(*NEW SERIES.*)

No. 18.

SCIENTIFIC MEMOIRS

BY

OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

HÆMOGREGARINA GERBILLI.

BY

LIEUT. S. R. CHRISTOPHERS, M.B., I.M.S.

ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA
BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT
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HÆMOGREGARINA GERBILLI.

IT has hitherto been believed that parasites belonging to the group of hæmogregarines are confined to cold blooded vertebrates, for, unless the parasite recently found in the leucocytes of the blood of dogs[1] is of this nature, no one has yet recorded the discovery of hæmogregarine parasites in either a bird or a mammal. There is also an undesirable gap in our knowledge of this interesting group of blood parasites, for we know little or nothing of the means by which they are transmitted from infected to healthy animals. I consider myself fortunate, therefore, in having recently found a characteristic hæmogregarine parasite in the blood of a mammal, a species of Indian field rat, kindly identified by Professor Henderson of Madras as *Gerbillus indicus*, and in having added interest to the discovery by finding that the parasite develops in the body of a louse which infests this animal. I propose to give in this paper a description of the parasite in the rat and of its developmental stages in the louse, so far as the details have been worked out up to the present time.

The Parasite in the Rat.

The examination of a large number of rats, of the species named, caught near Madras, shewed that infection with the parasite is very common: only very few of the rats examined were quite free from infection although the number of parasites present in some animals was much greater than in others. Young and medium sized rats were more frequently infected than adults. The number of parasites present in an animal was very constant over a considerable period of time. In the systematic examination of a number of rats the following results were obtained :—

Rat 1	Examined almost daily for 22 days.	Parasites always swarming.
Rat 2	Examined almost daily for 34 days.	Parasites always abundant.
Rat 3	Examined at intervals for 37 days.	Parasites always present but in small numbers.
Rat 4	Ditto	Sometimes no parasites found, at other times one or two after long search.
Rat 5	Ditto	Parasites always present but scanty.
Rat 6	Ditto	Parasites always abundant.
Rat 7	Ditto	Parasites always present but scanty.

Rat 8 . Examined at intervals for 12 days. Parasites always very abundant.

Fresh preparations.—In fresh preparations of the peripheral blood the characteristic parasites are seen lying quite motionless in spaces in, what appear to be, enlarged pale red cells. The enlarged corpuscles are oval, corresponding to the shape of the parasite and the cavity which it occupies. The affected corpuscles average 10μ in greatest length and 7μ in shortest diameter. Unaffected corpuscles in the same preparations vary from 5·5μ to 6·5μ. The substance of the corpuscle is of lighter colour than that of the normal red cell and stains less deeply with eosin. In some cases the enclosing red cell is hardly distinguishable. The space occupied by the parasite is clearly outlined and oval in shape. The parasite appears as a vermicule, possessing a short "tail" sharply flexed upon the body. A refractile mass (nucleus) is seen lying close to the bent end of the parasite, and one or two refractile granules are usually visible in the "tail." No pigment grains are present. (Fig. 1.)

In addition to the motionless intracellular forms, especially in certain preparations, a number of free bodies, exhibiting active movements, are conspicuous. These resemble the encysted forms, with the exception that the "tail" is no longer curved inwards. They traverse considerable distances by a rapid gliding motion, in which the "head" end is situated forwards. They also twist and turn among the corpuscles, using the "tail" extremity as a fixed point. When red cells are approached they cling to the body of the parasite and are forced quickly backwards. On reaching the hinder extremity they remain attached and are often dragged a considerable distance in this manner. Eventually they are liberated, but usually only after fresh cells have been similarly attached. (Fig. 7.) In many instances constricting waves are seen passing backwards along the body of the parasite, and it happens very commonly that forms in which all movements have ceased possess a moniliform shape, apparently due to the action of such constricting waves. Not infrequently forms were seen in the act of leaving the red cell. This process was accompanied by a bulging of the anterior portion of the parasite, which always protruded first from the cell. After exhibiting movement for a certain time the vermicules became quiescent and did not appear to become active again. In preparations kept under observation for considerable periods, both under the coverglass and as a hanging drop, only a minority of encysted forms were observed to exhibit any change.

Blood containing a very large number of parasites was also taken by means of a sterile syringe, wet with solution of sodium citrate, from the heart of a freshly killed rat and afterwards kept at a temperature of 24°C. Up to the third day no appearances suggesting development were seen.

In fresh films made from the liver and spleen forms were sometimes seen apparently lying in cells, but such were probably superposed. No developmental forms could be detected in these preparations.

Stained preparations.—In stained preparations of peripheral blood, made directly from the living animal, the parasite is seen in the encysted form only. Free vermicule forms are seen only in blood kept a short time *in vitro*, or in the organs, when these are examined at an interval after the death of the animal. In well stained specimens the characteristic parasites, having a blunt head and sharply flexed tail, are seen lying in spaces in enlarged and faintly stained corpuscles. (Fig. 2.) In imperfectly stained films the general aspect of the parasite is, with the exception of the absence of pigment, very like that of a malarial crescent. (Fig. 6.) This appearance is due to the "tail" not being differentiated from the body of the parasite. In specimens strongly stained with Romanowski's stain, (Leishman's modification) the space in which the parasite lies appears as a capsule owing to the presence of lines of stippling. When the capsule is stained in this manner it is difficult to differentiate the parasite clearly. A large chromatin mass is situated in the portion of the parasite just above the sharp curve at the tail. In some cases the mass extends so far back as to touch the origin of this structure, but usually a small area of protoplasm intervenes. The protoplasm is usually clear and free from granules except in the "tail" portion. This, nearly always, contains a variable number of small granules of chromatin, and also frequently stains a darker blue than does the rest of the parasite. In many instances a few chromatin granules are seen in the body of the parasite, both in front of and behind the chromatin mass. There does not appear to be any special significance in the arrangement of these granules.

Although certain forms were not so broad as others, no form that could be identified as young or developmental or in process of invading a red cell was seen. The chromatin mass in the attenuated forms stains intensely. It may be circular or elongated in shape. The protoplasm of these forms also appears denser and stains more darkly than in the bulkier parasites. The "tail" is conspicuous and more space is left for the parasite in the capsule. Every gradation is found between the fine forms and the swollen, apparently fully mature, forms. These more completely fill the capsule and the "tail" is sometimes so closely approximated to the body as to be distinguished only by its more granular appearance and darker staining. The chromatin mass is most frequently quadrangular in shape, and stains less densely than in the young forms. As a rule the strands form an open network. The young forms are almost invariably seen lying upon their sides. The swollen mature forms, though usually so situated, are not infrequently seen lying so that the "tail" (fig. 3) is either above or below the body of the parasite. They then appear oval in

outline, one extremity being clear, whereas the other shews a blue reticulum ("tail"). In such parasites the chromatin mass shews a marked tendency to form strands passing transversely. Occasionally the appearance is such as to suggest fission in a longitudinal direction. This may be evidenced by a partial splitting of the chromatin into two lateral masses and by a division of the protoplasm (fig. 4). Forms were sometimes found in which the protoplasm was very clear and free from granulations, though other forms in the neighbourhood were stained in the usual manner. No very certain differentiation of two sexual forms could be made out. A very common appearance was that of two forms lying in opposition. Undoubtedly this was most often accidental but in many cases the two forms were so exactly similar as to suggest that they had resulted from a process of fission. In every instance noted, however, each parasite possessed a separate capsule.

When films were made from a drop of blood which had been kept moist for some time, elongate vermicule forms were readily found. The chromatin mass, though elongated, was still situated nearer to the posterior extremity, and this portion of the parasite as heretofore contained most of the granules. A few fine granules were also usually present in the anterior portion of the parasite. Some of the free forms were very long and slender. (Fig. 8.)

Prolonged search for evidence of phagocytic action was made in several animals but in no instance was a parasite seen included in any form of leucocyte.

Examination of Viscera.—The tissues of two rats, whose peripheral blood contained very numerous forms, were examined with great care. Parasites were seen in almost all the viscera and in the muscles, but no forms not previously seen in the peripheral blood were distinguishable. The organs examined were the spleen, liver, bone-marrow, kidney, lungs, testes, thymus, thyroid, supra-renals, brain, muscles, and lymphatic glands. Parasites were most abundant in the spleen, less abundant in the liver and bone-marrow. In the other viscera they were for the most part present in small numbers only. In the spleen and liver, forms were seen which were thought at first to be included in white cells, but it is very probable that such forms were superposed.

The whole alimentary tract was laid open and scrapings of the mucosa taken at different levels. Both in fresh and stained films, exhaustive search was made for encysted forms in the epithelial cells but without result. In both rats a large number of protozoa including amœbae, flagellates and infusoria, were found. The epithelium of the gall bladder, trachea and urinary bladder was also examined but without result.

In addition to the two animals systematically examined, many spleen and liver smears were searched for developmental forms, but with the exception of occasional signs of longitudinal division no developmental stages were observed.

Effect of infection upon rats.—In the case of animals with severe infections no constitutional disturbance was noticed, but the blood shewed a certain degree of anæmia as evidenced by its pale colour. The percentage of the leucocyte values was little, if at all, altered even in animals very severely infected. Examples of counts of 500 leucocytes are the following:—

Rat 1.—Very severe infection.

Polymorphonuclear	40 per cent.
Large mononuclear	10 „
Small mononuclear	43 „
Mast cells	7 „

Rat 3.—Scanty infection.

Polymorphonuclear	43 per cent.
Large mononuclear	9 „
Small mononuclear	30 „
Mast cells	18 „

Rat 4.—Parasites only seen in very small numbers or absent.

Polymorphonuclear	43 per cent.
Large mononuclear	10 „
Small mononuclear	41 „
Mast cells	4 „

Development in the Louse.

An examination of a considerable number of the rats shewed that many of them were infested with a species of louse.* No fleas were found on any of the animals examined. It was thought probable that mosquitoes or the louse might transmit the infection. Preliminary experiments with "culex", "taeniorrhynchus" and "melanoconion", as genera likely to be concerned, were started, but were abandoned on finding evidence of transmission in the louse.

Description of the louse.

As a rule about a dozen full grown lice are found on infested animals. They chiefly frequent the long hair at the back of the neck and occiput, but they are found also in other situations. The lice are active and evade capture. Fully grown specimens measure 1·4 *m. m.* in length and reach a breadth of ·5 *m. m.*

The *head* is quadrangular in shape owing to the broad straight front and two large flat processes posteriorly. There is a marked constriction at its junction with the thorax.

The *antennæ* are five jointed. The basal joint is greatly developed, so that it forms a large globular mass. The third segment in the male has a

* Dr. Stephens to whom I sent specimens of the louse, has written to me that Mr. Newstead has kindly identified it as a new species of "*Hæmatopinus*."

spur-like process. All segments have a few short spines. There is a terminal papilla bearing a few short spines.

The *eyes* are absent.*

The *mouth* is situated upon the inferior surface of a papilla. The papilla carries six short curved spines arranged in a circlet. A second circle of similar but larger spines surrounds the papilla at a short distance.

At each of the posterior angles of the head, there is a large flat chitinous expansion, from which arises a single, very long, stout hair, and one or two small spines. The hair passes back as far as the abdomen. On the ventral surface of the head there are two medium sized spines arising near the base of the antenna.

The *thorax* is small. It is separated from the abdomen by a constriction. The posterior angles of the chief dorsal plate are thickened and carry a number of stout spines. Internally to the spines, a long hair resembling that on the head, passes backwards as far as the first segments of the abdomen. Ventrally there is a narrow sternal plate, on either side of which the coxæ of the three pairs of legs arise.

The *legs* consist of four large segments.

The *proximal joints* of the legs (coxæ) are oval and of large size. They bear on their anterior surface, especially at their distal extremities, some small spines. The *second joints* are small. They carry small spines anteriorly. The *third joints* are large and globose. They are armed with spines on their anterior surfaces. The *fourth joint* is large and triangular. It is smallest in the first pair of legs, and very large and massive in the third pair. The apex of the triangle articulates with the third joint. The anterior angle of the base is continued as a strong curved claw. The posterior angle forms a pad. Between the two is a hollow in which the shaft of the host's hair is held. The claw is jointed at its base. A second articulation is visible a short distance above this. The claws increase in size and massiveness from before backwards. That of the third legs is an extremely large mass of chitin shaped like a parrot's beak.

The *abdomen* consists of nine visible segments. The differentiation of the ninth segment is more readily made out in the female than in the male. Each of the first six segments carries on its posterior border, both ventrally and dorsally, a fringe of hairs. The seventh segment carries only two hairs on the dorsal surface. Laterally the segments carry long stout hairs, as well as short powerful spines. In the adult, the first segment carries a single hair. The 2nd, 3rd, 4th, 5th, 6th, 7th and 8th, each two hairs, of which those of the 2nd and 6th segments are the longest. The eighth segment in the male, carries a short hair laterally. In the female it carries a number of stout spine-like hairs. The ninth segment has, in the female, a slit-like opening guarded by two chitinous plates. In the male, a

* NOTE.—*Gerbillus indicus* is nocturnal in its habits and an extensive burrower.

horny papilla bears a circlet of short curved spines. On the dorsal surface of the papilla there is a large opening. In the male the penis forms a conspicuous object. It terminates in a horn-shaped process.

The *ova* measure ·48 *m. m.* in length. They are fixed to the hair about half way up the shaft. The attached end lies towards the base of the hair.

The young escape from the egg as forms closely resembling the adult. The abdomen, however, is very small and the hairs projecting from it proportionately very long. In the immature forms the abdominal segments carry a fringe of only four hairs instead of many as in the adult.

Structure.

The alimentary canal.—The alimentary canal consists of the following portions:—

Fore-gut . . .	Mouth.
	Chitinous pharyngeal pump.
	Oesophagus.
Mid-gut . . .	1st portion or descending part of mid-gut.
	2nd portion or ascending part of mid-gut.
	Pyloric ampulla with the origin of malpighian tubules.
Hind-gut . . .	Intestine.
	Rectum.

The mouth is very small. The opening is situated beneath the prow-like anterior portion of the head.

The *pharyngeal pump* is long and narrow. It passes through the head and into the thorax. It ends in the front part of the thorax in a number of chitinous bars.

The *oesophagus* passes backwards and upwards, surrounded by the large ganglionic masses. It is a delicate wrinkled tube much folded upon itself. It enters the large massive mid-gut with no appreciable proventricular fold.

The *mid-gut* is sharply bent upon itself, so that it forms a U shaped organ. The first portion is the largest and its walls are thickest. It is lined by large granular cells with large nuclei. In the distended organ the cells are flattened. In the empty viscus they are cubical. Externally there are longitudinal and circular fibres, arranged to form an open network. During life rythmical contractions are constantly taking place. The contents are blood, either unaltered in appearance, or partially digested. The mid-gut, especially the more massive first portion, occupies, when distended, most of the abdominal space not taken up by the ovaries.

The *pyloric ampulla** is a dilated portion of the mid-gut and is supplied with numerous circular muscles. Into it open four malpighian tubules. These enter two on each side, close together. Immediately beyond the malpighian tubules, a distinct dividing line between the mid-gut and the intestinal epithelum is seen.

The intestine is straight and thin-walled. There is no differentiation into large and small intestine. It passes from the muscular pyloric mass, close beneath the abdominal terga, to the rectum. The cells lining the cavity are small. It has a layer of circular muscle fibres, and outside this a number of longitudinal fibres. The nuclei of the latter form conspicuous objects in the fresh tissues.

The rectum consists of a distended globular portion, containing rectal papillæ, and a straight narrow portion leading directly to the anus at the apex of the ninth segment.

The salivary glands.—These are small oval structures, consisting of a single acinus, which rests upon the anterior portion of the mid-gut in the first abdominal segment. A duct, of delicate structure, leads forwards towards the pharyngeal pump. Each acinus consists of several large cells with a central clear very refractile space.

The malpighian tubules.—These resemble the tubules of insects generally and consist of cells with large oval nuclei. The cells are much drawn out and nuclei are situated at considerable intervals along the tube.

The generative organs.—The female organs consist of an ovary on each side opening into a short common oviduct. Each ovary is composed of five or six follicular tubes, each of which is attached to the body wall by a delicate apical strand of tissue, and opens into the oviduct of its own side. Each contains two or more egg follicles in different stages of development. In the adult louse two fully matured follicles, one lying on each side, occupy the greater part of the abdomen. All follicles, except the youngest, consist of a single layer of cubical or columnar cells surrounding an ovum and nurse cells. In the mature follicles the nurse cells which lie towards the apex of the follicle have become absorbed.

The spermatheca.—This is a large thin-walled chitinous sac, opening by a short tube near the anus. It may be empty or may contain masses of spermatozoa.

The male organs.—The testes are curious double peg-top shaped organs. Each opens by a narrow tube into a wide seminal vescicle, in which masses of spermatozoa are seen. The duct of the testes opens at a distance from the end of the seminal vescicle, so that this possesses a caecal extremity. The seminal

* Note.—A similar dilated portion of the alimentary canal is present in the mosquito. In the mosquito the dilatation is derived from the hind-gut; in the louse it appears to be derived from the mid-gut.

vescicles lie across the abdomen, and open together into a short straight tube surrounded with large muscle fibres. This tube in turn passes into the penis.

The fat body.—The fat body is well developed. In the head and thorax it forms pads lying between the muscles and the organs. A very conspicuous pad lies beneath the pharynx, and other masses are situated close to the salivary glands. In the abdomen, the fat body consists of a number of conical masses attached by their apices to the body wall, near the origin of the lateral hairs. The bases of the conical masses lie against the mid-gut and other viscera to which they give support. The cells which compose the fat body contain, as well as oil globules, masses of opaque granules apparently of a calcareous nature.

In addition to the fat body, certain very curious cells are seen free in the body cavity. On dissecting the abdomen, these are liberated and lie about on the slide, quite unconnected with the other tissues. They are oval or spindle shaped, measuring 140μ in length. At one or both poles they have a delicate spike-like process. They contain two nuclei and possess a fairly resistant cuticle, which is left as a shrunken structure when the cell contents have been extruded. In spite of the parasitic-like aspect of these cells, they appear to be normal tissue cells of the louse. Somewhat similar cells, though in less numbers, were found when dissecting a species of louse infesting calves. Certain groups of small, partially free, cells are also seen in the body cavity, especially near the terminal segments. The nature of these and of the large cells noted above is not clear.

The muscular system.—Powerful muscles are connected with the pumping organ and the large antennæ. They occupy much of the space in the head, not taken up by the large ganglia. The thorax is mainly muscular. The abdomen has certain small dorso-ventral muscle bundles, which when seen in optical section in the fresh louse are conspicuous refractile bodies. A band of muscle passes along each side of the body, and is attached to each segment near the origin of the long hairs. In the male, powerful muscles are seen in connection with the penis.

The tracheal system.—Two large spiracles of oval shape are situated just behind the spiny area of the thorax. From these, and smaller abdominal stigmata, tracheæ form loops with distributory branches for the tissues. The tracheæ are not very large or numerous.

The nervous system.—The ganglionic masses of the head are of great size. The thoracic ganglion is also a large and conspicuous object, especially in sections.

The circulatory system.—There is a pulsating chamber beneath the 6th and 7th abdominal terga, from which a dorsal vessel, also pulsatile, passes forwards over the mid-gut. Muscle fibres of a peculiar nature pass outwards from the chamber to the body wall.

Development of the parasite in the body of the louse.

In order that feeding experiments might shew unequivocal results, a young animal was selected in whose blood parasites were extremely abundant. As a cursory examination of the fur revealed no lice, a number were transferred from four older animals. They quickly disappeared among the hairs.

On the fourth day ova were noticed on the fur about the head. Two lice, presumably belonging to those placed on the rat, were removed for dissection. One was dissected immediately after removal, the other was left overnight in a moist chamber.

Dissection of the first louse shewed very numerous free vermicules in the mid-gut and intestine. A number were also scattered about the preparation, but may have been derived from the gut as this was ruptured. The vermicules exhibited active movements, mainly of lateral flexion. Very few encysted parasites were present.

No forms were seen in the salivary glands or ovaries.

A dry preparation was made from the mid-gut and its contents and stained with Romanowski's stain. Numerous large vermicule forms, in various attitudes, were seen among altered blood corpuscles. As many as a dozen vermicules were to be seen in single fields of the microscope. Compared with stained specimens of free vermicules from the peripheral blood of the rat, these forms appeared larger and finer. The chromatin masses, especially, were noted as extending through a greater portion of the parasite. The protoplasm of the vermicules was, in almost every case, free from granules and stained a clear blue. Some of the vermicules had assumed a characteristic crescentic form. (Fig. 9.)

Dissection of the second louse shewed many free vermicules collected in the pyloric ampulla (fig. 11). A motionless vermicule, which had become crescentic in shape, was detected in the body cavity in the neighbourhood of the rectum. In this dissection the alimentary canal was apparently free from injury. The vermicules in the gut shewed sluggish movements chiefly of lateral flexion. Only a single, still unchanged, encysted form was noted.

On the seventh day the rat was killed and the lice collected. Many young lice, apparently just hatched, were observed. Dissection of several of these shewed many very active vermicules in the gut. In the dissection of the larger specimens, made on this day, certain very striking large cysts undoubtedly of parasitic origin, lying free in the body cavity of the louse were found. The most developed cysts were of large size, measuring as much as 350μ in diameter, and being readily seen under a low power lying in the abdomen of the infected louse (fig. 20). They were most often seen in the anterior portion of the abdomen, but were present also in other

parts. On pulling apart the abdominal segments, they readily became free upon the slide, and were easily visible to the naked eye, as minute white spheres. Under the microscope, the large cysts were seen to be filled with a number of small oval cysts. Each of these again contained six to eight crescentic bodies lying as a rule slightly obliquely in the long diameter of the cyst. On the rupture of the smaller cysts, the crescentic bodies were liberated, and were seen to be sausage-shaped bodies with blunt extremities, measuring 15μ in length and 4μ in thickness. A refractile area (nucleus) was situated nearer one extremity than the other. The protoplasm was granular, but in both extremities clear areas resembling vacuoles were conspicuous. No movement was observed in the bodies. On rupture, the wall of the small cysts became folded so as to form elongated spindles of very regular pattern. In the empty cyst a small granular mass (reliquat) was visible. (Figs. 15 and 16.)

Younger stages of the large cysts were readily made out. All appeared quite free in the body cavity. The smallest cysts seen measured 10μ in diameter. They shewed a double contour, and a central clear refractile spot. Later stages are proportionately larger. In medium sized cysts a thick clear outer zone is present within the double contour of the cyst wall. The contents are granular, the granules sometimes being arranged in clusters. As maturation advances, the central granular portion becomes more opaque. At this stage the parasite has a characteristic aspect so that it can be detected, even without dissection, lying in the abdomen of the louse. The central granular mass is usually situated slightly excentrically, so that the clear zone is thicker at one side of the cyst than at the other. Before full maturation, the central mass becomes divided into oval bodies containing granular protoplasm arranged in a characteristic manner (fig. 17). Attempts to obtain satisfactorily stained specimens of the immature stages were not successful. (Figs. 12, 13 and 14.)

In stained films made from the ruptured mature cysts, beautiful specimens of the liberated sausage-shaped bodies were seen, but the collapsed and unruptured cysts remained unstained. Considerably nearer one extremity than the other, so that it divides the protoplasm in two unequal halves, there is a large granular densely staining chromatin mass, and the body of the parasite at this point shews a marked constriction. The protoplasm is granular but contains in either extremity a conspicuous clear area.

In all except two of eleven lice removed from the rat, cysts in some stage or other of development were found by dissection. In one louse three fully mature cysts, two medium sized cysts and three small cysts were found.

In none of the lice was any unusual appearance observed in the salivary glands or ovaries.

In addition to eleven lice which were dissected in the fresh condition, two were fixed in alcohol and reserved for the preparation of sections. Sections cu in paraffin were stained with hæmatein.

In one instance the mid-gut was found fully distended with recently drawn blood, the corpuscles of which were scarcely altered in appearance. Numerous vermicule forms were seen. They were especially abundant near the walls of the gut. One or two examples only were seen of forms still encysted in corpuscles. The vermicules were usually stretched out, so that they gave a vivid impression that they were travelling through the mass of closely packed red cells.

In the body cavity of the louse a large cyst was noted, lying between the wall of the mid-gut and the 5th and 6th abdominal terga. It contained numerous darkly stained bodies, shewing a central clear area and a nucleus. These bodies appeared to be the immature stages of the contained cysts, prior to the formation of the sickles. In the second specimen, the mid-gut was empty. No vermicules were seen in the lumen. A single vermicule was detected in the upper part of the intestine. The abdomen was distended with nearly mature ova. No unusual bodies were seen in these, nor in the salivary glands. No cysts could be detected in the sections.

The appearances to be expected, being now familiar, further dissections were made. A rat which had been placed in a separate cage, on account of the presence of numerous parasites in the blood, was noticed to have a few lice and a considerable number of ova on the hairs about the head. Three lice were removed and dissected with the following result :—

Louse . . .	1	*Nil.*
,, . . .	2	One medium sized cyst.
,, . . .	3	Two medium sized cysts and one small cyst.

The rat was kept under observation for five days and then killed. Ten lice were dissected with the following result : —

Louse . . .	1	*Nil.*
,, . . .	2	*Nil.*
,, . . .	3	One medium sized cyst.
,, . . .	4	Two fully matured cysts.
,, . . .	5	Two medium sized cysts.
,, . . .	6	*Nil.*
,, . . .	7	One fully matured cyst.
,, . . .	8	*Nil.*
,, . . .	9	*Nil*
,, . . .	10	Two large and one medium sized cysts.

A rat with a scanty infection was killed and the lice collected. Dissection of seven lice gave the following result :—

Louse . . .	1	*Nil.*
,, . . .	2	*Nil.*

Louse	3	One half grown cyst.
,,	4	*Nil.*
,,	5	*Nil.*
,,	6	*Nil.*
,,	7	*Nil.*

A rat in whose blood I had never found more than an occasional parasite, was found to be harbouring a certain number of lice. Nine adult lice only were obtained. No cyst was found in any of these.*

Behaviour of crescentic bodies when treated with intestinal juice.—A number of rats, which were kept together in a large cage, were found to have become almost free from lice in a comparatively short time. Several rats from the same batch, which had been placed in separate cages were, on the contrary, badly infested. It was thought possible that the animals might clean each other, especially as they were observed to snap up mosquitoes on the wing and to eat ants on the floor of their cages. The action of intestinal juice upon the sausage-shaped bodies was, therefore, tried. A louse, in which two mature cysts had been detected by low power examination, was carefully broken up in salt solution. The two cysts were obtained uninjured upon the slide. One was removed for a separate experiment. A rat was killed and the intestine opened a short distance below the duodenum.

A small quantity of intestinal fluid was placed upon the cyst, and the whole covered with a coverglass. Under a low power the large cyst was seen to have ruptured, and the small contained cysts to be scattered in the intestinal fluid. A small number of the latter had also ruptured, and the contained bodies were liberated. The exposed crescentic bodies exhibited some sluggish movements. The bodies in the cysts remained unchanged. The preparation was placed in the incubator and examined at the end of an hour. Most of the free bodies were motionless, and some appeared to have undergone dissolution. The coverglass was pressed, in order to rupture more of the cysts, and the preparation placed again in the incubator at 37°c. At the end of an hour, no changes were seen, and the bodies exhibited no movement. After twelve hours most of the free forms had disappeared.

Behaviour of the bodies in plasma.—Upon the other cyst, a drop of blood from the heart of the rat was placed, and the whole covered by a coverglass. The pressure of the glass ruptured the outer cyst but only a small number of the contained cysts were ruptured. After a very few minutes, a commotion was observed among some corpuscles, and a vermicule-like body was seen to be moving these about vigorously. Other bodies exhibiting active movements

* Note.—Since writing the above I have dissected lice from a considerable number of rats. Those from rats with numerous parasites in the blood consistently shew the cystic bodies, whereas lice from rats with very scanty or no infection are negative or contain the bodies in very small number only.

were also noticed. At first it was suspected that these were vermicules liberated from the red cells of the rat; but a cursory examination of the rat's blood shewed that, if present, parasites were scanty. It was also observed, that the active vermicules were present only in the neighbourhood of the ruptured cyst, and that they were distinctly larger than vermicules ordinarily seen in rat's blood.

Soon nearly all the liberated forms were seen to be exhibiting movements. The movements were not progressive, but consisted of a wriggling motion somewhat like that of the larvæ of blow-flies. Forms here and there were then noticed to be rotating one or more blood corpuscles, apparently by means of an invisible projection from the tail. This movement was so curious and significant, that the hope of some development in plasma was raised. The preparation was placed in the incubator at 37° for an hour. The bodies had all become quiescent, except one, which was rotating upon its own axis at considerable speed. The preparation was again placed in the incubator for an hour. On examination, it was found that the form just noted had also become quiescent, but that it had changed somewhat in shape so as to become egg-shaped with rather truncated ends. The forms still included in the cysts were apparently unchanged. With a view to liberate more of these, the coverglass was compressed with a needle. By this means most of the cysts were ruptured, and the contained forms set free. Almost all the forms so liberated, and many of those lying in partially ruptured cysts, at once began to exhibit the most active movements. It was then noted that the majority of the forms were going through the same extraordinary feat of rapidly rotating one or more red cells by means of their narrow extremity. The corpuscles, in many cases, were put in rotation whilst still separated by a distinct interval from the point of the parasite. Very often no movement of the parasite was seen, but in some cases the narrow extremity moved in a circular manner. As the process continued, the corpuscles became aggregated in masses of ten or twenty, the whole mass being rotated in some cases. In others the body of the parasite itself rotated, using the blood cells as a fixed point. For an hour the field of the microscope was filled with forms revolving upon their axis or causing red cells to revolve. The preparation was again placed in the incubator and not examined until next morning. All forms were then motionless, but had in most cases taken on the egg-shaped form already noted. A certain number of bodies had retained their original sausage shape. From the position of some of these, it appeared probable that they were forms which had failed to exhibit the movement of rotation. A stained specimen, made from this preparation, shewed oval forms, having a light reticular protoplasm and a central lightly staining chromatin mass. The constriction previously noted was no longer present, and

the appearance of the bodies was much changed. The forms measured in the fresh state 9·5μ in length and 5·5μ in breadth.

CONCLUSIONS.

So far as I am aware a hæmogregarine parasite has not been observed previously in either a bird or a mammal. The only mammalian parasite, which may possibly come under this designation, is that recently described by Captain James [1] and Dr. Bentley [2]. Except that this parasite appears to invade the polymorphonuclear leucocytes, there are some points of general resemblance between it and the parasite now under discussion. In the parasite of the field rat, the resemblance to a hæmogregarine, in every respect, is exact. The absence of pigment, the vermicule form, the curved "tail," the presence of a space in which the parasite lies, the occurrence of motile forms and their behaviour are all features characteristic of the group. The only appreciable difference between this form and typical members of the genus is due to the absence of a nucleus in the red cell of the host.

I have provisionally named the parasite *Hæmogregarina gerbilli*.*

The method of increase in the blood is not very clear. The only method of multiplication, of which any evidence was obtained, was longitudinal fission. Forms younger than the attenuated form of vermicule, if present, must be very rare. The absence of appearances shewing how the red cells are invaded and the early stages of invasion, is also very remarkable. The existence of multiplication forms in the intestinal epithelium is doubtful. As regards the forms seen in the louse, I have no doubt that they are extra-corporeal stages of *Hæmogregarina gerbilli*. This is of interest since although the transmission of many protozoa by biting insects has been demonstrated by experiment, the only instance, of which we have actual knowledge of the stages gone through in the carrier, is that relating to the developmental stages of certain parasites in the mosquito. The effect of intestinal secretion upon the sporozoits does not suggest ingestion by the mouth as a probable method of infection; but the extraordinary behaviour of the sporozoits in freshly drawn blood of the rat is, on the other hand, very significant. Further dissection of lice, kept for longer periods on infected rats may throw light on the method by which the sporozoits gain entrance to the blood.

* M. Laveran has very kindly given me his opinion upon the nature of this parasite. He writes as follows:—"*Comme vous me l' écrivez, il ne parait pas douteux que ces hématozoaires nouveaux doivent être rangés parmi les hémogrègariens.*"

PAPERS REFERRED TO IN THE TEXT.

[1] On a parasite found in the white corpuscles of the blood of dogs. By Captain S. P. James, I.M.S. Scientific Memoirs by Officers of the Medical and Sanitary Departments of the Government of India New Series, No. 14, March 1905.

[2] Preliminary note upon a leucocytozoan of the dog. By C. A. Bentley, M.B., D. T. M. H. Cantab. D.P.H., British Medical Journal, May 6th, 1905, page 988.

EXPLANATION OF PLATE.

Figure 1.—Forms seen in fresh preparations of the peripheral blood.
Figure 2.—Forms seen in films stained by Romanowski's stain.
Figure 3.—Oval form due to position adopted by a mature vermicule. The nucleus shews transverse bands of chromatin.
Figure 4.—Longitudinal fission form.
Figure 5.—Forms possibly arising from an act of longitudinal fission.
Figure 6.—Imperfectly stained form, shewing similarity to malignant tertian crescent.
Figure 7.—Vermicules in fresh blood preparation.
Figure 8.—Vermicules stained by Romanowski's stain.
Figure 9.—Vermicules in contents of mid-gut of louse. A single field.
Figure 10.—Dissection shewing alimentary canal of louse and contained vermicules.
Figure 11.—Pyloric ampulla containing numerous vermicule forms. Fresh preparation.
Figures 12 and 13.—Young forms of cyst.
Figure 14.—Form shewing clear outer area and granular contents.
Figure 15.—Fully matured cyst.
Figure 16.—Zygotes containing sporozoits.
Figure 17.—Zygotes in process of development.
Figure 18.—Sporozoits in normal saline.
Figure 19.—Sporozoits; stained preparation.
Figure 20.—Adult louse shewing a single fully matured cyst in abdomen.

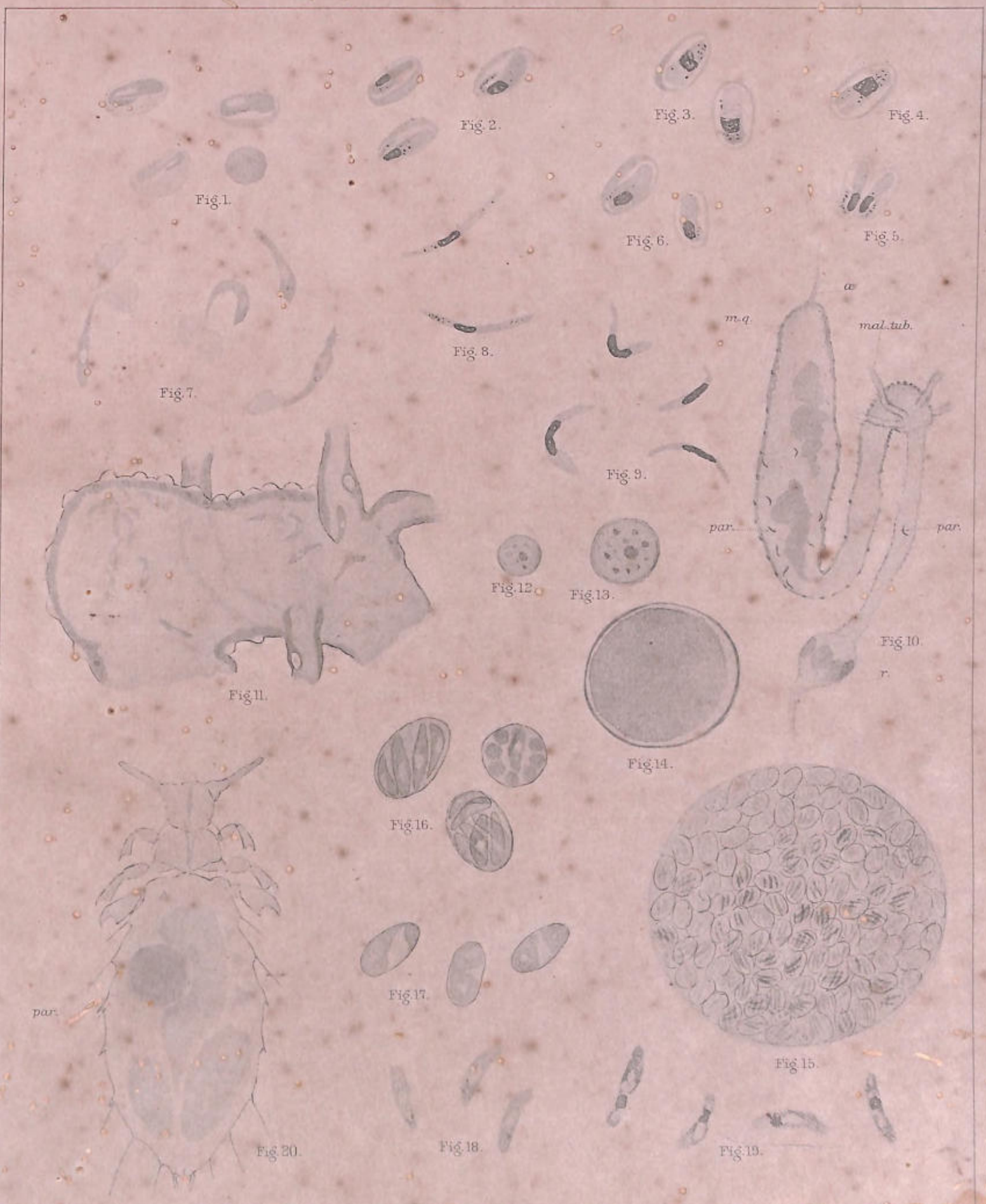
Fig. 1.
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Fig. 11.
Fig. 12.
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Fig. 15.
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Fig. 20.
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