–8 mm. in diameter. One end of a tube opens into the peritoneal cavity near the ovary; the other end opens into the superior lateral part of the uterine cavity. The tubes conduct the ova, discharged at ovulation, to the uterine cavity.

Four regions of the tubes are usually distinguished. Beginning with the ovarian end these are: (a) infundibulum; (b) ampulla; (c) isthmus; (d) uterine or interstitial segment. The *infundibulum* is funnel-shaped, and is formed of a number of processes or fimbriae. The *ampulla* is the longest of the segments and like the fimbriae is thin walled. It terminates in a relatively short segment which extends to the uterus, the *isthmus*. This segment is smaller in diameter and thicker walled than the ampulla. The last portion, *pars uterina*, is imbedded in the wall of the uterus. The mucosa of the infundibulum and ampulla is thrown into many tall folds with corresponding deep grooves. The lumen thus is very irregular in shape (Fig. 386). The folds progressively de-

FIG. 386. Cross section of Fallopian tube near fimbriated extremity, showing complicated foldings of mucous membrane. (Orthmann.)

crease in height towards the uterus and in the isthmus are low (Fig. 387). In the pars uterina there are but slight folds and the cavity reaches its smallest diameter, about 1 mm.

The wall of the Fallopian tube consists of three coats—mucosa, muscularis, and serosa.

The *epithelium* lining the Fallopian tubes is a simple columnar type, some cells of which are ciliated, whereas others are narrow, peg-shaped, and nonciliated (Figs. 388, 389). The height of the epithelium and the proportion of ciliated to nonciliated cells, although varying considerably even in neighboring regions of a tube, show changes which are correlated with the stages of the menstrual cycle. The epithelium during the first half (follicular phase) of the cycle is taller than it is in the second half which is under the influence of the corpus luteum. The relative number of nonciliated, peg-shaped cells also increases in the corpus luteum phase of the cycle. During pregnancy, the epithelium is quite low and there is an increased number of "peg" cells (Fig. 389). The epithelium secretes mucus and probably other substances which are necessary for the maintenance of the ovum during its journey down the tube.

The cilia beat toward the uterus. The beating of the cilia and the waves of muscular contraction transport the ovum through the tubes. No glands are present in the Fallopian tubes.

The stroma of the lamina propria is composed of a richly cellular connective tissue, quite compact in the isthmus, more loosely arranged in the high folds of the ampulla.

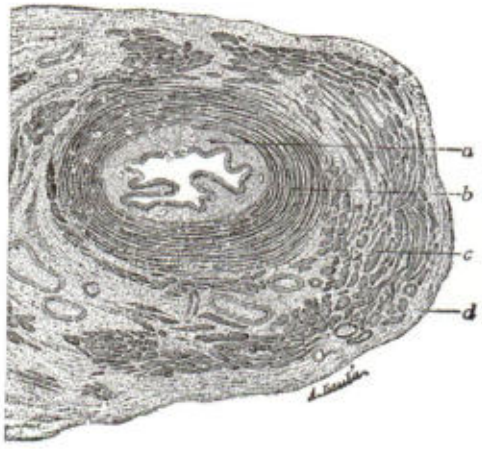

FIG. 387. Cross section of Fallopian tube near uterine end. *a*, Mucous membrane; *b*, circular muscle coat; *c*, longitudinal muscle coat; *d*, connective tissue of serous coat. (Orthmann.)

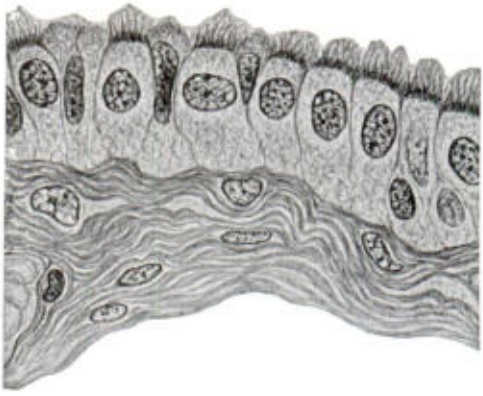

FIG. 388. Section of Fallopian tube removed at the middle of the menstrual cycle showing the characteristic type of epithelium present at that stage. × 1000.

FIG. 389. Section of Fallopian tube of a woman two and one-half months pregnant showing the type of epithelium characteristic of pregnancy and of the progravid stage of the menstrual cycle. × 1000.

The *muscularis* is thickest in the isthmus and gradually thins out toward the fimbriated end. It consists of a well developed inner circular layer, and a rather thin outer longitudinal layer. The latter is complete only in the isthmus. In the ampulla the longitudinal muscle bundles are discontinuous, and may be absent altogether in the fimbria.

The *serosa* has the usual structure of peritoneum.

The larger *blood vessels* run in the stroma along the bases of the folds. They send off branches which give rise to a dense capillary network in the stroma.

The *lymphatics* arise as relatively large lacunae in the stroma of the mucosal folds. These empty into narrower channels which pass through the muscularis and form a rich subserous net. The lymphatics drain into the upper lumbar lymph nodes.

The *nerves* form a rich plexus in the stroma, from which fibers pass to the blood vessels and muscular tissue and to the epithelial lining.

THE UTERUS

The uterus is a thick-walled pear-shaped organ, somewhat flattened dorsoventrally in its upper two thirds. It varies considerably in size, averaging some 7 cm. in length, 5 cm. in width at its upper (broadest) part, and 2.5 cm. in thickness. Its cavity conforms to the general shape of the organ, being very narrow dorsoventrally in the body of the organ, and more circular in the lower portion. The uterine tubes open into the superior lateral part of the uterine cavity, one on either side. The lower part of the cavity (the cervical canal) opens into the vagina.

Anatomically two main regions of the uterus are distinguished: (a) an upper *body* or *corpus* with its rounded dome-shaped top, the *fundus*, and (b) a narrower cylindrical *neck* or *cervix* whose terminal portion projects into the vagina as the *portio vaginalis*. The narrow zone of transition between corpus and cervix is known as the *isthmus*.

The wall of the uterus consists of three coats which from without inward are the serosa or *perimetrium*, the muscularis or *myometrium*, and the mucosa or *endometrium*.

The *perimetrium* is the peritoneal layer of the broad ligament, which covers the corpus and a portion of the cervix. It is firmly attached to the underlying muscularis and has the usual structure of a serous membrane.

Myometrium. The myometrium is a massive muscular coat, about 15 mm. in thickness, consisting of bundles of smooth muscle fibers held together by connective tissue. The disposition of the muscle fibers is quite complicated but in a general way three layers may be distinguished. The inner muscular layer, the *stratum subvasculare*, consists of fibers running longitudinally, i.e., parallel to the long axis of the organ. The middle layer, *stratum vasculare*, forms the bulk of the muscularis and is composed mainly of fibers running circularly or spirally. In the interstitial tissue are numerous large blood vessels, especially veins. The outer layer, *stratum supravasculare*, is relatively thin and composed of both circular and longitudinal fibers. The latter predominate and form a fairly distinct subserous layer which becomes continuous with the longitudinal muscle coat of the vagina. In the cervix the inner longitudinal layer is absent.

The muscle cells of the virgin uterus have a length of 40–90 μ, the variations conforming to definite phases of the menstrual cycle. The fibers are shortest in the first week after menstruation, and reach their greatest length in the fourth week of the cycle. During pregnancy the muscle tissue of the uterus is greatly increased. This is due partly to an increase in the

number, but mainly to the tremendous increase in the size of the muscle fibers which in the later stages of pregnancy may have a length of over 500 μ.

The interstitial tissue contains numerous blood vessels and consists of loosely arranged collagenous fibers and rather few connective tissue cells. Elastic fibers are found in considerable amounts in the outer layer of the muscularis and in the subserous connective tissue. The inner portions of the myometrium are relatively poor in elastic tissue. In the cervix elastic tissue is abundant.

Endometrium. The endometrium is lined by simple columnar epithelium which usually does not have any cilia. Numerous tubular glands are present each of which opens to the lumen without any intervening duct. There is no submucosa, and the mucosa is closely attached to the myometrium, the juncture between them being very irregular.

During the child-bearing period the

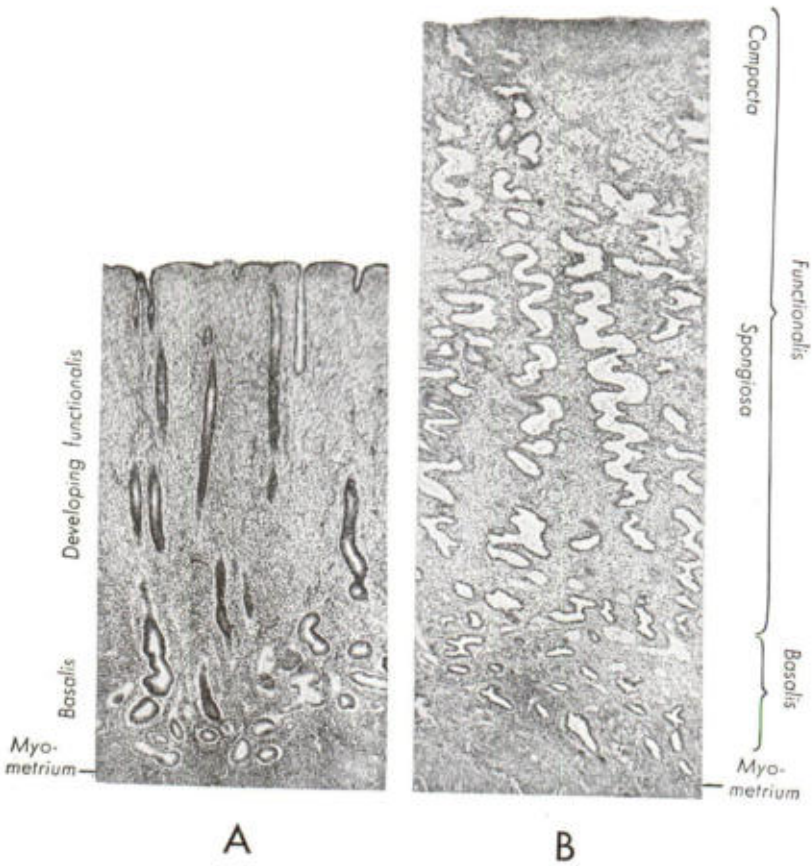

FIG. 390. Human endometria. *A*, Proliferative stage, about day ten of menstrual cycle. *B*, Late secretory stage, twenty-fifth day of cycle. Photomicrographs. × 30. The tissue of the secretory stage illustrated in this figure and the stages illustrated in Figures 391, 392, 393, 394 were supplied by Dr. Arthur Hertig

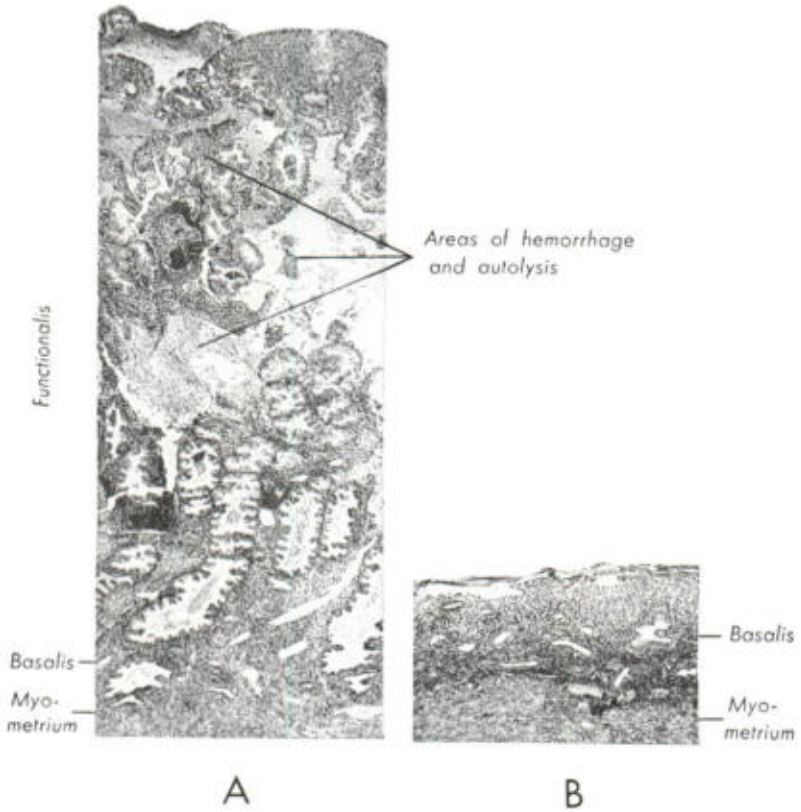

FIG. 391. Human endometria. *A*, Early menstrual phase. *B*, Termination of menstruation. Photomicrographs. × 30.

mucosa of the corpus and fundus passes through cyclic changes, each cycle closely related to the maturation of a Graafian follicle, the discharge of the contained ovum and the subsequent formation of a corpus luteum. In general these changes are in the nature of a preparation for pregnancy and consist of a hypertrophy of the glandular, vascular and interstitial elements of the mucosa. If the egg is fertilized and implantation occurs, the hypertrophy continues. If the egg is not implanted the hypertrophied layer breaks down, and the tissue débris together with a certain amount of blood is discharged as the *menstrual* fluid. With cessation of the flow regeneration occurs rapidly and a new cycle sets in, determined as before by a new ovarian cycle. A uterine bleeding or *menstruation* occurs typically at intervals of about 28 days and lasts for 3–5 days although there is great variability not only among different individuals but commonly in any one individual, also. The first day of menstruation is counted as the first day of the uterine or menstrual cycle.

Four stages, each of which has characteristic structural features, are distinguishable in the endometrium during an ovulatory menstrual cycle. It should be kept in mind, however, that the endo-

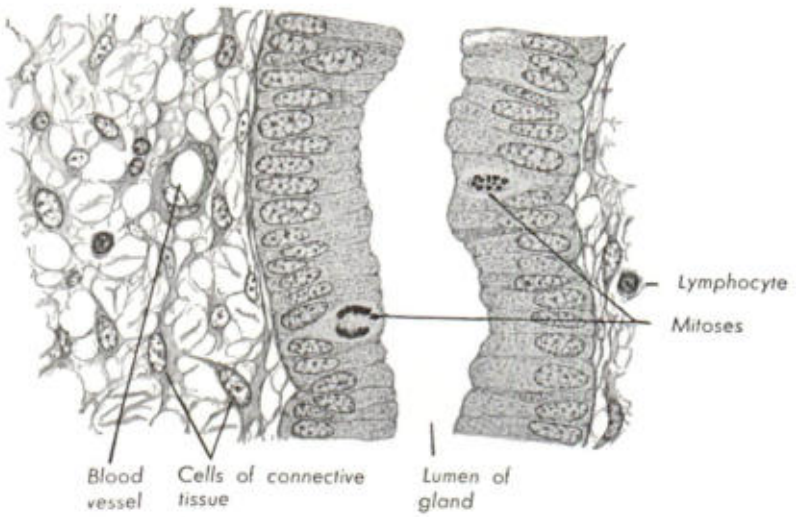

FIG. 392. A section of human endometrium in the proliferative stage (about day eleven) showing a part of a gland tubule and adjacent stroma. × 650.

metrium undergoes a continuous change, and that in each stage the structural features change somewhat so that a stage does not abruptly pass into the next. The indicated duration of each stage is based on a twenty-eight day cycle. (1) The *menstrual* stage occupies the first three to five days of the cycle during which time there is an external menstrual discharge. (2) The *proliferative* (estrogenic) stage begins with the termination of menstruation and extends to about the middle of the cycle, namely, to the thirteenth or fourteenth day. (3) The *progravid* or *secretory* (luteal or progestational) stage extends from the middle of the cycle to the twenty-sixth or twenty-seventh day. (4) The *premenstrual* stage is one or two days in length and is terminated by the appearance of visible external bleeding. The length of the proliferative stage is less constant than that of the others and variations in its duration are chiefly responsible for the varying lengths of the menstrual cycles.

A description of the changes occurring during the *proliferative* stage of the cycle will be given first. This stage is characterized by rapid regeneration of the endometrium from the narrow basal zone remaining after menstruation (Fig. 391, *B*). Epithelial cells from the remaining portions of the glands migrate and cover the raw surface of the mucosa. Numerous mitoses occur in cells of the glands and of the stroma. As the gland cells increase in number, they become tall and closely packed together (Fig. 392), and the glands increase in length. Although forked terminations of the glands are frequent, nevertheless, the glands remain relatively straight and are quite uniform in diameter (Fig. 390, *A*). Near the close of the proliferative stage some secretion appears in the basal ends of the gland cells. The stroma is formed of branching cells among which are reticular fibers. Blood vessels (coiled arteries) grow into the regenerating tissue, and, towards the end of the proliferative stage, a considerable degree of edema develops although it is not as pronounced as in the secretory stage. During the proliferative stage, the endo-

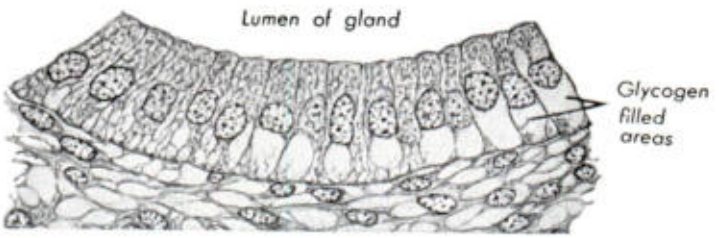

FIG. 393. A section of human endometrium in the early secretory stage. Nineteenth day of menstrual cycle. × 650.

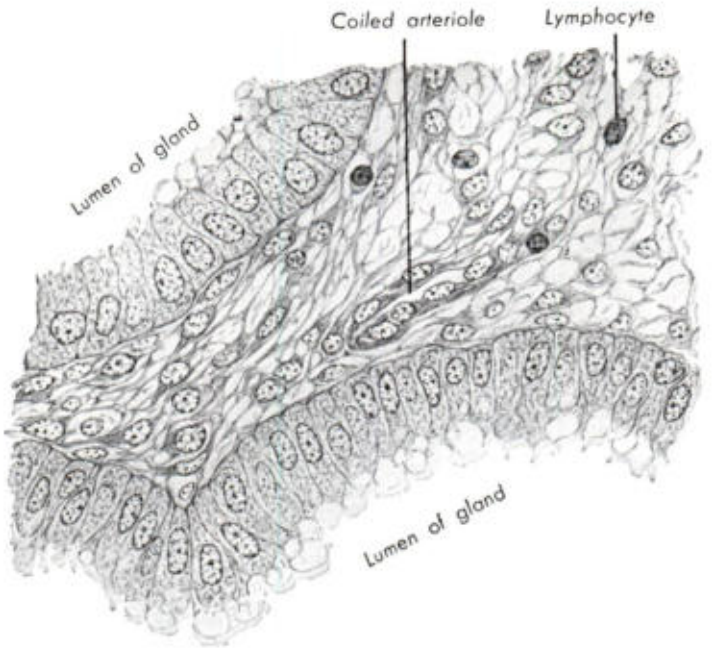

FIG. 394. A section of human endometrium in the late secretory stage. Twenty-fifth day of cycle. × 650.

metrium increases from a postmenstrual thickness of half a millimeter or less to two or three millimeters.

In the *secretory* stage of the cycle the endometrium hypertrophies, reaching a thickness of 4–5 mm. The increase is due not to mitotic activity but to hypertrophy of the gland cells and to an increase in edema and vascularity. The gland cells decrease in height and become broader (Figs. 393, 394). The glands assume a corkscrew shape and their lumina become large and irregular in diameter, giving them a characteristic appearance (Fig. 390, *B*). The edema increases and the coiled arteries grow nearly to the surface.

With the development of the secretory stage, several layers can be distinguished in the endometrium (Fig. 390, *B*). The *basalis* is the deepest layer and is relatively narrow. Its glands undergo little

or no change. This layer is not lost at menstruation or at parturition. The *functionalis* comprises all of the endometrium lying above (superficial to) the basalis. It undergoes periodic changes in the menstrual cycle and is lost at menstruation and at parturition. The functionalis is divided into two layers, a superficial layer, the *compacta*, and a deep layer, the *spongiosa* (Fig. 390, *B*). The *compacta* is relatively narrow. It has little edema and the portions of the glands which lie in it are quite straight. The *spongiosa* comprises the bulk of the endometrium. It is edematous, and the glands are tortuous and have large lumina, giving the zone a spongy appearance. It is emphasized that these zones become evident only with the development of the secretory stage.

During the secretory stage the gland cells undergo certain progressive secretory changes. With the onset of this stage both glycogen and mucin increase rapidly in the gland cells of the functionalis, and this secretion at first is localized largely in their basal portion. With the usual technical procedures the secretion is dissolved (Fig. 393). During the latter half of the secretory stage the secretion moves to the apical zone of the gland cells and the nuclei consequently become basally located (Fig. 394). The secretion, composed of glycogen, mucin and some fat,

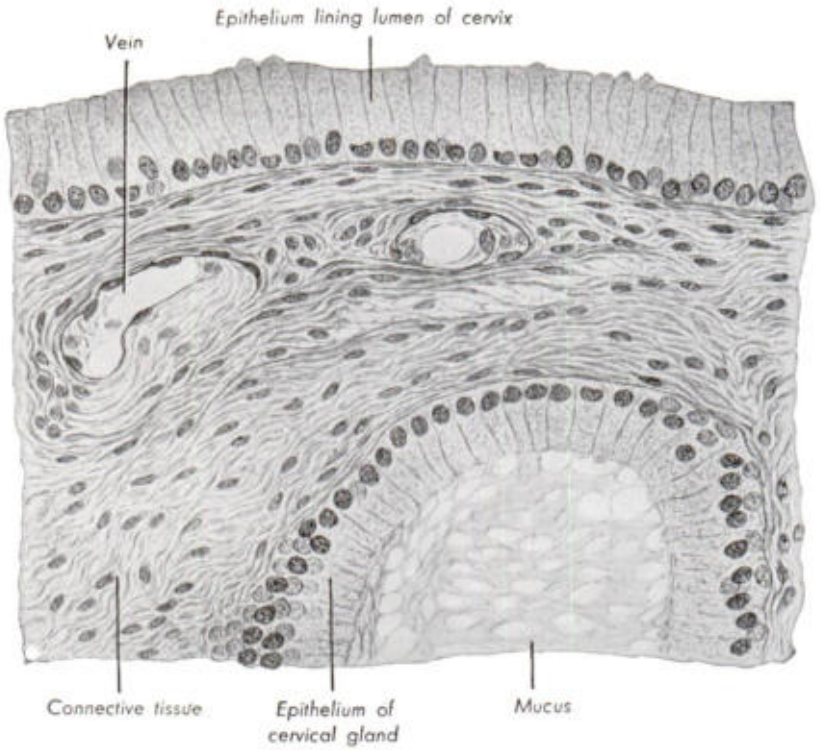

FIG. 395. Transverse section through the superficial portion of the cervical mucosa of a woman 31 years old. End of the second month of pregnancy. Therapeutic abortion. Stained with iron hematoxylin. × 554.

then appears in the lumina of the glands, thus terminating the secretory cycle of the cells of the glands.

In the *premenstrual* phase of the cycle important changes occur in the coiled arteries leading to a breakdown of the functionalis. In studies made on living endometrial transplants in the anterior chamber of the eye in monkeys, Markee observed that a constriction of the coiled arteries and vascular stasis occurred during the premenstrual period, producing a condition of anemia and anoxia. Finally blood escaped from the vessels. These observations assist in explaining the immediate factor responsible for the areas of hemorrhage and autolysis seen in sections of fixed tissue (Fig. 391, *A*). In the premenstrual stage there is also a decrease in edema and an infiltration of the stroma with leucocytes. The glands fragment, the surface of the endometrium breaks down, and blood and tissue débris appear in the uterine lumen.

During *menstruation* the functionalis is lost although there may be considerable variation in the amount of endometrial destruction. The coiled arteries undergo necrosis and some blood may spurt from them although most of the menstrual blood comes from veins. To this blood is added the secretion of the glands and the broken down tissue of the functionalis.

Thus in each uterine cycle a period of growth and proliferation is followed by one of secretory activity. In the first period a new functional layer is built up, with its glands and interstitial tissue. In the second the functional layer is transformed into a swollen nutritive membrane, ready for the implantation of the ovum. If the egg is not implanted, desquamation of the functionalis occurs, rapidly followed by regeneration of the epithelial surface. Menstruation thus indicates a "biological failure", a failure of the egg to be fertilized and implanted.

The mucosa of the *cervix* (Fig. 395) is somewhat thicker than that of the body and fundus of the uterus and shows numerous folds, the *plicae palmatae*. The stroma, also, is firmer and less cellular.

The lining epithelium consists mainly of high columnar mucous secreting cells, though ciliated cells may also be present. The numerous forked glands are much larger than those of the body of the uterus and are lined by tall mucous secreting cells. Closure of the mouths of some of the glands frequently occurs, leading to the formation of cysts of considerable size, the so-called *ovula Nabothi*. Near the external opening of the cervix, the simple columnar epithelium changes abruptly to a stratified squamous epithelium which likewise covers the external surface of the portio vaginalis. The cervix does not exhibit distinct menstrual changes.

Relation of Menstruation to Ovulation. As already stated the cyclic uterine changes are closely related to the ovarian cycles associated with ovulation. From the available evidence it is possible to relate, temporally, the specific phases of the two cycles. The postmenstrual proliferative changes correspond to the preovulatory period of maturation of the follicle. The secretory stage is always associated with the formation and growth of the corpus luteum, and lasts as long as the latter retains its full function. The beginning involution of the corpus luteum always marks the onset of menstruation. Ovulation occurs normally at the end of the proliferative period, i.e., at the interval, though the estimates of different investigators vary within relatively wide limits (from the eighth to the twentieth day of the cycle). Very precise and extensive data have been collected by Hartman on rhesus monkeys. Their sex cycles and reproductive processes are very similar to the human. In some three

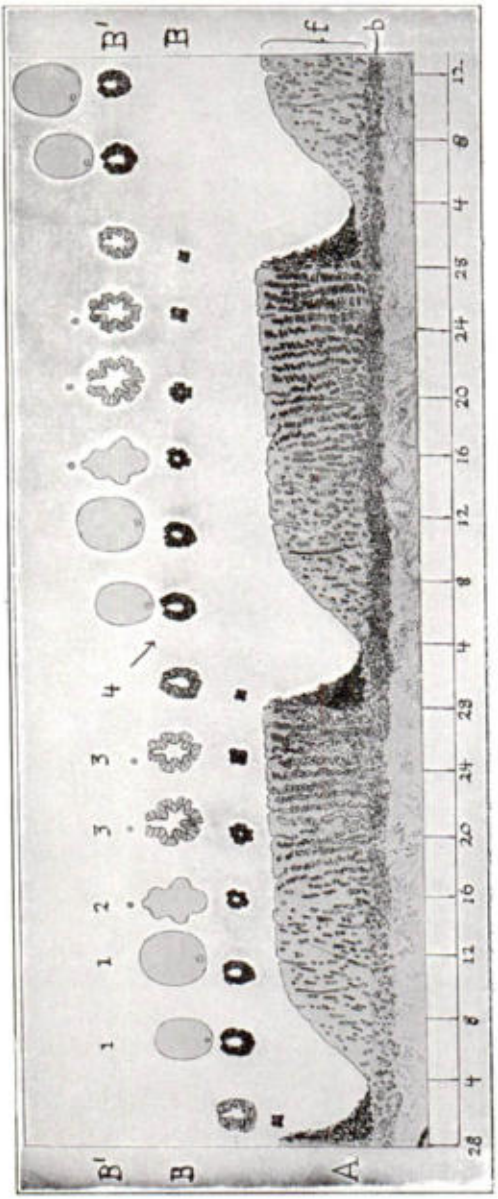

FIG. 396. Diagram illustrating relation of menstruation to ovulation. *A*, Cyclic changes in uterine mucosa; *B*, *B'*, ovarian cycles; *b*, basal layer; and *f*, functional layer of mucosa; *1*, *1*, maturing follicle; *2*, rupture of follicle and discharge of ovum (ovulation); *3*, *3*, corpus luteum in full function; *4* and remaining figures, degenerating corpus luteum. Numbers at base indicate days of menstrual cycle. (After Schroeder.)

hundred observations he found that ovulation occurred most frequently on days eleven, twelve and thirteen (dated from the first day of the preceding menstruation), although occasional instances of ovulation were found between the eighth and twenty-third day of the cycle. A schema of the temporal relations of two cycles is given in Figure 396.

Evidence secured from the human as well as from rhesus monkeys has revealed that the cyclical uterine changes are induced by estrogen, a product of the follicle and perhaps of other parts of the ovary, and of progesterone, a product of the corpus luteum. The endometrium after ovariectomy involutes but can be caused to undergo the proliferative changes by estrogen injections. Continued injections of this hormone will not cause a development of the secretory phases, progesterone administration being necessary to induce the development of this phase, but its injection must be preceded by estrogen treatments in order for it to act. It is established that the corpora lutea in women secrete estrogen as well as progesterone and that in the latter half of the cycle the uterus is under the influence of both of these hormones. A more highly developed secretory type of endometrium can be produced experimentally, if the progesterone injections are supplemented by estrogen administration. By the use of these two hormones, the endometrial changes characteristic of the normal cycle can be secured in ovariectomized women as well as in monkeys. The changes thus induced obviously correlate with the follicular and lutein phases through which the ovary passes in the complete ovulatory cycle.

Experimental work has revealed the rôle of the hormones in menstruation although it was confused for a time by the fact that during the summer months, rhesus monkeys, in captivity, do not ovulate or form corpora lutea but still exhibit periodic menstruation. Such cycles are known as *anovulatory cycles*. It has been demonstrated that bleeding will occur from a proliferative endometrium if the estrogen administration which induced this phase is stopped. However, if progesterone injections are commenced at the time the estrogen injections are stopped, menstruation does not occur but will take place a few days after the progesterone treatment is terminated. The institution of estrogen injections when the progesterone treatment is withdrawn, however, will not inhibit the expected menstruation from progesterone withdrawal.

Blood Vessels. Branches from each uterine artery penetrate to the middle (vascular) layer of the uterine muscle and then continue both ventrally and dorsally in this layer to the mid-line, forming the arcuate arteries. They anastomose with the branches from the other uterine artery. Two sets of branches arise from these arched arteries: (1) small branches which course peripherally and supply the supravascular (outer) part of the uterus; (2) larger branches which course centrally. Branches from the latter in turn form two systems. One set penetrates for a variable distance into the endometrium, depending on the stage of the cycle, is extremely coiled (coiled arteries) and terminates in a tuft of arterioles (Fig. 397). The other set supplies the inner layer of the uterine muscle and the basal part of the endometrium where branches from it anastomose with the branches of the coiled arteries. The endometrium is thus supplied by a basal and a superficial set of vessels. The basal set, as would be expected, does not undergo modifications with the stages of the cycle but the coiled arteries show pronounced changes. During the pro-

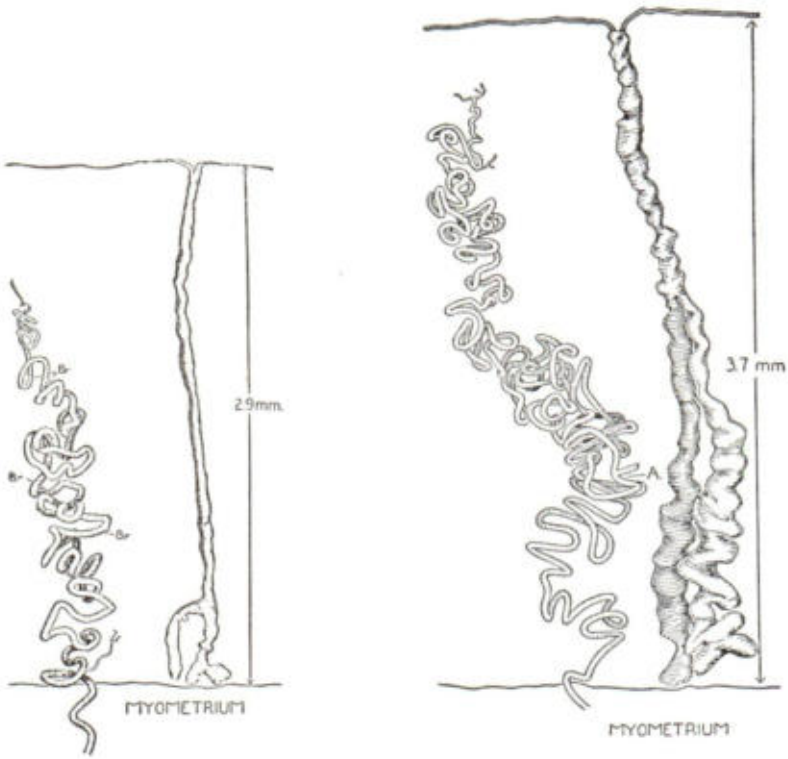

FIG. 397. Projection reconstructions of the coiled arteries and the glands in the endometrium of rhesus monkey (Old-world monkey). A, Early proliferative stage (early postmenstrual). B, Late progravid stage. (After Daron.)

liferative stage of the cycle they penetrate only one-half or two-thirds through the thickness of the endometrium but during the secretory stage they increase in extent and approach the surface and with the tissue loss at menstruation may protrude into the uterine lumen. Their peripheral part then undergoes necrosis. With the reparative postmenstrual process the vessels again grow. The menstrual blood, however, does not come mainly from the arteries but from the veins. Two mechanisms appear to operate to prevent the bleeding of the coiled arteries. One of these is the tortuosity of the vessels which would slow the flow. The other and more effective mechanism is arterial constriction, a constriction which is aided by localized thickenings or cushions, composed of longitudinal smooth muscle, on one side of the arterial lumen.

Menstrual fluid is composed of extravasated blood, desquamated tissue and the secretion of the uterine glands. The discharge does not clot for it is markedly fibrinolytic. It is also toxic as shown by injection into experimental animals.

Lymphatics. The lymph vessels are larger and more abundant in the uterus than in most other organs of the body. They are enlarged during pregnancy. All layers of the uterine wall have lymph vessels except the superficial part of the endometrium (compacta) which is devoid of them. During its cyclical changes

the endometrium exhibits pronounced changes in its water content and these changes are probably responsible for the unusually extensive lymph drainage.

Nerves. Both myelinated and unmyelinated nerve fibers occur in the uterus. The latter predominate and are connected with minute ganglia found in the upper vaginal wall near its junction with the cervix. These fibers supply the walls of the blood vessels and the muscle tissue of the myometrium. The myelinated fibers apparently run to the mucosa and form a scanty plexus beneath the epithelium. The distribution and endings of the mucosal nerve fibers have not been fully investigated.

The Uterus during Pregnancy

The ovum, if fertilized, becomes implanted in the endometrium at about the sixth day after ovulation. At this time, the endometrium has reached a highly developed secretory or progravid condition. It is thick and edematous, and the lumina and cells of the glands are large and contain glycogen, mucus and some lipid. Implantation may take place in any region of the uterine mucosa and even in the tubal epithelium. The uterine changes and correlated events in the ovary and ovum during the early phases of pregnancy in women are shown diagrammatically in Figure 398.

From the extensive studies of Hertig and Rock on a considerable series of ova and later stages, secured from timed hysterectomies, it appears that the following events occur subsequent to ovulation. Fertilization takes place before or soon after the ovum enters the uterine tube. At this time cumulus cells and the zona pellucida are still present. The migration down the uterine tube is leisurely, the ovum undergoing cellular division during its migration, and arriving in the uterus on the third day after ovulation. An ovum having two normal blastomeres was recovered from the mid one-third of the uterine tube 2–2½ days after the estimated time of ovulation, and one in an eight cell stage was recovered from the uterus on the third day. A blastula, also lying free in the uterine cavity, was recovered on the fourth day. It was found to consist of a thin epithelial membrane

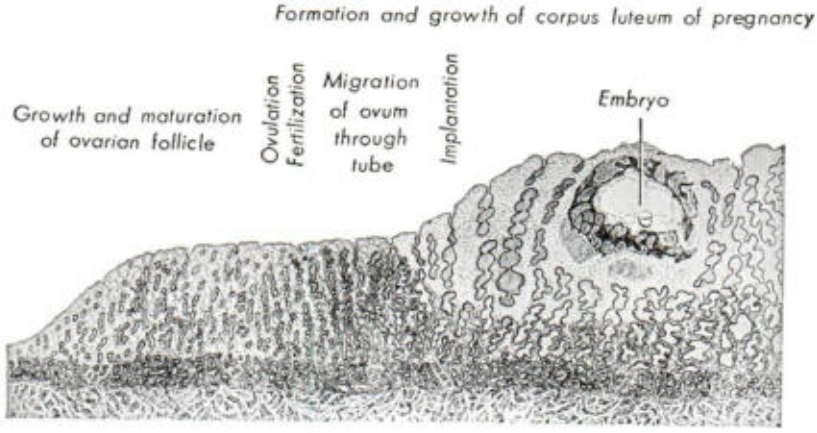

Fig. 398. A semidiagrammatic figure depicting the uterine changes and correlated events in the ovary and ovum. That part of the diagram showing the uterine changes has been taken from Schroeder's monograph.

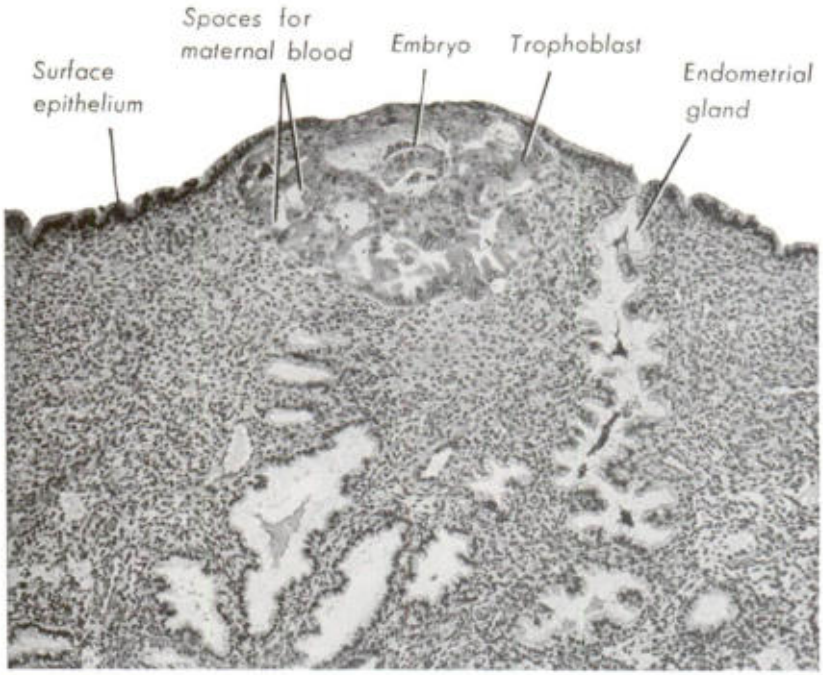

FIG. 399. Section through a human embryo nine and one-half days old. The embryo lies just beneath the epithelial surface of a twenty-six day secretory endometrium. × 100. (Courtesy of Doctors Rock and Hertig.)

and a small inner cell mass, destined to form the embryo. A blastocyst secured 7–7½ days after ovulation was implanted just beneath the surface of the endometrium, and the eroded epithelium had not, as yet, undergone sufficient repair to cover it. The chorionic membrane had proliferated; it is now known, because of its nutritive role, as the *trophoblast*. From the evidence summarized above, together with evidence from other fertilized but abnormal ova which have been recovered, it has been concluded that implantation occurs on about the sixth day after ovulation.

At somewhat later stages when the developing ovum is imbedded entirely within the endometrium, the trophoblast over the entire surface is found to have undergone growth (Fig. 399). The outer surface of the trophoblast shows irregular processes between which are irregular spaces or lacunae. In these spaces maternal blood which comes from the eroded coiled arteries of the endometrium will later flow. This is returned by the maternal veins.

The trophoblast, which in the early stages is syncytial in structure, has differentiated by the eleventh day (Hertig and Rock) into an epithelium consisting of two layers of cells, an inner layer, the *cytotrophoblast*, and an outer layer, the *syncytial trophoblast*. Beneath these is a stratum of mesenchyme which is destined to form the connective tissue component of the chorion (fetal portion of the placenta). From the irregularities on the outer surface of the trophoblast, finger-like sprouts or *villi* grow into the surrounding space. These, at first, are composed only of epithelial cords (primitive

villi). Later a core of embryonal connective tissue which is connected with the inner stratum of mesenchyme mentioned above, forms within them. They are then designated as secondary (chorionic) villi. In the core of embryonal connective tissue branches of the fetal vessels soon develop.

On the surface of the chorion facing the uterine lumen the villi do not grow as rapidly as on the deeply embedded surface. They disappear from the luminal surface at about the end of the first third of pregnancy leaving that surface smooth. It is named the *chorion laeve*. On the deeply-lying surface of the chorion, i.e., the surface facing the myometrium, the villi continue to increase and form the fetal component of the placenta. This portion of the chorion is designated the *chorion frondosum*. That part of the chorion to which the villi of this deep surface are attached forms a fairly firm plate-like structure and is called the *chorionic plate*.

The *endometrium* also undergoes important changes in pregnancy. It is called the *decidua graviditatis* because, except for the deepest layer, it is destined to be cast off at parturition. Thus, the

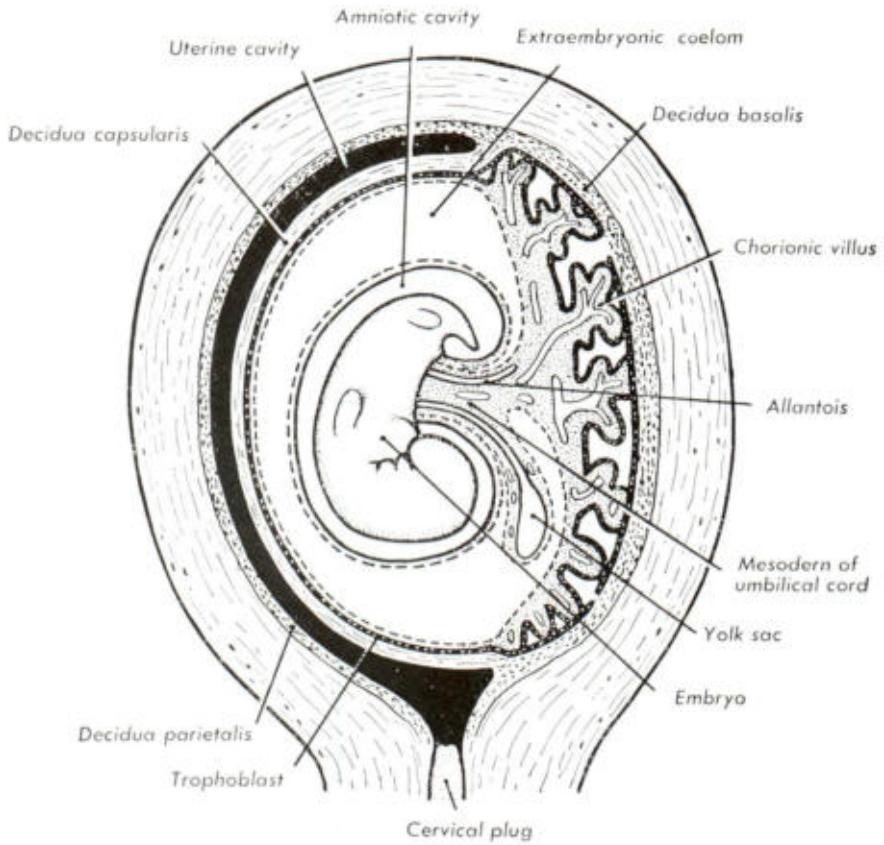

FIG. 400. Diagram of the formation of the fetal membranes in relation to the decidual membranes. (After Hamilton, Boyd, and Mossman.)

process at birth is not unlike menstruation except that it is much more cataclysmic and involves the loss of much more tissue.

In relation to the developing embryo, three regions of the decidua are distinguished: (a) the *decidua basalis* or *serotina*, that part of the mucosa lying

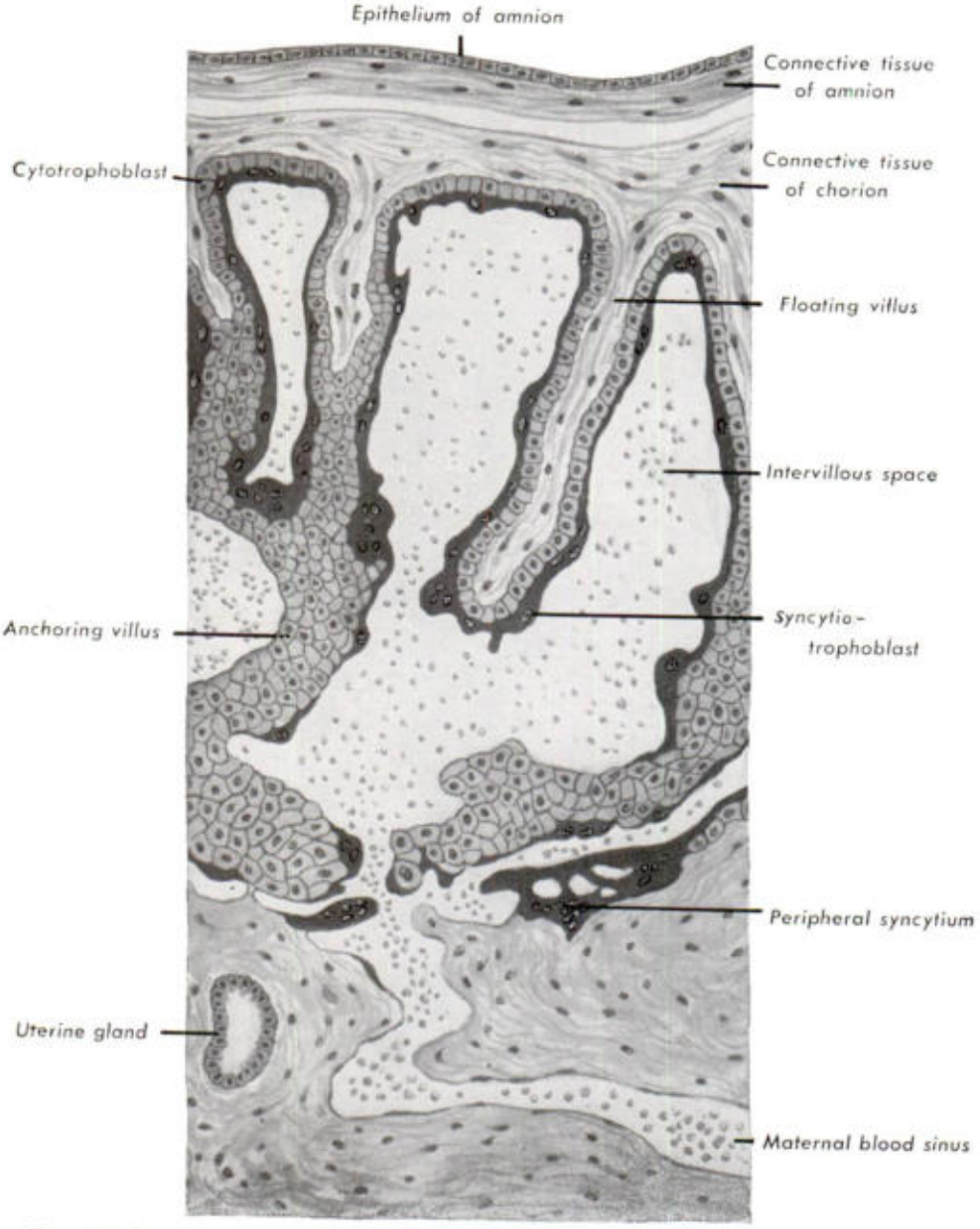

FIG. 401. Semischematic diagram of structure of fetal and maternal tissues of the placenta. (Redrawn and modified after Hamilton, Boyd, and Mossman.)

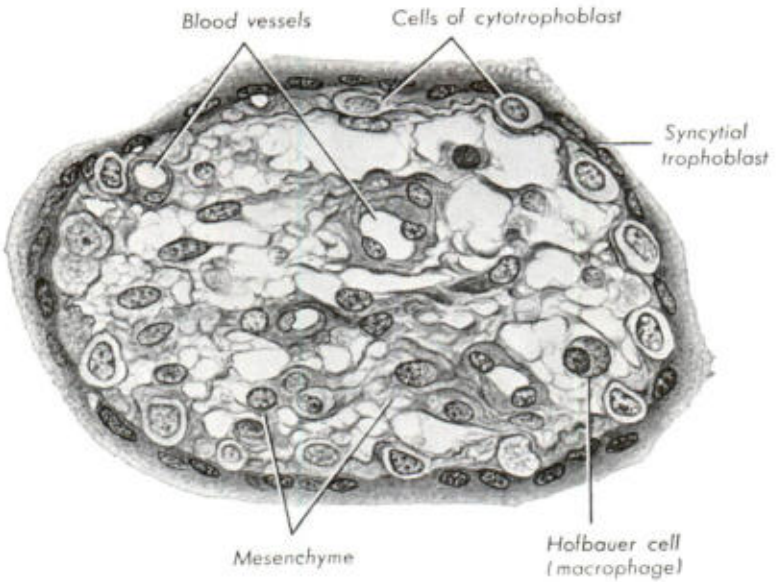

FIG. 402. Transverse section of a secondary (free) villus of a human placenta. From a pregnancy of four and one-half months duration. Therapeutic abortion. Masson's trichrome stain. × 775.

beneath the embryo, i.e., between the embryo and the myometrium; (b) the *decidua capsularis*, or *reflexa*, that part of the mucosa which lies between the embryo and the lumen of the uterus; (c) the *decidua parietalis* or *vera* which consists of all the remaining mucosa of the body and fundus of the uterus.

In the early part of pregnancy the endometrium increases in thickness. The glands enlarge and become more tortuous, and the cells of the endometrial stroma become large and rounded, forming characteristic cellular elements, the *decidual cells*. These will be described later. In the latter half or two-thirds of pregnancy the parietal decidua gradually becomes thinner and the glands become reduced to slit-like spaces. As the lumen of the uterus is obliterated by the growth of the fetus, the decidua parietalis and capsularis come in contact with each other. The capsularis degenerates and the parietalis, denuded of epithelium, fuses with the external fetal membrane, the chorion.

The Placenta. The mature human placenta measures about seven inches in diameter and one inch in thickness. It is usually circular but may vary a good deal in shape. The placenta reaches nearly its maximum diameter during the first half of pregnancy but continues to increase in thickness throughout most of the gestational period due to the growth of the villi.

The placenta consists of two components, a *fetal* and a *maternal*. Their development has been described briefly in the preceding section. The *fetal component* consists of a chorionic plate and branching processes or villi which arise from the chorionic plate and lie in the spaces through which the maternal blood circulates.

The *chorionic villi* are usually classified

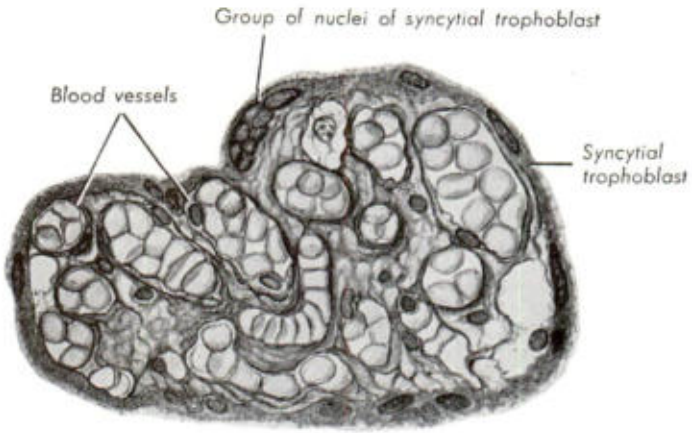

FIG. 403. Transverse section of a secondary villus of a human placenta at term. The blood vessels are engorged. Masson's trichrome stain. × 775.

into two types, *anchoring* and *free* or *floating* villi, the structure of the two being similar. The anchoring villi pass from the chorionic plate to the decidua basalis, thus, one of their functions is the anchoring of the chorionic plate to the decidua. They give origin throughout their length to branches which float in the blood-filled lacunar spaces between the fetal portion of the placenta and the decidua basalis and these are called, therefore, free or floating villi. The villi, as development proceeds, become very numerous. They also become increasingly irregular in shape, with many protuberances which correspond with the loops and coils of the capillaries which they contain. Their total surface area has been estimated to be six to seven square meters. Each villus has a central core of mesenchymal tissue and contains branches of fetal blood vessels. These receive their blood from the umbilical arteries and drain into the umbilical vein. They are covered by trophoblast, as is also the chorionic plate and the chorionic surface of the decidua basalis (Fig. 401).

The structure of the trophoblast differs somewhat according to the age of the embryo. During the first few days of pregnancy the cells are syncytial-like. Two cell layers then become differentiated, an inner cellular and an outer syncytial layer (Figs. 402, 404). Since the cells of the inner layer are discrete and well defined, this layer is named the *cellular trophoblast* or *cytotrophoblast*. The cells composing it are frequently called *Langhans cells*. The outer layer of the trophoblast, i.e., the layer next to the spaces filled with maternal blood, is plasmodial in nature and is named, therefore, the *syncytial trophoblast*. These two cell layers persist for approximately the first half of pregnancy. The cells of the cytotrophoblast then gradually decrease in number and, at term, they are very inconspicuous.

The cells of the *cytotrophoblast* are irregularly ovoid in shape and vary considerably in size (Figs. 402, 404). Their cytoplasm stains very lightly and contains some glycogen. Their nuclei are distinct and have a small amount of chromatin and distinct nucleoli. Beneath the cellular trophoblast is a delicate basement membrane of argyrophilic fibers. It is generally accepted that the cytotrophoblastic cells divide and give rise to the syncytial cells.

FIG. 404. The trophoblast of a secondary villus of a thirty-day human placenta. The syncytial layer is faintly vacuolated and has a distinct brush border. The cytotrophoblastic cells form a continuous layer. Subjacent to the basement membrane is a capillary which contains nucleated erythrocytes. Mallory's connective tissue stain. × 1600. (Courtesy of Doctors Wislocki and Bennett.)

FIG. 405. A portion of a human chorionic villus at term stained to show the argyrophilic fibers (reticulum). These fibers form the basement membrane beneath the syncytial trophoblast and also lie beneath the endothelium of the capillary. Cytotrophoblastic cells may be occasionally present at term but are not shown in the figure. × 1600. (Courtesy of Doctors Wislocki and Bennett.)

The *syncytial trophoblast* persists throughout pregnancy. It forms a narrow lamina in which dark staining nuclei are fairly regularly spaced in young placentae (Figs. 402, 404); in older placentae, knots or clumps of nuclei are of frequent occurrence (Fig. 403). No intercellular boundaries can be distinguished. The surface of the syncytial trophoblast varies considerably in structure. In some places a brush border is present whereas in other places hair-like or frond-like processes, suggestive of cytoplasmic streamers, can be observed. In the cytoplasm of the syncytium are vacuoles and granules which have been associated by investigators with absorption and excretion. Electron micrographs reveal in the cytoplasm many small vesicles of endoplasmic reticulum studded with RNA granules. The ribonucleic acid decreases in amount toward term. Many of the blood vessels (fetal) of the villi, especially in the latter two-thirds of pregnancy, lie very close to the surface of the villus (Figs. 403, 405), the syncytial trophoblast over them being attenuated and thin.

The *maternal component of the placenta* is formed by the decidua basalis. This comprises all of the endometrium beneath the fetal portion of the placenta except the deepest part which is destined to remain after parturition as in normal menstruation. In the decidua basalis and also in the decidua parietalis many of the connective tissue cells undergo a pronounced change. They hypertrophy and form large ovoid cells of somewhat irregular shape and are named *decidual cells*. They are one of the most striking features of the endometrium in the first half of pregnancy, for they are very numerous and some become relatively very large (Fig. 406). Some of them contain two or more nuclei. The nuclei are large and the chromatin sparse.

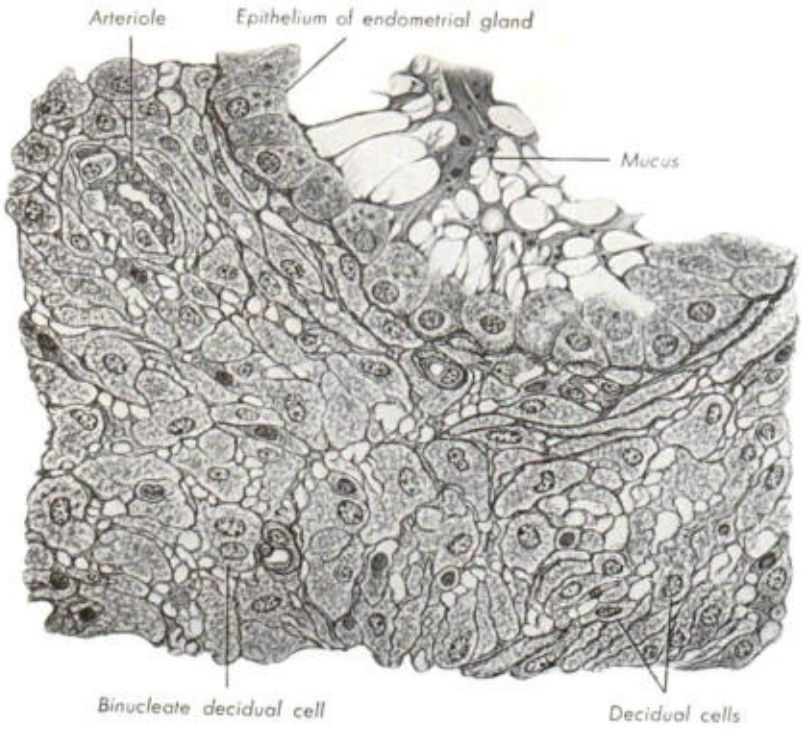

FIG. 406. Section through the decidua of a six-weeks human pregnancy. The numerous decidual cells and a part of an endometrial gland are illustrated. Therapeutic abortion. Masson's trichrome stain. × 450.

Nucleoli are present. The cytoplasm is vesicular or finely granular. Especially the smaller decidual cells contain large amounts of glycogen.

Some indication of decidual cell formation may be seen even in the terminal phase of a nonfertile menstrual cycle (predecidual reaction). With the onset of pregnancy, these cells rapidly develop and form a large component of the decidua in early pregnancy as stated above. They then regress and by the end of pregnancy are rarely present. Their function is obscure.

Function of the Placenta. An obvious function of the placenta is to transfer from the maternal to the fetal circulation the nutritive and other substances necessary for the development of the embryo. It also transfers waste products of fetal metabolism to the maternal circulation. Although the circulation of the mother and child are entirely separate, nevertheless, they are in close contiguity. The maternal blood circulates through the intervillous spaces and, thus, is separated from the fetal circulation only by the syncytial trophoblast, the cytotrophoblast (in the first part of pregnancy), a delicate basement membrane and the structures forming the walls of the fetal blood vessels.

The placenta also acts as a selective barrier against the transmission of certain substances from the maternal to the fetal circulation.

Another function of the placenta is the elaboration of hormones. It is known to secrete the steroids, *estrogen* and *progesterone*. If the ovaries are removed in

women, even early in pregnancy, abortion does not occur and the urinary excretion of estrogen and a degradation product of progesterone—pregnandiol—show but a temporary decrease. Estrogen and progesterone can be extracted from the placenta. The fetal part of the placenta also forms a hormone called *chorionic gonadotrophin* which differs, physiologically, from gonadotrophin of hypophyseal origin. This hormone begins to be formed, as judged by urinary assays, shortly after implantation and reaches a maximum in about two months, and then gradually decreases in amount. In cultures of placental tissue, gonadotrophin has been found to be elaborated, but no estrogen formation could be detected (Jones, Gey and Gey). Since the cytotrophoblastic cells were the predominant type in these cultures, it appears that they are probably the cells which form the gonadotrophic hormone. The site of elaboration of estrogen and progesterone has not been demonstrated by tissue culture. However, acetone-soluble ketones are present in the syncytium, a fact which suggests that this layer is probably the site of formation of the placental steroid hormones (Wislocki and Bennett).

Other Uterine Changes During Pregnancy. The changes described above involve the endometrium of the body and fundus of the uterus. A very pronounced hypertrophy of the smooth muscle of these regions of the uterus also takes place. The muscle fibers increase both in diameter and length. They may reach a length of half a millimeter. An increase in the number (hyperplasia) of the muscle fibers also takes place. Although the major uterine changes during pregnancy involve the body and fundus, some changes in the cervix also occur. The glands become more extensive and secrete copious amounts of mucus which forms a plug that occludes the cervical canal.

THE VAGINA

The wall of the vagina consists of three coats—mucosa, muscularis, and fibrosa (Fig. 407).

The *mucosa* shows transverse folds or rugae. It is lined by stratified squamous epithelium which rests on a moderately firm lamina propria. Many studies have been made from biopsy specimens in attempts to correlate changes in the epithelium with the menstrual cycle. The findings described, however, are not entirely harmonious due to the fact that there are variations in the structure of the epithelium in different parts of the vagina which make it difficult to establish the presence or absence of cyclical changes.

The vaginal epithelium is rich in glycogen which, in the primates, increases with the administration of estrogen. This has also been established in women and it has been fairly well determined that in the estrogen phase of the cycle, the vaginal fluid has a lower pH than at other times. This is attributed to the action of the lactic acid forming bacteria on the carbohydrate from the vaginal epithelium. The effect of estrogen on formation of glycogen and the consequent increase of acidity of the vagina is used clinically in the treatment of gonorrheal vaginitis in children.

In the tissue beneath the epithelium, lymphocytes and polymorphonuclear leucocytes are common. These invade the epithelium especially just before, during and just after menstruation, and appear as free cells in the lumen of the vagina.

In some lower forms the different types of free cells in the vagina show periodic changes in their proportions which are correlated with the ovarian cycle. The free cells consist of desquamated epithelial cells and leucocytes. The stage of the cycle is thus readily diagnosed by smears. This finding, first established by Stockard and Papanicolaou in 1917, has been of

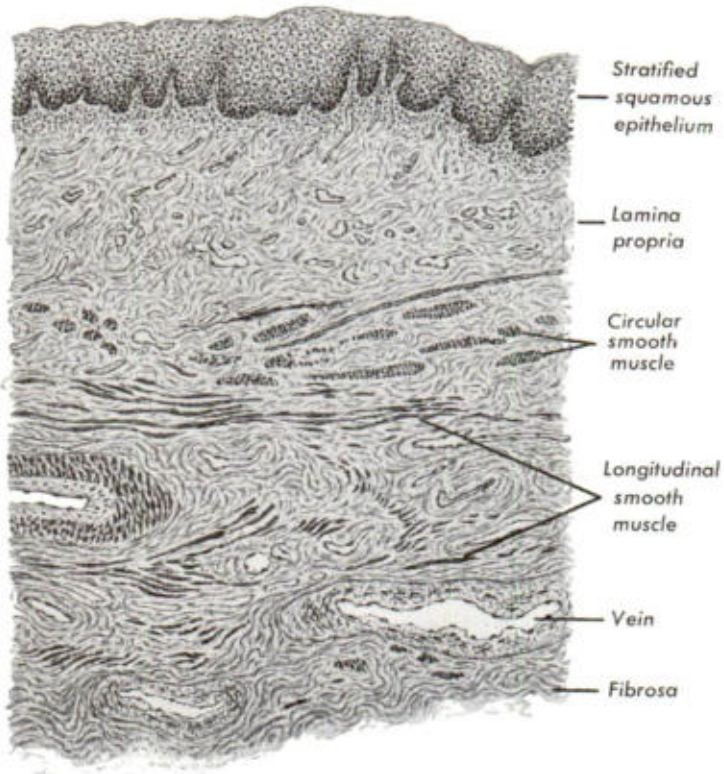

FIG. 407. Longitudinal section through the posterior wall of vagina. Human, age 50 years. Camera lucida drawing. × 35.

the greatest value in experimental work on reproduction. In the human, the vaginal smear changes are not as clearly marked nor as uniform as in some of the lower forms but are present as shown by Papanicolaou.

The lamina propria consists of loose connective tissue especially rich in elastic fibers, and contains diffuse lymphoid tissue and, more rarely, aggregations resembling solitary lymph nodules. A few isolated glands resembling those of the cervix may be found in the uppermost portion of the vagina. Elsewhere the vaginal wall is entirely devoid of glands, and the mucus found in the lumen is derived from the glands of the cervix. In the posterior wall of the vagina, the connective tissue papillae are especially high.

The *muscularis* consists mainly of bundles of longitudinally disposed smooth muscle fibers, which become continuous with the myometrium of the uterus. In the inner portion of the muscularis, circular bundles interlace with the longitudinal ones. The muscle bundles are separated by areolar tissue rich in elastic fibers. At the introitus there are skeletal muscle fibers in the vaginal wall.

The *fibrosa* consists of dense connective tissue with many coarse elastic fibers. It serves to connect the vagina with the surrounding structures.

The *hymen* is a thin semilunar fold which partially occludes the opening of the vagina into the vestibule. It has the same structure as the vaginal mucosa.

The larger *blood vessels* run in the deeper

portion of the mucosa, giving off branches which break up into capillary networks in the stroma and muscularis. These networks have a general direction parallel to the surface. The capillaries empty into the veins which form a plexus of broad venous channels in the muscularis. In the rugae there are large veins, thus giving them somewhat the character of erectile tissue.

An unusually well developed system of *lymph vessels* is present in the wall of the vagina.

The vagina receives both myelinated and unmyelinated *nerve fibers*. The latter are connected with scattered groups of sympathetic ganglion cells, and supply the muscle tissue and walls of the blood vessels. Sensory myelinated fibers arborize in the mucosa. Their terminals are not fully known.

EXTERNAL GENITALIA

The *vestibule*, into which the vagina and urethra open, is lined by a typical stratified squamous epithelium whose superficial layers are cornified. It contains numerous small mucous glands, the *glandulae vestibulares minores*, placed chiefly near the clitoris and opening of the urethra. They are similar in structure to the glands of Littré. The larger *glandulae vestibulares majores* or *glands of Bartholin*, analogous to the bulbo-urethral glands of the male, are placed in the lateral wall of the vestibule, their ducts opening close to the base of the hymen.

The *clitoris* consists mainly of erectile tissue similar to that of the corpora cavernosa of the penis. It is covered with a thin stratified squamous epithelium, underneath which is a papillated stroma rich in blood vessels and containing numerous sensory nerve fibers with highly specialized terminations, such as Meissner's corpuscles, Pacinian corpuscles, Krause's end-bulbs and others.

The *labia minora* which flank the vestibule are covered with a stratified squamous epithelium whose basal layer contains considerable pigment. The underlying, richly vascular connective tissue contains numerous elastic fibers, and sends tall slender papillae into the epithelium. In the stroma are found large sebaceous glands, not associated with hairs, and nerve endings similar to those of the clitoris.

The *labia majora* are folds of skin which cover the labia minora. They have the general structure of skin and consist of a stratified squamous epidermis and an underlying corium of fibroelastic tissue. On the outer side there are numerous hairs, sweat glands and sebaceous glands. On the inner side the epidermis is thinner and hairs are absent. The interior of the labia is filled with fatty tissue.

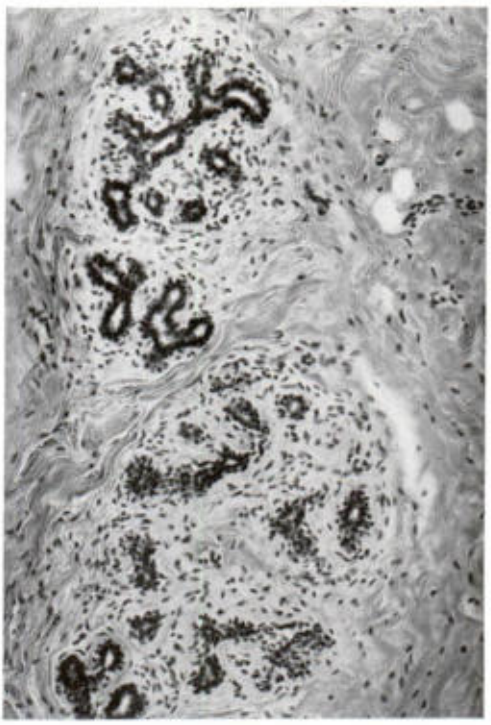

FIG. 408. Section through two lobules of an inactive mammary gland. Woman, 20 years of age. Photomicrograph. × 140.

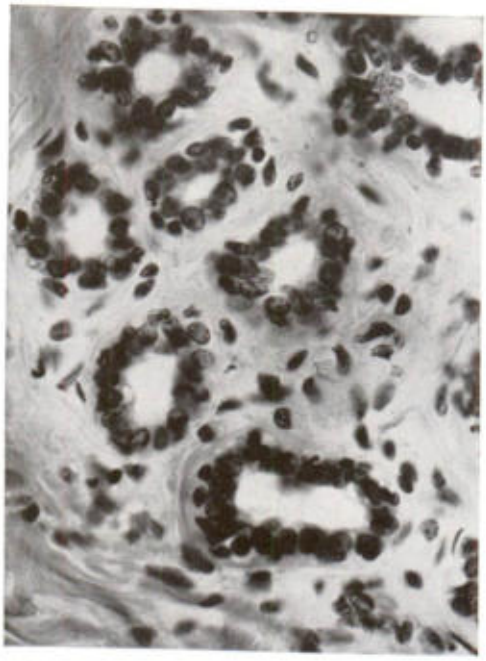

FIG. 409. Inactive mammary gland. Woman, 20 years of age. Photomicrograph. × 720.

THE MAMMARY GLANDS

The mammary glands are cutaneous in origin, developing within the superficial fascia (tela subcutanea). Like the large sweat glands, they have an apocrine type of secretion. A mammary gland consists of 15–20 lobes each of which is a compound gland with a duct (lobar duct) opening at the apex of the nipple.

Stroma. The stroma is both fibrous and fatty in nature. Surrounding or encasing the gland both on the superficial (except at the areola) and deep surface is a layer of fat. Fat is also present within the gland, the amount varying with the functional state. Extending from the dermis into the gland are rather dense fibrous strands (Cooper's ligaments) serving a suspensory function. The interlobar and interlobular connective tissue is also a dense type and forms septa between the subdivisions of the gland. The intralobular connective tissue, on the other hand, is fine and cellular (Fig. 408). The amount of connective tissue varies considerably with the functional state of the glands, being reduced in the lactating gland.

Ducts. There is one main duct for each lobe. These lobar ducts course through the nipple and open on the surface. Just beneath the nipple there is a local enlargement (sinus lactiferous). The ducts branch as in any compound gland, a terminal duct finally entering each lobule as an intralobular duct. The epithelium lining an intralobular duct is simple cuboidal. It increases in height as the ducts increase in size and it becomes stratified squamous near the opening onto the surface. Lying between the epithelium and basement membrane are myoepithelial cells which are more readily observed in the larger ducts.

Nipple and Areola. The skin of the nipple is pigmented, is somewhat wrinkled, and has tall connective tissue papillae. It has many sebaceous but no sweat glands or hairs. The areola, an area extending outward from the nipple for 1–2 cm., is pigmented, also, and has modified mammary glands (glands of Montgomery) whose ducts open through the skin of the areola. These glands which have some of the features of sweat glands, produce small elevations on the surface. Sweat and sebaceous glands and a variable number of coarse hairs are present, also. The subcutaneous tissue of the nipple and areola contains both radially and circularly coursing smooth muscle fibers.

Parenchyma. The parenchyma of the mammary gland has extreme variations in structure associated with its functional state. Variations are evident, also, among individuals in similar functional states.

The Inactive Mammary Gland. The glandular tissue in a nonlactating mammary gland of a sexually-mature nonpregnant woman, is sparse and con-

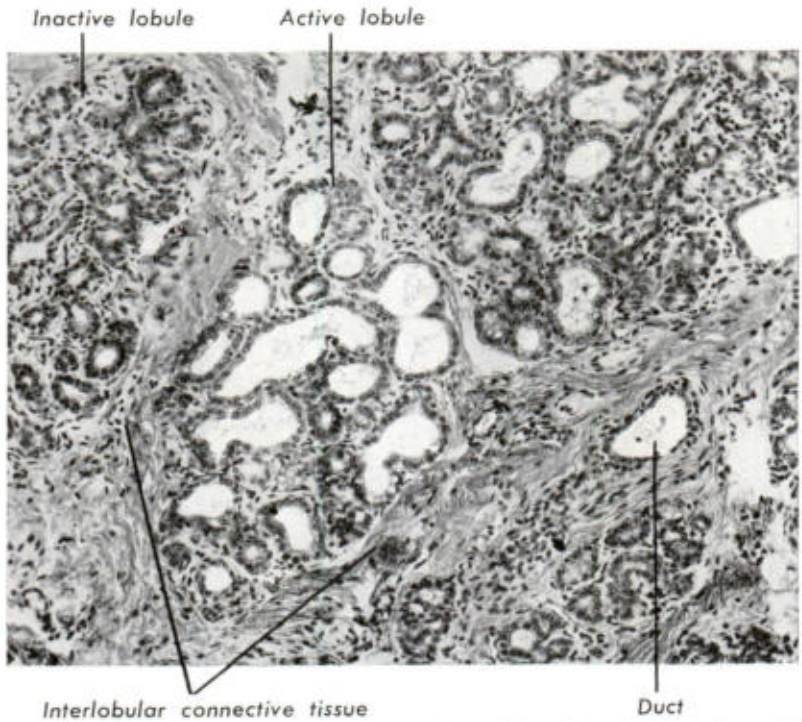

FIG. 410. Section of mammary gland showing active and inactive glandular tissue. Fourth day postpartum. Woman, 29 years of age. Surgical specimen. Photomicrograph. × 140.

sists of tubules which have the appearance of ducts. These are grouped together in lobules (Fig. 408). Many inactive glands show deviations from this normal structure, however, and a considerable percentage have, without any clinical symptoms, some degree of gross or microscopic cystic disease or other abnormalities, as for instance groups of secretory cells. The examination of many autopsy and surgical specimens is necessary in order to secure normal mammary gland tissue. For the incidence of pathological changes and review of the literature consult the paper by Frantz et al.

The Mammary Gland During Lactation. Throughout pregnancy the mammary gland undergoes extensive changes in preparation for lactation. The tubules, characteristic of the inactive gland, form buds which enlarge into alveoli. As this growth of glandular tissue proceeds, the fat and the intralobular and interlobular connective tissue decrease in amount, the latter forming septa in which the ducts are imbedded (Figs. 410, 411). The alveoli at the termination of pregnancy are large and irregular in shape, although there is frequently great variation in the degree of development among the lobules or even within a lobule (Fig. 410). The alveoli are lined by a simple cuboidal epithelium which rests on a delicate basement membrane. During secretion fat droplets form in the epithelium and coalesce at the free ends of the cells (Fig. 412). The fat is next discharged into the lumen by the constriction of the apical end of the cell and its detachment from the basal nucleated portion. The cell subsequently regenerates and a new cycle of secretion begins. With usual techniques the fat is dissolved, and the space which it occupied appears as a vacuole (Figs.

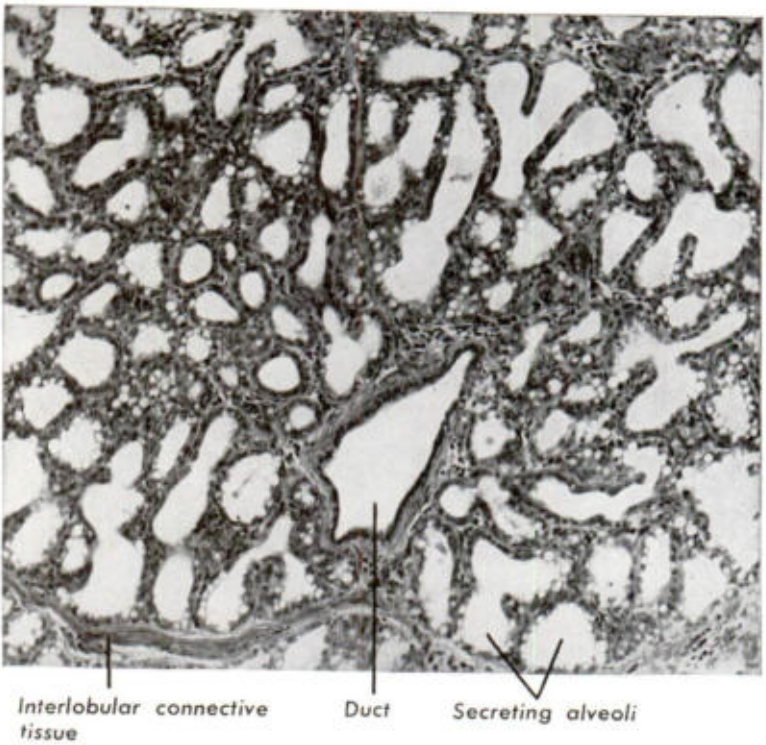

FIG. 411. Section of mammary gland showing uniform secretory activity. Photomicrograph. × 140.

411, 412), but it can be preserved and stained with proper reagents (Fig. 413).

After cessation of lactation the parenchyma of the mammary glands involutes, the alveoli decreasing in size until they become no longer recognizable. The connective tissue and fat again become abundant as the structure of an inactive gland is reassumed.

The mammary glands undergo progressive atrophy following the *menopause*. Some of the lobules and ducts may be obliterated; the connective tissue becomes increasingly dense and frequently hyalinized. Cystic dilatation of the ducts frequently occurs.

Milk consists of an albuminous fluid containing casein, milk sugar (lactose) and salts in which fat droplets are suspended. Milk is rich in calcium needed by the growing infant. Some cellular debris is also present.

Colostrum is the secretion formed during the first few days after parturition. It contains colostrum corpuscles which are large spherical or oval cells, filled with fat droplets of varying sizes. They are probably leucocytes or other wandering cells which have migrated into the alveoli and have taken up fat droplets by phagocytosis.

The Mammary Gland of the Male. There is little agreement as to the constitution of the male breast. Some mammary tissue always is present, but it attains its maximum development during early adolescence and then undergoes involution. The gland consists of ducts with usually no alveoli or lobulation. Under conditions of abnormal hormonal stimu-

lation, as in some testicular tumors, the male mammary gland may enlarge and develop extensively, a condition designated as gynecomastia.

Hormonal Control of the Mammary Gland. Although some development of the mammary gland occurs during childhood, growth characteristically is greatly accentuated during adolescence. At this time the gland comes under the influence of estrogen and progesterone secreted cyclically by the ovaries, a secretory process which in turn is dependent on hormones from the anterior hypophysis. During gestation when there is a continuous and prolonged production of both estrogen and progesterone by the ovaries and placenta the greatest development of the mammary glands takes place, alveoli and a presecretory condition being established.

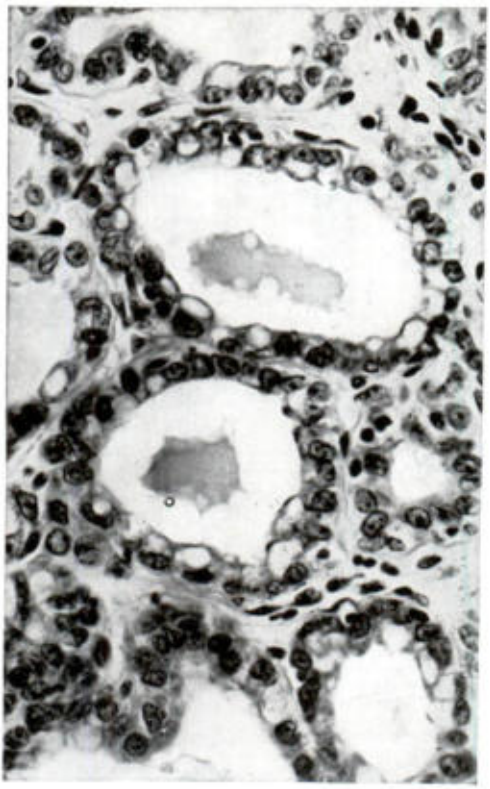

FIG. 412. Section of a few of the alveoli of a mammary gland in active secretion. Photomicrograph. × 720.

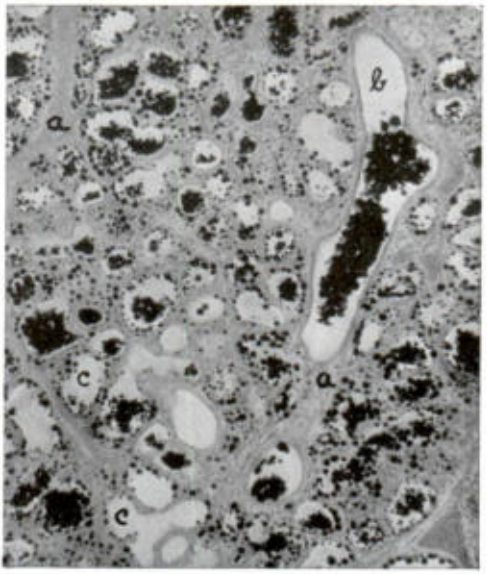

FIG. 413. Section through human mammary gland during lactation. Osmic acid preparation. × 85. Photograph. *a*, Interlobular connective tissue; *b*, duct; *c*, alveoli. The fat droplets are stained black.

The rôle of estrogen and progesterone in the development of a secretory condition of the mammary gland has been extensively investigated, experimentally. Both of these hormones are essential but the degree of response to each is subject to some species variation. In general, estrogen causes duct growth which is followed by the development of alveoli when progesterone is subsequently administered.

The influence of the anterior hypophysis on the mammary gland, aside from its indirect effect of stimulating ovarian secretion, has not been fully clarified. In those animals which do not abort following hypophysectomy during gestation (rats, mice, guinea pigs, rhesus monkeys) the mammary glands will attain a nearly

complete gestational development in the absence of the anterior hypophysis, and will briefly lactate. However, lactation is not maintained in the absence of the hypophysis. The anterior pituitary hormone *prolactin* (lactogenic hormone, luteotropin), which has been obtained in relatively pure form, is generally thought to function in the maintenance of lactation in the fully developed mammary gland. There is some evidence that other hormones, such as adrenal cortical hormones, may also be necessary for this function. Also, either a pituitary or a placental factor appears to be essential for full gestational development of the mammary glands, since a number of investigators have failed to produce this effect with administration of estrogen and progesterone in non-pregnant hypophysectomized animals.

One of the neurohypophyseal hormones, oxytocin, has a definite and pronounced effect on the lactating mammary gland, causing the contraction of the myoepithelial cells which surround the alveoli. This is the "milk ejection" or "milk letdown" effect. The secretion of oxytocin is initiated by nerve impulses reaching the hypothalamus, the most effective stimulus being suckling or similar stimulation of the nipple area. Some investigators also favor the possibility that oxytocin, reaching the anterior hypophysis by way of the blood stream, may be responsible for the release of anterior pituitary factors necessary for milk secretion.

It has not been definitely determined if structural changes occur in the human breast during the menstrual cycle. Evidence both for and against such changes has been advanced. It is difficult or impossible to secure material to determine if changes occur, not only because of the inherent variability among breasts of different individuals, but also because material must be secured either at autopsy, often after a debilitating illness, or from tissue removed surgically because of some pathological conditions. In an extensive, controlled series of rhesus monkeys (a form with menstrual cycles similar to the human) Speert has found that during the corpus luteum phase of the cycle there is lobular enlargement and dilatation of the alveoli. This supplies presumptive evidence that similar changes occur in women.

Blood Vessels. The blood supply of the mammary gland comes from several neighboring vessels—the intercostals, internal mammary and thoracic branches of the axillary arteries. These vessels subdivide and form a rich capillary plexus around the ducts and alveoli. The richness of the blood supply fluctuates with activity, being much greater in the active than in the inactive or the involuted gland. From the capillaries veins arise which accompany the arteries.

Lymphatics. The lymph vessels of mammary glands are numerous. An understanding of the course of these vessels is facilitated by keeping in mind that the gland arises from the ectoderm and grows into the underlying mesoderm and that in this underlying tissue there is, over the whole body, a plexus of lymph vessels. Thus, as the developing ducts grow deeply into the connective tissue, lymph vessels accompany them, and drain toward the surface into the subcutaneous lymph plexus. This is particularly well formed beneath the areola. From the subcutaneous plexus vessels pass to the axillary lymphatics and nodes along the pectoral muscles. There are accessory paths of drainage. Some vessels cross the mid-line, others follow the branches of the internal mammary artery and drain through the sternal nodes, and still others may drain into the abdominal lymph nodes. Because of the frequency of mammary carcinoma, the lymph drainage of this gland is of great importance.

Nerves. Both cerebrospinal and sympathetic nerves supply the gland, the larger trunks following the interlobar and interlobular connective tissue septa. The nerve terminals break up into plexuses which surround the alveoli just outside their basement membranes. From these plexuses delicate fibrils have been described passing through the basement membrane and ending between the secreting cells.

DEVELOPMENT OF THE URINARY AND REPRODUCTIVE SYSTEMS

During development three generations of urinary structures make their appearance. These in order of their succession are known as the *pronephros*, *mesonephros*, and *metanephros*. The first two are present only in the embryo in higher animals, but are important in furnishing the efferent duct system of the male reproductive organs. The metanephros, generally known as the kidney, forms the adult urinary organ in all the higher vertebrates (reptiles, birds, and mammals).

All three kidney generations arise from the intermediate cell mass or nephrotome, a mesodermal mass which connects the primitive somites with the lateral coelomic plates. In man only the cranial portion of the intermediate cell mass shows a definite segmentation corresponding to that of the primitive somites, and it is in this region that the pronephros develops. Below the tenth somite the segments of the nephrotome are so close together as to form a continuous cord of mesoderm, the nephrogenic strand, extending to the sacral region of the body. The upper longer portion of the strand (mesonephric strand) furnishes the mesonephros. The uriniferous tubules of the permanent kidneys are formed from the lower portion (metanephric strand).

The *pronephros* in man is a variable and rudimentary structure which has no urinary function whatever. In some embryos it may be entirely missing. It arises in the cranial segments of the nephrotome in the form of ridge-like condensations which may or may not acquire a lumen. The most anterior ridges or tubules are the most rudimentary and soon undergo involution. The caudal ones become somewhat longer and fuse at their lateral ends to form a duct, the pronephric duct, which grows caudally beyond the territory of the pronephros and ultimately empties into the cloacal portion of the intestine. The pronephric duct is placed lateral to the nephrotome, directly underneath the ectoderm. The greatest extent of the pronephros is seen in embryos of about 2.5 mm. while in embryos of 5 mm. involution of the tubules has definitely begun. All the tubules gradually disappear leaving only the pronephric duct.

The *mesonephros* or Wolffian body begins its development in embryos of 2.5 mm., just caudal to the pronephros. Cellular condensations appear in the mesonephric strand, which soon become vesicular by developing lumina. The vesicles elongate and are transformed into S-shaped tubules which then connect at one end with the pronephric duct. The latter is now called the mesonephric or Wolffian duct. The distal end of each tubule becomes invaginated to form a two-layered capsule which encloses a tuft of blood vessels, the glomerulus, derived from a branch of the aorta. The capsule together with the enclosed glomerulus constitute a Malpighian corpuscle.

The mesonephric tubules develop progressively from before backward and finally form a series extending from the cervical to the pelvic region of the embryo. By increase in number and length of the tubules each mesonephros comes to form a large structure projecting into the dorsal part of the body cavity. The greatest extent is reached during the fifth or sixth week.

From the sixth week on the mesonephros gradually atrophies, leaving finally only certain parts which differ in the two sexes. In the male 8–15 tubules in the cephalic portion persist as the ductuli efferentes, while a few in the caudal portion remain as the paradidymis and vasa aberrantia. The mesonephric duct is transformed into the ductus epididymidis, ductus deferens, and ejaculatory duct. In the female the mesonephric tubules disappear for the most part, only a few remaining to form the epoöphoron and paroöphoron, while the duct persists in part as Gärtner's canal.

Each *metanephros* or kidney begins in embryos of about 5 mm. as a hollow bud from the dorsal side of the mesonephric duct near its opening into the cloaca. This bud, the anlage of the ureter, grows dorsally and cranially into the metanephric blastema where it ends in a terminal dilation or ampulla, the primitive pelvis. The pelvis elongates in a craniocaudal direction and forms four to six branches which likewise terminate in ampullae. These branches are the primordia of the primary calyces. Each ampulla then divides into two to four secondary ampullae, and this process is repeated again and again until the whole system of collecting tubules are formed.

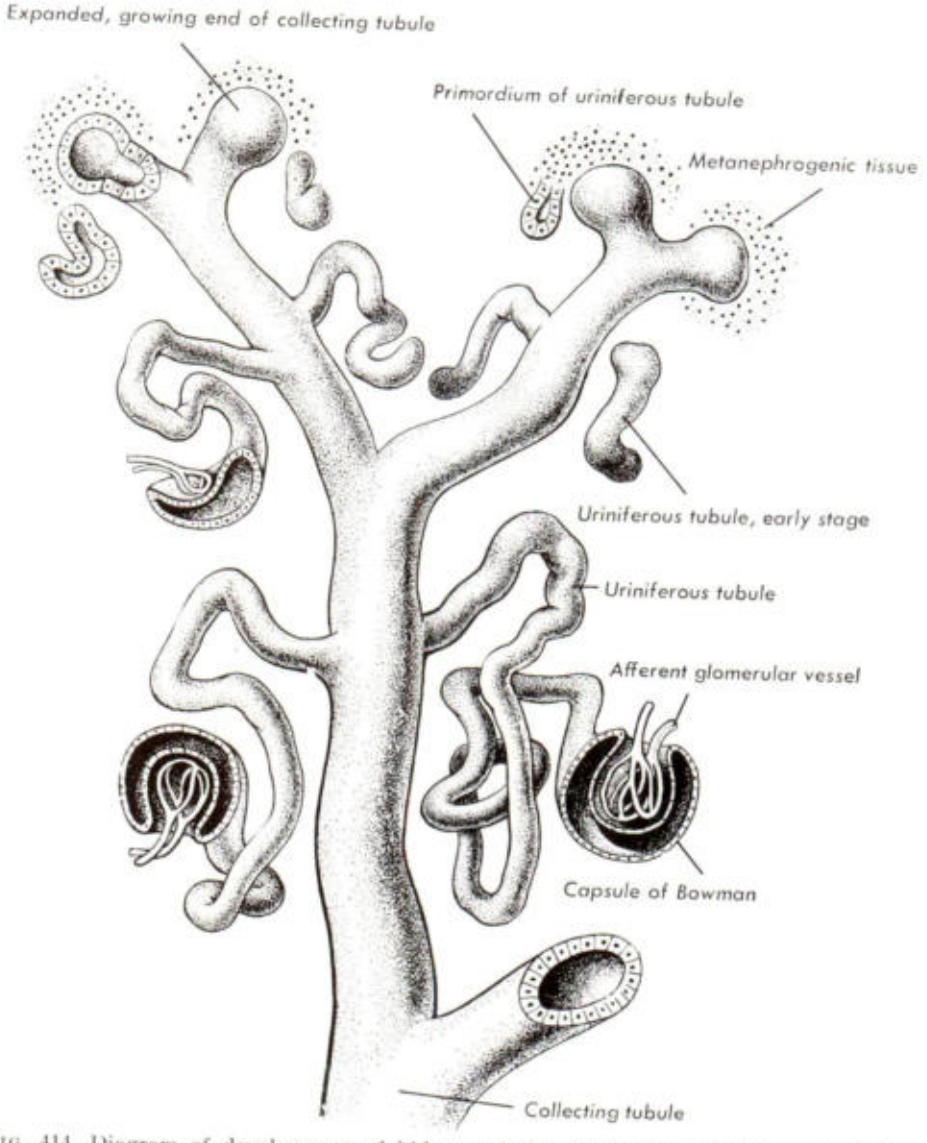

FIG. 414. Diagram of development of kidney tubules. Early differentiation of uriniferous tubules from metanephrogenic tissue is shown in the upper part of figure; later stages in the lower portion of the diagram. (Redrawn and modified from Corning.)

The nephrons, or uriniferous tubules proper, have an independent origin from the metanephric tissue which forms cap-like condensations around the growing ampullae of the collecting tubules (Fig. 414). Portions of the condensations acquire a lumen and detach themselves from the nephrogenic cap. Each vesicle elongates into an S-shaped tubule which secondarily establishes a communication with the collecting tubule. The place of junction becomes the arched or junctional collecting tubule. By further growth and histological differentiation, the S-shaped tubule gives rise to the convoluted tubules and loop of Henle, while the enlarged blind end becomes invaginated as Bowman's capsule to enclose a glomerulus (Fig. 414). Thus the two types of tubules found in the adult kidney have sep-

arate origins. The nephrons are derived from the metanephric blastema (metanephric strand). The ureter, pelvis, calyces, and all the collecting tubules are formed from the ureteric bud, an outgrowth from the mesonephric duct.

The *gonads* or sex glands make their first appearance on the mesial surface of the mesonephros as ridge-like thickenings of the celomic epithelium, the *genital ridges*, which at first extend from the mid-thoracic to the sacral levels. As development proceeds, the anterior portion retrogresses and the gonads become restricted to the lumbar region. The epithelial cells of the ridge proliferate and form a many-layered band composed of two types of cells—a large number of small cuboidal cells which stain rather intensely, and scattered between them larger spherical cells with vesicular nuclei and clearer cytoplasm, the primitive sex cells. The whole epithelial band is known as the *germinal epithelium* and is separated from the mesonephric tubules by a layer of embryonal connective tissue.

As the germinal epithelium continues to proliferate, irregular plugs or strands of epithelial cells, the *medullary* or *sex cords*, extend into the underlying connective tissue. These are likewise composed of the two types of cells. The deepest portions of the cords which lie closest to the mesonephric tubules, anastomose with one another to form the anlage of the *rete*.

Up to about the sixth week, development proceeds similarly in both sexes. This is the so-called "indifferent" period. Though sex is determined at a much earlier time by the constitution of the fertilized egg, histological differences between the male and female can not be observed during this period.

From the sixth week on, histological changes occur which lead to a definite differentiation of the gonads. In the *testis* a layer of embryonal connective tissue, the future *tunica albuginea*, grows in between the medullary cords and the germinal epithelium, and the latter becomes gradually reduced to a single layer of flat cells, the visceral layer of the tunica vaginalis. The medullary cords become more distinct, and elongate to form the convoluted *seminiferous tubules*. The deeper anastomosing portions of the cords unite with a number of mesonephric tubules to form the *rete testis* of the adult. As already stated the mesonephric tubules with which such union is established become the *ductuli efferentes*, while the mesonephric duct is transformed into the *ductus epididymidis* and *ductus deferens*. The larger sex cells of the cord either become smaller temporarily or according to some, entirely disappear, and the seminiferous tubules consist for a time of only the smaller cuboidal cells. From these are differentiated both the spermatogenic cells and the cells of Sertoli.

Differentiation of the *ovary* begins at a later period and differs considerably from that of the male. The medullary cords and rete formed during the indifferent period gradually disappear, though vestiges of the rete may remain in the stroma of the adult ovary. On the other hand, the germinal epithelium continues to proliferate so as to form a many-layered epithelial mass. The underlying connective tissue grows into this mass and subdivides it into a number of spherical or irregularly shaped cell clusters. At first the clusters consist of several larger egg cells scattered in a mass of small (follicular) cells. Later each cluster is broken up by invading connective tissue into several primary follicles, each containing a single egg cell surrounded by a layer of flattened follicular cells.

This process of epithelial proliferation with subsequent formation of cell clusters and primary follicles, continues to the end of fetal development. Near birth or soon after, the invading connective tissue reaches the surface layer, and as the albuginea separates the latter from the cortical layer containing the follicles. Further proliferation ceases, the germinal epithelium persisting as a single layer of cuboidal or columnar cells.

The primordia of the female genital ducts are the *Müllerian ducts* of the embryo. These begin in both sexes as celomic invaginations into the cranial portions of the mesonephric ridge, which then grow backward, running parallel and close to the mesonephric duct. Caudally the two Müllerian ducts approach each other and fuse to form a terminal unpaired tube which ends in the urogenital sinus (cloaca) between the openings of the mesonephric ducts. In the male the Müllerian duct degenerates leaving as vestiges the *appendix testis* and the *colliculus seminalis* (utriculus prostaticus, uterus masculinus). In the female the paired upper portions become the *Fallopian tubes* or *oviducts*, while the unpaired terminal portion forms the *uterus* and *vagina*.

Development of Mammary Glands. In animals with numerous glands, the beginnings of the mammary glands are represented by two ridges of thickened epithelium, the mammary lines, which extend from the axillary to the inguinal regions. At various points of these ridges epithelial proliferations form the prim-

ordia of future glands while the intermediate portions of the milk lines ultimately disappear. In man the milk line is poorly defined and of brief duration, and normally only one pair of glands develop. Each gland appears in the second month as a broad epidermal thickening in the region of the future nipple, produced by a proliferation and downgrowth of the germinative layer. The thickening spreads laterally to form a hemispherical mass whose convex surface is directed towards the derma. Externally the circular patch of skin, or mammary area, corresponding to the thickening sinks below the surface as the mammary pit.

Around the fifth month a varying number of secondary sprouts, the future lactiferous ducts and sinuses, grow down into the derma and there branch repeatedly, the branches ending in terminal swellings. At first the sprouts are solid but from the seventh month on lumina appear in various places and finally become confluent. This process of branching and canalization continues until birth, the histological picture being that of a prepubertal gland. The formation of the glandular alveoli does not take place until adolescence.

Soon after birth the shallow mammary pit is raised up above the surface by the proliferation of connective tissue. The central portion develops into the nipple which contains the openings of the lactiferous ducts. The remainder of the mammary area forms the areola distinguished from the surrounding skin by its hairlessness, pigmentation and thinness of epidermis.

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XXI

THE ENDOCRINE GLANDS

The endocrine glands, or glands of internal secretion, include a diverse group of tissues and organs widely scattered in the body. The criteria by which it is determined whether or not a certain tissue belongs to the endocrine system are largely physiological. The members of the group do, however, have certain anatomical features in common. They have no ducts; their secretions pass directly into the blood or lymph circulation. This feature led, early, to their designation as ductless glands. They almost invariably have a rich blood supply. Thus, in a blood-vascular injection of the pancreas, the endocrine portions, the islands of Langerhans, are clearly distinguishable from the adjacent exocrine tissue because of the great number of capillaries which they contain. The parenchyma of the endocrine organs is usually, although not invariably, formed of cells of epithelial or epithelioid character. In other anatomical features, the various endocrine glands differ widely from each other. Also, their embryological derivation differs greatly; they are derived from all three germ layers in the embryo.

Physiologically, the endocrine glands have certain similarities. Each endocrine secretes one or more specific substances, called hormones; each hormone has a specific effect upon a particular tissue or organ, or on the body as a whole. Since the hormones are secreted into the blood or lymph, they all reach all parts of the body. Only a limited part of the organism, however, may respond, as for example, a particular organ. The responsive structure, designated as the "target" organ or tissue, selectively utilizes the circulating hormone. This is in contrast to the transmission of nerve impulses which cause a response in a limited part of the organism because they are carried over definite discrete pathways to a limited area.

In some cases, it can readily be determined whether or not an organ has an endocrine function. Experimental ablation of the thyroid gland, for example, produces certain effects which are reversed by thyroid administration. Such is also the case, as can be shown both experimentally and clinically, with the anterior hypophysis, neurohypophysis, adrenal cortex, the parathyroids, the testes and ovaries (their endocrine portions only), and the islets of the pancreas. The specific hormones of some of the glands have been chemically isolated, analyzed and synthesized; the secretions of others have been extracted in a more or less highly purified state.

Whereas severe disabilities or even death may ensue following ablation of certain endocrine glands, this is not true of all endocrines. For example, removal of the adrenal medulla, whose hormone was the first one to be chemically analyzed and synthesized, results in no severe or even appreciable disabilities.

The general concept of internal secre-

tion has undergone considerable modification since the term was first introduced by Claude Bernard as a result of his work upon the liver. He spoke of glucose, which is liberated into the blood stream by this organ, as its internal secretion, in contrast to its external secretion, bile, which is carried from the liver by way of ducts. Since glucose has only a general nutritive function, it is not now included among the internal secretions.

Hormone, the term applied to the specific product of an endocrine gland, is also now used in a more limited sense than it was at one time. By derivation it means a "chemical excitant." Used in this sense, carbon dioxide would be a hormone since it is an excitant of the respiratory center. However, since carbon dioxide is a general product of tissue metabolism, it would not, according to current usage, be spoken of as a true internal secretion or hormone.

There are some tissues which have an endocrine function but which are not organized into definite glandular organs. The mucosa of the gastro-intestinal tract, for instance, secretes hormones which have effects on other structures, such as the exocrine portion of the pancreas, the muscle of the gall bladder, etc.

Although the follicles and corpora lutea of the ovary, the fetal placenta, the interstitial tissue of the testis and the islets of the pancreas elaborate hormones, it has seemed wise to include a description of them under the organs to which they belong.

THE HYPOPHYSIS CEREBRI

The *hypophysis* (pituitary gland) structurally and functionally is the most complex of the endocrine glands. It is derived in part from oral ectoderm, and in part from the brain with which it maintains its connections in the adult (Fig. 415). The major part of the gland (anterior and posterior lobes) lies, in most forms, in a bony fossa, the sella turcica. This part is ensheathed by the dura, an extension of which, the *diaphragma sellae*, roofs over the sella turcica. There is a small aperture in the diaphragma through which the pituitary stalk passes. The suprasellar portion of the gland includes the pituitary stalk and that portion of the hypothalamus known as the median eminence of the tuber cinereum. The

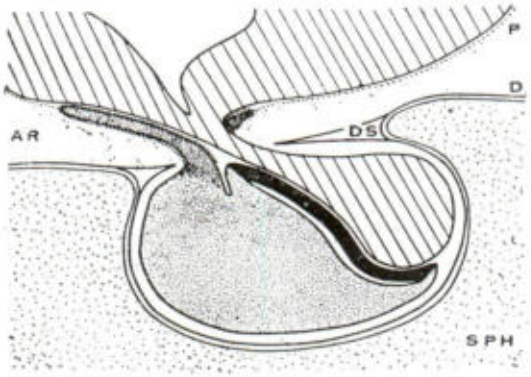

FIG. 415. Diagrammatic sagittal section of the hypophysis. Fine stipple, pars anterior; coarse stipple, pars tuberalis; solid black, pars intermedia; lined, neurohypophysis including the infundibular stem and median eminence; *ar*, arachnoid spaces; *d*, dura; *ds*, diaphragma sellae; *p*, pia mater; *sph*, sphenoid bone. (Atwell.)

TABLE 3

COMPONENTS OF HYPOPHYSIS AND THEIR DERIVATION

Derivation	Divisions	Components	Lobes of Pituitary Gland within Sella Turcica
Oral ectoderm	Adenohypophysis	Pars tuberalis	
		Pars anterior—Pars distalis	Anterior lobe
		Pars intermedia	Posterior lobe
Neural ectoderm	Neurohypophysis	Pars nervosa—Infundibular process (neural lobe)	Posterior lobe
		Infundibulum (neural stalk): infundibular stem	Posterior lobe
		Infundibulum (neural stalk): median eminence of tuber cinereum	Posterior lobe

infundibular stem forms the bulk of the pituitary stalk. The smaller component, the pars tuberalis, surrounds the infundibular stem and flares out onto the median eminence.

The table gives the various parts of the gland, their embryological derivation and the more common synonyms.

The pars nervosa and pars intermedia are intimately fused thus forming a single main division, the posterior lobe. Except at the periphery, this lobe is separated, in most species, from the anterior lobe by a cleft-like cavity, the vestigial lumen. In man, only remnants of this cleft are present. In the cetacea (whales, porpoise), in birds and in the armadillo, a pars intermedia is not present and there is no vestigial lumen. In them the neural lobe is encapsulated by the meninges and consequently is entirely separated from the anterior lobe.

In man the transverse dimension of the gland is its greatest, 1.2–1.5 cm. Its width is about 1 cm. and its depth about 0.5 cm. Its weight varies considerably but correlates better with stature than with body weight. In the human the weight of the anterior lobe increases with pregnancy and decreases slightly in old age. The average weight of the hypophysis in the male is about 0.6 gm. In multipara it may weigh more than a gram.

Pars Anterior. About seventy-five per cent of the hypophysis is anterior lobe. The parenchyma of this lobe is formed of anastomosing cords of cells between which are large capillaries (sinusoids). The number of different cell types making up the parenchyma of the anterior hypophysis has not been established. In routine preparations, three types of cells are readily distinguished. These are the chromophobes, acidophiles, and basophiles. The last two are sometimes collectively spoken of as chromophiles. By means of special staining techniques and histochemical methods, additional cell types within the acidophile and basophile groups may be demonstrated. As many as six different kinds of cells have been described in some species. Small masses of colloid material are occasionally seen within the cell cords of the anterior lobe.

The *chromophobes* (reserve or chief cells) are prone to appear in groups near the centers of the cords. When thus grouped, they were earlier described as nuclear

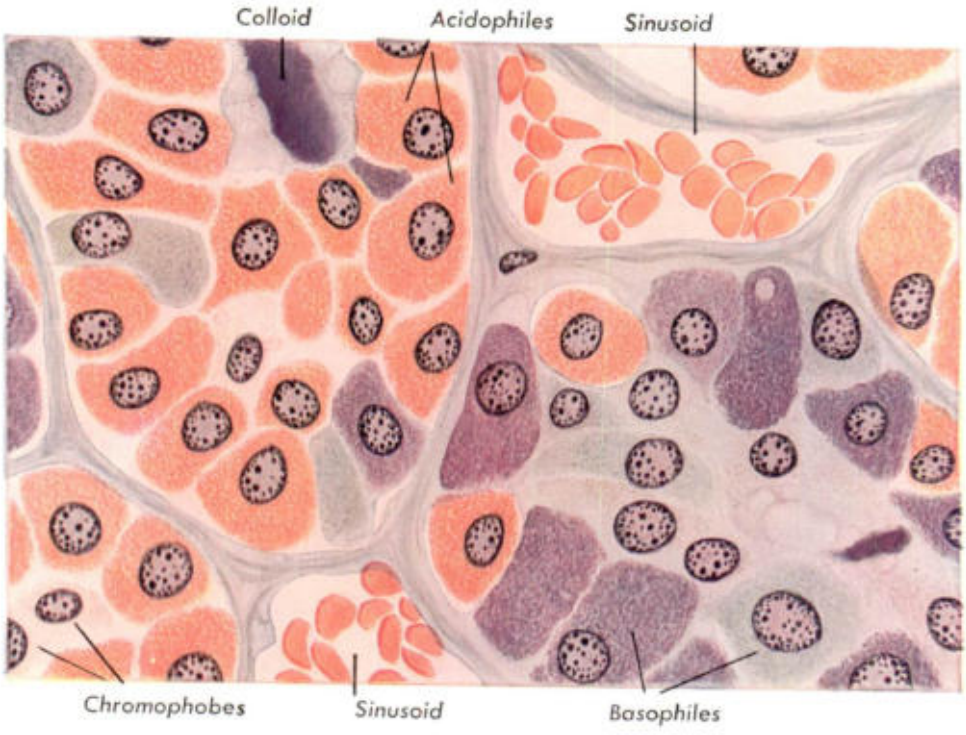

FIG. 416. Human anterior hypophysis. The section shows parts of several cords of cells containing acidophiles (orange), two types of basophiles (purple and green) and chromophobes. Fine connective tissue and sinusoids separate the cords. Masses of colloid are seen in the centers of two of the cords. A 4 μ section stained with aldehyde fuchsin-fast green-orange G. Woman, age 26. Camera lucida drawing. × 1200.

heaps (Kernhaufen). Their nuclei are surrounded by a small amount of diffuse, light-staining cytoplasm. The cell boundaries are not distinguishable in ordinary preparations. Secretory granules are not present or are but few in number.

The *acidophiles* are readily stained and in ordinary preparations are the most evident cell type (Figs. 416, 427). Their cytoplasm contains specific secretion granules. The cell membranes are distinct. The granules take the acid dyes but also are stainable with certain basic stains (safranin). Due to this fact some investigators have designated them as alpha cells. The acidophilic granules are spherical and uniform in size. They are resistant to alkalies and acids.

A modified type of acidophile, the *pregnancy cell*, accumulates in large numbers during pregnancy in some species, including the human. Typical chromophiles remain but many of the chromophobes enlarge and become filled with small, light-staining acidophilic granules. These cells become very numerous in late pregnancy causing an increase in the size of the anterior lobe. Following parturition a partial involution takes place. With each subsequent pregnancy there is again a development of pregnancy cells followed by an incomplete involution so that the anterior hypophyses of multiparae may reach weights of more than a gram.

The *basophiles* show considerable variation in their staining properties, both within an individual gland, and from one species to another. In the human hypophysis they are not difficult to stain with hematoxylin; in mice they are stained with great difficulty. The basophiles have definite cell membranes; their cytoplasm contains specific secretory granules which are not uniform in size or shape.

Two types of basophiles, designated as *beta* and *delta* basophiles, can often be distinguished according to their shape, their special staining properties, their distribution within the gland, or changes in the cells with different physiological states. In some forms, such as the rat, there is evidence associating each of the two types with the secretion of specific hormones. However, since there are pronounced species differences, it is not wise to generalize on the basis of experimental work in a single species. In the rat, in which the cytology of the anterior hypophysis has been studied most extensively, the beta basophiles are usually polyhedral or angular in shape, and have granules which are stained purple by the aldehyde fuchsin method of Gomori; they show pronounced changes following thyroidectomy or thyroid hormone administration. The delta basophiles of the rat are more rounded cells, with granules which do not stain with aldehyde fuchsin; they reflect changes in gonadal function, such as estrogen administration or castration.

Although it is tempting to try to separate the basophiles and name them according to their presumed function, the evidence at present does not justify such an attempt. Until more definite criteria can be used to distinguish different types of basophiles in species other than the rat, it seems preferable to retain the term *basophile* for purposes of general discussion.

Both the basophiles and the acidophiles contain a prominent Golgi apparatus which can often be seen as a clear area or ring near the nucleus even in routine preparations (Fig. 427). Mitochondria are also numerous. Since with certain technical procedures they stain similar to the granules of the acidophiles, they probably have been mistaken in basophiles for acidophilic secretory granules by certain investigators. This has led to the descrip-

tion of both acidophilic and basophilic granules in the same cell.

The concentration of granules in individual chromophile cells varies, with the result that some cells of each type stain more intensely than do others. The varying number of granules is correlated with the physiological state of the cell when fixed. As a cell liberates its secretion, it loses granules (degranulation), although in the earlier stages of this process the size of the cell body does not decrease. Such cells consequently stain less intensely than those which are in the storage phase.

The proportion of each of the cell types present in the cell cords varies greatly, not only in different regions of the anterior lobe but even in adjacent cords. In the human hypophysis, the basophiles are most numerous in the region of the midsagittal plane and anterolateral margin of the gland. The acidophiles are most numerous in the central and posterior part of each lateral half of the gland. A survey of much of the gland is necessary in order to determine the percentage of the various cell types.

The *lineage* of the cells of the anterior lobe is important in the interpretation of experimental work and tumor formation. Since mitoses are rare it is certain that few if any of the cells are destroyed when their secretion is liberated; thus the cells must pass through secretory cycles. It also seems certain that the chromophobes represent the non-secretory stage of the cycle. They form true reserve cells. As they pass into an active stage, granules begin to form in them. These granules are specific for the different cell types, acidophilic in some cells, basophilic in others. When a cell becomes engorged with secretory granules, it takes on the characteristics of a typical acidophile or basophile. The secretion is then liberated and the cell returns to the chromophobe type.

Investigations on the appearance of the Golgi apparatus in cells of the rat hypophysis have provided strong support for such a secretory cycle. In this species the Golgi bodies of the acidophiles and of the basophiles differ from each other in their morphology and position, and these two types are present in the chromophobes (Severinghaus). This has made it possible to differentiate between the chromophobes which will develop into each type of chromophile cell and to detect the various stages of granule formation and granule discharge. It seems certain, in this form, that the basophiles and the acidophiles have separate lineages and that neither transforms into the other type of cell. In embryonic development, however, these two types undoubtedly differentiate from the same cell type.

In other species studied, including man, there are not constant differences in the Golgi apparatus of the acidophiles and basophiles. Nevertheless, there is presumptive evidence for secretory cycles similar to those in the rat. Detailed cytological studies, including those with the electron microscope, have shown progressive stages in granule formation and depletion in the different cell types.

Pars Intermedia. In most of the mammals the pars intermedia forms a well-developed epithelial stratum lining the vestigial lumen dorsally. It is formed of several layers of polygonal cells which take the basic dyes and in which secretory granules have been described. In man and the apes the pars intermedia becomes reduced in amount and is rudimentary. It is composed of a thin stratum of cells and small vesicles which often contain colloid. Cellular prolongations from it extend into the neural lobe for variable distances (Fig. 417). Some of these become filled with colloid, or show cyst formation. Some of the cells of these epithelial outgrowths are small and pale staining;

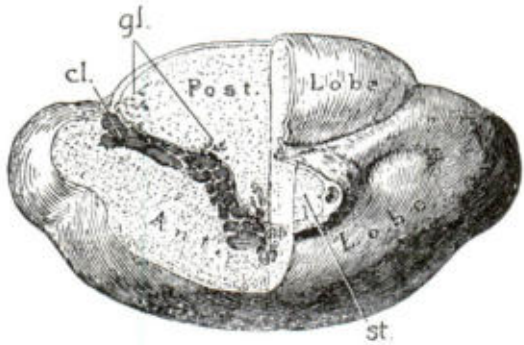

FIG. 417. Hypophysis of a one-year-old child viewed from above. The gland was reconstructed from serial sections. *cl*, cleft (vestigial lumen) with septa; *gl*, glandular ingrowths of pars intermedia into the neural lobe; *st*, stalk of pituitary gland. (Lewis and Lee.)

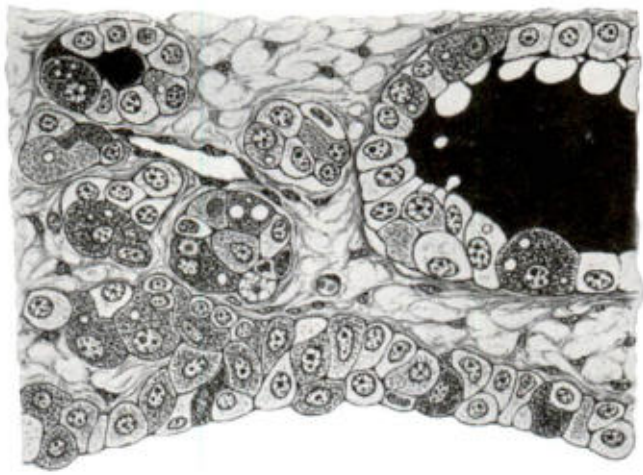

FIG. 418. Pars intermedia of adult human hypophysis showing the growth of cords of cells up into the neural lobe. The formation of vesicles with colloid (black) is shown in two of the ingrowths. The basophilic cells are dark. (Rasmussen.)

others stain deeply with the basic dyes (Fig. 418); mucous and ciliated cells are not uncommon.

Colloid is also frequently found in the vestigial lumen in certain species. In the ox it occurs either as a viscous fluid or a firm mass. The masses sometimes reach the size of a large pea. Tests have not revealed any physiological activity of the colloid. It contains iodine in very small amounts only. It thus differs from the colloid of the thyroid gland.

Neurohypophysis. The neural lobe of the hypophysis, or infundibular process, is, as indicated above, a downgrowth from the hypothalamic region of the brain. Both anatomically and functionally, it forms a part of a larger unit, the *neurohypophysis*. The neurohypophysis includes the infundibular process (neural lobe) and the infundibulum, which in turn includes the infundibular stem and the median eminence of the tuber cinereum. These regions are similar in that

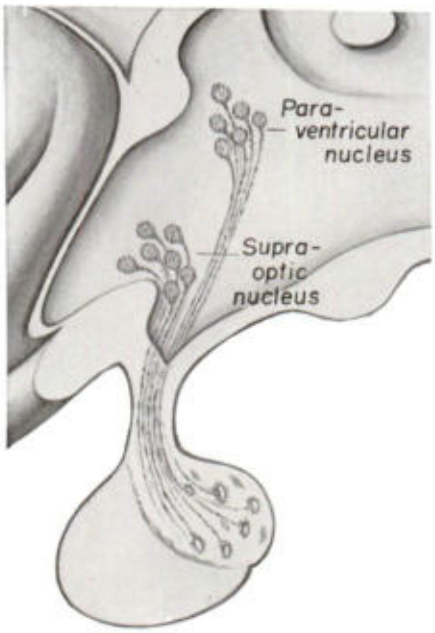

FIG. 419. Diagram of the hypothalamico-hypophyseal tract in the human. Nerve fibers from the supraoptic and paraventricular nuclei of the hypothalamus carry the neurosecretion to the blood vessels of the pars nervosa by way of the infundibular stem.

they possess the same type of cell, the same nerve and blood supply, and yield the same active substances upon extraction.

The cells of the neural lobe were named *pituicytes* by Bucy. In some respects, the pituicytes resemble the neuroglia cells found elsewhere in the central nervous system; i.e., they are small cells with ramifying processes but without the distinctive features of nerve cells. Unlike neuroglia cells, many of the pituicytes contain variable numbers of refractile droplets or granules in their cytoplasm. The granules are composed of neutral, unsaturated fat, and although they are not seen in routine histological preparations, they are easily stained with lipid dyes, including osmic acid. Some of the pituicytes also contain yellow-brown pigment granules, the number of which increases with age.

The nuclei of the pituicytes (Fig. 420) are round or oval with a fine chromatin network. The cytoplasm is drawn out into a variable number of processes which often end either on the walls of blood vessels or on connective tissue septa of the gland. In routine preparations, the cytoplasm surrounding the nucleus is barely discernible, and the processes cannot be followed at all. Between the cell bodies, the meshwork of interweaving processes stains faintly. The pituicytes may be blackened by special silver techniques, and their form thus determined. Romeis, who has made a thorough study of the cytology of the human neural lobe, distinguishes four types of pituicytes according to their morphological characteristics in silvered preparations. He has called them reticulopituicytes, micropituicytes, fibropituicytes and adenopituicytes.

Although the number of osmiophilic granules present in the pituicytes is closely correlated with the secretory state of the neurohypophysis, it is not accepted that the pituicytes are responsible for the elaboration of the secretory products. Certain nerve cells, those of the supraoptic and paraventricular nuclei located in the hypothalamus of the brain, are actually neurosecretory in nature. The active substances of the neurohypophysis appear to be elaborated in these nerve cells and to pass by way of their amyelinated nerve fibers to the neural lobe where the secretion is stored. After sectioning of the pituitary stalk in experimental animals, the stainable neurosecretory material is seen to accumulate in large masses in relation to the severed nerve fibers proximal to the cut.

The neurosecretory material, both in the nerve cells of the supraoptic and paraventricular nuclei, and in the nerve fibers

FIG. 420. Posterior hypophysis. A section from the pars nervosa, human, age 26. Nerve fibers containing neurosecretory material (deep blue) are cut in both cross and longitudinal sections. One of the longitudinal sections shows three bulging masses of neurosecretory material; it appears to terminate on the surface of a small blood vessel (lower center of field). Larger accumulations of neurosecretion (Herring bodies) appear at right. Nuclei of pituicytes are stained red. A 4 μ section, stained with chrome hematoxylin and phloxine. $\times$ 1200.

FIG. 421. Photomicrograph of a neuron from the human supraoptic nucleus. Both the nerve cell body and its axon contain granular neurosecretory material. Chrome hematoxylin stain. × 1200. (Courtesy of Dr. S. L. Palay.)

of the hypothalamico-hypophyseal tract, is stained by a number of methods, among them chrome hematoxylin (Figs. 420, 421). This stainable material of the neurohypophysis is believed to be a protein which is associated with the actual hormones, perhaps as a carrier. The amount of stainable material corresponds to the amount of hormone which can be extracted from the tissue, although the purified hormones themselves are not stained.

Most of the fibers of the hypothalamico-hypophyseal tract, which forms a major component of the infundibulum, terminate in various regions of the neural lobe, a few going to the pars tuberalis and the pars intermedia. They terminate in close association with the capillaries, often in a palisade arrangement along the wall of the blood vessel (Fig. 422). The terminal axons, as seen in electron micrographs, contain vesicles which are typical of nerve endings in general; in certain physiological states these vesicles may be filled with neurosecretory material (Fig. 423). The endothelium of the capillaries of the neurohypophysis is seen in electron micrographs to be fenestrated (Palay). This presumably allows for the free passage of the secretion into the lumen of the capillaries.

In the normal posterior lobe, there may frequently be found masses of a rather homogeneous material which stains the same as the neurosecretory material. These are known as Herring bodies (Fig. 420), and are interpreted as accumulations of the stored secretory material surrounding the nerve fiber terminals or as expanded portions of nerve fibers showing increased concentrations of the secretory material.

Pars Tuberalis. The pars tuberalis is a layer of cuboidal cells which covers the neural stalk and tuberal area of the brain. The cytoplasm of the cells is faintly basophilic, but secretory granules have not been described. In contrast to the pars intermedia, the pars tuberalis is quite vascular. The cells frequently form vesicles which contain colloid. In the pars tuberalis, especially at its upper and lower poles, groups of squamous cells have been described which probably are "rests" from the craniopharyngeal duct.

Pharyngeal Hypophysis. There occurs constantly in the vault of the nasopharynx, in man, a body of typical anterior lobe tissue. It has not been demonstrated in other species. This body lies in the mid-line and is elongated, its longitudinal axis being parallel to the sagittal plane of the body. It measures from 3.5 to 7 mm. in length by 1 mm. or less in diameter. The cells of the pharyngeal

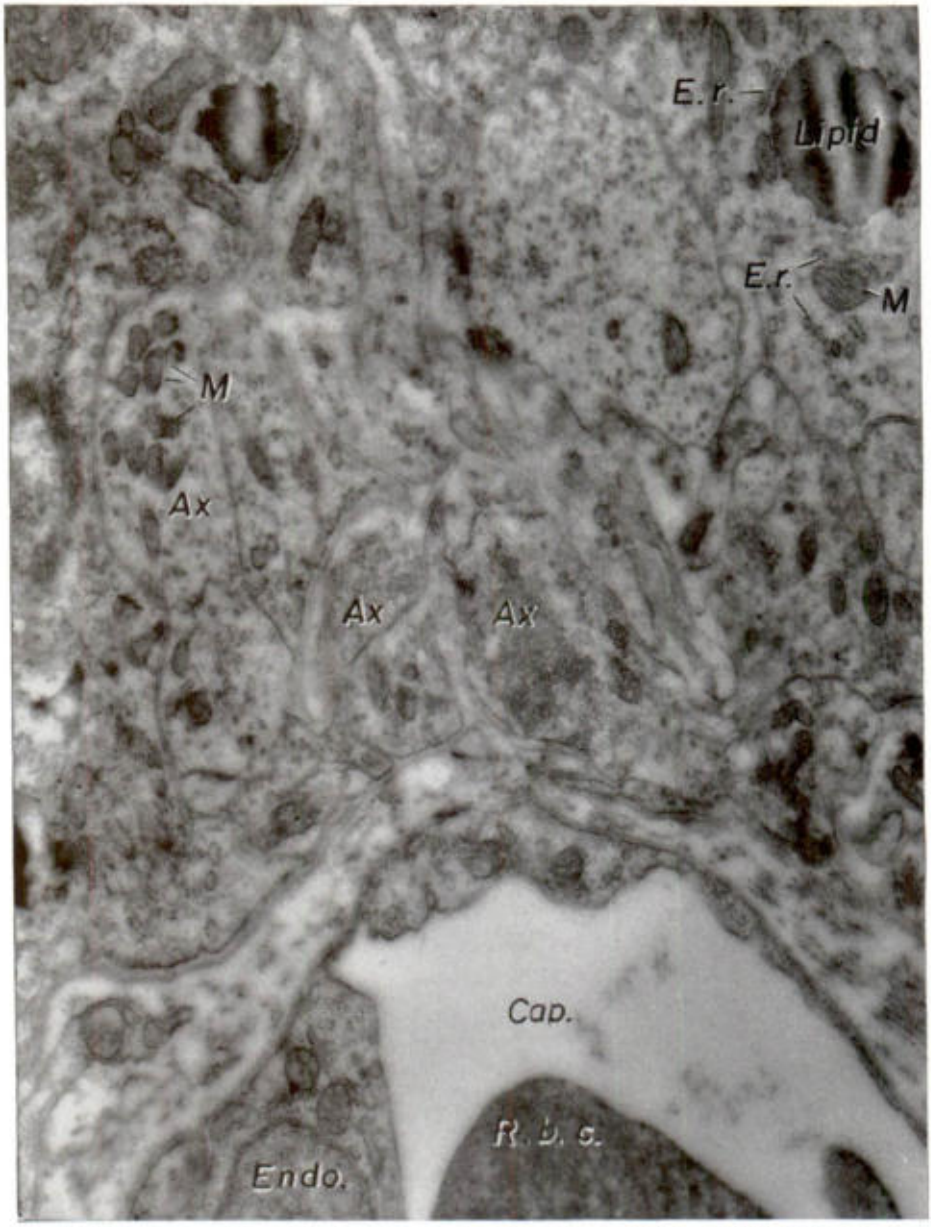

FIG. 422. Electron micrograph showing perivascular "palisade" in neurohypophysis of dehydrated rat. Nerve endings (terminal axons of hypothalamico-hypophyseal fibers) arrayed around capillary contain mitochondria and characteristic small vesicles. Neurosecretory material is lacking in the endings because of the dehydration of the animal. Pituicytes contain lipid droplets and endoplasmic reticulum. Separating the terminal axons from the capillary endothelium is a thin connective tissue space, and on either side of this is a thin basement membrane. *Ax.*, terminal axon; *M.*, mitochondria; *E.r.*, endoplasmic reticulum; *Cap.*, capillary; *Endo.*, endothelial cell nucleus. × 23,900. (Courtesy of Dr. S. L. Palay.)

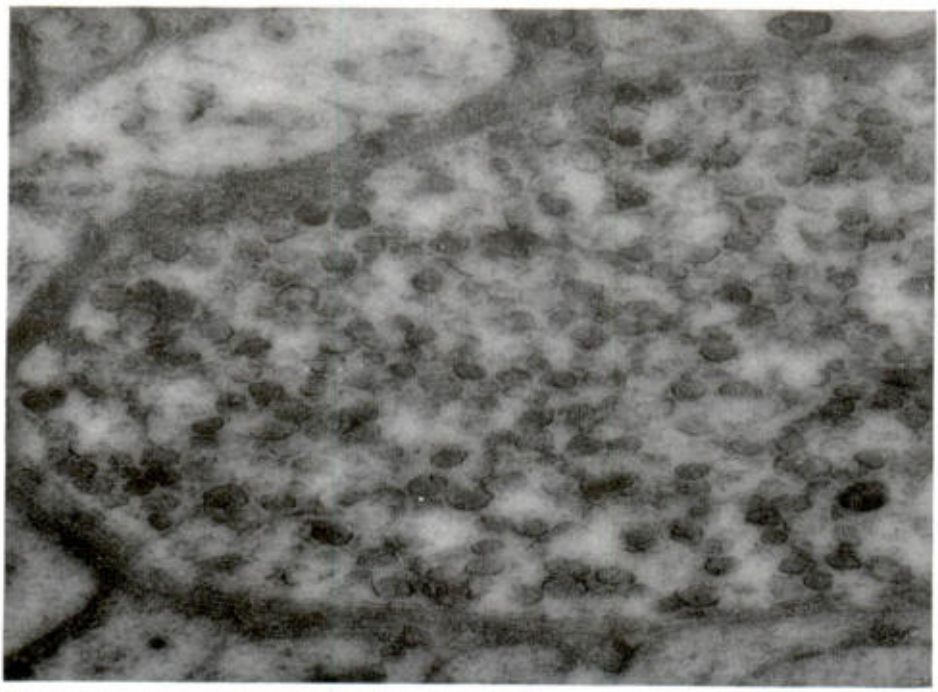

FIG. 423. Electron micrograph showing a portion of a nerve ending in the neurohypophysis of a hydrated rat. The terminal axon is filled with vesicles which contain a dense material, the neurosecretory substance. × 37,160. (Courtesy of Dr. S. L. Palay.)

hypophysis are structurally identical with those of the anterior lobe and parallel them in their differentiation and development but the experimental implantation of the pharyngeal hypophysis into animals has not revealed that it contains pituitary hormones.

BLOOD AND NERVE SUPPLY

The blood circulation of the hypophysis has been carefully studied by a number of investigators. Each of the two major lobes has its own independent blood supply. The anterior lobe is supplied by the superior hypophyseal arteries which arise from the internal carotids and circle of Willis. Some arterial twigs pass directly to the anterior lobe, there emptying into the sinusoids of the pars distalis. Other branches give rise to a capillary network in the infundibulum, i.e., the median eminence and infundibular stem; venous trunks draining from these capillaries traverse the stalk, largely in the pars tuberalis, to supply, with the arterioles mentioned above, the sinusoids of the anterior lobe. This system of vessels forms the *hypophyseal portal system*, arising in capillaries of the infundibulum, and ending in sinusoids of the pars distalis. Thus there is established a humoral connection by which hormonal substances may pass from the neurohypophysis to the anterior lobe. This is the only accepted connection between the brain and the pars distalis, and it forms an important functional pathway in the regulation of anterior lobe function.

The pars nervosa (infundibular process) receives its blood from the inferior hypophyseal arteries. Veins from both the anterior and posterior lobes drain into the cavernous sinuses.

The anterior lobe of the hypophysis is

richly supplied with sinusoids. The pars intermedia is avascular, being similar in this respect to other epithelia. The neural lobe is quite vascular, but its capillaries are much smaller than the sinusoids of the anterior hypophysis. Both the capillaries of the neurohypophysis and the sinusoids of the anterior lobe have been shown by electron microscopy to have a fenestrated type of endothelium.

Nerves. An innervation of the anterior hypophysis has not been demonstrated. The neurohypophysis is supplied by nonmyelinated nerve fibers which arise in the supraoptic and paraventricular nuclei. Reports that these fibers extend into the anterior lobe also, have not been substantiated. Nevertheless, many different lines of evidence point to the conclusion that there is some nervous control over anterior lobe function.

Section of the pituitary stalk in rabbits prevents ovulation after mating. In this form, as in the cat and ferret, ovulation occurs only after copulation. The inhibition of ovulation after stalk section is attributed to the interruption of the *neurohumoral pathway* from the hypothalamus to the anterior lobe. Coitus in the rabbit normally causes the release of certain anterior lobe hormones, the stimulus for this release being a substance which is produced by the brain and is transmitted to the anterior lobe by way of the hypophyseal portal circulation.

Regulatory controls by the brain over other functions of the anterior hypophysis by such a neurohumoral pathway have been postulated. However, neither the exact mechanisms nor the extent of these controlling influences have been determined.

Functions of the Hypophysis

The Anterior Hypophysis. The anterior hypophysis is complex functionally as well as structurally. It secretes a number of hormones to most of which the suffix *trophic* (*tropic*) is applied.

Although the two suffixes are often used interchangeably, their meanings are not the same. Trophic (to nourish) implies the nourishment of the target organ by the anterior pituitary. Tropic (turn towards) is more general, meaning "aimed at", and in the light of present knowledge is probably more applicable in most cases.

The multiplicity of the functions of the anterior hypophysis is shown by the disabilities which result from its surgical removal or destruction by disease.

Following hypophysectomy there is a cessation of general body growth, involution of the gonads (the testes or ovaries), the thyroid, and the cortex of the adrenal glands. Numerous secondary effects also result. For instance, there is involution of the accessory reproductive organs due to the inactivation of the gonads and the failure of their endocrine function. Due to the involution of the thyroid there is a lowering of the basal metabolic rate. The involution of the adrenal cortex results in a lowering of resistance to stress and a disturbance in carbohydrate metabolism. The anterior hypophysis is well named the master gland of the endocrine system.

The following hormones have been prepared from the anterior hypophysis in quite pure form as judged by their physiological activity and by their molecular homogeneity as shown by electrophoresis and ultracentrifugation: Adrenocorticotrophic hormone, ACTH; Thyrotrophic or Thyroid stimulating hormone, TSH; Follicle stimulating hormone, FSH (gonadotrophic); Luteinizing or Interstitial cell stimulating hormone, LH or ICSH (gonadotrophic); Prolactin or Luteotrophin; Growth hormone or Somatotrophin. Since these hormones are proteins, their structure has not been determined although suggestive chemical work has been done on the corticotrophic hormone.

The names of these hormones, in general, characterize their activities. Thus ACTH and TSH stimulate the adrenal cortex and thyroid, respectively.

The individuality of the action of FSH, ICSH and prolactin (luteotrophin) are not as well defined as in the case of ACTH and TSH. FSH stimulates follicular growth and the seminiferous tubules. Maturation of the follicle, ovulation and transformation of the granulosa into lutein tissue apparently require the action of ICSH (LH) also. Similar difficulties in determining the specific actions of the hormones are encountered in the male for although FSH stimulates the seminiferous tubules, ICSH will also stimulate spermatogenesis due to the androgen secreted by the interstitial cells. For experiments with the gonadotrophic hormones hypophysectomized animals usually are used. Prolactin, called also the lactogenic hormone, was originally so named because it was supposed to maintain or stimulate milk production. It will prolong the functional life of corpora lutea in hypophysectomized rats and, therefore, has also been named luteotrophic hormone. Growth hormone will reinstitute general body growth after hypophysectomy and will prolong skeletal growth in normal animals if the epiphyses have not closed.

The clinical use of the hypophyseal hormones has, in general, been disappointing except for the corticotrophic hormone whose therapeutic action is like that of cortisone. Although the administration of the hypophyseal hormones has not been as successful as was hoped, nevertheless, their secretion by the hypophysis is essential for the maintenance of a normal condition as demonstrated by the disabilities which result from destruction of the hypophysis.

The rate of hormone production by the target organs, in some way not as yet fully understood, influences the rate of secretion by the hypophysis itself, thereby maintaining a balanced normal condition. For instance, if the thyroid hormone (or iodide) is administered the activity of the thyroid decreases, due to lessened output of thyrotrophic hormone by the hypophysis. On the other hand if an antithyroid drug is administered, the formation of thyroid hormone thereby being inhibited, the thyroid will enlarge due to an increased secretion of the thyrotrophic hormone. Similarly, the administration of adrenocortical hormones or of the sex hormones (estrogen, androgen) decreases the hypophyseal output of adrenocorticotrophic or of gonadotrophic hormones. A balance is thus maintained by the reciprocal action on the hypophysis of the hormones secreted by the target organs.

Attempts to associate the secretion of the hypophyseal hormones with cell types have been, in part, successful.

Two chief lines of evidence associate the secretion of growth hormone with the acidophiles. In acromegaly and gigantism, tumors of acidophiles are almost invariably present. In a strain of mice showing hereditary dwarfism in a Mendelian ratio, the hypophyses of the dwarfs have been shown to have no acidophiles. Their hypophyses contain no growth hormone as shown by the most sensitive tests. Basophiles are present and sexual development is normal in the male although somewhat deficient in the female. The association of acidophiles with the secretion of growth hormone, however, does not eliminate the possibility that they also secrete other hormones. The abnormalities in acromegaly and in hypophyseal dwarfism involve more than general body growth.

The acidophiles are probably also the source of prolactin (luteotrophin). It has been shown in several species that during pregnancy there is an increase in the number and size of the acidophiles. The increase reaches a peak two to three days post partum, and there is correlated with it a comparable increase in the luteotrophin content of the glands. Histochemical evidence based on the demon-

stration of specific chemical groups known to be present in the purified hormone also points to the presence of luteotrophin in *some* of the acidophiles in the rat.

Evidence indicates that the follicle stimulating hormone (FSH), the thyroid stimulating hormone (TSH) and probably the luteinizing hormone (LH, ICSH) are secreted by the basophiles. Chemical analyses show that these hormones are rich in glycoproteins. Cytochemical studies reveal that the granules of the basophiles, but not of the acidophiles, contain glycoprotein thus associating the chemical nature of their granules with the constitution of the above hormones. Experimental procedures, such as thyroidectomy, gonadectomy and the injection of TSH and the sex hormones, combined with a study of alterations of the hypophyseal cell types, indicate that the delta basophiles probably secrete FSH and possibly LH also. For instance, after castration the delta basophiles are increased in number and may form a vacuolated, signet-ring type of cell in rats (castration cell). They decrease with estrogen administration. The response of the beta basophiles to alterations in thyroid function (e.g., decrease in number following thyroxine administration) indicates that, in the rat, the beta basophiles probably produce the thyrotrophic hormone. Thus, in this species, it is possible to designate the delta basophiles as "gonadotrophs," and the beta basophiles as "thyrotrophs." The cell type producing adrenocorticotrophic hormone is not, as yet, beyond the stage of conjecture.

It is generally believed that the chromophobes are nonsecretory for in chromophobe tumors there is usually hypopituitarism. In this condition the chromophobes have increased at the expense of the chromophiles.

The Neurohypophysis. It has been stated (p. 521) that the neurohypophysis includes the neural lobe, infundibular stem and median eminence of the hypothalamus. Extracts of the tissue composing this complex yield two fractions, both polypeptides, which have been purified, analyzed, and synthesized. One of these, *oxytocin*, stimulates the contraction of the uterine musculature during the latter part of pregnancy. It is used clinically in obstetrics for the induction of labor. Oxytocin also causes the ejection of milk from the lactating mammary gland by its specific action on the contractile myoepithelial cells of the mammary gland alveoli. The other fraction, *vasopressin* (antidiuretic hormone, ADH), inhibits diuresis through its action on the kidney tubules; it also raises blood pressure and, in large dosages, causes contraction of the intestinal and bronchial musculature.

THE THYROID

The thyroid consists of two lateral lobes and a connecting part, the isthmus. The lobes lie lateral to the superior part of the trachea and the inferior part of the larynx. The isthmus crosses anterior to the trachea at the level of the second to the fourth tracheal cartilages. A median process of varying size which arises from the isthmus or a lateral lobe, and extends cephalically, is frequently present. The thyroid has a connective tissue sheath formed by the deep cervical fascia. Beneath this is a delicate stratum of connective tissue, the true capsule of the gland. Delicate trabeculae and septa penetrate the gland substance, indistinctly dividing it into lobes and lobules.

The thyroid is an extremely labile gland and varies in size and structure in response to a large number of factors, among which are sex, nutrition, temperature, age, season, and the iodine content of the food, the latter being of great importance.

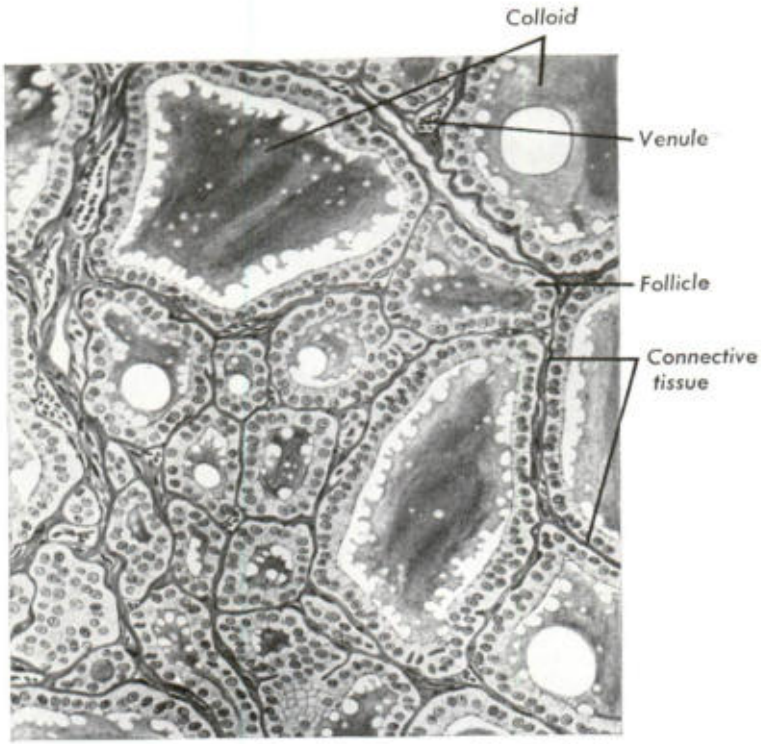

FIG. 424. Thyroid of human adult. (Stöhr.)

The structural unit of the thyroid is the follicle (acinus). These units are usually of microscopic dimensions but may become sufficiently large, as in colloid goiter, to be visible macroscopically. Follicles vary greatly in shape as well as in size but they are usually irregularly spheroidal. In highly activated glands they become extremely irregular in shape (Fig. 425).

A *follicle* consists of a layer of simple epithelium enclosing a cavity, the follicular cavity, which usually is filled with a gel-like material, *colloid*. The apical ends of the cells of the thyroid epithelium face inward, i.e., toward the follicular cavity. The basal ends of the cells rest on a delicate basement membrane outside of which is a rich bed of blood and lymph capillaries.

The *follicle cells* vary greatly in height depending on the activity of the gland. In a normal gland they are cuboidal (Fig. 424) but when a follicle is distended with colloid they become low cuboidal or even squamous. In highly active glands the cells are tall columnar (Fig. 425). The nuclei and cell membranes are distinct and terminal bars are present. The number of mitochondria and size of the Golgi complex vary with the functional state of the gland. In addition to the usual type of follicle cells, another type is occasionally present, the colloid cells of Langendorff. These are small, darkly staining cells with pycnotic nuclei, and often appear to be filled with colloid. They are degenerating cells.

The cavity of the thyroid follicle is filled with a semifluid or gel-like substance, the thyroid *colloid*. Observations of the undisturbed living gland or of a gland prepared by the freezing-drying methods reveal that only rarely are

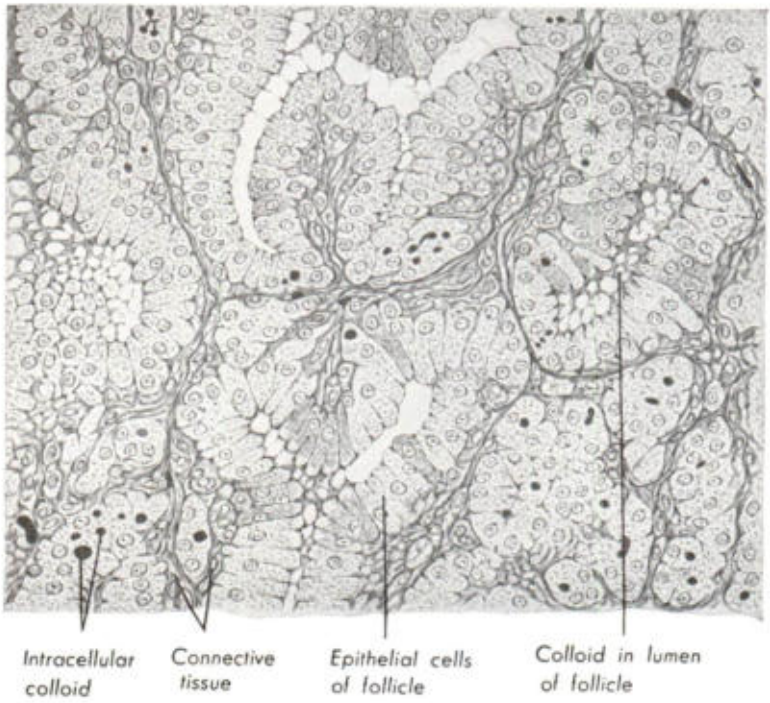

FIG. 425. Surgical specimen of human thyroid showing diffuse extreme hyperplasia. The patient was a woman with toxic goiter who had been treated with thiouracil and then operated upon without pretreatment with iodine. (Aranow *et al.*, Surg. Path. No. 88452. Ann. Surg. *124:* 167, 1946.) A surgical specimen. Heidenhain's azan stain. × 320.

vacuoles (spaces) present either within the colloid or between it and the abutting epithelium. However, the colloid is prone to undergo shrinkage with the ordinary technical procedures used in preparing slides for study. The shrinkage is frequently so pronounced that a continuous and often irregular space is present between the colloid and the epithelium (Fig. 424). Spaces or vacuoles are also present within the colloid. This is most commonly found in human glands. With better technique only small vacuoles are present at the periphery of the colloid. These are most numerous in activated glands (Fig. 427, *D*), due to the fact that the colloid has a different, and probably more fluid consistency in these areas. Even though the vacuoles are artifacts, nevertheless, they are useful in diagnosis. They indicate a changed consistency of the colloid associated with resorption, and consequently are frequently designated as absorption vacuoles. In highly activated glands the colloid not only is reduced in amount but has, in usual preparations, vacuoles throughout, often giving it a netlike or stringy appearance (Fig. 425).

Colloid in different glands or even in the follicles of a single gland may show tinctorial differences. In activated glands the colloid is predominantly basophilic, in inactive glands it is acidophilic.

Thyroid colloid not only varies in its physical properties but also in its chemical composition. It is always rich in nucleoproteins and contains thyroglobulin. *Thyroglobulin* is a glycoprotein which in turn contains several iodinated amino acids, some of which are active thyroid principles. Both the amount of thyro-

globulin in the colloid, and the degree of iodination may vary. For instance, in colloid goiter which is associated with hypothyroidism the iodine content is much lower than in the colloid of a normally functioning gland. The colloid also contains proteolytic enzymes. Occasionally desquamated or phagocytic cells are present in the colloid.

The *secretory process* of the thyroid involves the synthesis of the thyroid hormone, which is a component of the follicular colloid, and it also involves the transport of the thyroid hormone from the follicular cavity to the perifollicular capillaries. Both the formation of new secretion and the outward transport of the stored hormone may occur at the same time. The secretory processes of the thyroid thus are complex; they have been extensively studied but not as yet completely elucidated.

It has long been known that the normal thyroid is rich in iodine and that it will rapidly take up iodine from the circulation. It has also been shown that viable slices of thyroid tissue, but not of an homogenate, will rapidly take up tracer doses of iodine from the surrounding medium, as much as ninety per cent of the iodine being taken up by the slices within two hours. The major part of the iodine (over 90 per cent) is incorporated in follicles in an organic form. By the use of radioactive iodine (I^{131}) the incorporation of iodine in thyroid epithelium and in the colloid can be followed. Tracer doses of radio-iodine injected into normal rats will appear within the thyroid epithelium in an hour or less, following which it will appear in the colloid in twenty-four hours (Fig. 426). The rate of iodine uptake by the thyroid is influenced by the activity of the gland and by its previous iodine content. The circulating iodide is trapped by the epithelial cells, oxidized to iodine, and subsequently leaves the epithelial cells to become incorporated in the proteins of the colloid within the follicle.

Within the colloid, the iodine is present in the form of iodinated amino acids, chiefly the iodotyrosines and iodothyronines which are components of the larger molecule, thyroglobulin. Coupling of the iodotyrosines within the thyroglobulin molecule yields diiodo-, triiodo- and tetraiodothyronine (thyroxine),

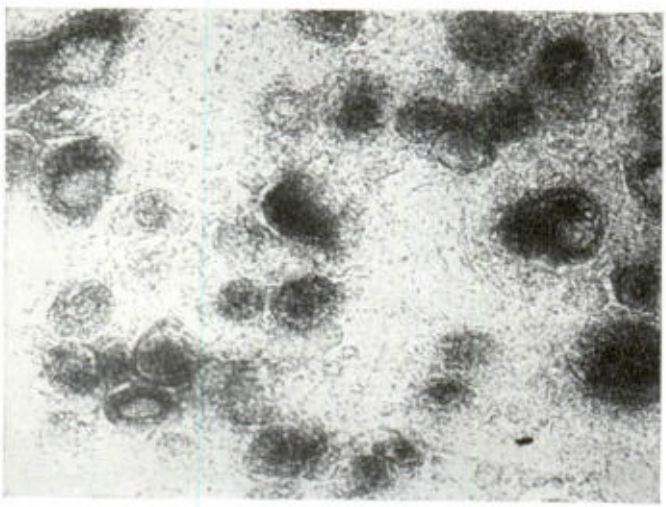

FIG. 426. Section through the thyroid of a rat which was injected with radioactive iodine. The section was placed on a photographic plate which was later developed. Photomicrograph.

which are the active hormonal principles of the thyroid gland. Enzymes are involved in this process of conversion.

The passage outward of the thyroid secretion into the perifollicular capillaries is difficult to follow. Evidence has been advanced that the thyroid cells can secrete in either direction—either into the follicular lumen or into the blood vessels. The discovery of enzymes in the colloid which can hydrolyze thyroglobulin, liberating the active hormonal products, together with the discovery that the enzymatic activity increases in thyroid glands activated by thyrotrophic hormone makes it seem probable that the smaller molecules resulting from hydrolysis may diffuse outward through the epithelium. Direct observations of thyroid glands transplanted into transparent ear chambers of experimental animals indicate that bits of colloid may be pinched off by the follicular cells, transported as droplets across the cells and discharged into adjacent capillaries.

Thyroxine circulating in the blood stream is bound to a plasma protein. Triiodothyronine, which has several times the hormonal potency of thyroxine, is not protein-bound, and thus may enter the cells of the body more readily. It is possible that circulating thyroxine is normally converted to triiodothyronine before being taken up by the cells peripherally.

The use of *tracer doses* of radio-iodine has found clinical application especially in two ways. The rapidity with which the radio-iodine is taken up by the thyroid (as determined by a Geiger counter) is an indication of the activity of the gland of a patient. In thyroid carcinoma, the site of metastases can frequently be determined by their radioactivity. Radio-iodine is also extensively used as a therapeutic agent in hyperthyroidism. Due to the radiation from the I^{131} taken up, part of the overactive gland tissue is destroyed. This reduction in functioning tissue thus substitutes for surgical removal.

Significant contributions correlating the functional activity of the thyroid with the size of the Golgi complex and number of mitochondria have been made. During the active formation of secretion, the Golgi apparatus increases in size and the mitochondria become less numerous. If there is only colloid depletion the Golgi apparatus does not enlarge and the mitochondria increase in number (Severinghaus).

Blood Vessels

The thyroid has a rich plexus of blood and lymph capillaries, which are in intimate relation to the follicular epithelium. The arteries at the places where they branch, frequently have localized pad-like thickenings beneath the intima. These do not encircle the vessels. They probably assist in shunting the blood to different parts of the gland as the arteries contract, and may also serve to reduce the pulse wave. Arterio-venous anastomoses are common. The architecture of the blood vessels, as well as the fact that all the follicles do not show the same degree of activity at any one time, indicates that there are fluctuations in the amount of blood received by different parts of the gland.

Nerves

A large number of nonmedullated nerve fibers are present in the walls of the thyroid arteries. Most of these terminate in plexuses around the blood vessels. Secretory fibers have not been unquestionably demonstrated.

Function

The main effect of the thyroid hormone is on the rate of metabolism of tissues in general. The ramifications of this effect are very extensive, however, extending to as diverse activities as the rate of absorption in the intestine, carbohydrate metabolism, the rate of the heart

beat, mental activity, general body growth, and many other effects. Hypothyroidism in the infant causes cretinism; in the adult it causes myxedema. The symptoms in both conditions are attributable to a lowered metabolic rate not only in the general body tissues but in other endocrine glands. Administration of thyroid has a striking therapeutic effect. The opposite condition, hyperthyroidism, leads to overactivity and sometimes is followed by exophthalmic goiter (Grave's disease). Surgical removal of a part of the thyroid, or the use of antithyroid drugs or radio-iodine reduces the metabolic rate but does not alleviate the exophthalmos when once established. The thyroid is not essential to life, although in early work the close topographical association of the parathyroids with it caused confusion on this point.

THE PARATHYROIDS

There are normally two pairs of parathyroids in mammals. Due to their origin from the III and IV pharyngeal pouches, respectively, they are designated as parathyroids III and IV. In man a member of each pair lies on the posterior surface of each lateral lobe of the thyroid near the arterial anastomosis of the inferior and superior thyroid arteries. Parathyroid III lies near the lower lobe of the thyroid, and IV lies somewhat more cephalad. These glands are somewhat flattened and measure some 6 mm. in length, and about half this in width. In man they are brown, but in some species they are white or but faintly colored. A connective tissue capsule separates them from the thyroid. Delicate septa give the gland a lobulated appearance in the adult.

The parenchyma is composed of anastomosing cords of cells between which are capillaries. This strand-like arrangement of the cells increases in distinctness with age due to an increase in the connective tissue.

Two types of cells compose the cords, (a) chief cells, (b) oxyphil cells (Fig. 427, *B*). The chief cells are of constant occurrence. They are sometimes divided into clear and dark types. The clear chief cells have vesicular, relatively large nuclei. Their cytoplasm takes a light stain and contains no granules. The dark chief cells have smaller nuclei and a very finely granular cytoplasm. They probably represent one stage in the secretory cycle of either the chief or the oxyphil cells.

The oxyphil cells do not occur in some animals (dog, cat, rat) and are not present in man until the end of the first decade of life. They are larger than the chief cells but usually have smaller and darker staining nuclei. Their cytoplasm stains deeply with the acid dyes, and contains fine granules. These cells may occur in small or large groups.

Both chief and oxyphil cells contain mitochondria and Golgi apparatus. The chief cells, in contrast to the oxyphil type, are rich in glycogen.

Small colloid follicles are of frequent occurrence in the parathyroid. This colloid has no relation functionally to that of the thyroid. In contrast to the thyroid, the parathyroid contains no more iodine than other tissues of the body.

Blood Vessels and Nerves

The parathyroids have an abundant blood supply but in vascular injections the capillaries do not appear to be as numerous as in the thyroid. In man there is said to be a plexus of veins at the periphery of the gland.

Nonmedullated nerves in small numbers occur in the parathyroid. They are probably vasomotor.

Function

No association between the different types of cells of the parathyroids and function has been established. These glands are important in the regulation of calcium metabolism. Removal of the

parathyroids causes a fall in blood calcium, nervous hyperexcitability, and spasms, leading to death. Calcium administration relieves these symptoms, and the injection of parathyroid extract, parathyrin, maintains the animals in good health. Such injections in normal animals raise the blood calcium, and excessive doses will cause death.

Removal of one or more of the parathyroids does not cause a compensatory hypertrophy of those which remain. In rickets the parathyroids enlarge. The parathyroids readily "take" in autotransplantation.

There is evidence that the parathyroids function independently of the anterior hypophysis, i.e., are not under the control of the hypophysis.

THE ADRENAL GLANDS

The adrenal glands are paired organs, one being situated close to the cranial pole of each kidney in the retroperitoneal tissue. Both are somewhat flattened, the left adrenal gland being crescentic, the right one more triangular in shape. The combined weight of the two glands is some 10–12 gm., the left usually being somewhat heavier than the right one. In most mammals they are more regular in shape than in the human, being ovoid or spheroid.

The adrenal glands are composite organs consisting both functionally and structurally of two distinct parts, the cortex (interrenal tissue) and the medulla (chromaffin tissue). In lower vertebrates these two tissues are not united into a common organ but are topographically separated. Correlated with the evolution of the higher vertebrates is a progressively closer association of the cortical and medullary components. In mammals the chromaffin tissue is surrounded by the interrenal tissue.

Grossly, in a section of the fresh human gland, an outer and broader yellowish zone of the cortex can be distinguished from a brownish-yellow inner zone. The medulla when unstained is white or gray.

The adrenal gland has a tough, connective tissue capsule in which small blood vessels course. From the capsule delicate trabeculae penetrate into the cortex although the main supporting tissue of the cortex is reticular fibers. From the capsule, capillaries and arterioles also penetrate into the gland.

The hilum is situated ventromedially (towards the vena cava) and is chiefly marked by the emerging thick-walled adrenal vein.

The arrangement and distribution of the cortex and medulla in the human do not have the uniformity characteristic of the adrenal glands of laboratory animals. This lack of uniformity may be due, in part, to the irregularity in the shape of the human adrenal. The line of

FIG. 427. Drawings of the normal anterior hypophysis and parathyroid of man, and the normal and activated thyroid of the rhesus monkey.

A, Anterior hypophysis of an executed man, 38 years old. Cords of cells are from the central part of the gland. In 3 of the acidophiles (pink), the Golgi area is stained bluish. In the basophile in the lower cord, a negative picture of the Golgi apparatus is shown as clear canals. Chromophobes are present in the middle of the central cord. The rather large amount of connective tissue shown in the drawing is characteristic of the human anterior hypophysis. A colloid mass (blue) is shown at the lower part of the inferior cell cord. Modified Masson stain. × 1250.

B, Parathyroid of normal human adult. The oxyphils are pink and the chief cells bluish purple. The spaces shown were filled with fat. Hematoxylin and eosin stain. × 350.

C, Thyroid of a normal adult rhesus monkey. A few peripheral and central colloid vacuoles are shown. Modified Masson stain. × 570.

D, Activated thyroid of a normal adult rhesus monkey. The thyroid was activated by injections of an anterior hypophysis extract. The epithelium is high and many absorption vacuoles are present. Colloid droplets and vacuoles are present in the thyroid epithelium. Modified Masson stain. × 570.

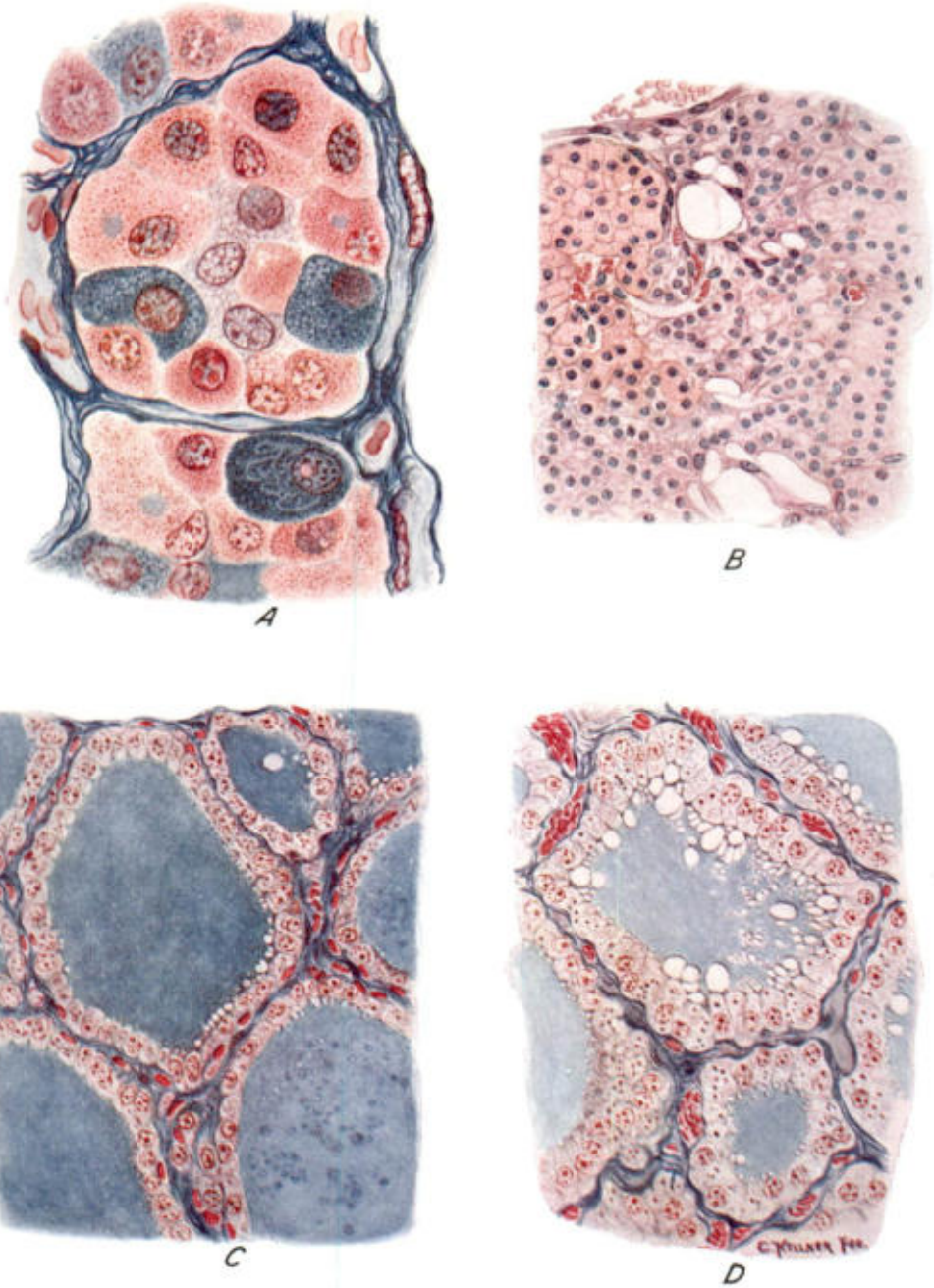

FIGURE 427

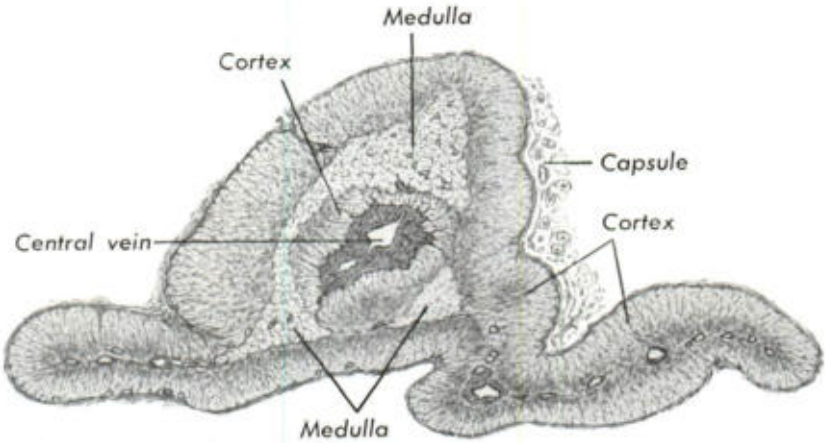

FIG. 428. A transverse section through the left adrenal of a man 72 years old. The adrenal was surgically removed in an attempt to give relief in prostatic carcinoma with metastases. Camera lucida drawing. × 6.

separation between the cortex and medulla is frequently very irregular, and in single sections, clumps of cortical cells may appear to be surrounded by medulla although a study of serial sections will reveal that these clumps are connected with the cortical zone. The distribution of the medulla also varies among different adrenal glands. It very often is absent in the "wings" of the glands, the cortical zones of the two sides abutting against each other with only some connective tissue and small veins separating them (Fig. 428). A study of sections through the gland near the emergence of the adrenal vein may reveal cortical tissue adjacent to the vein. This cortical tissue may be in part, at least, enclosed by medulla, outside of which are the peripheral cortical zones (Fig. 428). The cortex surrounding the vein appears to have been inverted into the gland with the outermost zone of the cortex lying next to the wall of the vein.

In the zonation of the cortex there is not as much uniformity in the human as in laboratory animals although the zones are always readily recognizable.

The Cortex. The cortex, classically, is divided into three zones due to differences in the cord-like arrangement of the cells composing it: an outer zone, the *zona glomerulosa*; a middle zone, the *zona fasciculata*; and an inner zone, the *zona reticularis* (Fig. 429). The zona fasciculata is by far the broadest of the three but is not uniform in structure throughout its full extent. Due primarily to the differences in the lipid content of its cells and the consequent differences in staining, the fasciculata may be divided into outer and inner zones (Fig. 429). There is no sharp demarcation between the cortical zones, each blending with the adjacent one.

In the *zona glomerulosa* the arrangement of the cords is such that ovoid groups of cells are formed. Within a group there is no cavity. The cells composing these groups are columnar and their nuclei stain deeply. Their cytoplasm often takes a basic stain, though lightly. They contain a few lipid droplets.

In the *zona fasciculata* the cords run parallel to each other and course radially toward the medulla in fairly straight lines. The cords usually are the width of two cells. The cells are cuboidal and frequently binucleate. The nuclei are more vesicular and achromatic than in the

glomerulosa. Especially in the outer two-thirds of the fasciculata, the cells are large and filled with lipid droplets, composed of cholesterol, fatty acids, and neutral fat. Cholesterol, which is more abundant in the adrenal than in any other tissue of the body, is largely concentrated into this zone. Much of the lipid is birefringent. Since with the usual technical procedures, the lipids are dissolved, the cells in the usual preparations have a spongy appearance and so are called "spongiocytes." When frozen sections are stained with a fat-dye or the tissue is fixed in osmic acid, the large amount of fat present nearly obscures the other structures in the cells.

In the *zona reticularis* the cords do not pursue a regular course but run obliquely toward the medulla, thus forming a network. The cords are but one cell in width. The cells, especially in the inner (juxtamedullary) part of the reticularis, contain much pigment. They frequently have deeply staining nuclei. The cytoplasm of some of the cells stains deeply, in others it stains less intensely. Evidences of degeneration can be seen in some of the cells.

The zonation described above is based on the arrangement of the cells of the cortex. From the structure of the cells, the cortex can be divided into four zones. The outer zone is the glomerulosa. The next zone, the zone of spongiocytes, includes approximately the outer two-thirds of the fasciculata. The third zone includes the inner third of the fasciculata and outer part of the reticularis. The fourth zone consists of the juxtamedullary part of the reticularis. In cats, Bennett has designated these zones as presecretory, secretory, postsecretory and senescent zones.

Extensive studies on the replacement of cells in the cortex, coupled with the obvious zonation, have given rise to the theory that new cells arise in the outer region (glomerulosa), move inward (through the fasciculata) as they pass through their period of secretory activity, and finally degenerate in the inner region of the cortex (reticularis). It has been stated repeatedly that in normal animals mitotic figures are most numerous in the zona glomerulosa and outer fasciculata. However, mitoses do not occur exclusively in the outer part, but are normally found to some extent throughout the cortex, including the reticularis. Also, by no means all of the degenerating cells are found in the reticularis, nor is the proportion of dead or dying cells in that zone high enough to justify the conclusion that the reticularis is formed exclusively of degenerating cells. An alternate theory has recently been proposed to explain the presence of distinct zones in the adrenal cortex of most species. There is considerable evidence that each of the three major zones is primarily concerned with the production of a particular group of hormones, each group having a different type of physiological activity (see p. 538).

The adrenal cortex is especially rich in cholesterol and ascorbic acid, the latter being present also in the medulla.

Medulla. The cells composing the medulla of the adrenal are ovoid and occur in groups or anastomosing cords. They contain fine granules which become brown when oxidized by such agents as potassium bichromate and are therefore called *chromaffin cells*. The granules also stain green with ferric chloride, yellow with iodine and brown with osmic acid. The cells do not show an equal content of granules so that some stain deeply and others stain lightly or remain colorless. Although in sections the cells of the medulla appear to have a random arrangement, each cell actually has one pole directed toward a vein, and one pole directed toward a capillary. The granules present in the cells are adrenin or a closely related substance. These granules

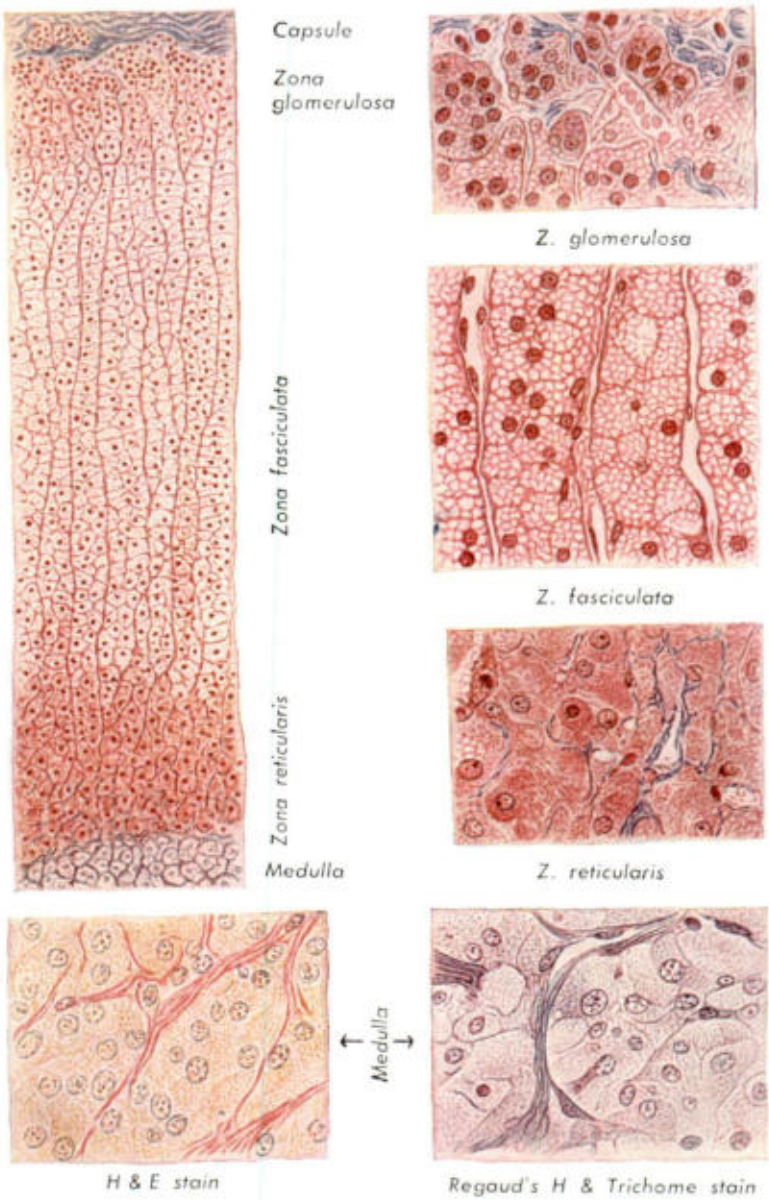

FIG. 429. Sections through the right adrenal of a man 35 years old. The adrenal was removed in an attempt to give relief from a testicular tumor with metastases. The tissue was fixed in Helly's fluid (formol Zenker). The section used for all the drawings, except the lower left one, was stained with Regaud's hematoxylin and Masson's trichrome. The low power (upper left) is × 205, the others × 435.

enter the veins and can be there identified by their staining reaction. Chromaffin cells are also called *pheochrome cells*.

Mitochondria occur in the cells of both the cortex and medulla. Electron microscopy reveals that the mitochondria of the adrenal cortical cells have a peculiar internal structure, with villous or saccular internal membranes instead of laminar as in most cells. According to some investigators the mitochondria contain lipid droplets and are concerned with the formation of the steroid hormones. A Golgi apparatus has been described in the cells of all the cortical zones and the medulla. In the cortex, there is an increase in the size of both the mitochondria and the Golgi apparatus with increased functional activity.

Blood Supply

The adrenal glands are highly vascular organs. The arterial supply is subject to great individual variation in the human but there are always a number, sometimes a large number, of small arteries to each gland. These commonly arise from the inferior phrenic, celiac and renal arteries. The adrenal arteries usually branch before entering the gland so that there are small branches entering the capsule at intervals over most of its surface. The vascular distribution in the gland has been described in detail in the dog (Fig. 430). From arteries which enter and course in the capsule three sets of branches arise. One of these sets gives rise to capillaries which supply the capsule. Blood from these is collected in the veins of the capsule. The second set supplies the sinusoids of the cortex which then empty into veins within the medulla. The third set sends arterial branches through the cortex which empty into the capillary plexus of the medulla (Fig. 430). The venules arising from the second and third sets drain into the vena cava through the medullary vein which is unusually thick-walled for a vein with many longitudinally directed smooth muscle fibers. In both the cortex and medulla the terminal network from the arterioles is in close relationship to the parenchyma. Histiocytes lie in the walls of the cortical sinusoids. Lymph vessels have not been described in the adrenal gland except in relation to the larger blood vessels.

Nerves

The adrenals are abundantly supplied with nerves. Most of these are derived from the sympathetic division of the autonomic nervous system and course through the splanchnic nerves. Some of the fibers are distributed to the cortex. The majority, however, pass to the medulla. The fibers which pass to the medulla have been described as being preganglionic. A few ganglion cells are present in the medulla.

Physiologically the innervation of the medulla has been better established than that of any other endocrine gland. Stimulation of the splanchnic nerves causes an outpouring of adrenin (epinephrine). Cutting the splanchnics (denervation) inhibits the secretory activity of the medulla.

Function of the Adrenal Glands

Attention was first attracted to the physiological importance of the adrenal glands by Addison in 1855. He described a fatal syndrome, since named Addison's disease, which resulted from a destruction of the adrenals by some disease process. Experimentally, work beginning with that of Brown-Séquard in 1856 has shown that the adrenal cortex, but not the medulla, is essential for life. Destruction of the *cortex* causes a loss of sodium and an accumulation of potassium so that diets high in sodium and low in potassium are helpful. There is a concentration of the

blood, a sluggish circulation and an increase in urea resulting from impaired kidney function. Carbohydrate stores are depleted and there is hypoglycemia with lowered resistance to insulin. There is a decreased resistance to stress (heat, cold, trauma, fatigue, etc.). Cortical extracts, first successfully prepared in 1930, are assayed on the basis of their ability to prolong life or to alleviate the other disabilities resulting from adrenalectomy.

Thus far forty-one crystalline compounds (steroids) of known constitution have been isolated from the adrenal cortex. Of these crystalline compounds, at least seven have been shown to have cortical activity in the tests so far used. In general, the active crystalline compounds can be divided into two categories as judged by their type of activity. Those in one category have an effect on electrolyte and water balance (mineralocorticoids); those in the other category have their greatest effect on carbohydrate metabolism (glucocorticoids). In addition there is a third group of steroids which includes the female sex hormones (estrone and progesterone) and several androgenic steroids. Certain adrenal tumors have a feminizing or a masculinizing influence. The cortical hormones (except possibly for the estrogens) may be synthesized from cholesterol in which the adrenal cortex is very rich.

There is some evidence that the mineralocorticoids are produced primarily in the zona glomerulosa, the glucocorticoids in the zona fasciculata, and the sex hormones in the zona reticularis.

The normal activity of the adrenal cortex is at least partially under the control of a hormone of the anterior hypophysis, the adrenocorticotrophic hormone (ACTH). Injection of ACTH causes a drop in the cholesterol and ascorbic acid content of the adrenal. This response is commonly used (with hypophysectomized rats) in determining the potency of ACTH extracts. The zona glomerulosa appears to be less under pituitary control than do the other cortical zones. After hypophysectomy the zona glomerulosa shows less atrophy than the remainder of the cortex, and electrolyte metabolism is less affected following hypophysectomy than after adrenalectomy. This is interpreted as evidence for the continued secretion of some mineralocorticoids by the zona glomerulosa even after hypophysectomy.

Clinical use of one of the adrenal steroids (cortisone) and also ACTH is quite extensive in some of the "collagen" diseases, for example in rheumatoid arthritis and rheumatic fever.

The *adrenal medulla* or chromaffin tissue is not essential to life. From it an extract (adrenin, epinephrine) is prepared which stimulates the sympathetic nerve terminations, causing a contraction of smooth muscle. Because of the contraction which it induces in the small blood vessels it is used locally, in surgery to reduce bleeding. The number of chromaffin granules in the medullary cells is an index of their secretory content. In depletion of the medulla the granules disappear and are formed again with a storage of secretion. The hormone of the adrenal medulla was the first hormone to be obtained in crystalline form (1901). It has since been discovered that what appeared to be a pure compound is in reality two closely related substances, epinephrine and norepinephrine. Both are produced by the adrenal medullary cells and the two compounds have somewhat different physiological effects. Norepinephrine is readily converted to epinephrine, and may serve as a normal precursor for epinephrine in the synthetic process within the cells.

Epinephrine will stimulate the secretion of ACTH by the anterior hypophysis as

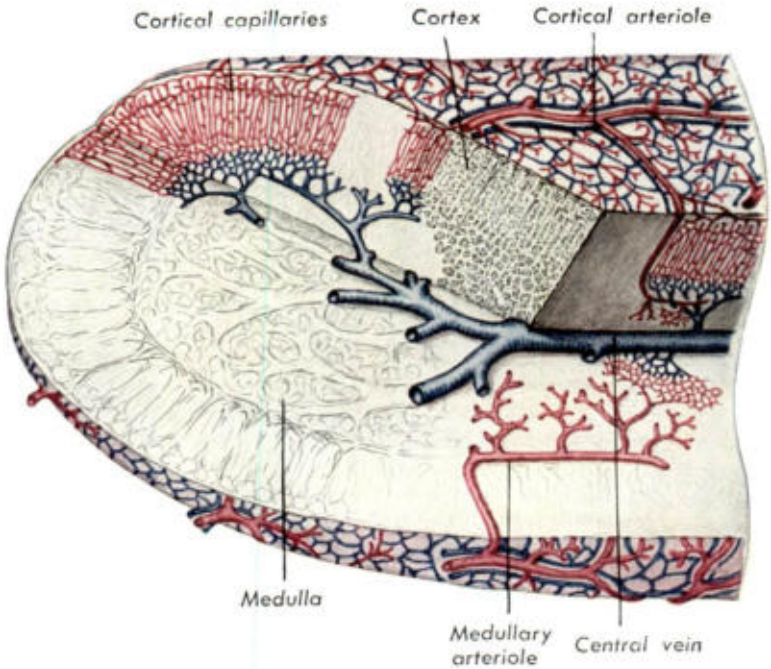

FIG. 430. Blood supply of adrenal of dog. One end, only, of the adrenal is shown. (After Flint.)

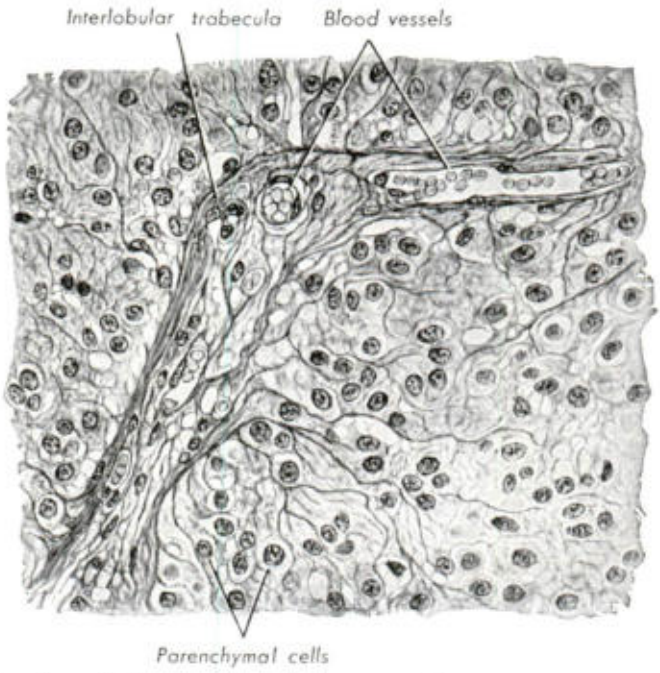

FIG. 431. Section through the pineal gland of a woman thirty-seven years of age. Hematoxylin and eosin. × 450.

shown by experimental procedures. There is thus indirectly a neural stimulation of the anterior hypophysis, for the adrenal medulla responds to sympathetic nerve stimulation, its secretion in turn activating the anterior hypophysis.

THE POSTNATAL INVOLUTION OF HUMAN ADRENAL GLANDS

The adrenal glands at birth, in the human, are relatively large bodies being about one-third the size of the kidneys, whereas in the adult they are about one twenty-eighth the size of the latter organs. Immediately following birth, the adrenals undergo a pronounced involution. In the first fourteen days of postnatal life this decrease amounts to one-third their birth weight, and in the first four months it amounts of one-half. The loss in weight is due to the degeneration of the inner part of the cortex. At birth the cortex consists of a narrow, outer zone which will proliferate and form the cortex of the adult and a massive inner zone which is destined to degenerate, the so-called "fetal cortex."

The adrenal glands attain their large prenatal size by a steady growth throughout intrauterine life. Their growth is proportionate, in this period, to general body growth.

THE PARAGANGLIA

Under the head of *paraganglia* are included groups of cells which are closely associated both anatomically and embryologically with the sympathetic nervous system. They are largely retroperitoneal, occurring in association with sympathetic ganglia. In shape and staining reaction the cells are similar to those composing the adrenal medulla. They are clear when unstained, become yellow with

chromic acid and its salts, and dark with osmic acid. The cells are oval or polyhedral and have a cord-like arrangement. They lie in close contact with capillaries. Because they are embryologically and structurally similar to the chromaffin cells composing the medulla of the adrenal, and have the same staining reaction it is assumed that they secrete adrenin (epinephrine).

The *aortic chromaffin bodies* (lumbar paraganglia, organs of Zuckerkandl) are relatively large, irregularly paired masses formed by a fusion of paraganglia. They are located retroperitoneally and lie ventrolaterally to the aorta at about the level of the origin of the inferior mesenteric artery.

THE PINEAL BODY

The pineal body or pineal gland (epiphysis cerebri) in man is a slightly flattened cone-shaped structure measuring 8 to 12 mm. in length and 5 to 8 mm. in width. Its base is constricted to form a peduncle by which it is attached to the roof of the third ventricle. The peduncle or stalk is hollow, with a narrow prolongation of the third ventricle extending up into it. Pia mater covers the pineal body except at its attachment and forms a capsule from which connective tissue trabeculae penetrate into the organ, incompletely dividing it into lobules (Fig. 431).

The lobules are composed of two main cell types, the parenchymal cells and the neuroglia. In routine preparations, the shape of the parenchymal cells is poorly defined. Their true shape is best observed in preparations stained by a special silver method devised by del Rio-Hortega. Material prepared by this method shows that the cells have irregular-shaped bodies from which a variable number of long slender processes arise (Fig. 432). These processes usually have club-shaped terminations. Many of the processes extend into the trabeculae and terminate near or on blood vessels. Processes of other cells, especially those located more deeply within the lobule, interweave to form a plexus.

FIG. 432. Section of a pineal gland of a boy. The parenchymal cells and their processes were impregnated with silver. The processes are shown extending into an interlobular septum. Collagenous tissue and glia are not shown with this technique. (After del Rio-Hortega.) Semidiagrammatic.

The cytoplasm of the parenchymal cells varies in amount, in some cells being scanty and in others abundant. The usual organelles, such as mitochondria, centrioles and Golgi apparatus, are present. In addition, the cytoplasm contains some ribonucleic acid, glycogen and alkaline phosphatase. Various secretion granules have also been described in the cytoplasm, but the secretory processes and the nature of the secretion, if any, have not been satisfactorily elucidated. The nuclei of the parenchymal cells are rather pale and vesicular, with a distinct nuclear membrane.

The glial cells are of the astrocyte type. They serve as a supporting element (Fig.

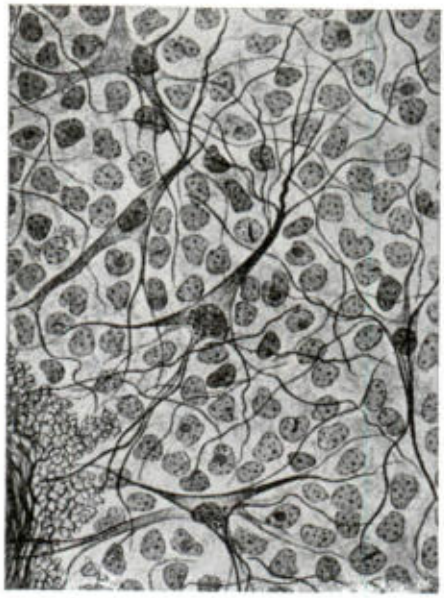

FIG. 433. Pineal gland showing the glial cells and their processes. The nuclei of the parenchymal cells are also shown. Silver preparation. (After del Rio-Hortega.)

433). These cells are relatively few in number, have smaller, darker nuclei than the parenchymal cells, and have delicate fibrous processes. Microglial (mesoglial) cells are also present and have a phagocytic function as in the central nervous system.

Brain sand (acervuli, corpora arenacea) is almost always present in the pineal body, even in infancy. These calcareous bodies usually lie in the connective tissue capsule and at the base of the organ although they may occur in other parts as well. When located within the organ the acervuli usually are surrounded by or are adjacent to areas rich in glia (Fig. 434). The concretions are mulberry-shaped and in section show concentric zones. They vary greatly in size as well as in the number present in different glands. The glial areas and acervuli are stated by some investigators to be due to degenerative changes of the gland.

The pineal body reaches its maximum

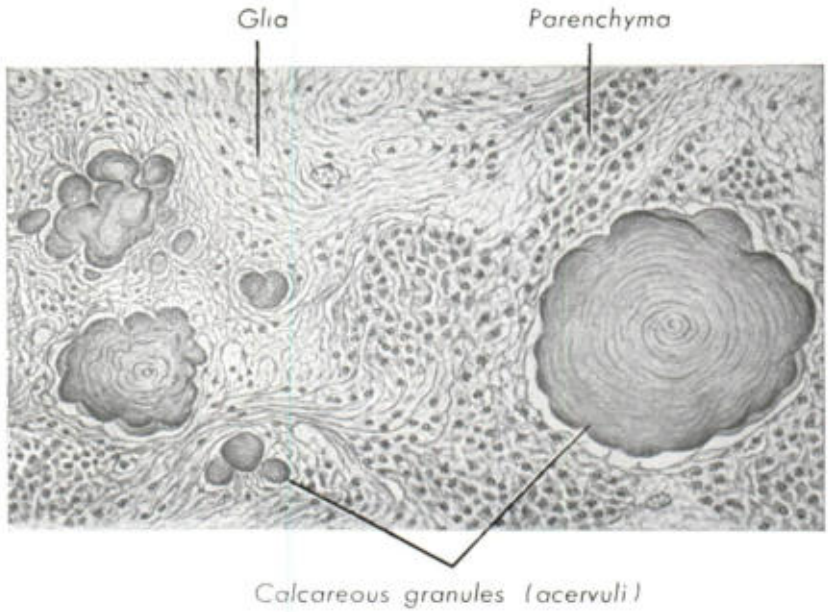

FIG. 434. Section through a region of the pineal gland of a woman thirty-seven years of age, showing calcareous granules (acervuli, brain sand). Hematoxylin and eosin. × 240.

development by the middle of the first decade or shortly thereafter. Later in life it shows regressive changes; these, however, are variable as regards the time of their appearance. Contrary to popular belief, the pineal body does not undergo involution at the time of puberty in man.

The pineal body contains nerve fibers but no mature nerve cells. It has a rich vascular supply. Substances appear to diffuse freely from the blood vessels into the stroma of the pineal body. This is not true of brain tissue in general, where there is a physiological barrier to diffusion, the hemato-encephalic or blood-brain barrier.

There has not as yet been unequivocal proof of specific functions of the pineal body. There is, however, some evidence, both experimental and clinical, which indicates that it may exert an inhibitory effect upon the genital organs, either directly or by way of the anterior hypophysis. Pinealectomy in experimental animals, and some tumors of the supporting elements of the pineal body (which presumably crowd out the parenchyma) in young boys result in precocious growth and development of the gonads. However, since a tumor may also impinge upon neighboring brain tissue, it is difficult to determine whether the effects are primarily pineal or neural in origin. Other possible functions are even less well substantiated.

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XXII

THE ORGANS OF SPECIAL SENSES[1]

THE EYE

The eyeball (*bulbus oculi*) constitutes the organ of vision. It is essentially a spherical structure, lightproof except for its transparent anterior surface (the cornea), and contains a system of refracting media with convex surfaces, which transmit light rays reflected from an object and bring them to a focus on a photosensitive surface (the retina) in the form of a small inverted image. The photosensitive elements (rods and cones) on which the image falls are thereby stimulated to nervous activity and send impulses over fibers of the optic nerve to the brain, where the image then enters the field of consciousness and the sensation of vision is experienced. Correct focus is accomplished by changing the curvature of one of the refracting bodies (the lens) through alteration of the tension exerted on it by the mechanism from which it is suspended (the ciliary body). Placed perpendicularly in front of the lens is an adjustable diaphragm (the iris), the aperture of which (the pupil) can be changed in diameter to regulate the amount of light admitted to the eye.

The eye is suspended by a series of ligaments in the bony orbit, which also contains the following structures: the extrinsic ocular muscles, which control movements of the eyeball; the lacrimal gland, which keeps moist its anterior surface; the nerves and blood vessels supplying the eye and orbital structures; and a considerable amount of connective tissue and fat, which fills in all otherwise unoccupied orbital space. Also associated with the eye are the lids and a duct system which drains the tears from the eye into the nasal cavity.

In form, the eyeball (Fig. 435) departs slightly from that of a ball; it is more accurately described as consisting of the segments of two spheres, unequal in size. The larger sphere forms the posterior five-sixths of the eyeball. The anterior one-sixth, which is the cornea, constitutes a segment of the smaller sphere and hence is more curved than the posterior part. The two segments are structurally continuous.

The wall of the eyeball consists of three

[1] Many of the original figures in this chapter were made from sections which were made available through the kindness of Dr. A. B. Reese of the Institute of Ophthalmology, Columbia-Presbyterian Medical Center, New York City. Figures 442, 443, 444, 446, 453, 454 and 460 were made from sections in the personal collection of Dr. Reese. Figures 440, 459, and 461 were made from sections in the collection of the Institute of Ophthalmology. Sections of the eye were also kindly loaned by Dr. R. Castroviejo of the Institute of Ophthalmology.

In the section dealing with the ear, some figures were made from slides generously loaned by Dr. E. P. Fowler, Jr., of the Department of Otolaryngology, College of Physicians and Surgeons. Acknowledgment of these has been made in the legends accompanying these particular figures.

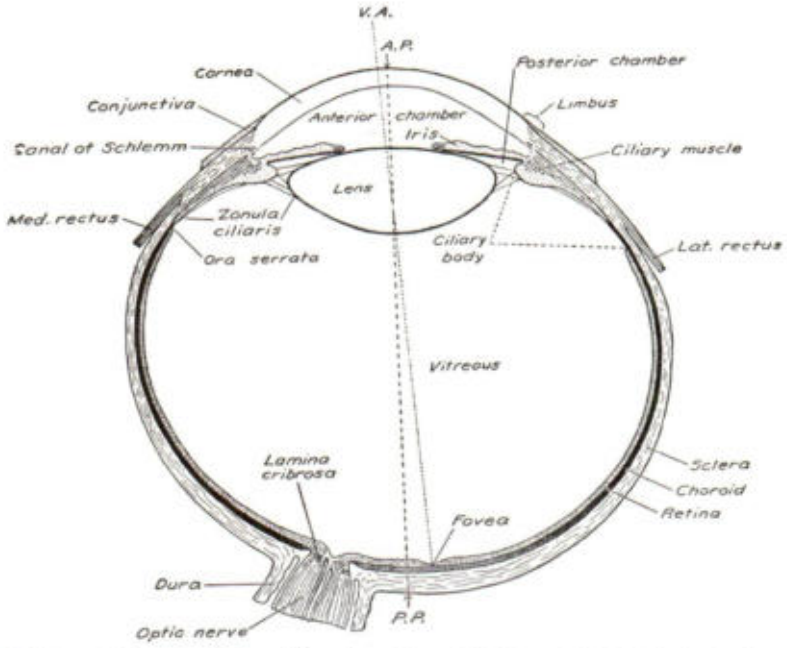

FIG. 435. Schematic horizontal meridional section of right eye. × 3. *A.P.*, Anterior pole; *P.P.*, posterior pole; *V.A.*, visual axis. (Redrawn and modified from Salzmann.)

concentric layers, or tunics, which enclose the transparent media through which light is transmitted to the sensitive retina:

1. The outer layer is the *tunica fibrosa*, comprising the *cornea* and *sclera*. The cornea forms the anterior portion of this tunic and is transparent. A fact not commonly appreciated is that most of the refraction of light takes place at the surface of the cornea rather than in the lens, the refractive power of the cornea being about two and one half times that of the lens. The remainder of the outer tunic is the sclera, a grayish-white tough protective coat, a part of which is seen through the overlying transparent conjunctiva as the "white" of the eye. The tendons of the extrinsic ocular muscles insert into the sclera. Posteriorly, it is perforated where the fibers of the optic nerve leave the eye, this area being known as the *lamina cribrosa*.

2. The middle layer of the eye is the *tunica vasculosa*, or *uvea*, which includes the *choroid*, *ciliary body* and *iris*, the last named having a central circular opening, the *pupil*. All three regions of this tunic are characterized by extreme vascularity and the presence of considerable amounts of pigment. In addition, the ciliary body and iris contain smooth muscle. That in the ciliary body serves as the muscle of accommodation; by its contraction, the tension on the suspensory ligament of the lens is relaxed and the lens assumes a greater curvature to bring the image of near objects into correct focus on the retina. The smooth muscle in the iris acts to regulate the diameter of the pupil.

3. The innermost tunic, the *tunica interna*, is represented chiefly by the photosensitive *retina* but also includes the *pigment epithelium layer* and the forward extension of elements of both of these layers to form the posterior layers of the ciliary body and iris. The nerve

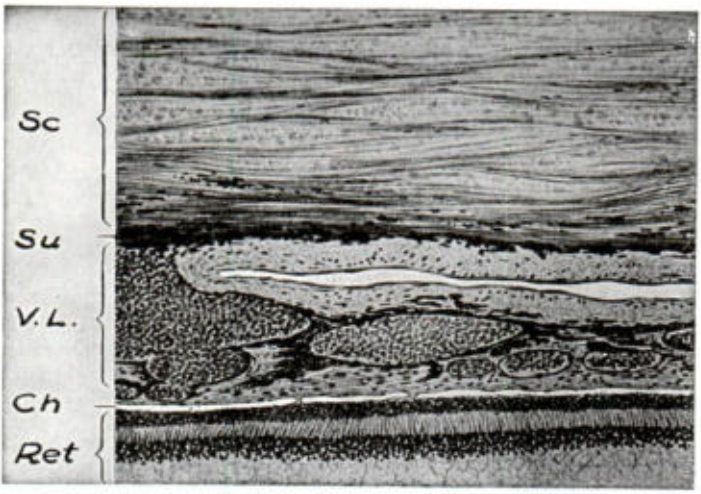

FIG. 436. Section through choroid and part of sclera. *Sc*, Sclera; *Su*, suprachoroid; *V.L.*, vessel layer; *Ch*, choriocapillaris; *Ret*, retina. (After von Möllendorff.)

fibers of the retina converge about 3 mm. medial to the posterior pole of the eye to form the *optic nerve*; consisting only of nerve fibers, this area constitutes a *blind spot*. Almost at the posterior pole is a small area of the retina known as the *macula lutea*, in the center of which is a depression—the *fovea centralis*. This is the region of most acute vision.

An understanding of certain commonly used descriptive terms facilitates discussion of the histology of the eyeball. Thus we speak of the anterior pole of the eye as being coincident with the midpoint of the cornea. A point diametrically opposite is the posterior pole. A line connecting the anterior and posterior poles is the geometric axis of the eye. This must be distinguished from the visual axis, which is a line joining the fovea centralis and the nodal point of the optic system. This latter point, which is the optical center, lies in the posterior part of the lens.

A section of the eyeball passing through the anterior and posterior poles is designated a meridional section. Most instructive of the various meridional sections is that which passes through the horizontal meridian. At an angle of 90 degrees to this plane is the vertical meridian, which divides the eyeball into a medial, or nasal, half and a lateral, or temporal, half. The equator of the eye is a circle taken equidistant from the two poles; sections parallel to this plane are called equatorial sections.

The terms "outer" and "inner" are used as follows: "outer" (or external) refers to that which is nearer the surface of the eyeball; "inner" (or internal) refers to that which is nearer the midpoint of the bulb. The terms, anterior and posterior, refer to points which are nearer the anterior or posterior poles, whether such points be in a sagittal plane or along a meridian.

Tunica Fibrosa

The Sclera

The sclera (Fig. 436), which forms the opaque posterior five-sixths of the protective outer tunic, is composed of dense fibrous connective tissue. It is thickest at the posterior pole (about 1 mm.) and gradually becomes thinner until it is only 0.3 mm. thick at the insertion of the recti muscles (Fig. 435). It is pierced by three sets of apertures, or *emissaria*, through which pass nerves, blood vessels and lymphatics. At the point of attachment of the optic nerve, the sclera becomes a sieve-like membrane, the *lamina cribrosa*, through which pass the optic nerve fibers.

Although the sclera and cornea are structurally continuous, their line of junction is marked externally by a slight circular furrow, the *external scleral sulcus*. On the inner surface of the sclera, close to its junction with the cornea, there is also seen a shallow furrow, the *internal scleral sulcus*. The posterior margin of this furrow projects slightly inward and forward to form the *scleral spur* (*scleral roll*), to which the ciliary body is attached (Figs. 442 and 446). The furrow itself is filled in by the *meshwork of the iris angle*. At the base of the furrow lies the *canal of Schlemm* (see p. 561).

Layers of the Sclera. For descriptive purposes, three layers of tissue may be designated in the sclera but they are in no sense sharply delimited from each other. From without inward, these layers are:

1. Episcleral tissue.
2. Sclera proper.
3. Lamina fusca.

1. The episcleral tissue is composed of loose collagenous and elastic fibers. Superficially, it is continuous with the loose tissue of *Tenon's space*; inwardly it merges with the sclera proper; anteriorly, it attaches the conjunctiva to the sclera. It differs from the sclera in having a relatively large number of blood vessels.

2. The sclera proper is composed of a dense feltwork of collagenous fiber bundles running parallel to the surface (Fig. 436). Near the cornea and around the optic nerve, they are disposed chiefly in an equatorial direction; elsewhere, they cross and interlace with each other in complicated fashion. Each bundle of parallel collagenous fibers is oval in cross section, about ten or twelve times as broad as it is thick. Interspersed with the collagenous fibers, and particularly at the periphery of the bundle, are numerous delicate elastic fibers. The fixed cells of the sclera are found between the fiber bundles. They are flat connective tissue cells having protoplasmic processes which join with those of other cells to form a syncytium.

The tendons of the ocular muscles are similar to the sclera in structure except that the fiber bundles are all parallel and there are many thick elastic fibers. At their insertions, the tendons continue directly into the sclera, the tendon bundles spreading out and interweaving among those of the sclera. The boundary between tendon and sclera is marked only by the cessation of the thick elastic fibers.

3. The lamina fusca represents a zone of transition between the sclera and the choroid. Here the elastic fibers increase in number and thickness, the collagenous bundles become smaller and a number of branched pigment cells appear (Fig. 436). These are characteristics common to the adjacent suprachoroid, which is the outer layer of the choroid coat.

It has already been mentioned that the sclera itself has relatively few blood vessels. The vessels present merely pass through the sclera to reach the uveal tract. Lymphatics are absent. The nerve supply of the sclera is derived from branches of the ciliary nerves.

The Cornea

As has been stated, the cornea constitutes the anterior one-sixth of the eye and is more curved than the remainder of the bulb. Viewed from in front, the cornea appears slightly elliptical, with a horizontal diameter of 12 mm. and a vertical diameter of 11 mm. Viewed from behind, the cornea is circular, the difference in the two aspects being due to the fact that the sclera overlaps the anterior surface of the cornea above and below more than it does laterally and medially. At their junction is the aforementioned external scleral sulcus. The zone of transition between the two, known as the *limbus* of the cornea, is about one millimeter in width and has histological fea-

FIG. 437. Section through human cornea. (From a section by Dr. R. Castroviejo.)

tures differing from the remainder of the cornea.

Layers of the Cornea. Beginning at the front surface, the following five layers are seen in the cornea (Figs. 437 and 440):

1. Layer of epithelium.
2. Bowman's membrane.
3. Substantia propria.
4. Descemet's membrane.
5. Endothelium.

1. The corneal epithelium is of the stratified squamous variety, five or six cell layers in thickness (50–100 μ). The cells of the deepest layer (*basal* or *foot cells*) are columnar in shape, each cell presenting a flat surface toward Bowman's membrane and a rounded head toward the next cell layer. The nucleus is slightly oval, the long axis lying at right angles to the surface of the cornea.

The cells of the next layer—the *wing cells*—are polyhedral in shape, convex anteriorly and concave posteriorly. From the concave inner surfaces, which fit over the rounded heads of the basal cells, processes of the cells (the wings) extend out to pass between the basal cells. The nuclei of the wing cells are oval, with the long axis parallel to the corneal surface. The cytoplasm is slightly darker than that of the basal cells.

Although the cells of the remaining layers are also polyhedral in shape, they become much flatter and their nuclei more oval. This tendency is evident in the third layer and the succeeding layers continue to show the transition to the very flat cells of the surface layer.

The surface cells have a very large surface area but are quite thin. In the region of the nucleus, which is much

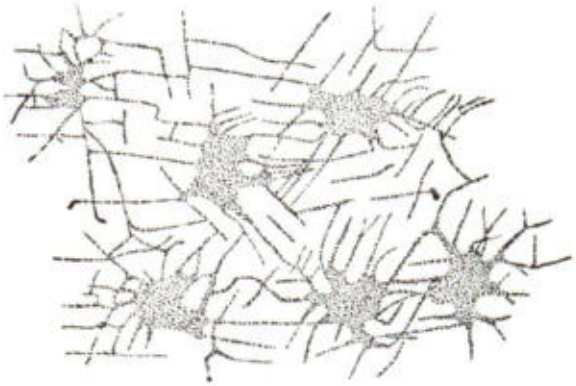

FIG. 438. Section of human cornea cut tangential to surface, showing corneal cells and their processes.

flattened, the cells are somewhat thicker but this thickening is always directed toward the deeper cell layers, leaving the outer surface of the cell perfectly flat. This accounts for the regularly smooth surface which is a feature of the cornea.

None of the cells of the corneal epithelium lose their nuclei, nor do they ever normally show keratinization. They adhere to one another by intercellular cement and by "intercellular bridges" as in the epidermis.

2. Bowman's membrane (the *anterior elastic membrane*) is a thin structureless membrane separating the epithelium and the substantia propria and ending abruptly at the limbus. The term "structureless" is here used in the sense that it appears to be completely homogeneous; neither fibers nor cell nuclei can be distinguished. Its anterior border, adjacent to the epithelium, is sharply defined. Posteriorly, it appears to merge with the superficial lamellae of the corneal stroma and may, indeed, be regarded as a modified portion of the latter. Bowman's membrane shows no regenerative capacity and is not a true elastic membrane.

3. The substantia propria (*corneal stroma*) forms about ninety per cent of the thickness of the cornea and is composed of connective tissue fibers and cells. The fibers, which constitute the predominant element, are arranged in very thin lamellae coursing parallel to the surface of the cornea. Each lamella is composed of delicate, straight, transparent, collagenous fibrillae. Isolated, such a lamella would appear as a broad thin band whose length equals the diameter of the cornea. Although the fibrillae composing any one lamella are arranged strictly parallel to one another, the different lamellae cross and interlace with each other at various angles. By special methods, fine elastic fibers may also be demonstrated.

Between layers of several such lamellae are found the cells of the corneal stroma. The fixed cells are connective tissue cells, very flat and thin, with flat nuclei and branching processes which make intimate contact with those of neighboring cells (Figs. 438, 439). In their arrangement, they resemble the osteocytes in a Haversian system of bone. They are also known as corneal corpuscles.

4. Descemet's membrane (*posterior elastic membrane*) is a true elastic membrane. Microscopically, it appears as a structureless highly refractile membrane, sharply delimited from the corneal stroma. It stains with elastic tissue stains,

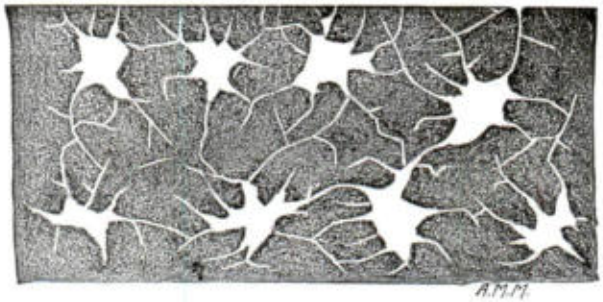

FIG. 439. Section of human cornea cut tangential to surface, showing anastomosing corneal cell spaces.

although not as intensely, for example, as the elastic membranes of arteries. Normally, Descemet's membrane is in a state of tension. Unlike Bowman's membrane, it shows the capacity to regenerate after injury.

5. The endothelium. The posterior surface of the cornea is covered by a single layer of cells which extend over the inner surface of Descemet's membrane. In cross section, the cells appear rectangular in shape, with oval nuclei. They resemble a low cuboidal epithelium more than they do the typical endothelium of blood and lymph vessels.

The cornea proper is entirely devoid of blood vessels, deriving its nutrition from the superficial marginal plexus of vessels, which will be discussed below.

It has a rich sensory nerve supply derived from the ophthalmic division of the trigeminal nerve. Small branches of this nerve enter at the periphery and branch extensively in the substantia propria as they course toward the surface and the center of the cornea. Near Bowman's membrane, this plexus proprius ends in a terminal net from which fibers, both individually and in bundles, pass as the corneal *rami perforantes* through pores in Bowman's membrane. Under the epithelium, they break up into finer branches which extend forward and terminate between the epithelial cells. Other nerve endings are found in the stroma and just under the epithelium at the limbus.

THE LIMBUS

Transitional between the cornea and the adjacent sclera and conjunctiva is a zone about 1 mm. wide known as the *limbus* (Fig. 440). The corneal epithelium, as it passes over into the limbus, increases in thickness up to ten or more cells. The surface cells retain the characteristics of those of the cornea, but the basal cells become smaller, with scanty cytoplasm and small dark-staining nuclei. Furthermore, the basal border of the epithelium becomes wavy in appearance. These changes are characteristic of the conjunctival epithelium with which the epithelium of the limbus is continuous.

The corneal stroma loses its regular lamellar arrangement, the fiber bundles assuming an irregular arrangement more like those of the sclera. Some elastic fibers are also found.

The only blood vessels which nourish the cornea are found in the limbus, where they form the superficial marginal plexus. These are in the superficial stroma and form a series of meridionally directed loops which extend to the border of Bowman's membrane.

Descemet's membrane and the corneal endothelium are continued over onto the

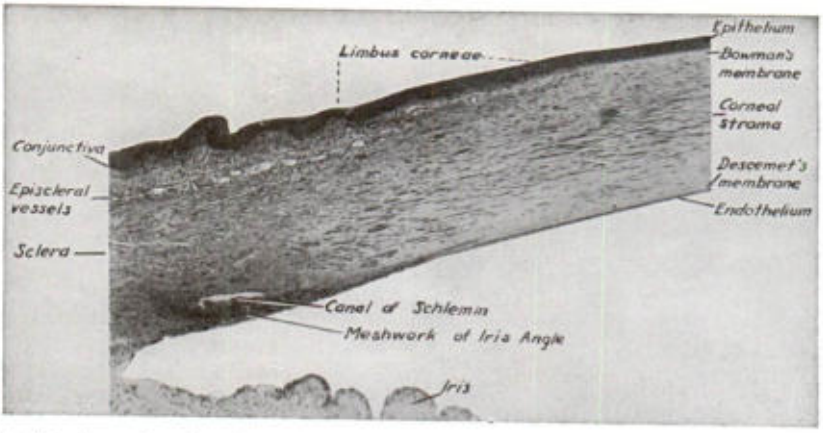

FIG. 440. Horizontal section through corneo-scleral junction of human eye, showing the cornea, limbus corneae and structures of the iris angle. (Photomicrograph.)

trabeculae of the scleral meshwork and the pectinate ligament (see p. 552).

Tunica Vasculosa (Uvea)

The tunica vasculosa comprises the *choroid*, *ciliary body* and *iris* and is characterized by the presence of numerous blood vessels and pigment cells. If this deeply pigmented coat be viewed after removal of the overlying sclera and cornea, it greatly resembles a grape (uva); hence the name *uvea*, or *uveal tract* is frequently applied.

The Choroid

The term choroid (chorioid) is derived from the resemblance of this layer, in vascularity, to the chorion of the fetus. It forms the posterior part of the uvea. Externally, it is separated from the sclera by a potential space, the *perichoroidal space*, across which delicate lamellae of the superficial layer of the choroid pass obliquely to blend with the lamina fusca of the sclera. The perichoroidal space, thus subdivided into numerous smaller but interconnected spaces, is limited anteriorly by the insertion of the ciliary muscle into the scleral spur. Posteriorly, it ends a short distance in front of the optic nerve. In life, this space is probably nonexistent; in the fixed specimen, it may appear (Fig. 442).

Internally, the choroid is intimately related to the pigment epithelium layer of the retina. When the retina is detached, this layer always remains firmly adherent to the choroid.

Layers of the Choroid. Histologically, the choroid (Figs. 436 and 447) may be divided into four layers, which from without inward are:

1. The suprachoroid.
2. The vessel layer.
3. The capillary layer.
4. The lamina vitrea.

1. Suprachoroid (*lamina suprachoroidea; epichoroid*). This, the superficial layer of the choroid, consists of delicate lamellae which course obliquely backward from choroid to sclera, bridging the perichoroidal space. Each lamella is composed of a very thin membrane showing occasional oval-shaped nuclei of fibroblasts. Supporting this is a plexus of elastic fibers which branch and cross each other in a reticular formation. In this meshwork are many chromatophores, large flat branching pigment cells with oval or irregular nuclei. The bodies and processes of these

cells are filled with black-brown pigment granules of melanin.

In the equatorial region and in front of it, smooth muscle fibers appear. Frequently, these are arranged in little star-shaped groups. The superchoroid is devoid of blood vessels.

When the choroid is stripped away from the sclera, this layer divides, one part remaining adherent to the lamina fusca of the sclera, the other to the choroid. In such preparations the torn lamellae of the latter portion account for the shaggy appearance of the external surface of the choroid.

2. The vessel layer (*stratum vasculosa*). This layer is sometimes regarded as being subdivided into an outer layer of large vessels (*Haller's layer*) and an inner layer of medium-sized vessels (*Sattler's layer*). Only in the thicker portions of the choroid can such a division be discerned. In the region of the fovea, only the smaller vessels are present; anterior to the equator, the small vessels merge with the capillary layer, leaving as a distinct layer only the large vessels.

The vessel layer consists of the blood vessels—arteries and veins—and a choroidal stroma. In their structure, the vessels conform to the description already given in the chapter on blood vessels. A characteristic of the veins, however, is their confluence to form four whorl-like patterns—the *vortices*. In each vortex, the veins unite in an ampulla from which a single vortex vein arises. The vortex veins (*venae vorticosae*) leave the eye through emissaria in the sclera, two superiorly and two inferiorly.

The choroidal stroma occupies the spaces between the vessels. Its lamellae resemble those of the suprachoroid, but the stellate chromatophores have longer and more slender processes, collagenous fibers appear and the elastic fibers become finer.

3. The capillary layer (*lamina choriocapillaris*), the only layer of the choroid which is not continued forward into the ciliary body, serves as the organ of nutrition for the outer layers of the retina. It consists of capillaries of wide bore arranged in a network disposed in one plane; i.e., the capillaries form a network in which the components are all in the same plane, rather than a feltwork. The capillaries themselves are unique in having lumina of sufficient width to accommodate several red blood corpuscles side by side, whereas in most parts of the body, capillaries are so narrow that the blood cells must pass through them in single file. Structurally they are simple endothelial tubes (Fig. 447).

The interspaces of the capillary net are filled in by a stroma of delicate collagenous and elastic fibrillae. Toward the vessel layer, pigment cells are lacking, connective tissue cells with vesicular nuclei are present and the stroma becomes continuous with that of the vessel layer. On the inner aspect of the capillary layer, a condensation of elastic fibrillae forms the outer lamella of the lamina vitrea.

4. Lamina vitrea (*lamina elastica, lamina basalis, membrane of Bruch*). This constitutes the innermost layer of the choroid and borders directly on the retinal pigment epithelium, which, indeed, contributes to its formation (Fig. 447). It is a noncellular membrane with a uniform thickness of 1 to 2 μ in which, under the most favorable conditions, two layers may be made out. The very thin *outer elastic lamella* has already been mentioned; the *inner cuticular lamella* is relatively thicker and appears homogeneous. It is considered to be a cuticular formation of the pigment epithelium.

Nerves of the Choroid. The ciliary nerves course in the perichoroidal space and give off fine branches which form plexuses in the suprachoroid and the

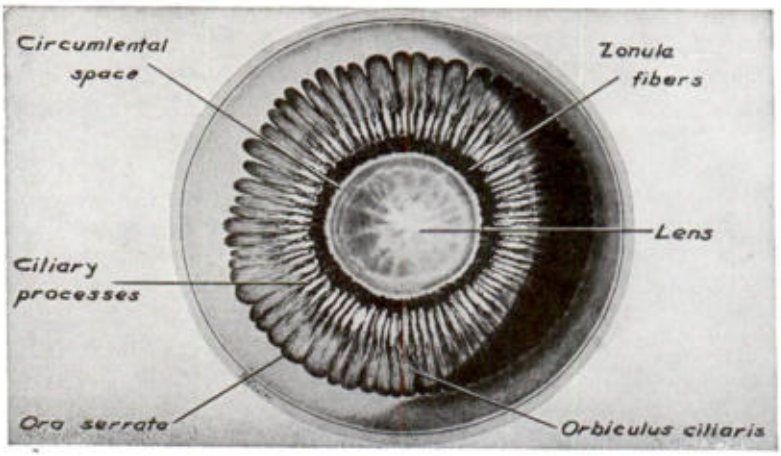

FIG. 441. Ora serrata, ciliary body, zonula ciliaris and lens as viewed from behind after removal of the vitreous body. (After Eisler.)

choroidal stroma. Multipolar ganglion cells associated with the plexuses are probably concerned with the sympathetic innervation of blood vessels.

Modifications of the Choroid. In certain of the teleost fishes a silvery membrane (*argentea*) is found between the suprachoroid and the vessel layer. It is formed by specialized cells containing crystals of guanine and extends into the iris, giving that membrane a characteristic silvery luster.

In most mammals, but not in man, a reflecting layer, the *tapetum lucidum*, is developed in the posterior region of the choroid. Lying between the choriocapillaris and the vessel layer, it consists either of several layers of flattened cells or several layers of fine fibers. The *cellular tapetum* is found in carnivores; the *fibrous tapetum*, in herbivores.

THE CILIARY BODY

The choroid extends anteriorly as far as the *ora serrata*, which is the anterior margin of the nervous portion of the retina (Fig. 453). In front of the ora serrata, the uveal tract is thickened to form the *ciliary body*. In its entirety, this forms a ring to which the suspensory ligament of the lens is attached and from which the iris takes origin. In meridional section, the ciliary body is triangular in shape, with its apex continuous behind with the choroid (Fig. 442). Its outer surface is separated from the sclera by the perichoroidal space. Its inner surface faces the vitreous body and lens, and shows a modification into two zones, which may be identified macroscopically (Fig. 441). The posterior two thirds appears darkly pigmented and is relatively smooth. This is the *orbiculus ciliaris*, or *pars plana*. The anterior third of the inner surface bears some 70 to 80 radially arranged ridges, the *ciliary processes*; this region is the *corona ciliaris* (*pars plicata*). The ciliary processes are pale in contrast to the darkly pigmented valleys between them.

The anterior surface or base of the ciliary body, from which the iris arises, faces toward the center of the cornea. Its outer edge is attached to the scleral spur. The greater part of the anterior surface is obscured by the overlying *meshwork of the iris angle*.

Layers of the Ciliary Body. In its

histological structure, the ciliary body represents a forward continuation of all the elements of the choroid except the capillary layer, plus certain layers continued over from the retina as the *pars ciliaris retinae*. Thus, from without inward, we find:

1. Suprachoroid and ciliary muscle.
2. Vessel layer.
3. Lamina vitrea.
4. Pigment epithelium } Pars ciliaris
5. Ciliary epithelium } retinae.
6. Internal limiting membrane.

1. The suprachoroid and ciliary muscle. Reference has already been made to the presence of smooth muscle fibers in the suprachoroid. In the ciliary body, this smooth muscle increases in amount so that the bundles of fibers form a smooth muscle mass of very appreciable bulk (Fig. 442). According to the directions in which they are disposed, we may distinguish three sets of fibers in the ciliary muscle:

a. The meridional fibers (longitudinal fibers, *Brücke's muscle*) are the outermost ones. They begin in the relatively sparse muscle stars of the suprachoroid in front of the equator and, increasing in amount, form bundles which run anteriorly to insert into the scleral spur (Fig. 446). Among the bundles of fibers are seen the pigmented suprachoroidal lamellae.

b. The radial fibers lie internal to the meridional fibers. They present a reticulated appearance because of the more pronounced character of the connective tissue stroma, which anteriorly is continuous with the meshwork of the iris angle.

c. The circular fibers (*Müller's muscle*) are continuous with the radial fibers and lie at the inner edge of the ciliary body. These fibers course in a circular direction around the ciliary body just posterior to the root of the iris. Immediately anterior to them is the *circulus arteriosus iridis major*, from which comes the arterial supply of the iris and ciliary processes.

The blood vessels of the ciliary muscle run in the interstitial tissue and are of small caliber. The arteries are branches of the long posterior ciliary and anterior ciliary arteries.

The ciliary muscle is innervated by parasympathetic fibers of the oculomotor nerve. Preganglionic fibers reach the ciliary ganglion by way of the short motor root; the postganglionic fibers are the axons of ganglion cells in the ciliary ganglion and course to the ciliary muscle through the short ciliary nerves. These postganglionic fibers are unique in that they are medullated, an exception to the general rule that postganglionic fibers are nonmedullated.

The action of the ciliary muscle is to relax the tension on the suspensory ligament of the lens, thus allowing the lens to assume a more convex form (see p. 572).

2. The vessel layer is similar in structure to that of the choroid, with the exception that there are fewer chromatophores and more collagenous fibers. Unlike the choroid, however, the vessels consist for the most part of veins, a condition brought about by the fact that the arteries supplying this region enter through the perichoroidal space and the ciliary muscle, leaving the vessel layer as the almost exclusive avenue of the returning veins. These drain the blood from the iris as well as the ciliary body and empty into the venous vortices of the choroid. The anterior part of the ciliary body is drained by the anterior ciliary veins.

In the orbiculus ciliaris, the vessels are arranged almost in a single layer. Further forward in the corona ciliaris, the vessels are disposed in several layers. The ciliary processes are formed by localized thickenings of the vessel layer (Fig. 443).

3. The lamina vitrea is a continuation

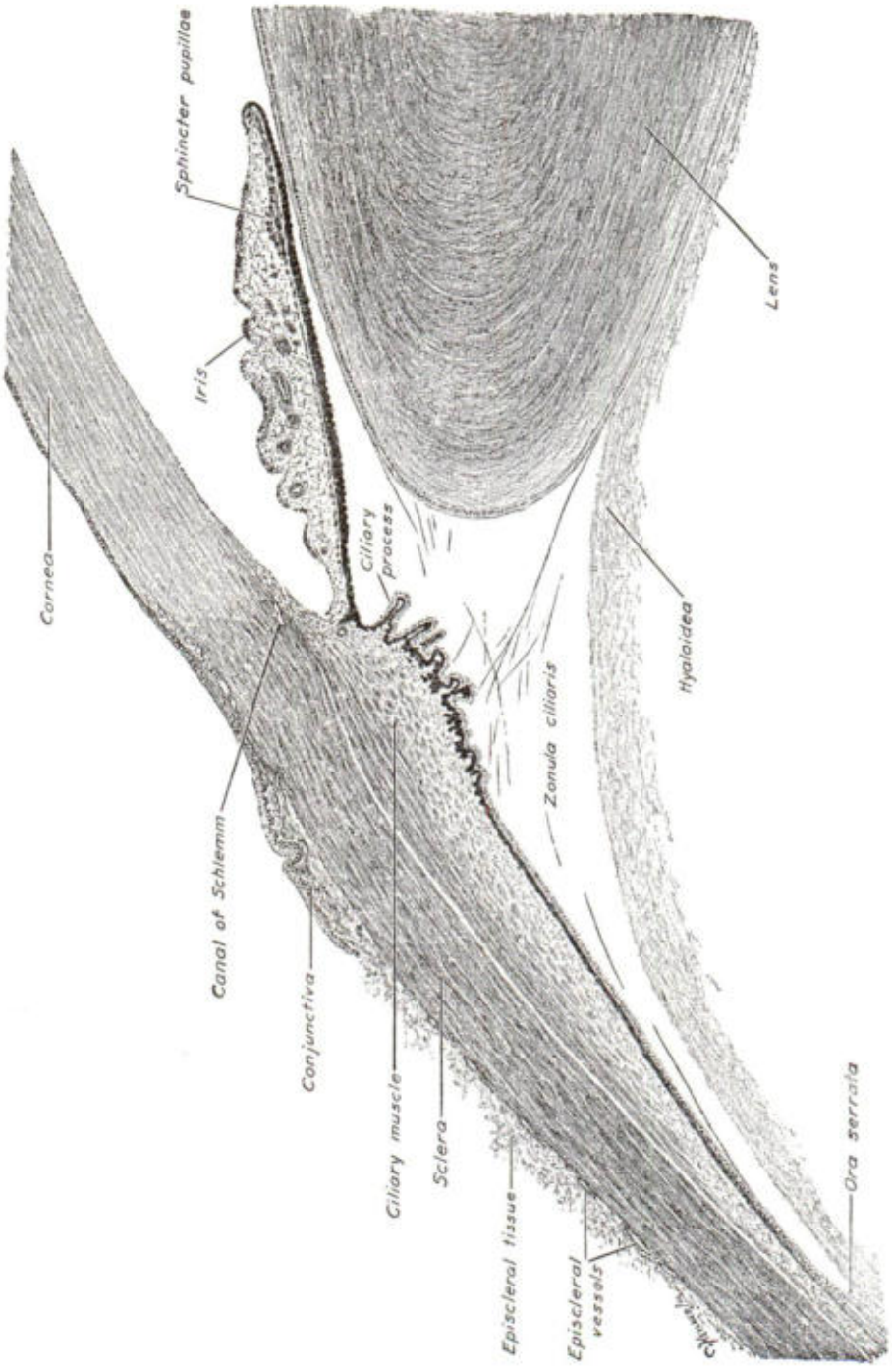

FIG. 442. Horizontal meridional section through anterior portion of human eye

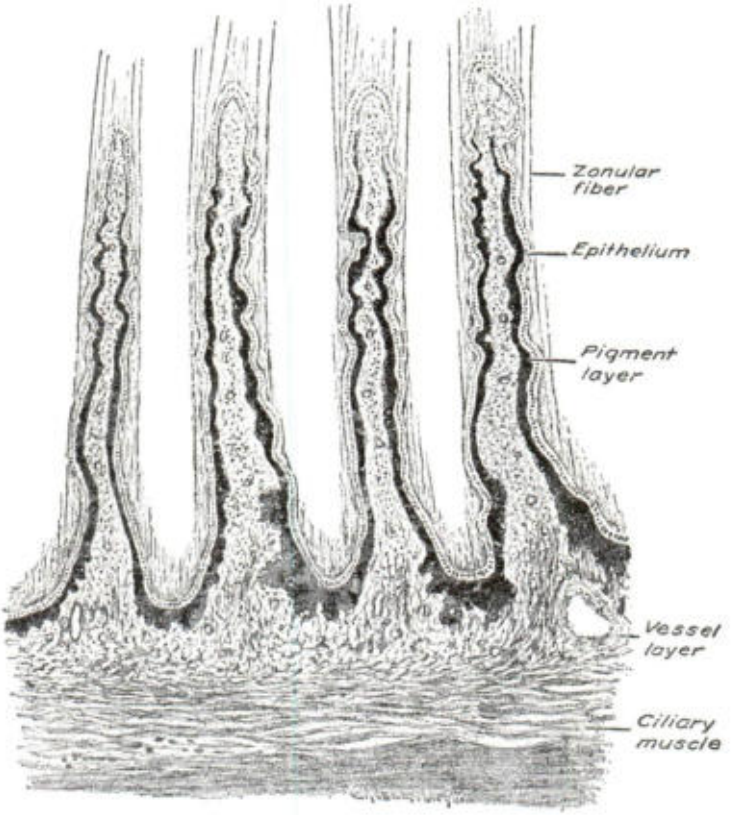

FIG. 443. Equatorial section through corona ciliaris of human eye

of the same structure in the choroid and thus is made up of an outer elastic lamella and an inner cuticular lamella. However, in the ciliary region a third layer, the interlamellar or intermediate connective tissue layer, is interposed between the two. The elastic lamella fades out at about the middle of the corona ciliaris; the collagenous fibers of the intermediate layer then merge with the stroma of the vessel layer. The cuticular lamella, on the other hand, is continuous to the root of the iris. Its surface shows a netlike arrangement of ridges, the reticulum of Müller.

4. The pigment epithelium is a forward continuation of the pigment epithelium layer of the retina. It is composed of a single layer of columnar cells resting on the cuticular lamella. The cells are so heavily filled with round melanin granules that cell borders cannot be distinguished, except on the summits of the ciliary processes, where the cells are more cuboidal and have less pigment (Fig. 443). This accounts for the characteristic white appearance of the ciliary processes.

5. The ciliary epithelium represents a continuation of the sensory portion of the retina. Over the inner surface of the ciliary body, this becomes a single layer of columnar epithelial cells, unpigmented except in the region of the iris root, where they acquire increasing amounts of pig-

ment and continue over the back surface of the iris as its pigment epithelium. Over the summits of the ciliary processes, the cells tend to become cuboidal in shape. Throughout, they are firmly joined to the underlying layer.

6. The internal limiting membrane is a very thin structureless membrane on the inner side of the ciliary epithelium. It is lacking on the posterior portions of the orbiculus.

The Iris

The iris is a thin circular diaphragm placed directly in front of the lens and having a circular aperture, the pupil, located slightly to the nasal side of its center. Its peripheral border (*ciliary border*, *iris root*) is attached to the anterior surface of the ciliary body (Fig. 446); its *pupillary border* rests on the lens. The iris, therefore, does not have the shape of a flat curtain but inclines forward from the ciliary body. Its shape is thus that of a low truncated cone. The iris divides the space between the cornea and the lens into two chambers which communicate through the pupil. In front of it is the *anterior chamber* of the eye; between it and the lens is the *posterior chamber*.

The anterior surface of the iris shows a division into two regions, an inner *pupillary zone* and an outer *ciliary zone*, which differ in structure and frequently also in color. The irregular circular line (about 1.5 mm. from the pupillary margin) which forms the junction between these two zones is known variously as the *collarette*, *iris frill*, or *angular line*. It marks the position of an underlying system of arterio-venous anastomoses, the *circulus vasculosus iridis minor*.

The pupillary zone is radially striated and near the collarette shows a number of depressions, the *pupillary crypts*, which may also occur in the neighboring part of the ciliary zone. At the pupillary margin is a dark border which represents the inner limit of the posterior pigmented epithelium.

The ciliary zone is marked by a series of fine radial striations formed by the blood vessels. In its outer half are a number of concentric circular furrows, the *contraction furrows*. Near the ciliary border, the *ciliary crypts* are seen. These are smaller than the pupillary ones.

Layers of the Iris. Like the ciliary body, the structural elements of the iris are derived from both the tunica vasculosa and the tunica interna. The following five layers can be distinguished:

1. Endothelium.
2. Anterior border layer.
3. Vessel layer (stroma).
4. Dilatator pupillae.
5. Pigment epithelium.

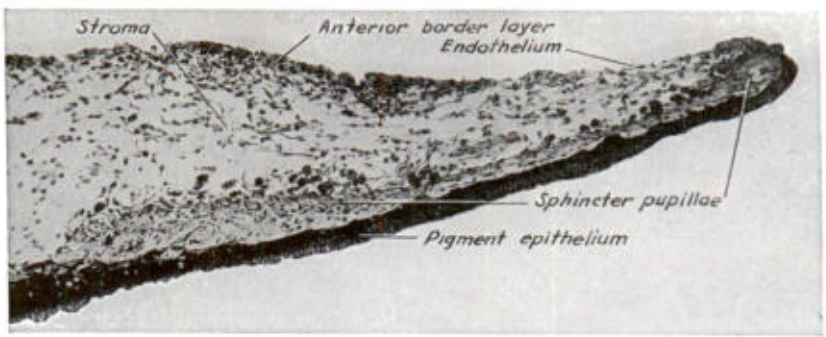

Fig. 444. Horizontal section through pupillary zone of human iris. (Photomicrograph)

The first three layers form the uveal portion of the iris. The latter two represent a forward continuation of the tunica interna and constitute the *pars iridica retinae*.

1. The endothelium covering the anterior surface of the iris is a very thin delicate layer which is difficult to demonstrate in sections (Fig. 446). The cell borders are best shown in surface views after treatment of the tissue with silver nitrate. The endothelium is lacking over the crypts.

2. The anterior border layer (Fig. 444) lies immediately under the endothelium and is the layer which determines the color of the iris. It is essentially a condensation of the iris stroma but has fewer collagenous fibers and no blood vessels. It is formed principally of *chromatophores*—branched connective tissue cells containing granules of yellowish-brown pigment. This layer is lacking over the crypts and is thin on the contraction furrows.

The color of the iris depends upon the thickness of the anterior border layer and the degree of pigmentation of its cells. In the brown iris, the layer is thick and the cells heavily pigmented. In the blue iris, the layer is thin and there is a minimum amount of pigment. Light striking it, therefore, will pass through this layer and the underlying stroma and be reflected from the darkly pigmented posterior epithelium. The reflected light appears blue. Since the pigmentation of the border layer and stroma in the white races is acquired chiefly in the first few years of life, it follows that in these races all eyes are blue at birth.

3. The vessel layer consists of a great number of blood vessels imbedded in a loose stroma of delicate collagenous fibrillae, with some elastic fibers and a number of stroma cells. Most of the latter are pigmented (chromatophores).

The arteries run inward in a radial direction from the greater circle (circulus arteriosus iridis major; see p. 555) and anastomose in the region of the collarette to form the lesser circle of the iris (circulus vasculosus iridis minor). The blood vessels of the iris possess a very much thickened adventitia; in the arteries the muscularis is thin and the intima poorly developed.

In the stroma of the pupillary zone there is a circular band of smooth muscle fibers, the *sphincter pupillae* (Fig. 444). This encircles the pupil and on contraction reduces the diameter of the opening. It is of ectodermal origin, derived from the pigment epithelium layer by a transformation of epithelial cells into smooth muscle fibers.

4. The dilatator pupillae (Fig. 446) is of similar origin but in this case the transformation is incomplete and the cells retain a double character (Fig. 445, *B*). The spindle-shaped cell body, with its oval nucleus, is moderately pigmented and has an epithelial character; from either end of the cell there extends a fiber-like process which is unpigmented and has the staining properties of smooth muscle. These "myoepithelial" cells, disposed between the iris stroma and posterior epithelium in a single sheet, thus give the appearance in section of a double membrane (Fig. 445, *A*). The anterior portion, bordering the stroma and composed of the radially directed fiber processes, has been called the *posterior border lamella* (*Bruch's* or *Henle's membrane*). The posterior portion, consisting of the pigmented cell bodies, forms the *anterior pigment layer* (*layer of pigmented spindle cells*). This and the pigment epithelium layer are often spoken of as the posterior epithelium of the iris.

In the region of the sphincter pupillae, the dilatator fibers are united to those of the sphincter. At the pupillary border,

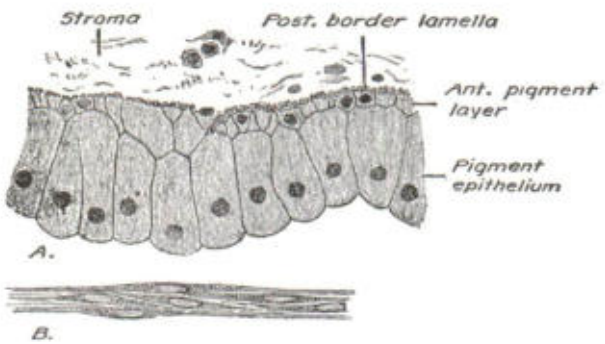

FIG. 445. *A*. Ectodermal layers of posterior surface of the iris. Transverse section, bleached. *B*. Surface view of elements of dilatator pupillae in middle of ciliary zone of the iris. Teased preparation. (Redrawn from Salzmann.)

the cells take on wholly the character of epithelium. Contraction of the dilatator muscle results in dilation of the pupil.

Together with the ciliary muscle, the sphincter and dilatator pupillae comprise the intrinsic muscles of the eye. The sphincter is innervated by parasympathetic fibers of the oculomotor nerve. These follow the same course as those described for the ciliary muscle (see p. 555). The dilatator pupillae is innervated by the sympathetic division of the autonomic nervous system. The preganglionic fibers arise from cell bodies in the *cilio-spinal center of Budge*, located in the upper three thoracic segments of the spinal cord. These fibers course to the sympathetic chain by way of their respective white communicating rami and continue upward to terminate in the superior cervical ganglion. Here are located the cells of the nonmedullated postganglionic fibers, which travel in the internal carotid nerve to the cavernous plexus, thence to the Gasserian ganglion and ophthalmic division of the trigeminal nerve, and finally by way of the nasociliary nerve and long ciliary nerves to reach the dilatator muscle.

5. The pigment epithelium. It has already been mentioned that the ciliary epithelium as it nears the root of the iris acquires increasing amounts of pigment. From the ciliary body, it is continued over the posterior surface of the iris as its pigment epithelium (Figs. 444 and 446). So densely pigmented is this layer that neither cell boundaries nor nuclei can be distinguished. If the pigment be bleached out, however, the cells can be seen as columnar or prismatic elements with round nuclei (Fig. 445, *A*). At the pupil, this layer bends slightly forward around the pupillary border as the *pigment seam* and becomes continuous with the epithelial cells of the anterior pigment layer.

THE IRIS ANGLE

The lateral borders of the anterior chamber have, in meridional section, an angular shape. This angle, known as the iris angle or the angle of the anterior chamber, is occupied by a loose spongy tissue, the *meshwork of the iris angle*. In section this tissue has the form of a triangle which fills in the scleral furrow and extends behind to the ciliary body (Figs. 440 and 446). The apex of the triangle is continuous with Descemet's membrane and the posterior lamellae of the cornea. Its base unites with the scleral spur and the anterior surface of the ciliary body; its outer border adjoins the tissues of the adjacent sclera and cornea and

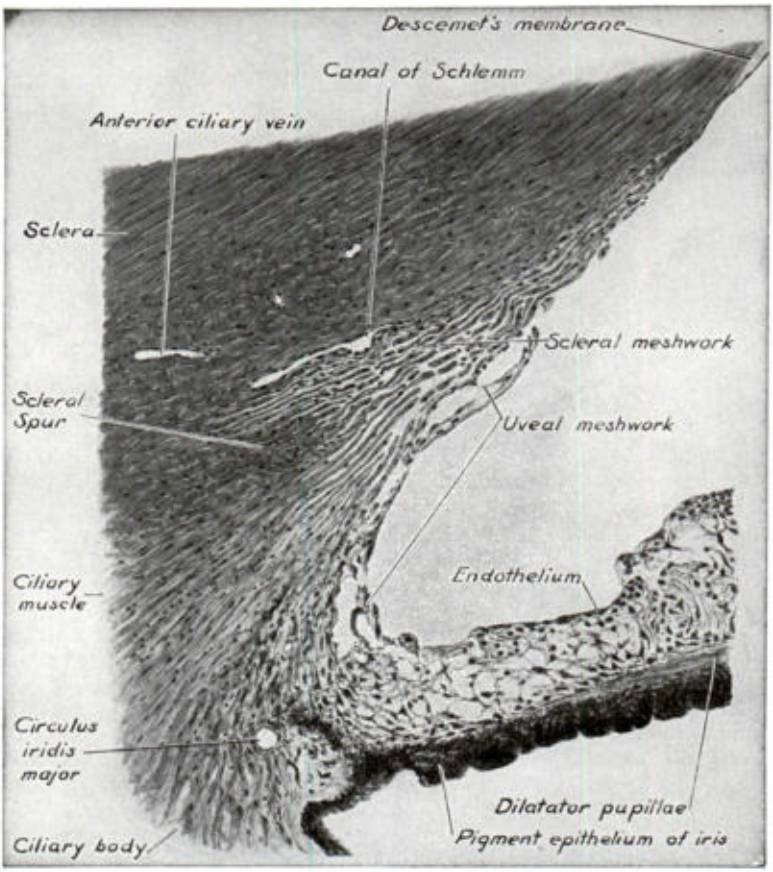

FIG. 446. Details of the iris angle. (Drawn from the same section as Figure 442)

forms the inner wall of the canal of Schlemm; its inner border bounds the anterior chamber.

The major portion of the tissue is known as the *scleral meshwork*. It is composed of a number of intercrossing flat trabeculae, each of which is made up of four elements. An inner core of collagenous fibrillae is covered by elastic fibers, outside of which is a thin homogeneous membrane having the same staining qualities as Descemet's membrane; it appears to be a continuation of the latter. The surface of a trabeculum is covered by a thin endothelium recognizable by its large oval nuclei. This layer is continuous with the endothelium of the cornea and the iris. The spaces of the meshwork are known as the *spaces of Fontana* and are in communication with the anterior chamber.

Along the inner border of the scleral meshwork, a thin surface layer of trabeculae forms the *uveal meshwork*, or *pectinate ligament*. Here the trabeculae are round instead of flat and they lack elastic fibers. At the root of the iris, their collagenous fibrillae blend with the stroma of the iris and their endothelial covering becomes continuous with that of the iris.

The *canal of Schlemm* also lies in the scleral furrow, close to its bottom. In meridional sections, it appears as one or

more endothelial lined oval spaces, just in front of the scleral spur and adjacent to the meshwork of the iris angle (Figs. 440, 442, and 446). Actually, it encircles the eye as a canal which irregularly divides into two or more branches which recombine only to divide again. It communicates peripherally by means of 20 to 30 small branches with the anterior ciliary veins in the neighboring scleral tissue (Fig. 446). It has no direct communication with the anterior chamber.

Together with the meshwork of the angle, Schlemm's canal forms a means of exit from the eye for the intraocular fluid. The aqueous humour passes readily through the spaces of Fontana and into the canal, although there are no direct openings in the latter. Particulate matter is caught by the trabeculae of the meshwork. Under normal conditions, only aqueous humour is found in Schlemm's canal. It has been suggested that the action of the ciliary muscle on the scleral spur provides a pumping mechanism for the canal.

The Retina

The retina forms the *pars optica retinae* of the tunica interna and is the part of the eye which transforms the stimulus of light into nerve impulses, resulting in the sensation of vision. Thus it might be considered that all other parts of the eye serve the purpose of assisting the retina in the proper performance of its highly specialized function.

Embryologically, the retina rises from the two layers of ectoderm which form the optic cup. The outer layer gives rise

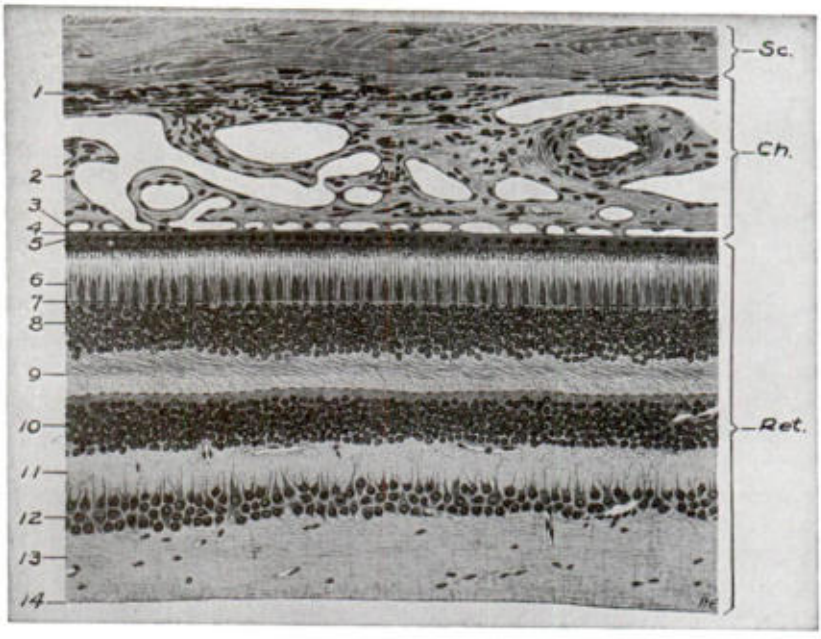

FIG. 447. Section through retina, choroid and sclera. *Sc*, Sclera; *Ch*, choroid, *Ret*, retina; *1*, suprachoroid; *2*, vessel layer; *3*, choriocapillaris; *4*, lamina vitrea; *5*, pigment epithelium; *6*, layer of rods and cones; *7*, external limiting membrane; *8*, outer nuclear layer; *9*, outer plexiform layer; *10*, inner nuclear layer; *11*, inner plexiform layer; *12*, ganglion cell layer; *13*, nerve fiber layer; *14*, internal limiting membrane. (After Eisler, from a preparation by Stieve.)

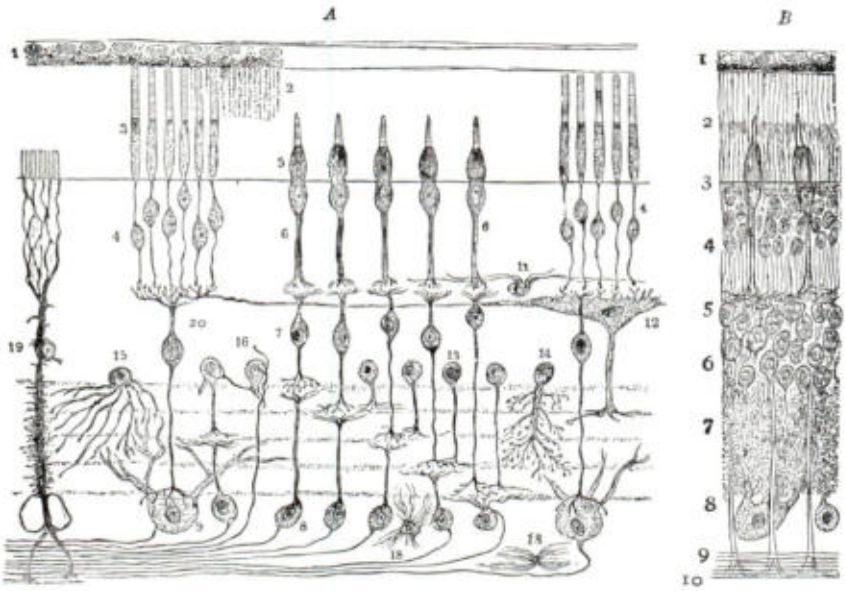

FIG. 448. *A*. Scheme of retina as shown by Golgi method. *B*. Section of retina to show layers as demonstrated by hematoxylin-eosin method.

A:—*1*, Pigment epithelium; *2*, processes of pigment epithelium cells; *3*, rods; *4*, rod nuclei and fibers; *5*, cones; *6*, cone fibers; *7*, cone bipolar cells of inner nuclear layer; *8*, smaller cells of ganglion cell layer; *9*, larger ganglion cells; *10*, ganglion cell axons forming nerve fiber layer; *11* and *12*, horizontal cells; *13*, *14*, *15* and *16*, amacrine cells; *18*, neuroglia cells; *19*, Müller's fiber; *20*, rod bipolar cell of inner nuclear layer.

B:—*1*, Pigment epithelium; *2*, layer of rods and cones; *3*, external limiting membrane; *4*, outer nuclear layer; *5*, outer plexiform layer; *6*, inner nuclear layer; *7*, inner plexiform layer; *8*, layer of ganglion cells; *9*, nerve fiber layer; *10*, internal limiting membrane.

to the pigment epithelium; the inner layer forms the remainder of the retina. In pathological detachment of the retina, or as generally occurs in fixed and preserved specimens, the primary embryonic cavity between the two layers becomes reformed and the two parts of the retina separate, the pigment epithelium remaining adherent to the choroid. The strictly nervous portion is firmly attached to underlying structures at only two regions—at its scalloped anterior margin, the ora serrata (Figs. 442 and 453) and at the optic disc, where the nerve fibers pass through the wall of the bulb to form the optic nerve (Fig. 454). Detached from the pigment epithelium, it is a thin delicate membrane which in life is transparent.

Layers of the Retina. Except at the optic disc (optic papilla), the fovea centralis and the extreme periphery, the retina consists of ten layers (Figs. 447 and 448), which, from without inward, are arranged as follows:

1. Pigment epithelium.
2. Layer of rods and cones.
3. External limiting membrane.
4. Outer nuclear (or granular) layer.
5. Outer plexiform (or molecular) layer.
6. Inner nuclear (or granular) layer.
7. Inner plexiform (or molecular) layer.
8. Ganglion cell layer.
9. Nerve fiber layer.
10. Internal limiting membrane.

1. The pigment epithelium is a single layer of cells, about half as high as

they are broad and hexagonal in surface view. The outer portion of the cell (i.e., toward the choroid) contains the spherical nucleus and only a moderate amount of round pigment granules. The inner portion is filled with dark needle-shaped pigment granules, which also extend into fine cytoplasmic processes arising from the inner surface of the cell and reaching down between the outer members of the visual cells. The pigment is a melanin known as *fuscin*.

A modification of the pigment epithelium is seen in the alligator, where, in the dorsal quadrant of the retina, the pigment epithelial cells are heavily laden with crystals of guanine, leaving only an inner fringe of melanin granules. This arrangement constitues a *retinal tapetum*.

In certain animals (e.g., frog) the position of the pigment granules is determined by the illumination of the eye. In the dark or in low light intensities, the pigment is condensed in the cell body and the cell processes are devoid of it. In bright light, the pigment granules stream out into the cell processes and form a protective dark curtain, as it were, around the rod outer segments. This phenomenon of pigment migration has not been demonstrated in mammals, including man.

The pigment epithelium is intimately related to the cuticular lamella of the choroid. It is only loosely attached to the rest of the retina.

The nature and significance of the remaining layers of the retina will be better understood if it is realized that the stratification depends upon the location of three sets of neurons and their relation to each other (Fig. 448, *A*). These neurons, together with certain supportive elements of ectodermal origin, make up the bulk of the retina. Considering them in the order of their conduction of a nervous impulse, we find that the first neuron is represented by the photoreceptors, the rod and cone cells. These form not only the layer of that name but also the outer nuclear layer, which consists of the nuclei and much reduced cell bodies of these neurons. From each cell a fiber is continued inward in the outer plexiform layer, where it makes a synaptic junction with the dendrite of the second neuron, the bipolar cell. The nuclei of the bipolar cells lie in the inner nuclear layer; their axons pass inward in the inner plexiform layer, where they effect synapse with the dendrites of the ganglion cells. The relatively large cell bodies of the latter form the ganglion cell layer; their long axons course in the nerve fiber layer to the optic disc, where all the axons converge to form the optic nerve. These fibers are continuous to the brain, where the great majority end in the lateral geniculate body, from which another neuron system carries the nerve impulse to the visual areas in the occipital cortex.

From the above brief description, it is evident that the retina consists mainly of layers of nerve cells alternating regularly with layers formed by their processes. It is further evident that the true percipient elements, the rods and cones, lie furthest removed and turned away from the light stimulus, which to affect them must first pass through all the intervening layers (except at the fovea). Also, the nervous pathway at first travels directly toward the source of the stimulus before turning to course inward to the brain. Thus the retina is an upside-down sense organ. This *inverted* retina is characteristic of all vertebrates.

In addition to the nerve cells, certain supportive elements, the *supporting fibers of Müller*, contribute to the formation of the several layers. They will be described later.

Further consideration will now be given to the individual layers of the retina.

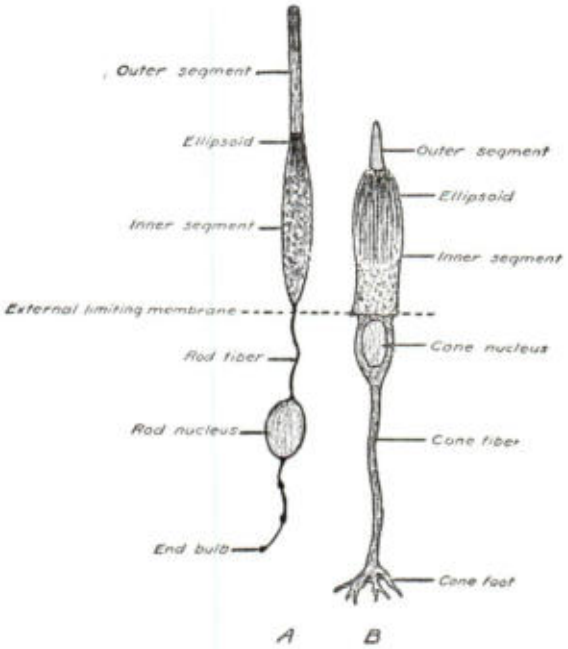

FIG. 449. Human rod (*A*) and cone (*B*). (After Greeff)

2. The layer of rods and cones lies between the external limiting membrane and the pigment epithelium, facing the latter. The rods and cones, which are the outer light sensitive portions of the rod and cone visual cells, are arranged in parallel fashion, perpendicular to the surface, and under low powers present the appearance of cilia.

THE RODS. These are long slender cylindrical elements, 40 to 60 μ in length and 2 μ in diameter. Each rod shows a division into an *outer* and an *inner segment* (Fig. 449, *A*). The outer segment is the longer and more slender of the two and is highly refractile. It shows delicate longitudinal furrows on its surface and may have transverse striations; possibly the latter are artifacts.

The inner segment of the rod is slightly thicker, finely granular, and stains more deeply. At its outer end is a longitudinal arrangement of fibrils, the *fiber apparatus* or *ellipsoid*. From its inner end a slender filament passes as the *rod fiber* through the external limiting membrane to the outer nuclear layer. Here it enlarges to form the *rod granule* which contains the nucleus, and then is continued as a fiber into the outer plexiform layer. The fiber terminates here in a small knob-like ending, the *end bulb*.

If a retina be freshly removed from an eye which has been kept in the dark, it will, upon examination in subdued light, appear purplish-red in color. On exposure to light, the color will rapidly fade. This color is due to the presence in the rod outer segments of a substance called *visual purple*, or *rhodopsin*. It is a substance intimately bound up with the function of the rods, which are primarily concerned with vision under conditions of low light intensities. In the light, visual purple bleaches; it regenerates in the dark.

THE CONES. Except in the fovea centralis (see p. 568) the cones are bottle or flask-shaped members which also consist of outer and inner segments (Fig. 449, *B*). The outer segment is the smaller and is conical in shape. The inner segment has a bulbous form. Its structure is similar to that of the rod inner segment but the ellipsoid is much larger. The inner segment is continuous with the *cone fiber*, which passes through the external limiting membrane with a diameter hardly less than that of the inner segment. Just under this membrane, as the *cone granule*, it contains the cone nucleus and then is continued as a relatively stout fiber into the outer plexiform layer. Here it ends in an expansion with numerous short lateral branches—the *cone foot*.

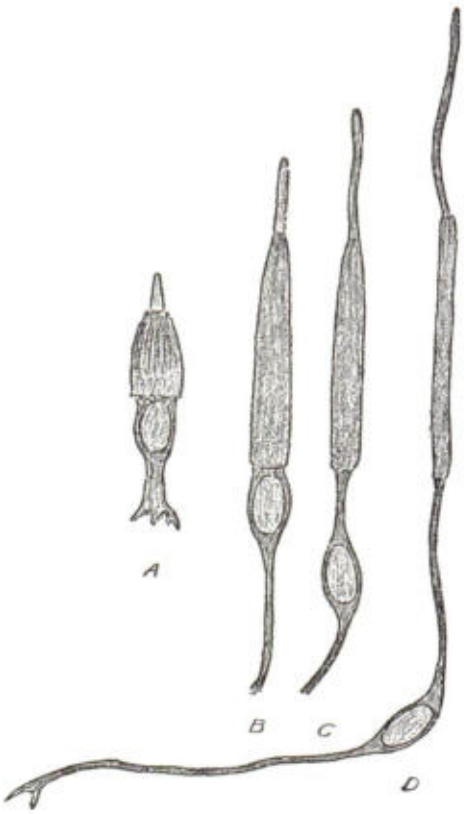

FIG. 450. Cones from different areas of the human retina. *A*, From near the ora serrata; *B*, from periphery of macula lutea; *C*, from the macula lutea; *D*, from the fovea centralis. (After Greeff.)

The cones present a great variation in their form (Fig. 450). In the fovea, they are very long and shaped like rods. They do not, however, contain rhodopsin. In the peripheral regions, the cones are shorter and broader, so that at the ora serrata the cones are but 25 μ long, with conical outer segments measuring only 6 μ in length. These peripheral cones attain diameters up to 7.5 μ. Cones have a higher threshold of sitmulation than rods and are concerned with vision under conditions of higher intensities of illumination. They are the instruments of color vision.

3. External limiting membrane. This is a thin sieve-like membrane formed by the chief supporting elements of the retina—Müller's fibers.

4. The outer nuclear layer appears to consist solely of the nuclei of the visual cells. However, a delicate mantle of cytoplasm can be made out around each nucleus. The rod nuclei are smaller and round and stain intensely. The cone nuclei are slightly larger, oval in shape, and stain less intensely. As has been stated, the latter are situated close to the external limiting membrane.

5. The outer plexiform layer is a reticular meshwork formed by the terminal fibers of the rods and cones and the arborizations of the dendrites of the bipolar cells. The fibers of several rods are here related to the dendritic ending of one bipolar cell. In the case of the cones, however, a single cone fiber is associated with a single bipolar cell.

6. The inner nuclear layer is thinner than the outer nuclear layer but is similar in appearance. It contains the nuclei of the bipolar cells, certain association neurons, and the nuclei of Müller's sup-

porting fibers. The bipolar nuclei are surrounded by a thin layer of cytoplasm. The association neurons are the *horizontal cells* and the *amacrine cells*; they serve to interconnect various regions of the retina. The nuclei of Müller's fibers have an appreciable amount of cytoplasm around them. In general, the nuclei of this layer are arranged in three zones: an outer one of horizontal cell nuclei, a middle one of bipolar cell nuclei and an inner one in which the nuclei of the amacrine cells predominate.

7. Inner plexiform layer. This consists of the processes of the amacrine cells, the axons of the bipolar cells and the profusely branched dendrites of the ganglion cells.

8. The ganglion cell layer is composed of a single layer of typical multipolar ganglion cells, among which are scattered neuroglia cells. Branches of the retinal blood vessels are also present. The ganglion cells are variable in size (11 to 30 μ) with clear round nuclei containing one or more prominent nucleoli. The cell bodies possess Nissl granules.

9. The nerve fiber layer consists of the axons of the ganglion cells. These nonmedullated fibers are arranged in bundles which run parallel to the surface of the retina and converge at the optic disc to form the optic nerve. Between the bundles are numerous fibrous neuroglia cells (*spider cells*) and rows of Müller's fibers. Also present in this layer are the retinal blood vessels.

10. The internal limiting membrane is a thin membrane of hyaline nature, formed by apposition of the expanded bases of Müller's fibers. It separates the retina from the vitreous body.

In the above description, frequent references to the supporting (or radial) fibers of Müller have been made. These fibers form the chief framework of the retina. They are elongated elements of ectodermal origin which course outward through all the retinal layers and give off numerous processes (Fig. 451). Their cell bodies and nuclei lie in about the middle of the inner nuclear layer. Their expanded bases form the internal limiting membrane and at their outer ends they fuse to form the external limiting membrane. In the nuclear and plexiform layers, they give off numerous processes which form a neuroglial meshwork supporting the nervous elements. In the nerve fiber layer and the ganglion cell layer, they very seldom give off processes and are here most readily distinguishable.

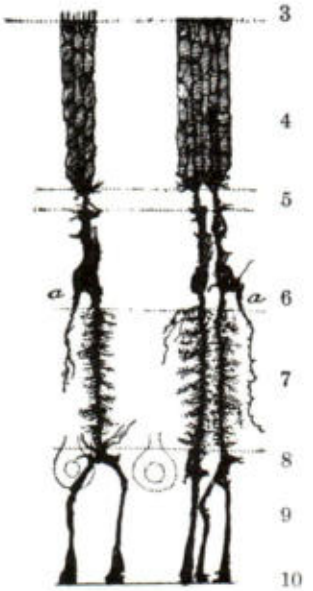

FIG. 451. Two Müller's fibers from retina of ox, showing relation to layers of the retina. *3*, External limiting membrane; *4*, outer nuclear layer; *5*, outer plexiform layer; *6*, inner nuclear layer; *7*, inner plexiform layer; *8*, layer of ganglion cells; *9*, nerve fiber layer; *10*, internal limiting membrane. (After Cajal.)

Blood Vessels of the Retina. The layer of rods and cones, the outer nuclear layer and the outer plexiform layer are

devoid of blood vessels. Their nourishment comes from the choriocapillaris of the choroid. The remaining layers of the retina are supplied by a system of retinal vessels derived from the central retinal artery, which enters the eye in the optic nerve. The larger arteries lie in the nerve fiber layer, with finer branches in the ganglion cell and inner plexiform layers. Two capillary networks are formed, one in the nerve fiber layer and another which extends as far as the outer border of the inner nuclear layer. The retinal veins follow the course of the arteries.

The Macula Lutea and Fovea Centralis. Very close to the posterior pole, the retina undergoes a localized modification of its layers to form a funnel shaped depression. In this area, called the macula lutea because of its yellow color, the inner layers of the retina are spread apart, leaving in the center a small pit, the fovea centralis (Fig. 452). Here the retina is reduced to a layer composed entirely of closely packed, long, slender cones (Fig. 450, *D*). In the center of the fovea centralis, in an area which is about 0.5 mm. in diameter, the cones are only 2.5 μ in thickness. Their length is variously given as 60 μ to 85 μ. Although they more closely resemble the rods in shape, their cone nature is established by the absence of visual purple and by the fact that they terminate in cone feet rather than in the end bulbs characteristic of rods. Their long thin cone fibers course obliquely from the cone nuclei in the floor of the fovea towards the thickened layer of bipolar cells in the margin. The ganglion cell layer is also much thicker in the macular region.

The fovea centralis is the region of greatest visual acuity and serves the function of central vision. The fact that the cones are extremely slender, making it possible for more cones to occupy this region, may partly explain the greater visual acuity of the fovea. The spreading apart of the inner retinal layers to allow the light to reach the photoreceptors unobscured and the absence of capillaries from the center of the fovea may also be contributing factors.

Outside the foveal region, rods appear among the cones and increase in number until three or four rods intervene between individual cones. Near the ora serrata, the rods and cones become shorter and broader and there is a relative increase in the number of cones. It has been estimated that there are 7,000,000 cones in the retina; of this number, 13,000 are said to be in the macular region and 4,000 in the fovea centralis. Estimates of the number of rods vary from 75,000,000 to 170,000,000.

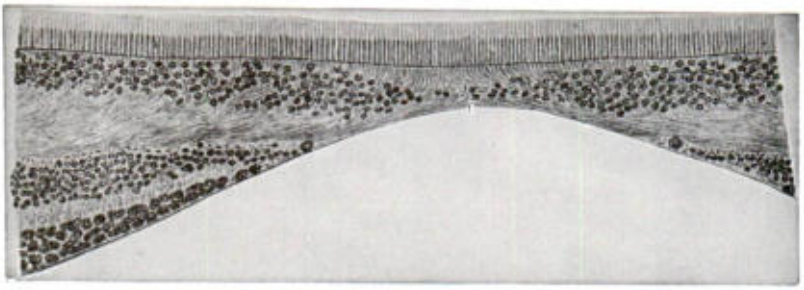

FIG. 452. Section through center of fovea centralis. Note that the outer nuclear layer is represented in the fovea by only a few scattered cells. (After Eisler.)

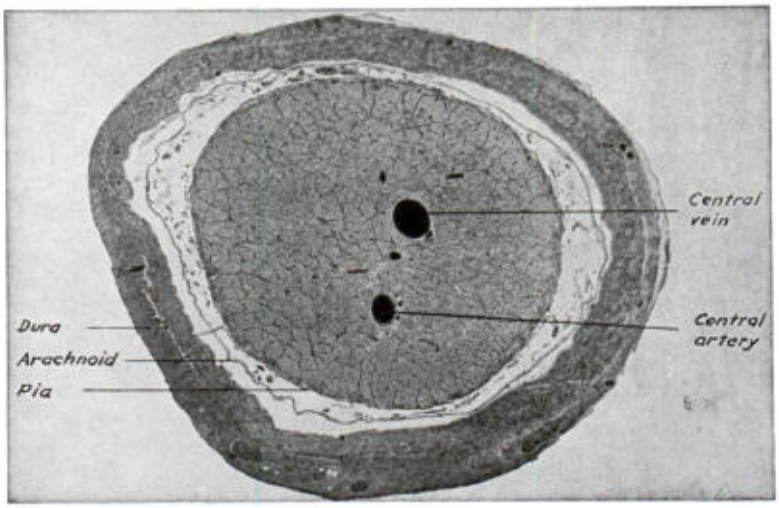

FIG. 455. Cross section of human optic nerve. (Photomicrograph)

be found in younger persons. The cysts are always confined between the external and internal limiting membranes.

The Optic Nerve

The nerve fibers of the retina converge at the optic disc and turn outward to pass through the sieve-like *lamina cribrosa* of the sclera as the optic nerve (Fig. 454). In this intraocular portion, the nerve has a structure similar to that of the nerve fiber layer; i.e., bundles of nonmedullated fibers surrounded by neuroglia cells.

Immediately behind the lamina cribrosa, the nerve fibers acquire myelin sheaths, with a consequent increase in diameter of the entire nerve. Since the optic nerve actually is a tract of the brain rather than a true peripheral nerve, it is not surprising that it should resemble the brain in its coverings and its histological characteristics. Although the fibers are myelinated, they do not possess neurilemma sheaths, in which respect they are like nerve fibers within the brain.

A cross section of the medullated orbital portion of the optic nerve (Fig. 455) shows it surrounded by three connective tissue sheaths: an outer *dura*, an inner *pia* and an intermediate *arachnoid*, which divides the *intervaginal space* into a *subdural space* and a *subarachnoid space*. These sheaths are continuous with the corresponding meninges of the brain; at the bulb, they become continuous with the sclera.

From the pial sheath, connective tissue trabeculae pass into the nerve and together with the neuroglial elements form a system of septa which enclose groups of nerve fiber bundles. Between the bundles are glia cells whose processes and fibers penetrate between the individual nerve fibers.

In the anterior part of the nerve, for a distance of about a half inch behind the eye, the septa are united to a central core of connective tissue, the *central supporting tissue strand*. This carries the *central artery* and *central vein* of the retina. Small branches from these vessels course in the septal system to supply the nerve itself.

The Lens

Structure of the Lens. The lens (Fig. 456) is a transparent and somewhat

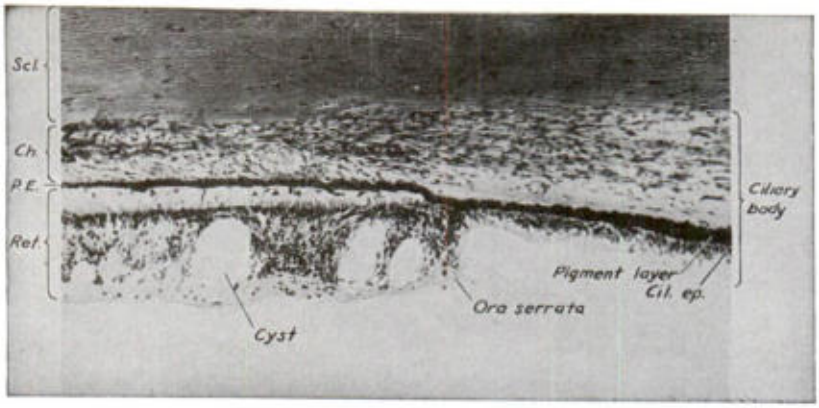

FIG. 453. Section of human retina at the ora serrata. *Scl.*, Sclera; *Ch.*, choroid; *Ret.*, retina; *Cil. ep.*, ciliary epithelium. (Photomicrograph.)

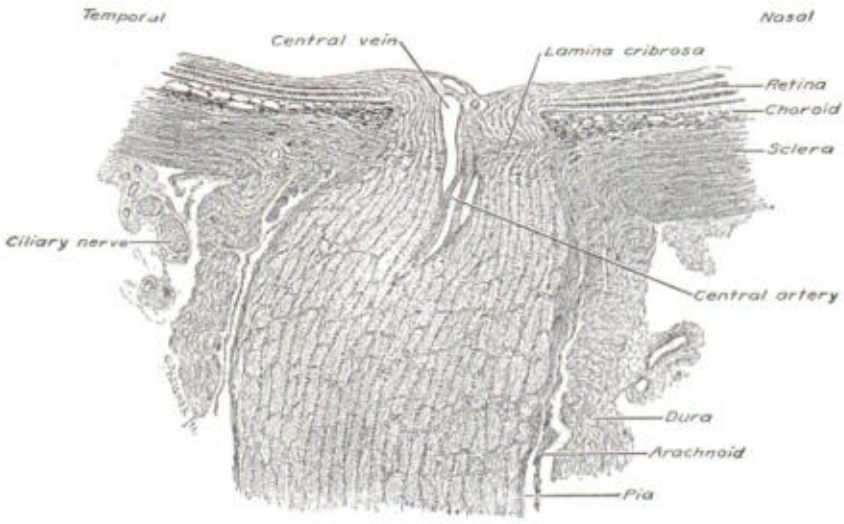

FIG. 454. Horizontal section of human optic nerve head

The Ora Serrata. The scalloped anterior border of the retina is known as the ora serrata (Fig. 453). Here the retina ends abruptly, its margin forming a step which may be rounded, angular, or even overhanging. Approaching this region, the rods and cones become shorter and thicker (Fig. 450, *A*), the nuclear layers become thinner and the ganglion cell and nerve fiber layers cease altogether. There is a corresponding increase in the number of neuroglia cells and Müller's fibers.

At or close to the ora serrata, there are frequently found in the retina cystic spaces of various sizes (Blessig's cysts). This condition, known as peripheral cystoid degeneration (Iwanoff's retinal edema), is regarded as physiological. Although most marked in old people, it is not necessarily a senile change and may

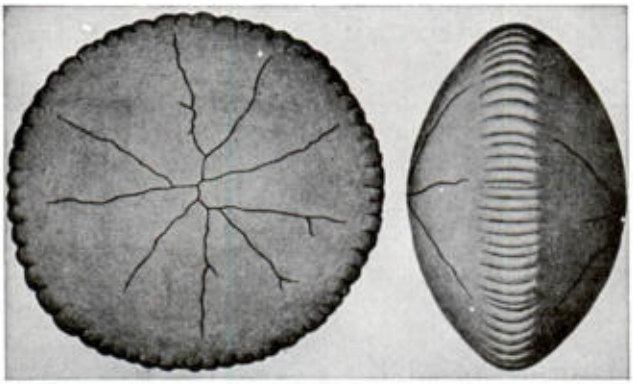

FIG. 456. The lens, viewed from behind and from the side. (From Eisler, after Rabl).

FIG. 457. From section through margin of lens, showing longitudinal sections of lens fibers and transition from epithelium to lens fibers. (Merkel-Henle.) *a*, Lens fibers; *b*, capsule; *c*, epithelium.

plastic biconvex body situated between the iris and the vitreous body. Its posterior surface has a greater convexity than the anterior surface (Fig. 435). Three structural components make up the lens:

1. The capsule.
2. The anterior epithelium.
3. The lens substance.

1. The capsule is a structureless highly elastic membrane ensheathing the lens. It is of varied thickness in different parts of the lens but is always thinnest at the posterior pole. On either surface a zone concentric with the equator serves for the insertion of the zonular fibers of the suspensory ligament (Fig. 459).

2. The anterior epithelium is a single layer of cuboidal cells on the anterior lens surface, just under the capsule. There is no posterior epithelium, these cells having gone to form the primitive lens fibers during embryonic development. At the equator, or margin, the cells are elongated and arranged meridionally in rows (Fig. 457). This is the region where new lens fibers are constantly being formed and the cells themselves may be regarded as young lens fibers.

3. The lens substance consists of long flat prismatic elements, the *lens fibers*, united by an amorphous cement substance. The primitive fibers formed from the embryonic posterior epithelium were directed straight from front to back in the lens. Succeeding fibers were formed superficially by a growth and metamorphosis of the epithelial cells at the equator, a process which continues throughout life. Consequently, these lens fibers come to be arranged meridionally in layers; the lens

substance, therefore, consists of concentric lamellae of fibers. The older and deeper fibers lose their nuclei. The amorphous cement substance which binds the lens fibers together is slightly more abundant where the ends of the lens fibers meet each other. It therefore forms a *central strand* in the axis between anterior and posterior poles of the lens, and from this a series of branching partitions which divide the lens fibers into sectors. These lines of separation, the *lens sutures*, are evident on the anterior and posterior lens surfaces as a star-shaped figure, the *lens star*.

On the basis of their consistency, we differentiate between a softer superficial *cortex* and a harder central lens *nucleus*.

The Zonula Ciliaris

The *zonula ciliaris* (*zonule of Zinn*, *suspensory ligament*) is a system of delicate homogeneous fibers which form a fairly thick band radiating from the equatorial zone of the lens capsule to the inner surface of the ciliary body, thereby fixing the lens in place (Fig. 442). Many of the fibers arise from the orbiculus ciliaris and sweep forward over the surface of the ciliary body to the lens capsule. Others come from the corona ciliaris. They arise in the valleys between the ciliary processes (Fig. 443) and are closely applied to the sides of the latter as they course radially inward to the lens.

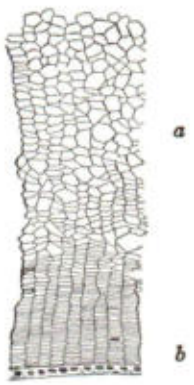

FIG. 458. From cross section of lens, showing transverse sections of lens fibers and epithelium. (Merkel-Henle.) *a*, Lens fibers; *b*, epithelium.

The zonular fibers are inserted on the lens capsule in two main zones—one in front of the equator and the other just behind it. The fibers which insert on the anterior capsule are the thicker ones.

Recalling the arrangement of the smooth muscle fibers of the ciliary muscle and its attachment anteriorly to the scleral spur, it becomes evident that contraction of this muscle will cause the ciliary body and choroid to be pulled forward, while the ciliary processes will at the same time be displaced toward the equator of the lens. The result of both actions will be to relax the tension normally maintained on the zonular fibers. The highly elastic lens capsule, in turn released from this tension, is thus enabled to mould the plastic lens cortex to a more spherical, or convex, form.

This constitutes the process of accommodation, by which images of near objects are brought to correct focus on the retina. It should be noted that in a state of rest, the ciliary muscle is relaxed and the zonula ciliaris and lens are under tension.

The Vitreous Body

The vitreous body occupies the space between the lens and the retina. (This space is not the posterior chamber.) In the fresh condition, the vitreous is a transparent firm jelly-like body which is particularly adherent to the retina in the region of the ora serrata and at the optic disc. On its anterior surface is a broad shallow depression, the *patellar fossa*, which accommodates the posterior convexity of the lens. Through its axis, from the optic disc to the patellar fossa, runs the *hyaloid canal*, which marks the site of the fetal hyaloid artery. In fixed prepa-

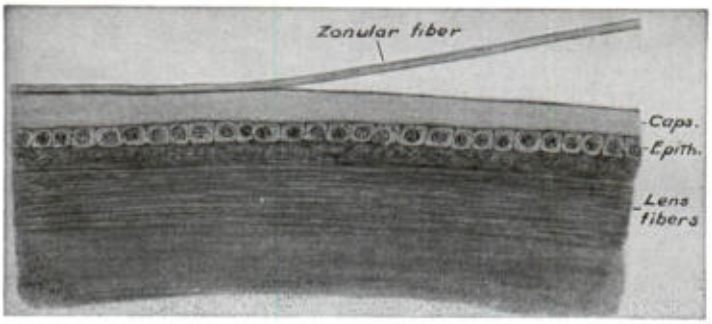

FIG. 459. Portion of anterior surface of equatorial zone of the lens, showing attachment of zonular fiber to the capsule. *Caps.*, Anterior capsule of lens; *Epith.*, lens epithelium.

rations, the vitreous appears to have a fibrillar structure. The peripheral condensation of the vitreous seen in such sections is considered by some authorities to be a surrounding membrane, the *hyaloidea*.

The Eyelids

The eyelids are essentially movable folds of skin which protect the eye both from injury and from excessive light. Each lid is covered by a thin skin, which on the posterior surface is modified to form a transparent mucous membrane, the *conjunctiva*. This lines the lid as the *palpebral conjunctiva* and is reflected on to the anterior surface of the eye up to the cornea as the *bulbar conjunctiva*. The reflection forms a deep recess known as the *fornix*. The form of the lid is maintained by a tough fibrous *tarsal plate*. In the connective tissue between this and the anterior surface are the palpebral fibers of the *orbicularis oculi muscle*. Associated with the free margin of the lid are the *eyelashes*, or *cilia*, and certain small glands. The relation of these structures to each other is best seen in a vertical section of the upper lid as shown in Figure 460. The following description applies to the upper lid but the lower lid is similar in all main respects.

The skin is very thin. It is provided with many fine downy hairs, with which are associated small sebaceous glands. Numerous small sweat glands and pigment cells are also present. The subcutaneous layer is a loose connective tissue, rich in elastic fibers but containing no fat. It is loosely adherent to the underlying muscle.

The *orbicularis oculi* is a thin oval sheet of skeletal muscle which covers the lids. It is innervated by the facial nerve and its action is to close the eye by bringing the lids together. The muscle bundles are disposed concentrically and are loosely united to the underlying tarsal plate by the submuscular connective tissue. Tendon fibers of the superior levator palpebrae muscle pass through the latter tissue, either to insert on the lower anterior surface of the tarsal plate or to attach to the skin of the lid. This muscle acts to raise the lid.

The *tarsal plate*, or *tarsus*, is a curved plate of dense white fibrous connective tissue with some elastic fibers. Its lower free border extends to the lid margin; to its upper border is attached the involuntary *superior palpebral muscle* (*muscle of Müller*). Embedded in the tarsal plate are a number of simple branched alveolar glands, the tarsal or *Meibomian glands*. These sebaceous glands are arranged in a single row with their long axes perpendicular to the lid margin.

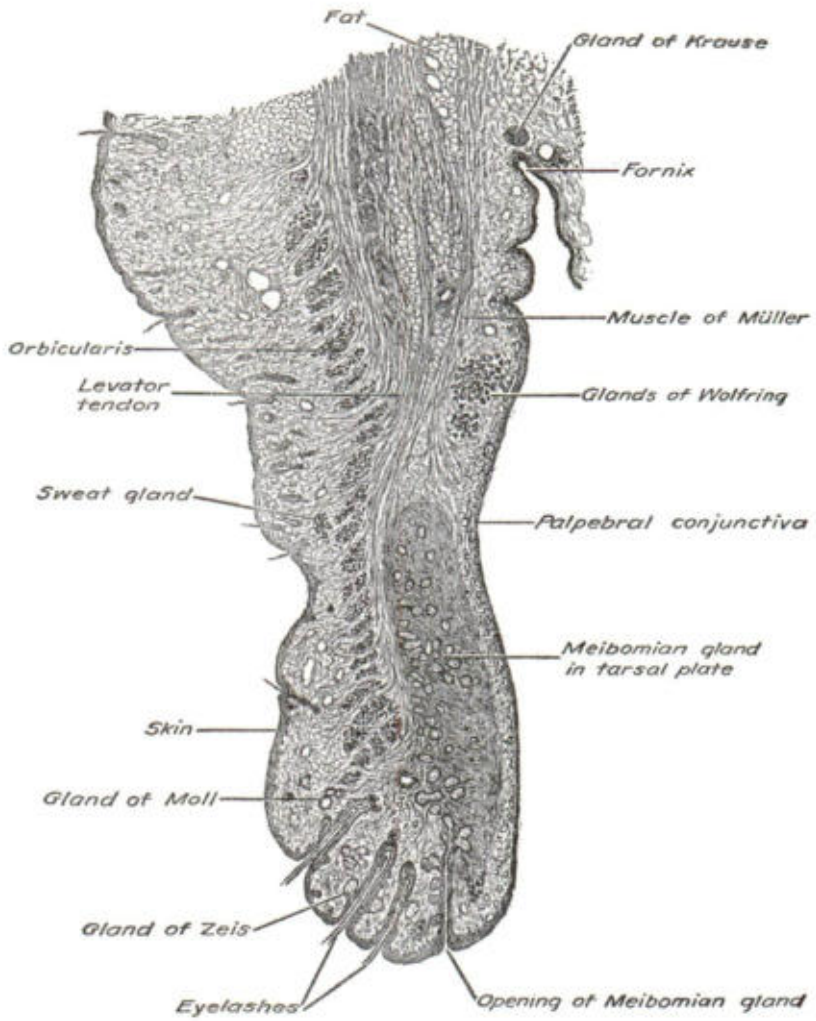

FIG. 460. Vertical section through human upper lid

Each Meibomian gland consists of a long straight central duct surrounded by numerous alveoli which open into it. The ducts are lined by simple cuboidal epithelium and open on to the lid margin by a series of minute orifices. The fatty secretion of these glands lubricates the edges of the lids, preventing them from sticking together and also helping to form a water-tight seal when the lids are closed.

In the lid margin are the eyelashes, or cilia, arranged in two or three irregular rows. They are short heavy curved hairs. Their follicles extend obliquely up to the tarsal plate and show the structure typical of hair follicles elsewhere in the body, with the exception that they lack erector

muscles. The large sebaceous glands associated with them are known as the *glands of Zeis*. Between the follicles are large spiral sweat glands, the *glands of Moll*.

The conjunctiva consists of an epithelium and a connective tissue substantia propria. At the lid margin, the epithelium has the stratified squamous character of the epidermis with which it is continuous. Over the tarsal plates (palpebral conjunctiva), it becomes reduced to two layers of cells; the surface cells are tall columnar and the deeper ones, flat cuboidal. In the region of the fornix a third layer of cells appears. Toward the limbus, the epithelium of the bulbar conjunctiva gradually increases in thickness, the superficial cells becoming flatter and the deep cells more cuboidal. At the limbus it is again stratified squamous. Throughout the conjunctiva, but particularly in the fornix and bulbar region, goblet cells occur in the epithelium.

The substantia propria consists of a thin layer of fine connective tissue fibers in which a profuse infiltration of lymphocytes occurs. This is especially marked in the region between the upper border of the tarsal plate and the fornix. Over the tarsal plate, the substantia propria firmly ties down the epithelium; elsewhere, it merges with the richly elastic subconjunctival connective tissue.

In the subconjunctival tissue above the upper border of the tarsal plate are several small tubulo-alveolar glands, the *glands of Wolfring*. Their ducts open on the surface of the conjunctiva.

The *glands of Krause* are small ac-

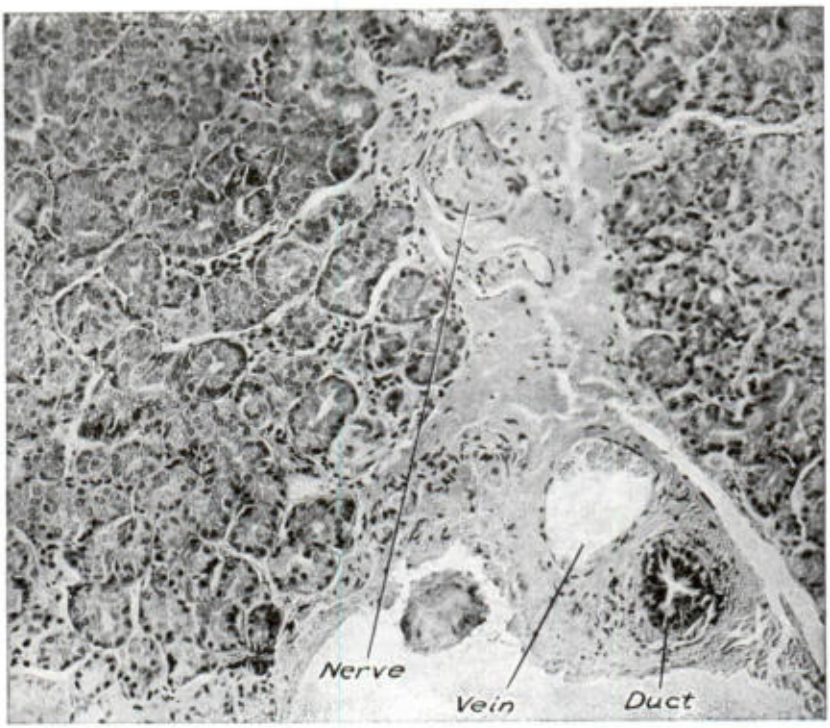

FIG. 461. Human lacrimal gland. (Photomicrograph)

cessory lacrimal glands which lie in the loose connective tissue beneath the fornix conjunctivae. Their ducts open into the margin of the fornix.

The Lacrimal Gland

The lacrimal gland lies in the superior temporal region of the orbit, just within the orbital margin. It is divided into a *superior* and an *inferior lobe*, which are continuous around the lateral horn of the aponeurosis of the levator muscle. It secretes the tears, which empty into the conjunctival sac through ten or twelve ducts opening just in front of the superior fornix.

The lacrimal is a compound tubuloalveolar gland of serous type (Fig. 461). It bears considerable resemblance to the parotid gland, differing slightly in that the secretory cells of its terminal alveoli are more columnar in form. Between them and the basement membrane are numerous basal or basket cells (see p. 363). The smaller excretory ducts are lined by a single layer of cuboidal cells; the larger ducts have a double-layered epithelium.

The stroma consists of loose connective tissue which blends peripherally with the surrounding structures. In the adult, considerable lymphoid tissue occurs in the stroma.

THE EAR

The organ of hearing comprises the *external ear*, the *middle ear* and the *internal ear* (Fig. 462).

The external ear consists of the auricle, or pinna, and the external auditory meatus, a canal which leads into the temporal bone and ends at the tympanic membrane. The latter forms the lateral wall of the tympanic cavity, which, with the auditory (Eustachian) tube, comprises the middle ear. The tympanic cavity lies within the temporal bone and contains the chain of auditory ossicles, or ear bones, with which are associated two small muscles. The internal ear consists of a complex arrangement of fluid-filled membranous chambers and canals (the membranous labyrinth) which are interconnected and lie within similarly shaped cavities in the temporal bone (the osseous labyrinth). The internal ear is related to the middle ear by means of two small apertures in the medial wall of the middle ear. One of these, the oval window, houses the base of the stapes, the smallest of the auditory ossicles. The other, the round window, is closed by a membrane, the secondary tympanic membrane. Within the membranous labyrinth are certain localized areas of neuroepithelium, which comprise the true sensory mechanism and in which are the endings of the auditory nerve.

The external ear serves to receive the sound vibrations from the air, the middle ear transmits the vibrations, and the internal ear transforms them into nervous impulses which are conducted to the brain by the fibers of the auditory nerve. In addition, certain parts of the internal ear serve as proprioceptive organs concerned with equilibration.

The External Ear

The *auricle* or *pinna* consists of an irregularly shaped plate of elastic cartilage covered by connective tissue and skin. The cartilage is richly cellular; its perichondrium contains many elastic fibers. The skin covering the auricle is typical thin skin. On the anterior concave surface, it is firmly united to the subjacent perichondrium. On the posterior convex surface, there is a subcutaneous layer.

Fine hairs and numerous large sebaceous glands are present in the skin of the auricle. On its posterior surface, sweat glands also occur.

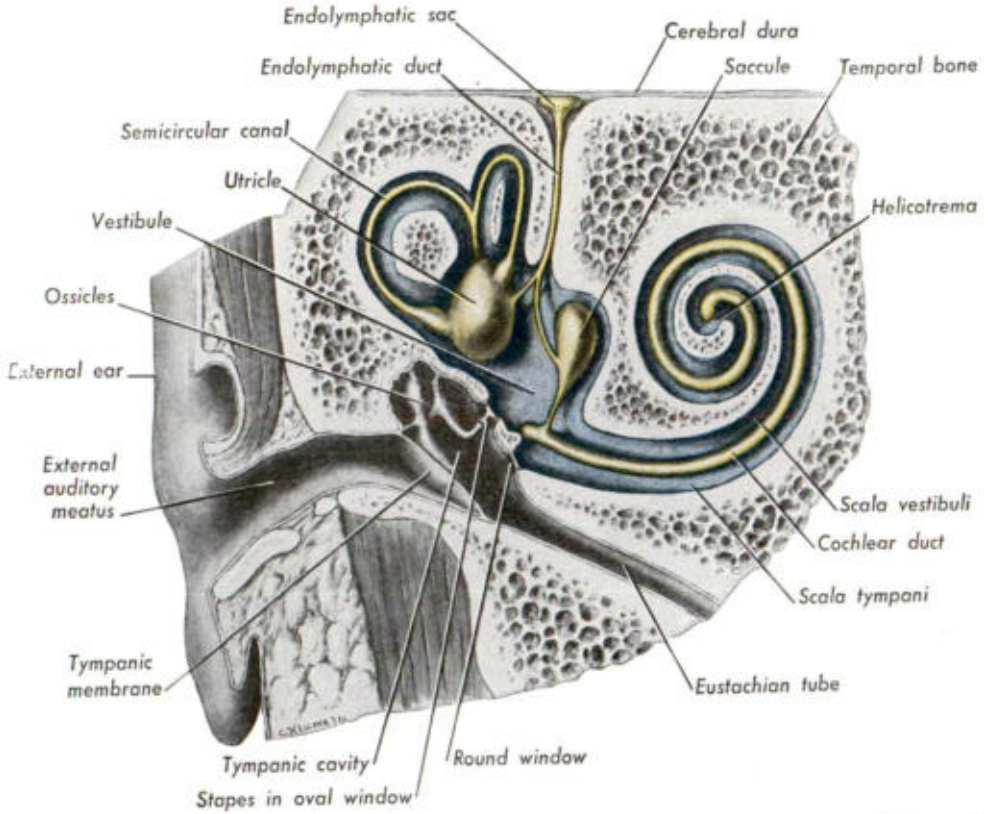

FIG. 462. Diagrammatic drawing to show the relationship of the structures of the external ear, middle ear and internal ear. No attempt has been made to maintain correct proportions. The perilymph within the osseous labyrinth is shown in blue; the endolymph within the membranous labyrinth is represented in yellow.

The cartilage does not extend into the *lobule*, which is composed of connective tissue and fat.

The *external auditory meatus* is a slightly S-shaped canal leading from the auricle to the middle ear and separated from the latter by the tympanic membrane. It consists of an outer *cartilaginous part* and an inner *bony part*. In the outer portion, its wall is formed by elastic cartilage, continuous with that of the auricle. The wall of the inner portion is formed by the temporal bone. In both parts, the wall of the meatus is lined by skin which is continuous with the skin of the auricle.

In the cartilaginous portion of the meatus, the skin is thicker and contains large stiff hairs, which guard against the entrance of foreign objects. Associated with them are large sebaceous glands. Another type of gland, the *ceruminous gland*, is peculiar to the skin of the meatus. These are simple coiled tubular glands which open upon the surface of the skin by long narrow ducts. The secretory cells are columnar and contain numerous brown pigment granules and fat droplets. The secretion of these glands, plus that of the sebaceous glands and desquamated epithelial cells, constitutes the ear wax, or *cerumen*.

In the bony portion of the meatus, the skin is thinner and adheres closely to the periosteum. The hairs are small and fine and, together with the sebaceous glands, are found only along the superior wall of the canal.

The Middle Ear

The middle ear (Figs. 462 and 463) consists of the *tympanic cavity*, which communicates with the nasopharynx by means of the *auditory* (*Eustachian*) *tube*. The tympanic cavity is a small narrow laterally-compressed chamber lying within the temporal bone. It comprises the tympanum proper, or *atrium*, which is the part of the cavity mesial to the tympanic membrane, and the *epitympanic recess*, or *attic*, which lies above the level of the tympanic membrane. Posteriorly, the tympanic cavity communicates, through the *tympanic antrum*, with the *mastoid cells*, which are air filled cavities in the mastoid process of the temporal bone. In front, the tympanum leads into the auditory tube.

The lateral wall of the tympanum is formed almost entirely by the *tympanic membrane*, which makes an angle of about 55 degrees with the lower and anterior walls of the external auditory meatus. The periphery of the membrane is fixed firmly by fibrocartilage in a groove (the *tympanic sulcus*) in the surrounding bony ring. Superiorly, however, the bony ring is notched, so that a small area of the membrane remains lax. This is the *pars flaccida*; the remaining and greater part of the membrane is the *pars tensa*.

The inner wall of the tympanum is formed by the lateral wall of the osseous labyrinth. It bears a rounded eminence, the *promontory*, which marks the position of the first, or basal, coil of the cochlea. Somewhat above and behind this is an oval aperture, the *oval window* (*fenestra vestibuli*), into which fits the base of the stapes. Behind and below the promontory is a funnel-shaped recess which leads to a second aperture in the bone, *the round window* (*fenestra cochleae*). This is closed by the thin *secondary tympanic membrane*.

Extending across the tympanic cavity is a chain of three small bones, the *auditory ossicles*. These, from without inward, are the *malleus* (hammer), the *incus* (anvil) and the *stapes* (stirrup). The *manubrium* (handle) of the malleus is firmly attached to the tympanic mem-

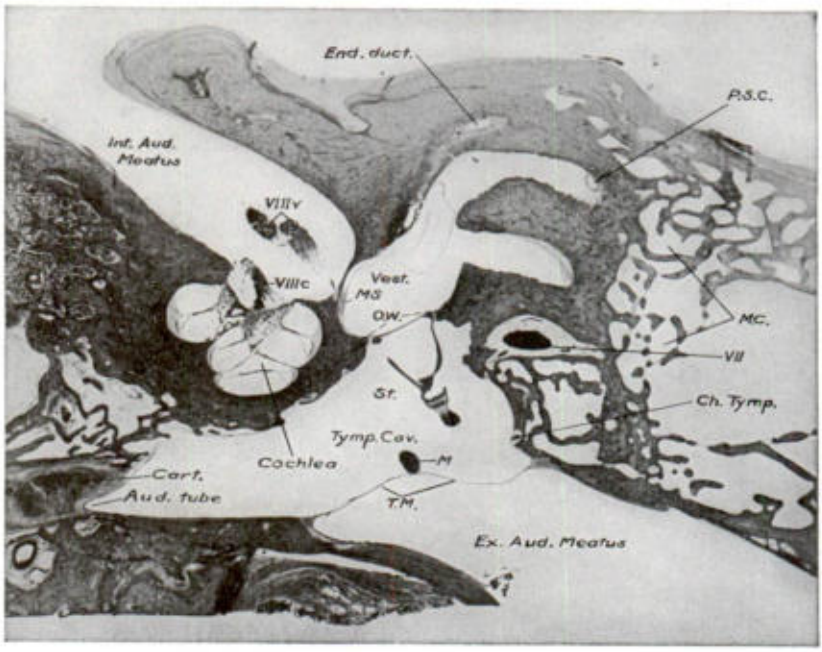

FIG. 463. Section through left temporal bone of man (age 17 years). *Aud. tube*, Auditory (Eustachian) tube; *Cart.*, cartilage of auditory tube; *Ch. tymp.*, chorda tympani nerve; *End. duct*, endolymphatic duct; *Ext. Aud. Meatus*, external auditory meatus; *Int. Aud. Meatus*, internal auditory meatus; *M*, manubrium of malleus; *M.C.*, mastoid air cells; *M.S.*, macula sacculi; *O.W.*, oval window; *St.*, stapes; *T.M.*, tympanic membrane; *Tymp. cav.*, tympanic cavity; *Vest.*, vestibule; *VII*, seventh cranial (facial) nerve; *VIIIc*, cochlear division of eighth cranial (auditory) nerve; *VIIIv*, vestibular division of eighth cranial nerve. (Photomicrograph of a section from the collection of Dr. E. P. Fowler, Jr.)

brane. The head of the malleus lies in the epitympanic recess, where it articulates with the head of the incus. The long process of the latter bends sharply near its end to articulate with the head of the stapes. The base of the stapes is firmly fixed to the border of the oval window by a ring of elastic fibers.

Associated with the auditory ossicles are several ligaments and two small muscles, the *tensor tympani* and *stapedius* muscles.

The *chorda tympani* is a small branch of the facial nerve which passes across the tympanic cavity on its lateral wall, directly over the manubrium of the malleus.

Lining the tympanic cavity, and investing all the structures contained within, is a mucous membrane, the *tympanic mucosa* (Fig. 463). This consists of a thin connective tissue tunica propria covered by simple squamous epithelium. On the bony walls of the tympanic cavity, it is firmly attached to the underlying periosteum. It is continued as the lining of the tympanic antrum and the mastoid air cells and forms the inner surface of the tympanic membrane.

The tympanic mucosa also invests such structures as the auditory ossicles and muscles and the chorda tympani nerve. The reason for this condition is

better appreciated when it is recalled that the tympanic cavity arises embryologically as a distal enlargement of the first branchial pouch which subsequently grows around and embraces, so to speak, the ossicles and muscles, which are developed from the mesenchyme of the first and second branchial arches.

The Tympanic Membrane. This is a thin semitransparent structure in which three layers can be distinguished. The outer *cutaneous layer* is composed of very thin skin in which the epithelium is reduced to a stratum germinativum one or two cells thick, over which is a thin loose stratum corneum. Along the manubrium of the malleus, the skin is thicker and possesses a subcutaneous layer. The inner *mucous layer* of the tympanic membrane is composed of the tympanic mucosa. Here it is very thin and consists of a single layer of flat squamous epithelium on a sparse tunica propria. Between the two surface membranes is the *substantia propria*, which forms the main mass of the tympanic membrane. It consists of two layers of tendon-like collagenous fiber bundles. The fibers of the outer layer are disposed in a radial manner from the manubrium of the malleus outward to the fibrocartilaginous ring. The inner fibers course in a circular direction and are most numerous near the periphery. Both layers are lacking in the pars flaccida.

Blood is supplied to the tympanic membrane by two sets of vessels, an external set derived from the vessels of the external auditory meatus and an internal set from the vessels of the middle ear. These give rise to capillary networks in the skin and mucous membrane, respectively, and anastomose by means of perforating branches at the periphery of the membrane. From the capillaries the blood passes into two sets of small veins, one extending around the periphery of the membrane, the other following the handle of the malleus.

The larger nerves run in the substantia propria. From these, branches pass to the skin and mucous membrane, beneath the surfaces of which they form plexuses of fine fibers.

The Auditory Ossicles. The malleus, the incus, and the stapes are composed of compact bone arranged in lamellae with scattered Haversian systems. Over their articular surfaces, they are covered by hyaline cartilage, which also occurs in patches on the base of the stapes and the manubrium of the malleus. The stapes alone contains a marrow cavity. The periosteum which covers the auditory ossicles is fused with the tunica propria of the overlying tympanic mucosa.

The Muscles. The tensor tympani and the stapedius muscles consist of striated skeletal muscle fibers (Figs. 464 and 465). The tensor tympani lies for the most part in a canal just above the auditory tube in the anterior wall of the tympanic cavity. It ends in a tendon

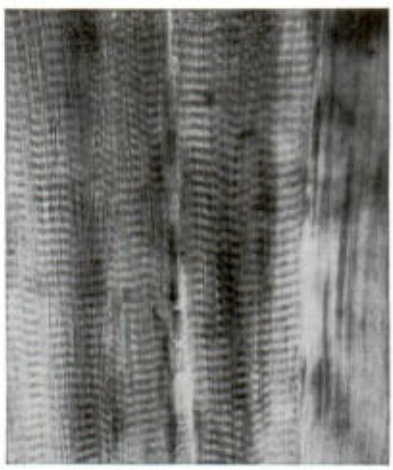

FIG. 464. Longitudinal section of muscle and tendon fibers of human tensor tympani muscle. (Photomicrograph.)

which bends sharply around the end of the bony canal and passes laterally across the tympanic cavity to be inserted into the manubrium of the malleus, near its upper end. When this muscle contracts, it draws the manubrium inward and thus makes tense the tympanic membrane. It is innervated by the fifth cranial (trigeminal) nerve.

The stapedius muscle lies within a small conical bony projection, the *pyramidal eminence*, on the posterior wall of the tympanic cavity. Its tendon passes through a minute aperture in the summit of the eminence and is inserted into the posterior surface of the neck of the stapes. The stapedius muscle is supplied by the seventh cranial (facial) nerve.

Action of Middle Ear Structures. Since the function of the middle ear is primarily to transmit vibrations through the oval window to the inner ear, it is not surprising to find its structures arranged and working according to mechanical principles. The tympanic membrane is a vibrating membrane and as such might be expected to possess a greater resonance to one or more specific frequencies of vibration. However, its shape and structure are such as to render it free from this defect and it forms an aperiodic membrane whose response is no greater to one frequency than to another.

FIG. 465. Longitudinal section of muscle and tendon fibers of human stapedius muscle. (Photomicrograph.)

The arrangement of the auditory ossicles is such that in effect they form a lever whose two arms are in the ratio of 3 to 2, with the longer arm formed by the malleus and the shorter by the incus and stapes. The result is that the vibrations transmitted to the oval window by the base of the stapes are reduced in amplitude by one-third but are increased in intensity by one-half. The extent of the excursion of the base of the stapes in the oval window is only about 0.04 mm.

The tensor tympani muscle by its normal resting tonus serves to keep the tympanic membrane in a taut condition. The stapedius muscle is believed to be concerned with maintaining the satisfactory articulation of the ossicles. In addition to these functions, it has been shown experimentally in the cat that these muscles contract reflexly in response to loud sounds, thereby reducing the amplitude of the vibrations transmitted to the internal ear. Perhaps in this manner they serve to protect the delicate mechanism of the cochlea from the injurious effects of too loud sounds.

The Auditory (Eustachian) Tube. This is a flattened canal leading from the anterior wall of the tympanic cavity to the nasopharynx. In its upper extent, near the tympanum, it is surrounded by a bony wall. Below this osseous part, the wall is formed partly by a cartilaginous plate and partly by a fibrous membrane.

The cartilaginous plate is a long triangle in form, with its apex firmly attached to the medial end of the osseous part. In cross section, the cartilage appears shaped like a hook, and forms the wall

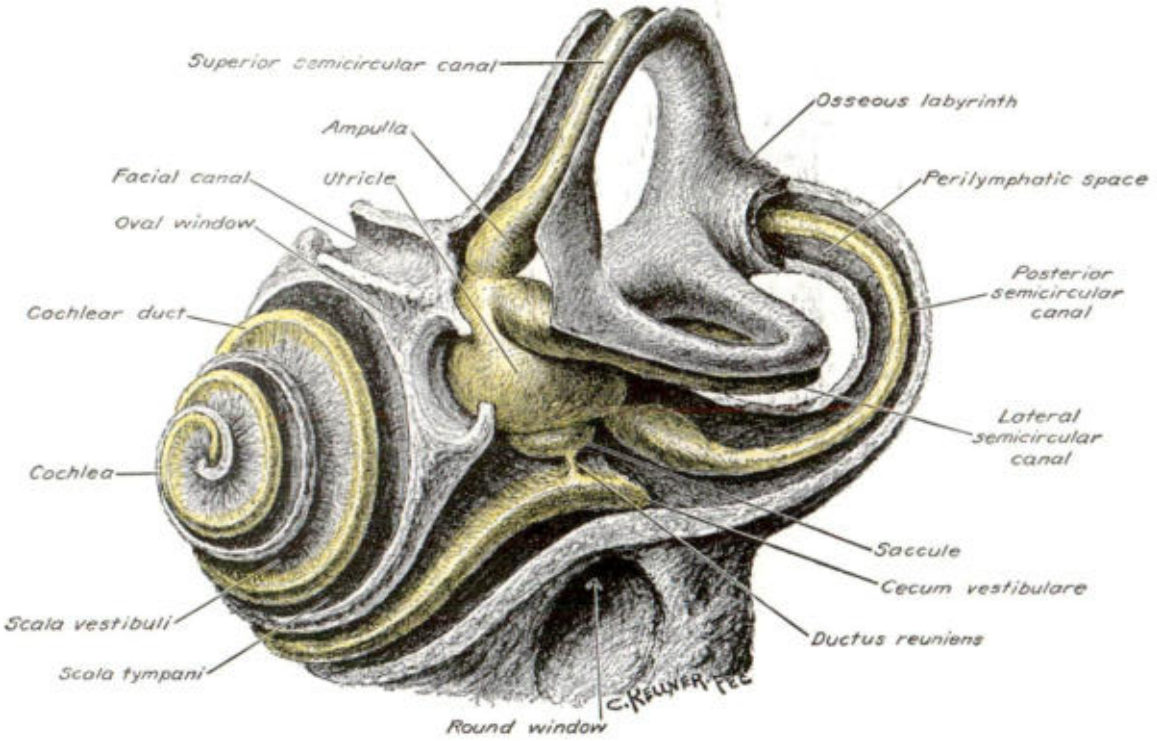

FIG. 466. The osseous and membranous labyrinths of the left side, as seen from the lateral aspect. (Based on a model by Tramond but considerably modified.)

of the posterior surface, the upper margin and the superior portion of the anterior surface of the canal. The remainder of the wall is formed by the fibrous membrane.

At its upper end, the cartilage is of the hyaline variety (Fig. 463). In its lower portion, patches of elastic cartilage occur in the hyaline matrix.

The mucosa which lines the auditory tube consists of a connective tissue tunica propria covered by ciliated columnar epithelium. In the bony part, the mucosa is thin and firmly united to the underlying bony wall. The epithelium is a low ciliated columnar type. In the cartilaginous part of the tube, the mucosa is loose and the epithelium is of the pseudostratified ciliated variety. Goblet cells occur near the pharyngeal opening, as well as tubulo-acinar mucous glands.

The auditory tube serves as a means of equalizing the air pressure on the inner surface of the tympanic membrane with the pressure existing outside. The tube is opened during the act of swallowing.

The Internal Ear

The internal ear is contained in the petrous part of the temporal bone and consists of a series of connected bony walled chambers and passages containing a similarly shaped series of membranous sacs and canals (Figs. 462 and 466). These are known as the *osseous labyrinth* and the *membranous labyrinth*, respectively. Intervening between the two is a space, the *perilymphatic space*, which contains the fluid *perilymph*. Within the membranous labyrinth there is also a fluid, the *endolymph*.

The Osseous Labyrinth. Although the osseous labyrinth does not occur as a separate entity, nevertheless the bone of its walls is of a harder texture than that surrounding it and with care the latter can be dissected away and the form of the osseous labyrinth revealed. Seen thus, it consists of an ovoid central chamber, the *vestibule*, from which are given off three *semicircular canals* and the *cochlea*. The vestibule is separated from the middle ear by a plate of bone which contains two openings—the oval window and the round window. A narrow canal extending from it to the posterior surface of the petrous part of the temporal bone is known as the *vestibular aqueduct*. The three semicircular canals are so arranged that their respective planes lie perpendicular to each other. Thus there are two vertical canals and one horizontal canal. The vertical ones comprise the *superior* (anterior) and the *posterior* (inferior) canals; the horizontal canal is known as the *lateral*, or external, canal. The lateral canals of the two sides of the head lie in the same horizontal plane. The vertical canals are so oriented with respect to the sagittal plane of the head that the plane of the superior canal of one side is approximately parallel to the plane of the posterior canal of the opposite side.

Just after leaving the vestibule, each canal presents a dilatation, the *ampulla*. The lateral canal has a return opening into the vestibule; the superior and posterior canals have a common return opening, the *crus commune*. Thus there are five openings from the vestibule into the semicircular canals.

Anteriorly, the vestibule opens into the bony cochlea, a conical structure consisting of an osseous axis around which winds a spiral canal. The apex of the cochlea is directed forward, outward, and downward.

The walls of the osseous labyrinth are composed of compact bone, with a periosteum which has a covering of mesenchymal epithelium.

The Membranous Labyrinth. This consists of a connected series of sacs and canals whose walls are formed of a fibrous connective tissue lined internally by simple squamous epithelium of ectodermal origin. In general, the membranous labyrinth has the same form as the osseous labyrinth in which it is contained. However, that part enclosed within the osseous vestibule is divided into two sacs (Figs. 462 and 466). The larger of these, the *utricle*, is an elliptical sac lying in the upper posterior part of the vestibule. From it are given off the membranous semicircular canals. In front of the utricle is the smaller spherical *saccule*, from the lower part of which a short and narrow canal, the *ductus reuniens*, connects with the membranous cochlea, or *cochlear duct*. The utricle and the saccule are connected by the *utriculosaccular duct*, the two parts of which converge and continue backward through the vestibular aqueduct as the slender *endolymphatic duct*. Under the dura of the posterior surface of the temporal bone, this duct terminates in a blind enlargement, the *endolymphatic sac*.

The membranous labyrinth only partially fills the space within the osseous labyrinth. At places, it lies close to the periosteum of the osseous wall, with which its connective tissue layer then blends. For the most part, however, it lies suspended in the perilymph by a number of connective tissue trabeculae which pass from the periosteum to the membranous wall (Fig. 470).

Except for certain special neuroepithelial areas, which will be discussed separately, the wall of the membranous labyrinth consists of an outer thin tunica propria and an inner lining epithelium. The tunica propria is composed of delicate connective tissue fibers and stellate fibroblasts. Its outer surface and the trabeculae which suspend it from the periosteum are covered by a single layer of epithelial cells of connective tissue origin. The epithelium of the membranous labyrinth is a single layer of flat squamous cells resting on a basement membrane.

In certain definite regions, the wall of the membranous labyrinth is considerably modified to form the true sensory areas. In these areas, the epithelium

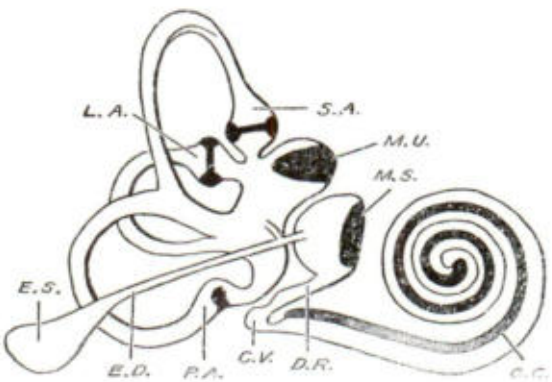

FIG. 467. Diagram of left membranous labyrinth viewed from the medial aspect. The neuroepithelial areas are shown in black. *C.V.*, Caecum vestibulare; *D.R.*, ductus reuniens; *E.D.*, endolymphatic duct; *E.S.*, endolymphatic sac; *L.A.*, ampulla of lateral semicircular canal; *M.S.*, macula sacculi; *M.U.*, macula utriculi; *O.C.*, organ of Corti in cochlear duct; *P.A.*, ampulla of posterior semicircular canal; *S.A.*, ampulla of superior semicircular canal. (Redrawn from Schaffer.)

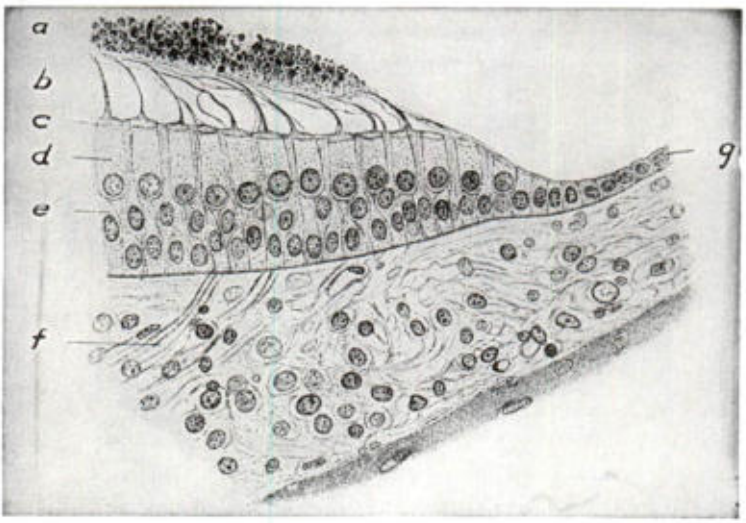

FIG. 468. Vertical section of the edge of the macula sacculi of a guinea pig. *a*, Otolithic membrane; *b*, hairs of the hair cells; *c*, cuticle; *d*, hair cell; *e*, sustentacular cell; *f*, nerve fibers; *g*, indifferent epithelium. (From Schaffer, after von Ebner.)

takes on a special complexity and among its cells the fibers of the acoustic (VIII) nerve terminate. There are six such neuroepithelial areas in each labyrinth: one in each macula, the *macula utriculi* and the *macula sacculi*; one in each ampulla, the *cristae ampullares*; and one in the cochlear duct, the *organ of Corti* (Fig. 467). The last named is the only one concerned with the function of hearing; all the others have to do with equilibration.

The Maculae. The maculae represent local thickenings of the membranous walls, each covering an area about 3 mm. by 2 mm. in extent and forming an elevation into the endolymphatic space. The epithelium here is of the columnar type, in which two kinds of cells may be distinguished—the *sustentacular* or *supporting cells* and the *hair cells*.

The Sustentacular Cells. These are tall columnar elements resting on the basement membrane. Their basal portions, containing the oval granular nuclei, are broader than their upper portions, which pass between the hair cells. The free surface of each sustentacular cell bears a cuticular plate (Figs. 468 and 469). At the edge of the macula, the sustentacular cells show a gradual transition into the simple squamous epithelium characteristic of the remainder of the membranous labyrinth.

The Hair Cells. These cells occupy the outer part of the epithelium. Each hair cell has the shape of a rounded flask. The large, oval, deeply-staining nucleus lies toward the base of the cell. The free surface is covered by a cuticular plate, through which passes a single long tapering process composed of a bundle of fine nonmotile cilia. These so-called hairs of the hair cells extend outward from the epithelium for 20 to 25 μ to penetrate a peculiar membrane which covers the surface of the macula.

This membrane, the *otolithic membrane*, consists of a gelatinous substance containing a great number of small bodies—the *otoconia* or *otoliths*. The otoconia are minute crystals composed chiefly of

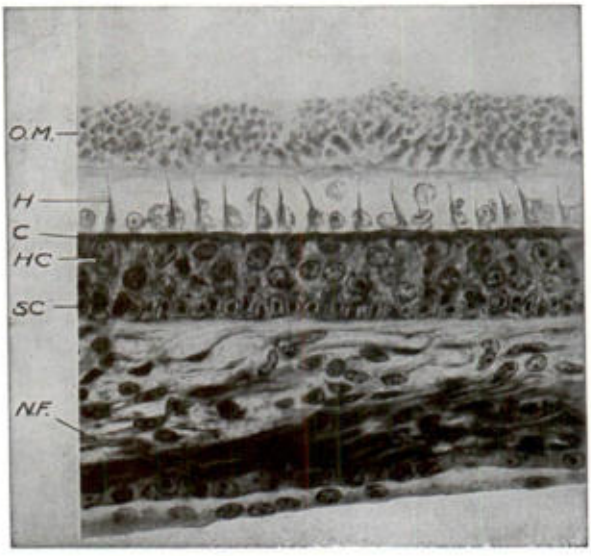

FIG. 469. Vertical section of macula of guinea pig. *OM*, Otolithic membrane; *H*, hairs of hair cells; *C*, cuticle; *HC*, hair cells; *SC*, sustentacular cells; *NF*, nerve fiber. (Photomicrograph.)

calcium carbonate. The otolithic membrane is separated from the free surface of the epithelium by a narrow fluid-filled space, in which there may often be seen faintly staining globules containing a finely granular substance (Fig. 469).

In the macular region, the tunica propria is firm and considerably thickened. It is closely united to the underlying periosteum of the bony vestibule.

The hair cells are intimately related to fibers of the vestibular nerve, which is part of the eighth cranial nerve. The vestibular nerve enters in the internal auditory meatus (Fig. 463), in which its vestibular ganglion is located. From the ganglion, the medullated fibers pass through the bony labyrinth and enter the thickened tunica propria of the macula. Here they lose their myelin and the naked axis cylinders pass through the basement membrane and terminate around the hair cells. They form nests of fine branches closely applied to the surfaces of individual hair cells.

According to current opinion, the maculae are concerned with static equilibrium, i.e., the position of the head in space.

The Semicircular Canals. Each semicircular canal forms an arc which is slightly more than a semicircle. It does not occupy the center of the osseous canal but lies against the periosteum of the outer, or convex, border, to which it is attached (Fig. 470). Connective tissue trabeculae from the wall of the osseous canal also serve to anchor it in the perilymphatic space.

The histological structure of the canals does not vary essentially from that described for the remainder of the membranous labyrinth. Certain special features are presented, however, in the structure of the cristae ampullares, the sensory areas which are found in each ampulla.

In each crista ampullaris, the membranous wall is thickened to form a ridge, placed transversely to the long axis of the canal. This ridge consists of the connective tissue tunica propria,

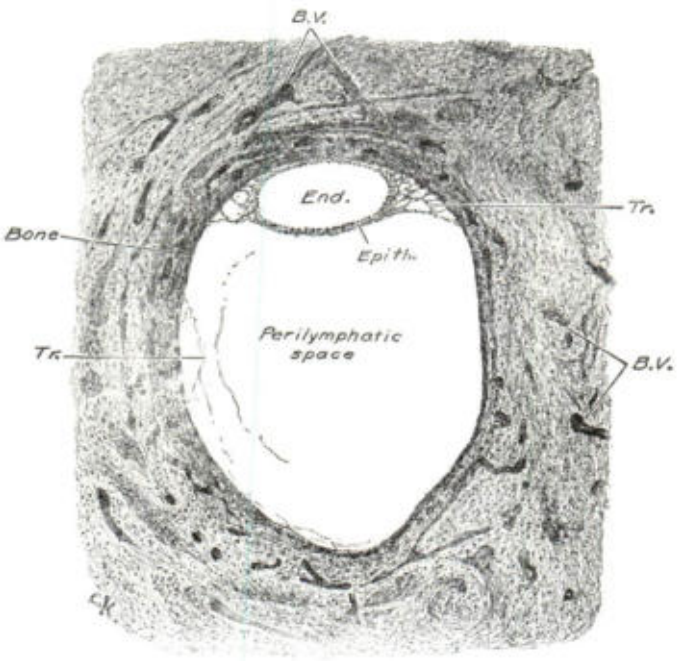

FIG. 470. Cross section of superior semicircular canal of 15-year-old girl. *BV.*, Blood vessel; *End.*, endolymphatic space; *Epith.*, epithelium; *Tr.*, connective tissue trabeculae in perilymphatic space. (From a section in the collection of Dr. E. P. Fowler, Jr.)

containing many nerve fibers and blood vessels and surmounted by a specialized columnar epithelium.

As in the maculae, the epithelium consists of two types of cells—the *sustentacular cells* and the *hair cells*. They are similar to those of the maculae.

A description of the histology of the crista is rendered difficult by the fact that we do not know how closely the appearance of fixed preparations resembles the structure in its living condition. To be true, this objection is valid in regard to many body structures, but in the case of the crista ampullaris there may be reason to believe that the classical description of the organ as seen in fixed preparations does not apply accurately to its condition in life.

In fixed preparations, the epithelium of the crista ampullaris is seen to be surmounted by a tall, rounded, longitudinally striated mass, the *cupula* (Fig. 471). This is supposed to be a gelatinous structure which is separated from the epithelium by a narrow space containing endolymph. The long tapering hairs of the hair cells pass through this space and penetrate for some distance into the cupula, each hair occupying a narrow canal filled with endolymph. The hairs in the middle of the crista stand perpendicular to its surface. Those toward the border tend to be inclined in the direction of the median plane of the cupula.

The above is briefly the description of the crista as seen in fixed and stained preparations. On the other hand, Bowen, studying the crista ampullaris in the living condition in fishes, finds that no structure resembling the cupula can be

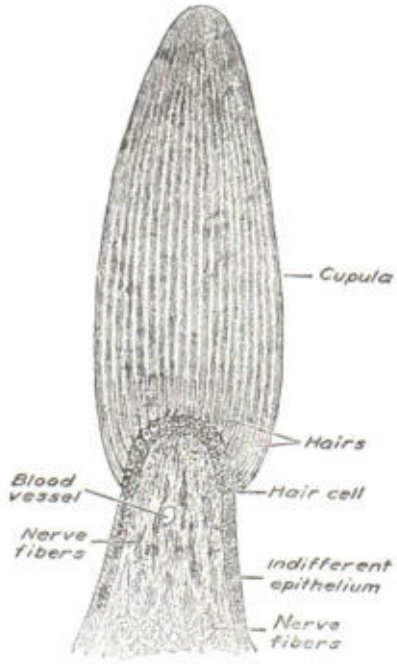

FIG. 471. Crista ampullaris of newborn child. (Redrawn from Schaffer.)

seen. The ampullar space distal to the sensory hairs is filled by a thin mucilaginous fluid. Under the microscope, it can be observed that when fixing reagents are applied to the crista, the sensory hairs quickly shorten and this fluid forms a coagulum which assumes the shape commonly associated with a cupula. According to this investigator, the structure seen in fixed preparations is an artifact. Dohlman, however, has recently taken motion pictures which show the piston-like movements of the cupula following India ink injection of the labyrinth in a teleost fish.

The fibers of the vestibular nerve terminate in the epithelium of the crista in much the same manner as they do in the maculae. The naked axis-cylinders pierce the basement membrane and form basket-like nests around the hair cells.

The cristae ampullares are supposed to be concerned with dynamic equilibrium. They are stimulated by movements which involve any amount of rotation.

The Cochlea. The osseous cochlea consists of a conical axis of spongy bone, the *modiolus*, around which winds a spiral bony canal. This canal in man makes about two and one half turns and ends at the rounded tip of the cochlea, which is known as the *cupula*. The base of the modiolus forms the bottom of the internal auditory meatus, through which the fibers of the cochlear nerve pass and enter the modiolus (Figs. 463 and 472). These fibers are processes of the bipolar ganglion cells of the spiral ganglion, which is lodged in the *spiral canal of the modiolus*.

Projecting from the modiolus partly across the osseous canal of the cochlea is a shelf of bone, the *osseous spiral lamina*. This follows the spiral turns of the cochlea and ends at the cupula in a hook-shaped process, the *hamulus*. Along the outer wall of the canal, opposite the osseous spiral lamina, is a projection of thickened periosteum, the *spiral ligament*. A connective tissue membrane, the *membranous spiral lamina*, bridges the space intervening between the spiral ligament and the osseous spiral lamina. Thus the osseous canal of the cochlea is divided into two spirally parallel parts, an upper *scala vestibuli* and a lower *scala tympani*.[2]

From the thickened periosteum covering the upper surface of the osseous spiral lamina, there arises a thin membrane, the *vestibular membrane* (membrane of Reissner), which extends obliquely outward to the upper part of the spiral liga-

[2] The terms "upper" and "lower" are here used arbitrarily as if the cochlea were oriented with its base downward and its apex upward. "Inner" and "outer" are used to designate directions with respect to the axis of the cochlea, the modiolus.

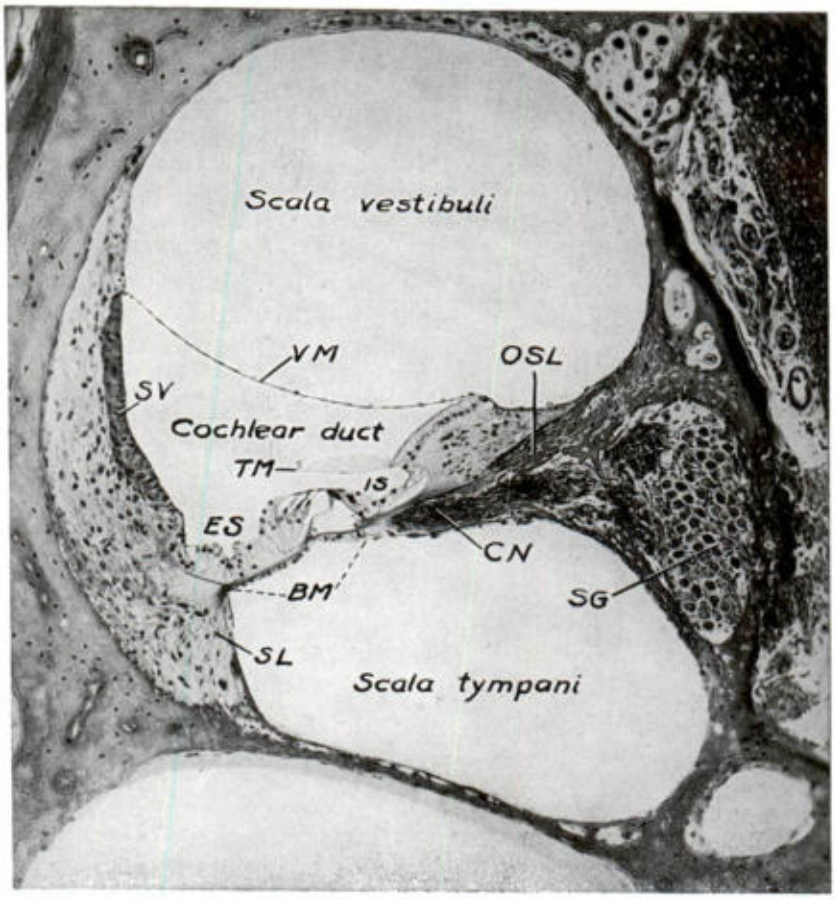

FIG. 473. Radial section through basal turn of cochlea of the guinea pig. *BM*, Basilar membrane; *CN*, cochlear nerve; *ES*, external spiral sulcus; *IS*, internal spiral sulcus; *OSL*, osseous spiral lamina; *SG*, spiral ganglion; *SL*, spiral ligament; *SV*, stria vascularis; *TM*, tectorial membrane; *VM*, vestibular (Reissner's) membrane. (Photomicrograph.)

thus allowing a division of its walls into upper, outer and lower.

The upper or vestibular wall is formed by the vestibular membrane. This consists of a thin central lamina of connective tissue covered on either side by simple squamous epithelium. That on the upper surface is continuous with the mesenchymal epithelium of the scala vestibuli; on the lower surface, the epithelium is continuous with that of the cochlear duct and is of ectodermal origin. In man, the vestibular membrane is nonvascular.

The outer wall of the cochlear duct is formed by the *spiral ligament*, a thickening of the periosteum which appears triangular in cross section (Fig. 473, *SL*). The outer part of the spiral ligament, nearer the osseous wall, consists of dense fibrous tissue. In its inner part, the connective tissue is more loosely arranged. The crest of the spiral ligament forms the *crista basilaris*, which serves for the at-

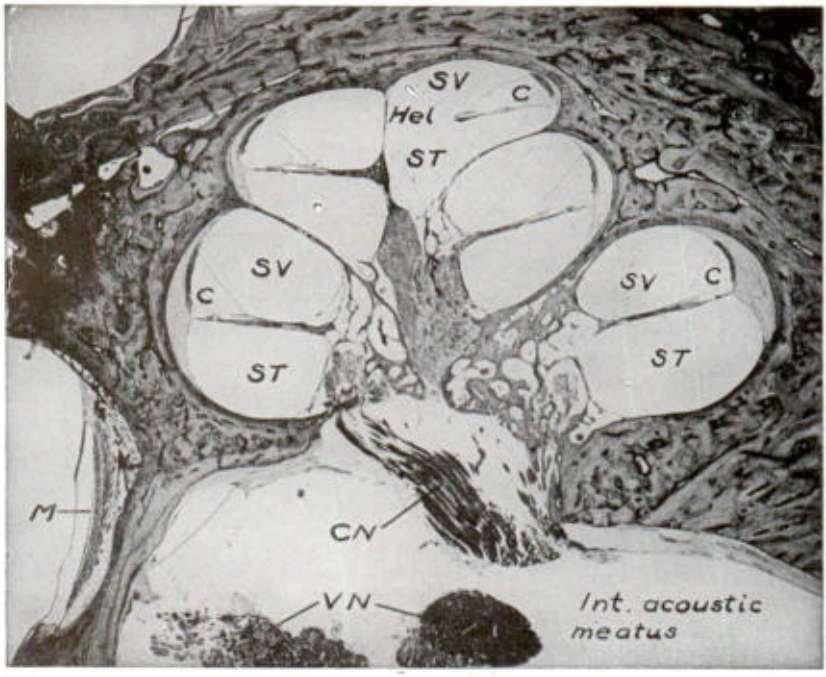

FIG. 472. Axial section of human cochlea and edge of sacculus (15-year-old girl). *C*, Cochlear duct; *CN*, cochlear division of auditory nerve; *Hel.*, helicotrema; *M*, macula sacculi; *ST*, scala tympani; *SV*, scala vestibuli. (Photomicrograph of a section from the collection of Dr. E. P. Fowler, Jr.)

ment. This membrane thus forms the roof of a canal, triangular in cross section and lying between the two scalae. This is the *cochlear duct*, or *scala media*. It is the membranous canal of the cochlea.

The Scalae. These are perilymphatic spaces. The scala vestibuli arises in the vestibule and is in close relation to the oval window (fenestra vestibuli). It follows the spiral course of the osseous canal to its apex, where it communicates with the scala tympani through a small canal, the *helicotrema* (Fig. 472, *Hel.*). The scala tympani follows a course parallel to the scala vestibuli and ends at the round window (fenestra rotundum, fenestra cochleae), which is closed by the secondary tympanic membrane. This is a thin but strong fibrous membrane, covered on its outer surface by the tympanic mucosa and on its inner surface by the lining epithelium of the scala tympani.

With the exception of the area covered by the spiral ligament, the bony walls of the scalae are lined by a thin periosteal connective tissue covered with mesenchymal epithelium.

The Cochlear Duct. This is a narrow membranous tube which communicates with the sacculus through the ductus reuniens (Figs. 466 and 467). Its lower end forms a blind pouch known as the *cecum vestibulare*, which lies between the oval and round windows. At the apex of the cochlea, its closed end is known as the *lagena* or *cecum cupulare*. The cochlear duct is triangular in transverse section,

tachment of the membranous spiral lamina. It contains dense converging fibers and relatively few cells. A short distance above the crista basilaris, there is a slight ridge, the *spiral prominence*. This contains blood vessels, the larger of which is a vein, the *vas prominens*. The prominence is covered by a single layer of deeply staining cuboidal epithelial cells.

The depression between the crista basilaris and the spiral prominence is known as the *external spiral sulcus*. Its surface is covered by cuboidal epithelium.

That part of the spiral ligament lying between the spiral prominence and the vestibular membrane is the *stria vascularis*. The epithelium of this region is relatively thick and is of the pseudostratified type. A basement membrane is lacking. The subepithelial connective tissue is richly supplied with capillaries which send loops into the epithelium, giving the unusual appearance of a vascular epithelium.

It has been suggested that the endolymph of the cochlear duct is secreted by the stria vascularis.

The lower or tympanic wall of the cochlear duct has an extremely complex structure. It is formed by the outer part of the osseous spiral lamina and the whole of the membranous spiral lamina, together with certain special modifications of their epithelial and connective tissue layers.

On the outer border of the osseous spiral lamina, a thickening of periosteal connective tissue forms an elevation known as the *limbus spiralis* (Fig. 474). Peripherally, the limbus terminates in an upper and a lower projection, known respectively as the *vestibular lip* and the *tympanic lip*. They partially enclose a groove, the *internal spiral sulcus*. The connective tissue of the limbus is firm and unusually cellular.

The vestibular membrane attaches to the upper surface of the limbus. Lateral to this point of attachment, the limbus is covered by columnar epithelium, whose surface bears a cuticular formation which appears to be continued into the *tectorial membrane* (Figs. 473 and 474). In surface view, the limbus is seen to bear a series of radial ridges which overhang the margin of the vestibular lip and form a series of processes known as the *auditory teeth of Huschke*.

The tympanic lip projects slightly farther outward than the vestibular lip. Its upper surface forms the floor of the internal spiral sulcus and is lined by a single layer of clear flat cuboidal cells, which continue to the vestibular lip as the lining of the sulcus. The lower surface of the tympanic lip is covered by a thin layer of epithelium continuous with that lining the scala tympani.

Continued directly from the tympanic lip is the *basilar membrane*, which extends outward to the crista basilaris of the spiral ligament. The basilar membrane consists of fine straight unbranched fibers, the *basilar fibers* or *auditory strings*, embedded in a sparse homogeneous ground substance. It may be divided into an inner *zona arcuata* (or *zona tecta*) and an outer *zona pectinata*, the limits of which are defined by the overlying epithelial structures.

The basilar membrane is completed by a *vestibular covering layer* and a *tympanic covering layer*. The former is thin and consists of a homogeneous substance containing a few connective tissue cells. The tympanic covering layer is thicker and consists of cellular connective tissue. In the zona arcuata, it contains blood capillaries which form a spiral vessel, the *vas*

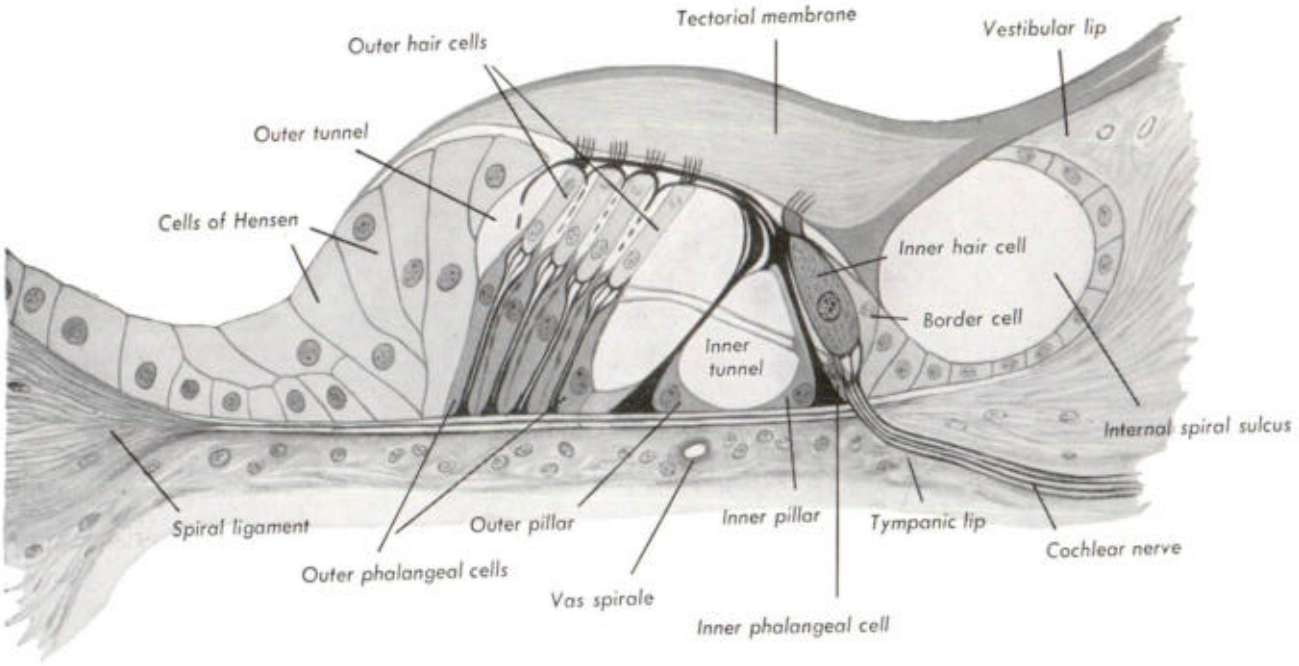

FIG. 474. Radial section of human organ of Corti, from the upper end of the first coil. (Redrawn and modified from Held, with assistance from Dr. E. P. Fowler, Jr.)

spirale, directly under the inner tunnel of the organ of Corti.

The breadth of the basilar membrane varies in the different turns of the cochlea. It is greatest at the apex, gradually diminishing toward the base until its narrowest extent is reached in the proximal end of the basal coil.

Cells of the Organ of Corti. On the upper surface of the basilar membrane, an arrangement of epithelial cells forms a complex structure which is the sensory part of the organ of hearing. This structure is the spiral *organ of Corti*. It extends the entire length of the cochlear duct with the exception of a short distance at either end. Named in their order from within outward, the following specialized cell types comprise the organ of Corti (Fig. 474):

1. The border cells.
2. The inner hair cells.
3. The inner phalangeal cells.
4. The inner and outer pillar cells.
5. The outer phalangeal cells (cells of Deiters).
6. The outer hair cells.
7. The cells of Hensen.

The last named are continuous with the cells of Claudius, which extend over the remainder of the basilar membrane to the spiral ligament. All except the hair cells may be considered as sustentacular or supporting cells. The hair cells are intimately related to the endings of the cochlear nerve and are neuroepithelial cells.

1. The border cells are slender columnar elements which rest on the tympanic lip and form a single row along the inner side of the inner hair cells. Their surfaces are provided with a cuticle.

2. The inner hair cells are larger than the outer hair cells, being broader and slightly longer. They form a single row occupying only the upper part of the epithelial layer. The rounded base of each cell rests on the adjacent supporting cells. The cytoplasm is finely granular. The surface of the cell bears a cuticular plate and a number of short stiff hairs which are in contact with the tectorial membrane.

3. The inner phalangeal cells are arranged in a row along the inner surface of the inner pillar cells. Their bases rest on the basilar membrane. The nucleus lies in the lower portion of the cell, which is continued as a slender process to the surface. Here it ends in a small cuticular plate. When viewed from above, this cuticular plate has a shape not unlike that of the phalanges, the finger bones.

4. The inner and outer pillar cells each consist of a broad curved protoplasmic base which contains the nucleus, and an elongated body or pillar which represents a condensation of deeply staining rigid tonofibrils. The thickened end of the pillar away from the base is known as the head and its free surface is covered by a cuticular plate to which the tonofibrils are attached. The head of the outer pillar presents a convexity on its inner side, which fits into a corresponding concavity on the head of the inner pillar, the heads of opposite pillars thus "articulating" with each other. From their articulation, the pillars diverge, so that their bases, which rest on the basilar membrane, are widely separated. There are thus formed by the pillars a series of arches which enclose a triangular canal, the *inner tunnel* or Corti's tunnel. This is crossed by delicate nerve fibers. As the outer pillars are the larger, they are fewer in number, the estimated number in the human cochlea being 3800 of the outer pillars and 5600 of the inner pillars.

5. The outer phalangeal cells (*cells of Deiters*) are the supporting elements for the outer hair cells, one for each cell.

Like the hair cells, therefore, their number varies in different regions of the cochlear duct. They form three rows in the basal coil, four in the middle coil, and five in the apical coil. Between the innermost of the outer phalangeal cells and the outer pillar there is a space, the *space of Nuel*.

Each outer phalangeal cell is a long columnar element whose expanded base rests on the basilar membrane. At the level of the bases of the hair cells, each phalangeal cell bears a facet, or indented shelf, which receives the base of the hair cell whose support it furnishes. The cell is continued as a slender process which passes to the surface through the space between adjacent hair cells. The round nucleus lies in the broader part of the cell.

Running through the cytoplasm of the phalangeal cell is a bundle of rigid tonofibrils which stand out conspicuously in sections. They are similar to the fibrils described in the pillar cells. The bundle is expanded at its base, and at the level of the hair cells the fibrils divide into two groups. One group terminates in a cup-shaped arrangement in the facet which lodges the hair cell. The other group of fibrils continues on between the hair cells and spreads out at the surface into a flat cuticular plate. The cuticular plates of each row of cells interdigitate with those of the next row. Between them are the outer ends of the hair cells. In sections of the cochlear duct, these bundles of fibrils are seldom included in their whole extent but appear as a series of unconnected dark segments in the spaces between the hair cells.

6. The outer hair cells occupy the superficial third of the epithelium of the organ of Corti. They are columnar cells, the free surface of each bearing a number of short sensory hairs which are in contact with the tectorial membrane. The rounded bases of the cells are supported by the phalangeal cells. The nucleus occupies the lower portion of the hair cell and below it the granular cytoplasm stains more deeply than that of the remainder of the cell. The terminal arborizations of the fibers of the cochlear nerve are closely applied around the bases of the hair cells.

The number of rows of outer hair cells varies, there being three rows in the basal coil of the cochlea, four rows in the upper end of the basal coil (Fig. 474) and in the middle coil, and five rows in the apical coil.

7. The cells of Hensen are tall columnar elements which form the outer border cells of the organ of Corti. They are arranged in several rows on the basilar membrane lateral to the outer phalangeal cells. The base of each cell is narrower than its upper part, which contains the nucleus. The outer cells decrease in height and pass into the cells of Claudius. The space between the outer phalangeal cells and the cell of Hensen is known as the *outer tunnel*.

The Cells of Claudius. These cells are cuboidal in shape and have a clear cytoplasm. They line the lower surface of the external spiral sulcus.

In certain areas of the basal coil, small clumps of cells having a deeply staining finely granular cytoplasm intervene between the basilar membrane and the cells of Claudius. These are the *cells of Boettcher*.

The cuticular plates of the sustentacular cells collectively form a cuticular membrane which is termed the *lamina reticularis*. It covers the organ of Corti and in surface view appears as a mosaic pierced by regularly arranged rows of holes in which the heads of the hair cells are inserted.

The Tectorial Membrane (*Corti's membrane*). This appears as a continuation of the cuticular covering of the columnar cells of the limbus spiralis. Toward the edge of the vestibular lip, this cuticular formation becomes thickened into a striated gelatinous structure which overhangs the internal spiral sulcus and extends outward as far as the cells of Hensen. The upper surface is more convex than the lower one.

The tectorial membrane is particularly susceptible to distortion from the action of fixing reagents. Therefore it is usually shrunken and separated from the processes of the hair cells in fixed preparations. In the living condition it is in contact with the processes of the hair cells and it has been suggested that the processes of these cells are stimulated by movements of the tectorial membrane which are related to vibrations in the endolymph.

Innervation of the Organ of Corti. The cochlear division of the auditory nerve enters the axis of the modiolus from the internal auditory meatus and divides into a number of branches. From these, numerous fibers radiate to the osseous spiral lamina, in the base of which they enter the *spiral ganglion* (Fig. 473).

The cells of the spiral ganglion are peculiar, in that while of the same general type as the spinal ganglion cell, they maintain their embryonic bipolar condition throughout life. Their central processes follow the already described course through the modiolus and thence through the internal auditory meatus to their terminal nuclei in the medulla. Their peripheral processes also become myelinated and pass outward in bundles in the osseous spiral lamina. From these are given off branches which enter the tympanic lip of the limbus, where they lose their myelin and pass through the *foramina nervosa* (minute apertures in the tympanic lip) to their terminations in the organ of Corti. In the latter, the fibers run in three bundles parallel to the inner tunnel. One bundle lies just inside the inner pillar beneath the row of inner hair cells (Fig. 474). A second bundle runs in the tunnel to the outer side of the inner pillar. The third bundle crosses the inner tunnel (*tunnel fibers*) and turns at right angles to run between the outer phalangeal cells. In Figure 474, these fibers appear as small dots between the phalangeal cells. From all of these bundles of fibers, delicate terminal fibers are given off which end in branching telodendria around the bases of the hair cells.

Physiology of the Auditory Mechanism. Remembering the action of the middle ear structures (p. 580), it is evident that sound vibrations which impinge upon the tympanic membrane will cause it to vibrate at the same frequency. The movement consequently imparted to the auditory ossicles will serve to move the base of the stapes in and out of the oval window at the same frequency as that of the stimulating sound. Since the fluid perilymph on the other side of the oval window lies in a chamber with rigid bony walls and is itself incompressible, it follows that the inward movement of the stapes will produce a pressure within the perilymph which can be relieved only by a compensating outward movement of the secondary tympanic membrane covering the round window.

Two avenues for the transfer of this change in pressure are available. It could travel the length of the scala vestibuli and pass by way of the slender helicotrema to the perilymph of the scala tympani, thence to be relieved by the outward bulging of the secondary tympanic

membrane of the round window. Since the helicotrema is a canal of very narrow dimensions, this route would obviously be effective only for very slow pressure changes and could hardly accommodate the rapid changes produced by vibrations up to 20,000 cycles per second.

Hence it is believed that the alternate route is travelled and the pressure change in the perilymph of the scala vestibuli is transmitted across the vestibular membrane of Reissner to the fluid endolymph of the cochlear duct. This causes a displacement of the basilar membrane toward the scala tympani and consequently the pressure is transmitted to the perilymph of the scala tympani and released at the round window. Thus a sound vibration of a given frequency would cause movements of the basilar membrane of equal frequency.

Recalling the arrangement of the epithelial cells on the basilar membrane, it is evident that the vibratory motion of this membrane will move the hair cells in relation to the tectorial membrane. Since the hairs of the hair cells are normally in contact with the tectorial membrane, their relationship with the tectorial membrane would be altered by movements of the basilar membrane. This might constitute the effective stimulus for the hair cells, with consequent activity which would be translated into nerve impulses in the associated nerve fibers. In following through this theoretical chain of events, it should be noted that stimulation of the hair cells would occur on the upward movement of the basilar membrane, which would coincide with the outward movement of the stapes in the oval window.

Although the above may represent a reasonable description of the chain of events initiated by sound vibrations and resulting in stimulation of the hair cells, it fails to account for the fact that the auditory mechanism is capable of differentiating between vibrations of different frequencies—in other words, *pitch* or *tone*. Considerable clinical and experimental evidence has accumulated to indicate that the organ of Corti functions as a differentially responsive structure which possesses specific resonances localized in different regions. It has been shown that high tones affect particularly the responsive structures in the basal end of the cochlea and that as the stimulus frequency is reduced, the region which is maximally affected becomes progressively nearer the apex. Thus, the receptors for low tones are localized at the apical end of the cochlea.

Exactly which part of the organ of Corti is the resonant structure is not certainly known but it is most generally considered to be the basilar membrane. It may not be without significance that the basilar membrane is narrowest in the basal coil and gradually increases to its maximum width in the apical coil.

THE ORGAN OF SMELL

The olfactory organ consists of the olfactory portion of the nasal mucosa. In this connection it is, however, convenient to describe briefly the *olfactory bulb* and the *olfactory tract*.

The Olfactory Mucosa. This can be distinguished with the naked eye by its brownish-yellow color, in contrast with the reddish tint of the surrounding respiratory mucosa. The epithelium is pseudostratified columnar, and is considerably thicker than that of the respiratory region. The surface cells are of two kinds: (1) sustentacular cells, and (2) olfactory cells (Fig. 475).

(1) The *sustentacular cells* are the more numerous. Each cell consists of three parts: (*a*) A superficial portion which is

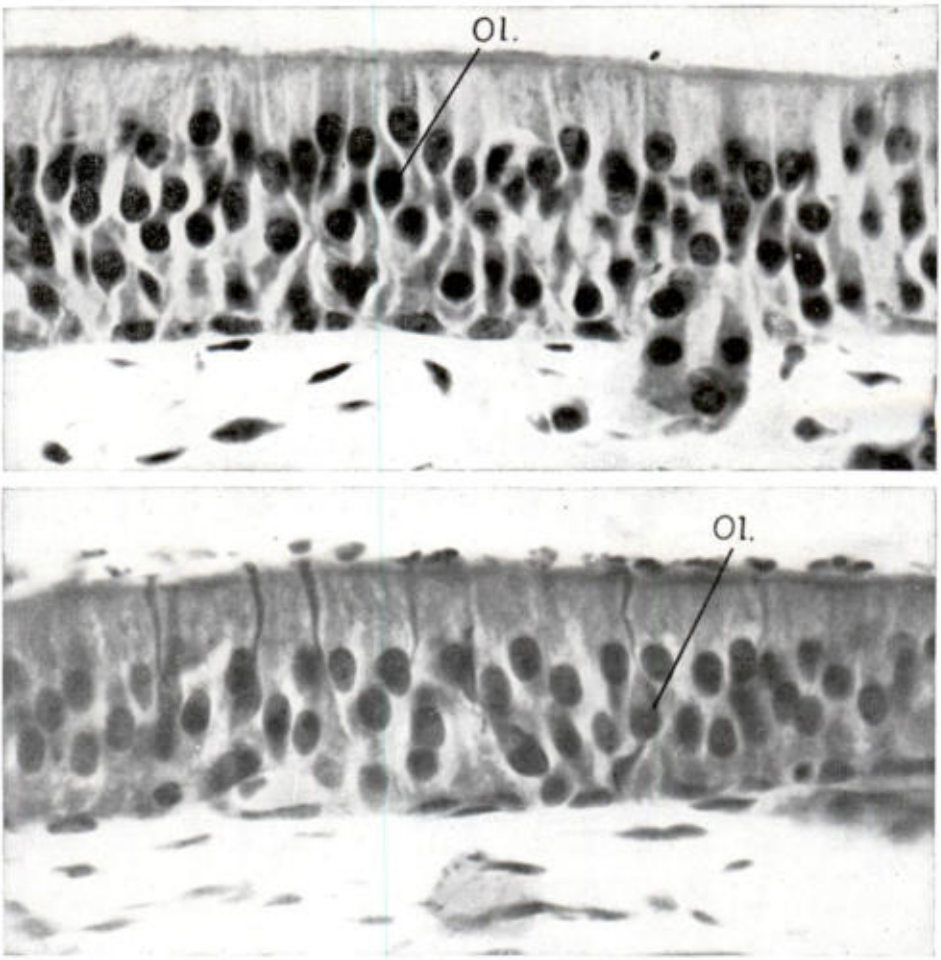

FIG. 475. Photomicrographs of the olfactory mucosa showing the olfactory receptor cells (*Ol.*) between the sustentacular cells. The preparation shown in the upper figure was stained with hematoxylin and eosin; the lower one was treated with silver which blackened the processes of the olfactory cells (bipolar neurons). × 1100. (From preparations by Dr. George W. D. Hamlett.)

broad and cylindrical and contains pigment and granules arranged in longitudinal rows. The cells have well-marked, striated, thickened free borders, which unite to form the so-called *membrana limitans olfactoria*. (*b*) A middle portion which contains an oval nucleus. As the nuclei of these cells all lie in the same plane, they form a distinct narrow band, which is known as the *zone of oval nuclei*. (*c*) A thin filamentous process which extends from the nuclear portion down between the cells of the deeper layers. This process is irregular and pitted by pressure of surrounding cells. It usually forks and apparently anastomoses with processes of other cells to form a sort of protoplasmic reticulum.

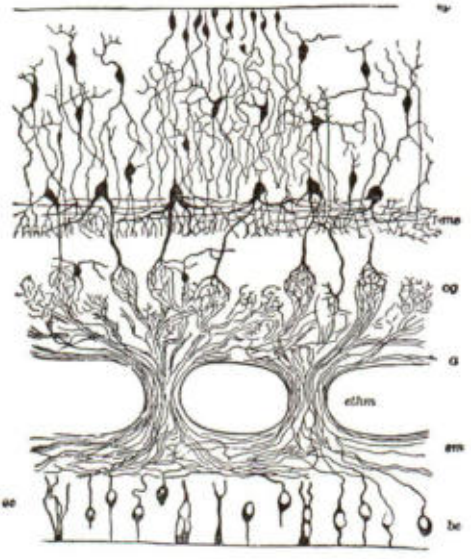

FIG. 476. Diagram of structure of olfactory mucosa and olfactory bulb. (Ramón y Cajal.) *bc*, Bipolar cells of olfactory mucosa; *sm*, submucosa; *ethm*, cribriform plate of ethmoid; *a*, layer of olfactory fibers; *og*, olfactory glomeruli; *me*, mitral cells; *ep*, epithelium of olfactory ventricle.

(2) The *olfactory cells* lie between the sustentacular cells. Their nuclei are spherical, lie at different levels, and most of them are more deeply placed than those of the sustentacular cells. They thus form a broad band, the *zone of round nuclei*. From the nuclear portion of each cell a delicate process extends to the surface, where it ends in several minute hair-like processes. From the opposite pole of the cell a longer process extends centrally which, as a centripetal nerve fiber of one of the olfactory nerves, passes through the cribriform plate of the ethmoid bone (Fig. 476, *ethm*) to its terminal nucleus in the olfactory bulb. The olfactory cell is thus seen to be of the nature of a bipolar ganglion cell with a short peripheral and a longer central process and is not a neuroepithelial cell but an analogue of the spinal ganglion cell, being the only example in man of the peripherally placed ganglion cell found in certain lower animals.

Between the nuclear parts of the olfactory cells and the basement membrane are the *basal cells*. These are small nucleated elements, the irregular branching protoplasm of which anastomoses with that of neighboring basal cells and of the sustentacular cells to form the peculiar protoplasmic reticulum already mentioned.

The *basement membrane* is not well developed.

The *stroma* consists of loosely arranged collagenous fibers, delicate elastic fibers and connective tissue cells. Embedded in the stroma are numerous simple branched tubular glands, the *glands of Bowman* (Fig. 475). Each tubule consists of a duct, a body, and a fundus. The secreting cells are large and irregular and contain a yellowish pigment, which with that of the sustentacular cells is responsible for the peculiar color of the olfactory mucosa.

The Olfactory Bulb. This is a somewhat rudimentary structure analogous to the much more prominent olfactory brain lobe of some of the lower animals. It consists of both gray matter and white matter arranged in six fairly distinct layers. These from below upward are as follows: (*a*) The layer of olfactory fibers; (*b*) the layer of glomeruli; (*c*) the molecular layer; (*d*) the layer of mitral cells; (*e*) the granule layer; (*f*) the layer of longitudinal fiber bundles. Through the center of the last-named layer runs a band of neuroglia which represents the obliterated lumen of the embryonal lobe. The relations of these layers to the olfactory neuron system are as follows:

The layer of olfactory fibers (Fig. 476, *a*) consists of a dense plexiform arrangement of the axons of the above-described olfac-

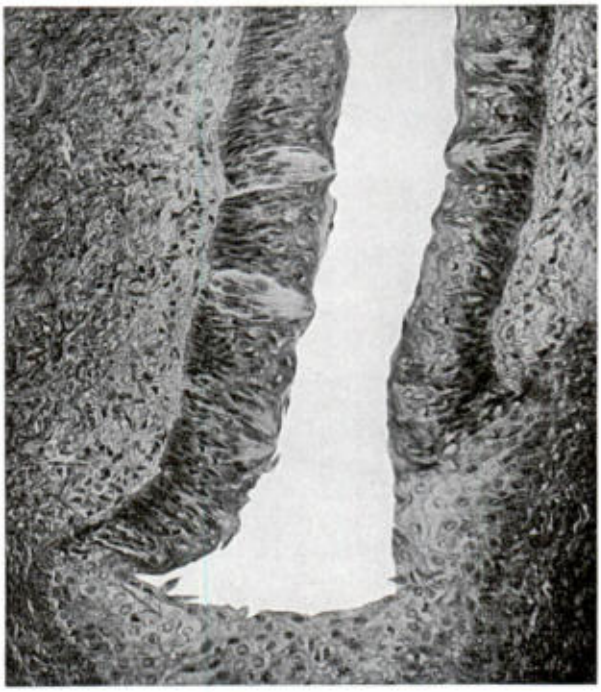

FIG. 477. Section through trench surrounding circumvallate papilla. Several taste buds are shown. (Photomicrograph; human.)

tory cells. From this layer the axons pass into the layer of olfactory glomeruli where their terminal ramifications mingle with the dendritic terminals of cells lying in the more dorsal layers, to form distinctly outlined spheroidal or oval nerve fiber nests, the *olfactory glomeruli* (Fig. 476, *og*). The latter mark the ending of neuron system no. I of the olfactory conduction path.

The molecular layer contains both small nerve cells and large nerve cells. These send their dendrites into the olfactory glomeruli. The smaller cells appear to be association neurons between adjacent glomeruli. The axons of the larger cells, the so-called brush cells, become fibers of the *olfactory tract*.

Of the mitral cells (Fig. 476, *mc*), the main dendrites end in the olfactory glomeruli, while their axons, like those of the brush cells, become fibers of the *olfactory tract*.

In addition to the fibers which pass through it (axons of mitral and of brush cells), the granular layer contains numerous nerve cells. Many of these are small and apparently have no axons (amacrine cells). Their longer dendrites pass toward the periphery, their shorter dendrites toward the olfactory tract. Larger multipolar cells, whose axons end in the molecular layer, also occur in the granular layer.

The layer of longitudinal fiber bundles consists mainly of the centrally directed axons of the mitral and brush cells. These fibers run in distinct bundles separated by neuroglia. Leaving the bulb they form

the olfactory tract by means of which they pass to their cerebral terminations.

The brush cells and mitral cells with their processes thus constitute neuron system no. II of the olfactory conduction path.

THE ORGAN OF TASTE

The organ of taste consists of the so-called taste buds of the lingual mucosa. These have been mentioned in connection with the papillae of the tongue and under sensory end-organs.

The taste buds are found in the side walls of the circumvallate papillae (Fig. 477), of some few of the fungiform papillae, in the mucosa of the posterior surface of the epiglottis, and especially in folds (foliate papillae) which occur along the posterolateral margin of the tongue.

The taste bud (Figs. 477 and 478) is an ovoid epithelial structure embedded in the epithelium and connected with the surface by means of a minute canal.

Each taste bud consists of two kinds of cells, neuroepithelial cells or taste cells and sustentacular cells (Fig. 478). The taste cells are long, delicate, spindle-shaped cells which occupy the center of the taste bud, each ending externally in a hair-like process, which usually projects through the inner pore as the *taste hair*. The inner end of the cell tapers down to a fine process, which may be single or branched. The sustentacular cells are long, slender cells which form a shell several cells thick around the taste cells.

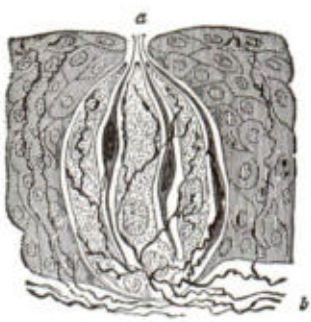

FIG. 478. Taste-bud from side wall of circumvallate papilla. (Merkel-Henle.) *a*, Taste pore; *b*, nerve fibers, some of which enter the taste-bud—intragemmal fibers—while others end freely in the surrounding epithelium—intergemmal fibers.

Sensory fibers of the facial and glossopharyngeal nerves (Fig. 478, *b*) end within the taste buds in a network of varicose fibers—*intragemmal fibers*. These terminal branchings of the nerve are applied directly to the surface of the taste cells. Other sensory terminals of the same nerves end freely in the epithelium between the taste buds. These are finer and smoother than the intragemmal fibers and are known as *intergemmal fibers*.

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