(*NEW SERIES.*)

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OFFICERS OF THE MEDICAL AND SANITARY DEPARTMENTS

OF THE

GOVERNMENT OF INDIA.

THE THEORY AND PRACTICE OF ANTI-RABIC IMMUNISATION.

BY

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AND

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Price Annas 12 or 1s. 2d.

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THE THEORY AND PRACTICE OF ANTI-RABIC IMMUNISATION.

ALMOST every Pasteur Institute has its own method of treatment differing from that of other Institutes and from Pasteur's original scheme. These differences show that immunity can be obtained by the administration of protective material in amounts which may vary within wide limits. In the light which research has thrown upon the problems of immunity in recent years we may ask ourselves which of the various methods of anti-rabic treatment is to be regarded as best combining accuracy of dosage with the establishment of protection. The enquiry may even be pushed further and include a consideration of the advisability or otherwise of adopting an entirely new method of procedure—one more conformable with that accepted as satisfactory for anti-bacterial immunisation. Some of the differences in treatment as at present practised are more apparent than real. Thus, in one place the rabbits used are smaller and yield in consequence thinner spinal cords. Drying will be more rapid in the case of the thinner than the thicker cords, and therefore larger doses of the former (*i.e.*, greater lengths or weights) will correspond to smaller doses of the latter. Again, the character and type of patient treated has to be taken into account. Patients of comparatively poor physique and deficient stamina will have to be treated more carefully and with smaller doses than robust patients lest they be disturbed in general health by the treatment and so require an enforced and undesirable rest from inoculation. There are many other reasons which singly or collectively serve to justify the adoption of particular formulæ of treatment; and it would be satisfactory if some consensus of opinion were reached at all events on such points as the following:—

(1) The effect which various degrees of desiccation or other treatment to which rabies material may be subjected has upon its infectivity or upon its vaccinal power, and the expression of this effect in terms of some definite unit.

(2) What total amount in units of fixed virus should be administered to a patient under given conditions and within a given period of time in order to produce as rapidly and satisfactorily as possible a maximum power of resistance to rabies infection.

(3) The nature of the immunity produced by anti-rabic inoculation.

We have made an attempt in the following pages to state facts, new and old, which may tend to some extent to simplify opinions on these points. We believe that a great many of the divergencies manifested in the different schemes of different Institutes would be annulled if in each case the total quantity of virus given within a certain period of time were looked upon as the ultimate standard to which all treatment formulæ had to conform. At all events statistics show that the success obtained in treatment by any one Institute does not differ greatly from that obtained by any other, however diverse in appearance the schemes employed. From this fact alone we would infer that the particular scheme employed in any one particular place is not so important as the total quantity of fixed rabies virus which is administered in a given time. Now, whether we agree or not with the view [1 & 2] that slow drying of rabies material simply results in diminution of the quantity of living virus contained in it, it is still unquestionably the case that smaller quantities of less altered rabies virus are the equivalent of larger quantities of more altered virus, however the alteration (desiccation, heat, glycerine, etc.) may have been brought about. We shall enter into this question later in all its bearings upon anti-rabic immunisation. As a result of the fact just stated it may be asserted that ample opportunity is afforded—and verily it has been made full use of—to construct a variety of schemes of treatment according to individual idiosyncrasy without involving any final difference in the dose administered to the patient. An illustration will make our meaning clear. A cord which has been drying for five days is still infective though not so infective as a cord which has been drying for only three days. Quantitatively expressed this might conceivably be equivalent to saying that 1 grm. of three-day cord was equal in strength to 2 grms. of the same cord after five days drying, or that a given quantity of three-day cord will produce exactly the same effect, other things being equal, as twice the quantity of five-day cord. We can then understand how it may come about that in one Institute a certain quantity of three-day cord might be administered to a patient whilst in another Institute, in the same circumstances, twice the amount of five-day cord might be given and with equal effect. This simple fact we think goes far to explain many of the divergencies in different schemes of treatment. These divergencies might be reduced to conformity if we were able to give definite values in units to definite quantities of rabies cord dried* for definite lengths of time or to definite dilutions of fresh rabies virus.

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[1] Pasteur, *Lettre sur la Rage ; Ann. de l' Inst. Past.*, 1887.

[2] Höyges' Art. '*Lyssa*' in Nothnagel's "*Specielle Pathologie und Therapie*," pp. 148, 161.

* We use the term 'dried' for convenience to express the idea of alteration in rabies virus, however it is brought about, instead of repeating on each occasion the various processes, such as desiccation, heating, use of glycerine, etc., by which that alteration is effected.

We should then be able to say that for a light case or a severe case at least so many units must be given within a certain period. We have endeavoured—as we show later—to obtain a basis on which a calculation of this sort may be made. Meanwhile we may exemplify our meaning by taking an arbitrary value and applying it to the elucidation of the simplest of all the schemes of treatment—that of the late Prof. Höyges. The next step will be to correlate the scheme of Höyges with other schemes which proceed on a different basis, and then we should finally be in a position to arrive at some general conclusion.

Let us say then, for the sake of our example, that 0·2 cc. of a 1 per cent. emulsion of fresh rabies material is equal to 1,000 units,—quite an arbitrary value of course—then 0·2 cc. of a $\frac{1}{1,000}$ emulsion = 100 units and 0·2 cc. of a $\frac{1}{10,000}$ emulsion = 10 units. From this we can give an exact value to Höyges' schemes, light and intensive.

Three cubic centimetres (the dose administered) of a $\frac{1}{10,000}$ dilution will be equal to 150 units, and so on. Thus in Höyges' light scheme the patient receives on the first day 887·5 units; on the second 800 units; and so on for the succeeding days.

TABLE I.

HÖYGES' LIGHT SCHEME.[1]

Day.	Dilution.	Dose.	No. of units.
1st . . .	$\frac{1}{10,000} + \frac{1}{8,000}$ $\frac{1}{6,000} + \frac{1}{5,000}$	3 cc. + 3 cc. 3 cc. + 3 cc.	887·5
2nd . . .	$\frac{1}{5,000} + \frac{1}{2,000}$	3 cc. + 2 cc. . .	800·0
3rd . . .	$\frac{1}{2,000} + \frac{1}{1,000}$	2 cc. + 1·5 cc. . .	1,250
4th . . .	$\frac{1}{1,000} + \frac{1}{500}$	1·5 cc. + 1 cc. . .	1,750
5th . . .	$\frac{1}{200}$	1 cc.	2,500
6th . . .	$\frac{1}{6,000} + \frac{1}{5,000}$ $\frac{1}{2,000}$	3 cc. + 3 cc 2 cc.	1,050
7th . . .	$\frac{1}{2,000} + \frac{1}{1,000}$	2 cc. + 1·5 cc. . .	1,250
8th . . .	$\frac{1}{1,000} + \frac{1}{500}$	1·5 cc. + 1 cc. . .	1,750
9th . . .	$\frac{1}{200}$	1 cc.	2,500
10th . .	$\frac{1}{6,000} + \frac{1}{5,000}$ $\frac{1}{2,000}$	3 cc. + 3 cc. 2 cc.	1,050
11th . . .	$\frac{1}{2,000} + \frac{1}{1,000}$	2 cc. + 1·5 cc. . .	1,250
12th . . .	$\frac{1}{1,000} + \frac{1}{500}$	1·5 cc. + 1 cc. . .	1,750
13th . . .	$\frac{1}{200}$	1 cc.	2,500
14th . . .	$\frac{1}{100}$	1 cc.	5,000
		TOTAL .	25,287·5

[1] Deutsch & Feistmantel "*Impfstoffe und Sera*," p. 154.

But it is obvious that the amounts in units given in this scheme may be made up in a variety of ways. Thus instead of the patient receiving 3 cc. of $\frac{1}{10,000}$, $\frac{1}{8,000}$, $\frac{1}{6,000}$, and $\frac{1}{5,000}$ dilutions on the first day, he might receive instead two doses (3 cc. each) of $\frac{1}{9000}$ and two doses (3 cc. each) of $\frac{1}{5,500}$ dilutions. In fact the whole treatment might be carried through with the use of a comparatively small number of dilutions—say $\frac{1}{4,000}$, $\frac{1}{1,000}$, $\frac{1}{200}$—and yet be exactly equivalent amount for amount with that set forth in the more complicated scheme. Further we may ask, is there any advantage in making numerous inoculations where one or at most two would suffice? Why give on the first day 3 cc. of $\frac{1}{10,000}$, $\frac{1}{8,000}$, $\frac{1}{6,000}$, $\frac{1}{5,000}$ instead of what is exactly equivalent, two inoculations, 4·87 cc. of $\frac{1}{5,000}$ and 4·005 cc. of $\frac{1}{5,000}$, or, simpler still, one inoculation, 3·55 cc. of $\frac{1}{2,000}$? In this the analogy with other diseases in which immunity is produced by artificial inoculation may help us. It has been observed (Bannerman), for instance, that the immunity prophylactically obtainable against plague is greater when the dose of inoculation material is divided up and inoculated in several places. Such an idea too is in harmony with the conception that anti-bodies are largely produced by those body cells which are in the neighbourhood of the inoculation.[1] Each new place inoculated constitutes a new factory of anti-bodies, whilst within certain obvious limits the more of these there are and the more cells directly stimulated to produce anti-bodies the greater will be the amount of anti-body production and the greater will be the resistance set up against the originally contracted infection. The endeavour of the operator must be to induce the maximum amount of anti-body production and to avoid any chance of the excessive development of that "negative phase" condition in which the person inoculated is rendered less rather than more resistant to attack. In spite of the fact, however, that there is some evidence to show that several small inoculations in different parts of the body produce a better or more rapid immunity than does the same total amount inoculated in fewer places, we find the general practice, *e.g.*, in the cases of plague, typhoid fever, cholera, staphylococcus infection, is to give only two or three properly interspaced injections. We may therefore legitimately ask ourselves whether it would be possible to apply this method likewise to the prophylactic treatment of rabies and whether this would be as good a method as that of the daily inoculation which is at present the only one practised. It would be a great boon to patients if, for example, they received one injection upon arrival at the Pasteur Institute and a final one, say, 10 days later. The question which must certainly be asked is whether the procedure would be safe or not. We have given massive

[1] Wassermann and Citron *Deutsch. Med. Woch.*, 1905, No. 15, p. 573, *Zeitschr f. Hyg.*, May 19th, 1905, p. 331.

doses of fresh rabies virus to various animals with no ill effect. We have also produced immunity to subdural inoculation in dogs by as few as one, two, and four subcutaneous inoculations (separate trials) of fresh rabies virus (*vide* pp. 23-24). These experiments we refer to later, but in any case we go no further in this paper than to afford our testimony and give our support to those workers who advocate a diminution of the number of anti-rabic inoculations with an increase in their daily strength.

Another point of discussion which is suggested by an examination of Höyges' scheme of treatment (and it is equally suggested by other schemes) is the necessity for returning to a weaker dose after having once reached a stronger. Would there, for example, be any serious apprehension of danger or of insufficient protection if Höyges' light scheme were altered as follows?

TABLE II.

Day of inoculation.	Number of units given.
1st	800
2nd	887·5
3rd	1,050
4th	1,050
5th	1,250
6th	1,250
7th	1,250
8th	1,750
9th	1,750
10th	1,750
11th	2,500
12th	2,500
13th	2,500
14th	5,000
TOTAL	25,287·5

It is said that it is important that a patient should be introduced as quickly as possible to virulent material. This, however, does not seem to us to be sufficient reason for a return immediately thereafter to less virulent, or, in the case of Höyges' scheme, to smaller amounts of virus. In Höyges' scheme the strongest dose given is 1 cc. of $\frac{1}{100}$ dilution (5,000 units). It can scarcely be that a $\frac{1}{100}$ dilution is *per se* more dangerous than a $\frac{1}{2,000}$ dilution; for 0·5 cc. of $\frac{1}{100}$ dilution (2,500 units) is equal as regards content in virus to 4 cc. of $\frac{1}{2,000}$ dilution. We consider, therefore, that some such alteration of Höyges' scheme as that given above (Table II) would be as satisfactory as the scheme in general use, or, if it were essential to hasten the earlier stages, it might be altered somewhat as follows:—

TABLE III.

Day of inoculation.	No. of units.
1st	800
2nd	1,000
3rd	1,000
4th	2,000
5th	2,000
6th	2,000
7th	2,000
8th	2,000
9th	2,000
10th	2,000
11th	2,000
12th	2,000
13th	3,000
14th	4,000
TOTAL	27,800

Again, when we consider that the intensive scheme (Höyges') results in the administration in 11 days of an amount (24,287·5 units) very nearly the same as the total amount given in 14 days in the light scheme (25,287·5 units), we may well ask whether some 25,000 or 26 000 units administered in 10 or 11 days

might not suffice for a light form of treatment and so save the patient the necessity of waiting longer. Let the patient have 25,000 units in 11 days and then depart. This idea (rapid immunisation) has been referred to by other authors, and has actually been put into practice (Ferrans,[1] Nitsch [2]). It is one to which we think earnest consideration should be given.

We may now sum up our position so far as we have yet discussed it.

(1) The variations among different schemes of treatment are probably to a large extent only apparent and do not necessarily imply any great divergence of opinion. Anti-rabic treatment consists in the administration of rabies virus in various amounts with the view of producing what is probably entirely an anti-microbic immunity* as distinguished from an anti-toxic. We have taken as an example one scheme and shown how an infinite number of variations in the method of its application are possible. The same applies to other schemes, only that it is a little more difficult in them to demonstrate the fact owing to the quantitative relations between the different strengths of vaccine not being so easily estimated.

(2) The justification of the administration of small doses frequently repeated possibly lies in this, that by so doing a greater area of anti-body producing cells is brought into action.

(3) It is possible and might even be advantageous so to alter the method of anti-rabic treatment as to bring it into line with the prophylactic treatment of plague, typhoid fever, etc. Such a method as this, even though not applied in the case of human beings, might be eminently serviceable in India for the treatment of the lower animals. The difficulty here is that delay might occur in obtaining the necessary material of inoculation. In such a case, if available, the brain of the biting animal might serve for the administration of a first subcutaneous injection. In some ten days, when a second injection would be due, the specially prepared fixed virus from a Pasteur Institute would have had time to arrive. Of course it might not always be possible to obtain the brain of the biting animal. In that case if the requisite material arrived late from the nearest Pasteur Institute, it might be necessary to adopt the method of intravenous inoculation of fixed virus which Roux and Nocard,[3] Protopoff,[4] Moncet[5] and others have shown to be of service in the rapid

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[1] Ferrans using fresh rabies material has reduced the period of treatment in ordinary cases to five days—*Handbuch der Path. Mikr.* Kolle & Wassermann Suppl. Art. '*Lyssa*' (Frosch.), p. 647.

[2] Nitsch "*Remarques sur la méthode pasteurienne du traitement préventif de la rage*" Extr. Bull de l' Inst. Past., 1906, p. 1057.

[3] *Ann. de l' Inst. Pasteur*, 1888, p. 311.

[4] " " " " " 1888, p. 462.

[5] " " " " " 1902, p. 398.

* To be yet more fully taken up later with the experimental evidence.

production of immunity to rabies. As a purely prophylactic measure, in anticipation of the possibility of being bitten by a rabid animal, anti-rabic inoculation is not accepted as expedient in the case of human beings. In India, however, where every year numbers of valuable dogs and other animals develop rabies, the question of a yearly inoculation of fresh rabies material as a preventive might commend itself to owners. This would be the more feasible if, say, one inoculation per year should prove to be sufficient to establish and maintain a satisfactory degree of immunity and yet be itself quite safe. The point requires more experimental work (*vide* pp. 23—24) than we have been able to devote to its elucidation.

We have now considered our subject from a general and more or less introductory point of view, and may now proceed to set forth certain experimental investigations carried out by us with their results. We shall consider—

I. The infective power of dried rabies spinal cords relatively to fresh rabies virus.

II. The rate of desiccation of rabies spinal cord by the ordinary procedure and its bearing upon the use of the cord for immunising purposes.

III. The reduction of systems of immunisation to uniformity.

IV. The effects obtained by the use of massive doses of fresh fixed virus in anti-rabic immunisation.

V. The question of the existence or non-existence of a rabies toxin.

VI. The standard fixed virus and how to maintain it.

I.—The infective power of dried rabies spinal cords relatively to fresh rabies virus.

1.—*Degree of infectivity of rabies virus a function of loss of weight in water by drying.*

By infectivity here we understand the power of producing rabies in an animal and the capability of that disease to be propagated by further inoculation into other animals. In many infections other than rabies the term minimum lethal dose is a familiar one. *A priori* there would seem to be no reason why this measure should not be employed as a standard one in the case of rabies also. But the death-point is variable even in the case of fixed virus, and therefore the criterion of 'fixity' of rabies virus has by universal consent been taken to be, that symptoms should supervene in a given time (6·7 days) after the date of subdural inoculation. In our experiments we make use of the term minimum infective dose (M. I. D.) and indicate thereby the smallest amount

of rabies virus which we have found under certain definite conditions to be capable of producing distinct paresis in a rabbit within six or seven days after subdural inoculation. It will be readily understood that very exact numerical results are almost impossible of attainment, where we are dealing—as in the case of rabies—with an entirely unknown virus. The actual amount of the organism present is only determinable by indirect means. We have, however, been able to obtain certain limits of infectivity of rabies virus, variously treated, which to us appear to be very much more definite than any which have hitherto been published. Within certain limits of unavoidable error, which for practical purposes may be neglected, we hold that we have been able to demonstrate a method by which any system of treatment may be approximately estimated in standard units and so become capable of direct comparison with any other system. We proceed to describe our method and the results obtained in detail.

Technique.—The amount of rabies emulsion used for subdural inoculation has been invariably 0·2 cc. of a definite dilution. The virus was fixed virus. The animals used were all rabbits; the inoculation made subdurally. The preparation of the emulsion in the case of fresh rabies material needs scarcely any description. A definite quantity of the fresh rabies material is ground up as finely as possible in a mortar; to it is added a sufficiency of physiological salt solution to make a 1 per cent. emulsion; this is strained through wire gauze of 0·3 mm. mesh. From the emulsion so made others are obtained by simple dilution. Where the emulsions were of greater strength than 1 in 100 the same procedure was followed.

In the case of dried rabies material the making of an emulsion of definite strength is not so simple a matter. Suppose we were to take the spinal cord of a rabies rabbit and divide it up into small lengths of equal weight. One length would suffice for the preparation of an emulsion of fresh virus. The other lengths each after a definite period of drying would be ground up to make their respective emulsions. The quantity of fluid added to each of these latter would be exactly the same as in the case of the fresh virus, irrespective of the fact that drying had diminished their weight. All these emulsions would then be exactly comparable. But as the spinal cords used in our experiments are dried in entire length and not in these small pieces of originally equal weight, we must make a calculation to determine what the weight of any given dried portion would have been in the fresh condition. Then we make up our emulsions as if the portion possessed this latter weight; otherwise our results would not be comparable. An example will make this clear. The weight of a fresh spinal cord when removed was 4·0282 grm. A portion weighing 0·153 grm. was removed for the test of the infectivity of fresh rabies virus. The remainder was then 3·8752 grm. This remainder weighed only 1·9986 grm. after one day's drying, and a portion

weighing 0·1079 grm. was then removed from it for the test of infectivity of one-day dried cord. The weight, therefore, of the portion taken would, in its fresh state, have been

$$\frac{3{\cdot}8752}{1{\cdot}9986} \times 0{\cdot}1079 = 0{\cdot}2092 \text{ grm.}$$

and it is on the basis of this latter weight, and not of 0·1079 grm., that the necessary dilutions are made. This calculation is essential to any method of estimation in which more or less dried material is to be compared with fresh moist material.

Results.—Altogether 128 rabbits were used in the test. Of these a very large number were expended in trials with fresh rabies material, as it was some considerable time before a definite decision upon limits could be reached. The numbers were—

Fresh material	91
One-day dried cord	4
Two-day dried cord	7
Three-day dried cord	13
Five-day dried cord	10
Nine-day dried cord	3
TOTAL	128

(1) *Fresh material.*—It was exceedingly difficult to get any definite limits in this case. The M. I. D. ranged from certainty at $\frac{1}{8,000}$ dilution up to tolerable certainty through successively greater dilutions to $\frac{1}{40,000}$. The possibilities of experimental error here were very great, especially in the high dilutions. A $\frac{1}{20,000}$ dilution contains only ·00001 grm. of rabies material per 0·2 cc. of emulsion used, and therefore a still less quantity of the pure rabies organism. It is not easy, then, to differentiate between such small amounts as, say, 0·2 cc. of $\frac{1}{20,000}$ and 0·2 cc. of $\frac{1}{16,000}$ dilution of rabies material containing respectively ·00001 grm. and ·0000125 grm. of solid matter in suspension. Some of our later trials were more definite than the earlier, and we found, *e.g.*, that whereas 0·2 cc. of a $\frac{1}{8,000}$ dilution produced, upon subdural inoculation, rabies in the fixed time of 6 or 7 days, the same amount of $\frac{1}{10,000}$ dilution and $\frac{1}{15,000}$ dilution gave rabies with an incubation period of some 9 days or longer or did not give rabies at all. We decided therefore to fix our unit for fresh rabies material at 0·2 cc. of $\frac{1}{8,0}$ dilution with this qualification that dilutions very much higher would in many instances afford examples of the M. I. D. in quantities of 0·2 cc.

(2) *One-day cord*—

Number of experiments 4.

Dose 0·2 cc.

i. Dilutions of $\frac{1}{4,000}$ and $\frac{1}{1,000}$. In all, four tests made: paresis was distinct by the 6th or 7th day.

Limit reached—*nil*. M. I. D. not greater than 0·2 cc. of a 4000-fold dilution.

(3) *Two-day cord*—

Number of experiments 7.

Dose 0·2 cc.

i. Dilutions $\frac{1}{1,000}$; $\frac{1}{500}$; $\frac{1}{250}$; 6 experiments. In all cases paresis by the 6th or 7th day.

ii. Dilution $\frac{1}{2,000}$; 1 experiment. Paresis after 34 days.

Limit—0·2 cc. of a 1,000-fold dilution.

(4) *Three-day cord*—

Number of experiments 12.

Dose 0·2 cc.

i. Dilutions $\frac{1}{200}$; $\frac{1}{100}$; $\frac{1}{50}$; 8 experiments. In all cases paresis by the 6th or 7th day.

ii. Dilutions $\frac{1}{500}$; $\frac{1}{1,000}$; 4 experiments. Paresis in 6 to 13 days.

Limit—0·2 cc. of a 200-fold dilution.

(5) *Five-day cord*—

Number of experiments 10.

Dose 0·2c.c.

i. Dilutions $\frac{1}{50}$; $\frac{1}{100}$; 8 experiments. In all cases paresis by the 6th or 7th day.

ii. Dilution $\frac{1}{200}$; 2 experiments. Paresis doubtful; certainly well over the limit of 6 or 7 days.

Deaths after 23 and 33 days.

Limit—0·2 cc. of a 100-fold dilution.

(6) *Nine-day cord*—

Number of experiments 3.

Dose 0·2 cc.

Dilutions $\frac{1}{10}$ and $\frac{1}{5}$.

Result—no rabies; progressive emaciation; death.

Limit (if any)—some concentration greater than is represented by 0·2 cc. of a 5-fold dilution.

The M. I. D's obtained with fresh, three-day, five-day, and nine-day dried material give us four well separated points on a curve convex to axes representing respectively the degree of dilution of the material used and the time of desiccation undergone. This number of points, well interspaced as they are, is sufficient to enable the curve to be plotted with fair accuracy. We have, however, as described in the text, also carried out a certain number of experi-

ments with one-day and two-day cords, which, although limits have not been reached in both cases, still go to confirm the results obtained with those other experiments in which limits were obtained. We think certain conclusions may be drawn from these results.

These are—

(1) That emulsion of nine-day cord is little if at all infective in a dose of 0·2 cc. of a 1 in 5 emulsion.

(2) That emulsion of five-day cord is infective in minimal time in a dose of 0·2 cc. of 1 in 100 emulsion, but becomes less so or not at all in a dose of 0·2 cc. of 1 in 200 emulsion.

(3) That in the same way the M. I. D. for an emulsion of three-day cord is 0·2 cc. of a 1 in 200 emulsion.

(4) That the M. I. D. of two-day cord is not less than 0·2 cc. of a 1 in 1,000 emulsion and probably not so great as 0·2 cc. of a 1 in 2,000 emulsion.

(5) That the M. I. D. of one-day cord is not less than 0·2 cc. of 1 in 4,000 emulsion and almost certainly not greater than 0·2 cc. of 1 in 8,000 emulsion (the lower accepted limit of fresh material).

(6) That fresh material is infective (M. I. D.) in a dose of 0·2 cc. of a 1 in 8,000 dilution and may be so in considerably higher dilutions even up to 1 in 40,000, but that with such high dilutions the experimental errors become so great as to preclude any more exact fixation of the M. I. D.

Although these observations may be wanting in mathematical exactness, still we are enabled to deduce from them certain conclusions regarding the infectivity of rabies material according as it has undergone desiccation to a greater or less degree. It is evident that by the third day of drying the rabies cord has lost very much of its original infectivity. Thus, let us take as our M. I. D. of fresh material 0·2 cc. of 1 in 8,000 dilution. The fall in infectivity is a rapid one. If 0·2 cc. of 1 in 8,000 fresh material be the M. I. D., then the M. I. D. of three-day cord is at least 40 times greater, whilst that of five-day cord is about 80 times greater. By the 9th day the cord has lost all or nearly all infectivity. The infectivity between the 5th and 9th days of drying diminishes very slowly and gradually. Our results may now be plotted for the sake of clearness in the shape of a curve (fig. I). The numbers along the vertical axis represent therelative degrees of dilution which the particular rabies material corresponding to a given amount of desiccation can undergo and still remain fully infective. Thus, for example, fresh undried material may be diluted eight times more than two-day dried material and will then be infective to an equal extent.

The curve (fig. I) shows the very sharp drop in infectivity which takes place in the first three days and the very gradual diminution thereafter. The inferences which it would seem justifiable to make from a particular study of this curve are—

(1) Sharp differences in infectivity between fresh, one, two, and three-day cords.

(2) A difference of infectivity amounting to about one half between three-day and five-day cords.

(3) Exceedingly gradual diminution in infectivity from five-day cord down to little or none in the nine-day cord.

Are we to conclude from the non-infectivity of nine-day cord that it is of no service in immunisation? The answer to this question depends on the nature of the material which is left after the death of the living virus and the usefulness of this non-living substance. We take up this subject in full later (*vide* existence of rabies toxin, p. 27).

II.—The rate of desiccation of rabies spinal cord by the ordinary procedure and its bearing upon the use of the cord for immunising purposes.

The material used for desiccation was caustic potash in sticks. The rabies cords were dried in the usual way over caustic potash, in air, in the dark, and at a temperature of 22°—24°C. The following is a typical instance—one of six observations—of the results obtained :—

TABLE IV.

Day of drying.	Weight.	Temperature.
0.	4·0282 grm.	...
1.	2·0775 ,,	22°—24°C.
2.	1·623 ,,	,,
3.	1·4401 ,,	,,
4.	1·3814 ,,	,,
5.	1·3540 ,,	,,
6.	1·3389 ,,	,,

TABLE IV.—*Continued.*

Day of drying.	Weight.	Temperature.
7.	1·3297 grm.	22°—24°C.
8.	1·3193 ,,	,,
9.	1·3127 ,,	,,
10.	1·3103 ,,	,,
11.	1·3057 ,,	,,
12.	1·3018 ,,	,,
13.	1·3002 ,,	,,

The loss of weight continues milligramme by milligramme for about 60 days. It is of course a loss of weight of water, for the solid basis material does not lose weight. We may plot the figures given above in the form of a graph (fig. II).

We now proceed to compare the curve of drying with the curve of infectivity (fig. III). But before doing so we may observe that in instituting this comparison it is the loss of weight of water only in desiccation which is taken into account; that is to say, that the solid unchanging basis material is excluded from the comparison. Moreover, infectivity with the doses which we used disappeared by the 9th day, but the loss of weight of water was not actually complete until about the 60th day. We may conclude from this, that the small amount of water remaining in the cord from the 9th day onward is insufficient to keep the virus infective. We may state this perhaps more correctly as follows :—The quantity of living virus which may possibly be present in the cord by virtue of the small amount of water remaining at the 9th day, is an insufficient amount in the doses used to produce infection. This statement amounts to this, that the very small quantity of water remaining in the rabies cord after the 9th day may be neglected so far as it affects the question of infectivity in our comparison. This quantity represents, therefore, so much dead weight and may be added to the dead weight of basis nerve-material already alluded to. We may show this in fig. II, by moving the abscissa upwards so as to cut the curve of desiccation where it meets the ordinate corresponding to the 9th day of drying. This is equivalent to saying that the *practical zero* from our point of view is reached by the drying curve on the 9th day. We utilise this fact in making the calculation of the actual loss of weight of water which is to be the subject of comparison with loss of infectivity. Thus in fig. II the area below the line A B C represents the dead weight of solid basis nerve-materal, which does not undergo any change in weight, *plus* the small

amount of non-effective water which is present after the 9th day of desiccation. We now proceed to plot the two curves on the same chart, reducing the figures in each case so as to make the graph start from unity. In this way we obtain fig. III. Our figures for the drying curve are averages obtained from four different sets of observations. The two sets of figures to be plotted are here given. It is to be noted that the weight of water remaining in the cord after the 9th day together with that of the solid basis material of the cord has been duly deducted from the actual weights obtained, and the result is shown in column 3 as 'loss of water.'

TABLE V.

(1) Desiccation.	(2) Infectivity.	(3) Loss of water.
Fresh	1·2	1·2
1st day	...	0·392
2nd day	0·125	0·115
3rd day	0·025	0·049
5th day	0·0125	0·013
9th day	0·0	0·0

These curves (fig. III), we think, show a very marked correspondence, experimental error being allowed for. Both curves show a marked and rapid fall to the third day and both show extremely gradual subsidence after the fifth day of drying. We believe, *cæteris paribus*, the two curves to be identical and indeed that they represent two parallel effects of the same causal action.

If we take this case as proven we may state this proposition:—

The rate of loss of infectivity of rabies cord undergoing slow desiccation is directly proportional to the rate of loss of water contained in that cord.

In other words, as slow desiccation means death of the rabies virus, and as we consider dead rabies virus to be comparatively speaking valueless for immunisation, we may state here provisionally this corollary to the above proposition—

The immunising *power of a given portion of rabies cord is a function of the unkilled remnant of rabies virus which is contained in that cord.*

If this be so, it follows that a cord which has been drying for nine days or longer has little or no value for immunisation. With regard to less dried cords a greater or smaller quantity will be required to produce a sufficient immunity to rabies infection according to the greater or less degree of desiccation. The experiment which we give in detail below gives, by means of a particular instance, support to this view and, by extension, to the view that immunisation against rabies might conveniently be carried out with a cord which had dried for a fixed time only, instead of with a graded series of dried cords. In our experiment we show that whereas nine-day cord has probably little immunising power, five-day cord has considerable power and might probably be used by itself[1] as the immunising substance from commencement to termination of a course of treatment. It may be as well to say here that, even if such a method as this were elevated into the position of a system of anti-rabic immunisation, we should still regard it only as a temporary compromise between the use of dried material and of fresh rabies virus. The method suggested by Nitsch amongst recent authors we regard likewise as simply a step towards the more general use of fresh material.

Experiment.—Two dogs were inoculated in the abdominal region, the one with nine-day cord alone, the other with five-day cord alone. After prolonged treatment with this material and after allowing a definite interval for the full development of immunity, these two dogs along with a control dog were proved by subdural inoculation. The following table gives all details together with the results obtained :—

TABLE VI.

Dog.	Weight.	Cord used for immunising.	Total amount used.	Number of days occupied in the immunising process.	Subdural inoculation after an interval of	Amount of dose inoculated subdurally.	Result.	Remarks.
I.	20 lbs.	5-day	3'4045 grm.*	20 days	27 days	0'3 cc. of a 5 per cent. emulsion.	Remained well.	Immune.
II.	18 lbs.	9-day	3'3809 grm.*	Do.	Do.	Do.	Developed rabies 9 days after subdural inoculation.	Not immune.
III. control.	21'5 lbs.	...	...	...	Inoculated subdurally at same time as I and II.	Do.	Developed rabies 8 days after subdural inoculation.	Not immune.

* This is *actual* weight of cord used in its dry state.

[1] Since this was written we find that Nitsch has recommended a method of treatment in which a commencement is made from five-day cord. Extract *Bull de l'Inst. Past.*, 1906, p. 1, 057.

The five-day inoculated dog showed itself entirely immune to the subdural infection. The nine-day dog and the control dog both succumbed to rabies, the former showing paresis nine days after subdural inoculation and the latter eight days after. The nine-day cord inoculations therefore produced no effective immunity, and it would seem that the incubation period of the disease had not been to any degree extended.

We have spoken above of "slow desiccation" (p. 16); this is merely in a comparative sense to distinguish it from the rapid desiccation of rabies material in vacuo and over sulphuric acid[1] by means of which, within 24 hours, a powder may be obtained which, although dry, still retains its infectivity. This phenomenon is not unknown in the case of other organisms, *e.g.*, vaccine virus, Staphylococcus pyogenes aureus, *B.* diphtheriæ. The periods to which such organisms will survive drying vary greatly. We have not made very many experiments to compare the infectivity of rapidly desiccated rabies virus with fresh material, but such as we have made would lead us to the opinion that, although the potency of the former may not be denied, it is yet very greatly less than that of the latter.

III.—An attempt at reduction of different systems of treatment to a uniform standard by means of a unit of comparison obtained by experiment.

We have to some extent worked out the correspondence between the dried cord system and the fresh rabies material system. We have seen that 0·2 cc. of $\frac{1}{8,000}$ dilution is by our method the M. I. D. for fresh rabies cord material, while 0·2 cc. of $\frac{1}{200}$ dilution is somewhere near the M. I. D. for three-day cord and 0·2 cc. of $\frac{1}{100}$ dilution for five-day cord. We have also found that 0·2 cc. of $\frac{1}{5}$ dilution of nine-day cord had no infectivity. A point which has to be taken into account in estimating these values is the varying virulence of different regions of the central nervous system. Thus it is said[2] that cortical material s 10 times more virulent than spinal cord. Except in some of our earlier experiments with fresh material we have used spinal cord material throughout. We may now take the values which we hav ound as actual and endeavour to interpolate, by means of curve No. 1 (fig. I), other values for those days of desiccation for which no observations at all are available. Thus roughly for the 4th day we take the mean value of the 3rd and 5th days: so also for the 7th day the mean value between the 5th and 9th days: for the 6th and 8th days the mean values between the 5th and 7th days and the 7th and 9th days, respectively.

[1] Vansteenberghe *Compt. Rend. Soc. de Biol.* t. LV. 1903, pp. 1646-47.
[2] Frosch quoting Nitsch *Handbuch f. Path. Mikr.* Supplement, 2nd part, Art. *Lyssa*, p. 646.

Let then the M. I. D. by this calculation be for

(1)	Fresh rabies material	...	0·2 cc. of	$\frac{1}{8,000}$	dilution.
(2)	3rd-day desiccated material	...	,,	$\frac{1}{200}$	,,
(3)	4th-day ,, ,,	...	,,	$\frac{1}{150}$	,,
(4)	5th-day ,, ,,	...	,,	$\frac{1}{100}$	,,
(5)	6th-day ,, ,,	...	,,	$\frac{1}{75}$	,,
(6)	7th-day ,, ,,	...	,,	$\frac{1}{50}$	,,
(7)	8th-day ,, ,,	...	,,	$\frac{1}{25}$	,,
(8)	9th-day ,, ,,	...	,,	0	,,

We do not proceed further than the 9th day because we regard the immunising value of material which has been drying for nine days or more may be neglected in practice. Let now 0·2 cc. of $\frac{1}{100}$ dilution of fresh rabies material be our arbitrary standard for the comparisons of infectivity, and equal to 1,000 units; then 0·2 cc. $\frac{1}{8,000}$ dilution is obviously equal to 12·5 units. So with the other M. I. D's, which are of course all equal to one another, *e.g.*, 0·2 cc. of $\frac{1}{200}$ dilution three-day material = 12·5 units and so also 0·2 cc. $\frac{1}{100}$ dilution five-day material = 12·5 units. The estimation of Höyges' system of immunisation is easy upon the basis of our arbitrary standard or indeed any arbitrary standard. With the Pasteurian methods we must know what the strengths of the emulsions used are. In the Kasauli Pasteur Institute 1 cm. in length of dried cord constitutes a dose for an adult person. This is emulsified in 3 cc. of physiological salt solution and the resulting emulsion is one of, on an average, 5 per cent.* Under this system the dose for an adult is 3 cc. of a 5 per cent. emulsion throughout the whole course. Calculated on this basis the following two systems (light schemata) work out in units to—

25,028 units . Kasauli.
25,287 ,, . . Höyges.

The respective schemata are here given.

---

* 0·15 grm. in 3 cc.

TABLE VII.

III. HÖYGES' METHOD LIGHT SCHEME.[1]

Dilution.	Dose in cc.	Units.
1 m. $\frac{1}{10,000} + \frac{1}{8,000}$	3 — 3	887·5
e. $\frac{1}{6,000} + \frac{1}{5,000}$	3 — 3	
2 m. $\frac{1}{5,000}$	3	800
e. $\frac{1}{2,000}$	2	
3 m. $\frac{1}{2,000}$	2	1,250
e. $\frac{1}{1,000}$	1·5	
4 m. $\frac{1}{1,000}$	1·5	1,750
e. $\frac{1}{500}$	1	
5 m. $\frac{1}{200}$	1	2,500
6 m. $\frac{1}{6,000} + \frac{1}{5,000}$	3 — 3	1,050
e. $\frac{1}{2,000}$	2	
7 m. $\frac{1}{2,000}$	2	1,250
e. $\frac{1}{1,000}$	1·5	
8 m. $\frac{1}{1,000}$	1·5	1,750
e. $\frac{1}{500}$	1	
9 m. $\frac{1}{200}$	1	2,500
10 m. $\frac{1}{6,000} + \frac{1}{5,000}$	3 — 3	1,050
e. $\frac{1}{2,000}$	2	
11 m. $\frac{1}{2,000}$	2	1,250
e. $\frac{1}{1,000}$	1·5	
12 m. $\frac{1}{1,000}$	1·5	1,750
e. $\frac{1}{500}$	1	
13 $\frac{1}{200}$	1	2,500
14 $\frac{1}{100}$	1	5,000
	TOTAL	25,287·5

[1] Deutsch & Feistmantel *Impfstoffe und Sera*, p. 154.

TABLE VIII.

II. Pasteurian Treatment.

Kasauli scheme.

One centimetre of cord is emulsified in 3 cc. sterile physiological salt solution. Weight of rabbits about 1,500 grms.

Day of desiccation.	Dose.	Units.
1 14—13	3 cc.	
2 12—11	,,	
3 10— 9	,,	
4 8— 7	,,	703·125
5 6	,,	700·00
6 5	,,	937·50
7 5	,,	937·50
8 4	,,	1,351·25
9 3	,,	1,875·00
10 7— 6	,,	1,168·75
11 5	,,	937·50
12 4	,,	1,351·25
13 3	,,	1,875·00
14 6	,,	700·00
15 5	,,	937·50
16 4	,,	1,351·25
17 3	,,	1,875·00
18 5	,,	937·50
19 4	,,	1,351·25
20 3	,,	1,875·00
21 5	,,	937·50
22 4	,,	1,351·25
23 3	,,	1,895·00
	Total	25,028·125

In Paris the rabbits used are 2 to 2½ kilos. weight, whereas those used at the Kasauli Institute weigh on the average about 1½ kilos. In Paris, then, length for length, cords would weigh more and dry less quickly than in Kasauli. The result of this difference in weight probably is that the emulsions used in treatment are of a higher concentration than those used in Kasauli and so a larger dose is given. The doses used in Paris represent in all probability a greater number of units than would be given by the assumption that the emulsions used were exactly equivalent to those used in Kasauli. We have not therefore attempted to estimate the Paris scheme. We have to note here that this estimation of systems takes no account of dead vaccinal material separate from living virus as we do not consider this to have any immunising value (*vide* p. 27). Further, we take no account of the fact that a cord such as nine-day cord although non-infective in a dose of 0·2 cc. of ⅕ dilution might be to a small extent infective, and therefore immunising, in a dose of 3 cc. of this dilution. However, we believe that no very great error is introduced by this last omission. To return to our comparison of systems, we might lay down, supposing our original assumptions to be somewhere near the truth, that 25,000 units given in 14 to 20 days or very probably in a considerably shorter period of time would be sufficiently protective for light anti-rabic treatment. This computation has, as we have said, special reference to the Kasauli mode of procedure, taken in conjunction with that of Höyges. In exactly the same way if we compared these systems dose for dose instead of as a whole we should conclude that 3 cc. of a 5 per cent. emulsion (that ordinarily used in Kasauli) of three-day cord was as nearly as possible intermediate in strength between 1 cc. (Höyges' dose) of $\frac{1}{100}$ fresh rabies emulsion and 1 cc. of $\frac{1}{300}$ fresh rabies emulsion. Similarly 3 cc. of a five-day cord emulsion would hold the relation to 1 cc. of $\frac{1}{200}$ fresh emulsion of 1·5 : 2. The relations, therefore, of these different doses mentioned would be 4 : 3 : 2 : 1·5.

4= 1 cc. $\frac{1}{100}$ fresh in material.
3·= 3 cc. 5 per cent. three-day cord.
2·= 1 cc. $\frac{1}{300}$ fresh.
1·5= 3 cc. 5 per cent. five-day cord.

Other systems might be estimated after the same fashion.

IV.—Massive Doses of fresh Fixed Virus in Anti-rabic Immunisation.

We have referred in our introduction to the possibility of immunisation against rabies by giving large doses in 2, or at most 3, injections properly interspaced. As we use here fresh living virus only it would be necessary to be sure that there was no danger in the procedure of giving rise to the disease which it was intended to ward off. We have at different times and for different purposes had occasion to give considerable doses of fresh virus to animals by subcutaneous injection.

In no single case did rabies result from the procedure. The emulsion of virus was always given in the loose abdominal subcutaneous tissue—after the fashion of anti-rabic inoculation. In the case of some of the dogs thus treated which were afterwards inoculated subdurally we found that immunity had been produced. The following table gives in a succinct form the doses used and the results of subsequent subdural inoculation when that was practised.

To show—

(1) That massive doses of fresh fixed rabies virus may be given to certain animals subcutaneously without danger of infection.

(2) That a small number of inoculations or even a single, large inoculation of such material can confer immunity to subdural infection.

TABLE IX.

Series.	Animal.	Weight.	No. of inoculations of fixed virus.	Total quantity fixed virus injected.	Duration of inoculations.	Subdural inoculation.	Remarks.	Result.
I	Dog 1 . .	25 lbs. .	4	0·495 grm. .	4 days .	...	In Höyges' method of immunisation the strongest dose given contains only 0·01 grm. fixed virus.	Remained well.
	Dog 2 . .	15 lbs. .	4	0·46 grm. .	„ .	25th May 1906—twenty-one days after last immunising dose with 0·2 cc. of 2 per cent. fixed virus emulsion.	0·2 cc.	Ditto.
	Small brown monkey 1.	...	4	0·22 grm. .	„ .	Ditto	...	Showed signs of rabies nine days later.
	Monkey 2 .	...	4	„ .	„ .	...	...	Remained well.
	Monkey 3 .	...	4	„ .	„ .	...	...	Ditto.
	Goat 1 . .	...	...	15th August 1906 to 19th August 1906, 0·845 grm.s 15th September 1906, ½ brain rabies rabbit. 8th October 1906, ½ brain rabies rabbit. 23rd October 1906, ½ brain rabies rabbit. 8th November 1906 whole brain rabies rabbit. 3rd December 1906, whole brain. 3rd January 1906, whole brain. 27th January 1906, whole brain, and so on at intervals of about a month.	Still continuing.	...	...	Ditto.

Series.	Animal.	Weight.	No. of inoculations of fixed virus.	Total quantity fixed virus injected.	Duration of inoculations.	Subdural inoculation.	Remarks.	Result.
III	Dog 3 . .	18½ lbs. .	1	½ rabbit brain fixed virus.	...	34 days after last immunising dose 0·3 cc. of 3 per cent. fixed virus emulsion. 26th February 1907.	...	Remained well.
	Dog 4 . .	8 lbs. .	2	¾ brain rabbit fixed virus.	(1) ¼ brain 24th Jan. 1907, (2) ½ brain, 3rd Feb. 1907.	23 days after last immunising dose 0·3 cc. of 5 per cent. fixed virus. 26th Feb. 1907.	...	Ditto.
	Dog 5 (control)	16 lbs. .	...	...	...	26th February 1907, 0·3 cc. 5 per cent. fixed virus.	...	Developed rabies nine days later.

From these experiments it will be seen that we were able to immunise a dog (No. 2) with as small a quantity as 0·46 grm. given in four injections. Dogs 3 and 4 were immunised to subdural inoculations with one and two injections respectively. In the first series of experiments it is evident that the amount given to the small brown monkey (No. 1) was insufficient to produce immunity to subdural injection. As this monkey was injected with the same amount and from the same emulsion as dog 2 it serves to some extent as a control in the particular series (Series I) of experiments in which it was used. The doses given subcutaneously may be regarded as massive doses, and in no case was there any evidence of harm resulting in either dogs, monkeys, or goat. We may infer therefore that in this small number of cases massive subcutaneous injections of fixed rabies virus did not produce infection but immunity to subdural inoculations and that this immunity was achieved in one case by one single inoculation. There are also numerous published instances in which massive doses have been given and no harm resulted—often indeed they have been preliminary to the subsequent trial of the immunity produced. Thus Krasmitski[1] relates having administered 5-8 cc. of thick emulsion of fresh fixed virus material intraperitoneally and 8 and 20 cc. (two separate occasions) subcutaneously in rabbits without harm resulting. Rabbits, however, are by no means immune to subcutaneous injection of fixed virus and a considerable number will develop rabies after such a procedure, owing, it is said,[2] to the impossibility of avoiding wounding muscle. The dog and the sheep would seem less susceptible than the rabbit. Roux and Nocard[3] injected 5 cc. of rabies emulsion (? strength) intravenously

[1] *Ann. Past.*, 1902, p. 403.
[2] Helman *Ann. Past.*, 1889, p. 19.
[3] *Ann. Past.*, 1888, p. 347.

in a sheep without effect. Helman and others have injected intraperitoneally and subcutaneously large doses of rabies virus without producing any signs of intoxication or of rabies.

More recently Nitsch[1] injected himself subcutaneously with the emulsion made from a fresh piece of rabies cord (858th passage—fixed virus) of 4-5 mm. length, without any harm.

V.—On the existence or non-existence of a rabies toxin together with a consideration of the concurrent symptoms and sequelæ of anti-rabic treatment.

The proofs of the existence of a rabies toxin as given by Babes[2] are—

1. The premonitory fever in rabies is a specific fever.

2. The leucocytosis apparent in nerve ganglia and in the circulating blood can be due only to the action of a toxin.

3. The hemorrhages observable in the central nervous system can result only from injury to tissues acted on by a toxin.

4. Filtered rabies virus or killed rabies virus injected in large quantity brings about death in test animals not by rabies but by marasmus.

From the argument which we develop in the following pages it will be seen that we offer another explanation of the disturbance resulting from inoculation of rabies virus. We do not consider that the reasons set forth above negative our proposition.

A good deal of attention has been drawn of late years to some of the symptoms which may accompany or follow anti-rabic treatment in their graver manifestations, particularly by Remlinger. The symptoms following or accompanying anti-rabic treatment are, in our own experience, local reaction, local irritability, headache, neuralgia, pains in the limbs, pains in the back or in joints, fever, urticarias (local and generalised), dysphagia, giddiness, lassitude, anorexia, nausea and, last and worst, paralysis of varying gravity. The former ideas with regard to these were that the symptoms—at least the graver symptoms—might be due (1) to a partial attack of rabies proceeding from the original infection which was rendered more or less abortive and non-fatal (for these symptoms rapidly disappear) by the anti-rabic treatment, or (2) to alcoholism in a person undergoing anti-rabic treatment, or to (3) other conditions which need not here be specified (*vide* article by Remlinger).[3]

Remlinger has shown by an exhaustive analysis of a large number of collected cases that these explanations will not answer. His belief, as far as we can gather, is that here we have special evidence of a rabies toxin as distinguished

[1] Quoted Art. "*Lyssa.*" *Handb. f. Path. Mikr.* Kolle & Wassermann, 2nd Supplement, p. 646.

[2] Quoted Art. "*Lyssa*" (Marx) *Handb. f. Path. Mikroorg*, 1904. Kolle & Wassermann, p. 1284.

[3] *Accidents Paralytiques au cours du traitement antirabique. Ann. Past., No. 10*, 1905, p. 639.

from the living virus. He directly incriminates, and we believe justly so, the inoculation material as the active cause of these symptoms. Where we do not follow him is in regard to his assumption that the symptoms must be due to a rabies toxin. The sum and substance of his argument on this point we conceive to be this (1) The existence of a rabies toxin is not to be doubted. (2) In rabies we have an instance of a disease accompanied by paralysis. (3) One of the gravest of the sequelæ of anti-rabic inoculation is a paralysis. Therefore it would seem justifiable that, as undoubtedly the same virus is present in both instances, the symptoms must in both cases be due to the same cause. But as in the second of the two instances it may practically be said that cure is the invariable rule, therefore in this case we can only be dealing with the rabies toxin and not directly with the living rabies virus as cause of the condition. The argument is based on this, that in the case of two occurrences where similar effects are produced and where in both of them the same antecedent condition exists, that condition is probably the cause of the effect. But this argument may be pushed too far, and the conclusion can only be accepted as amounting to a probability, not a certainty. The possibility remains open of there being more than one invariable antecedent, and in that case we have to consider the different claims of each to be regarded as the cause of the condition.

Now we believe, in opposition to the opinions hitherto set forth, that there is evidence for the view that the nerve material derived from the rabbit either *per se* or through its decomposition products (? neurin, cholin, etc.) may be the cause of the symptoms which we are discussing. Sir A. E. Wright has made us familiar with the idea of poisoning effects due to a too rapid succession of inoculations of material, in itself comparatively little toxic, but capable of producing cumulative effects unless recovery and reaction are allowed to take place between successive injections. The condition known as "serum disease" is also very interesting in this connection. Here "the phenomena of the disease are to be interpreted as events in a process of immunisation by which the organism purges itself from the foreign serum."[1] What the exact relationship may be between the state illustrated by the serum disease which is termed anaphylaxia and that "negative phase" condition so much emphasized by Wright, it is difficult to say. It is very probable that they are both manifestations of one and the same cell state. However this may be, we are able to state that some of the symptoms of the "serum disease" are identical with those which we have seen to follow anti-rabic inoculations. In this method of treatment we are compelled to inject large quantities of foreign albuminoids (nerve substance) day after day. May we not be dealing from start to finish here, both as regards

[1] Wright, *Clin. Journal*, May 16th, 1906, p. 72.

trivial effects and grave, with a condition of anaphylaxia due to this foreign material and not to a rabies toxin? We believe this to be so. But what proof is there for the suggestion which we now put forward? That proof is of an indirect rather than a direct nature, but then so also is the proof of the existence of a rabies toxin. We take up the subject under the following heads:—

A.—The probable non-existence of a rabies toxin—extracellular or intracellular.

B.—Toxicity of non-rabic foreign nerve-material.

C.—Comparison of effects of treatment by the methods of Pasteur, Höyges, and others and conclusions to be drawn therefrom.

D.—Existence of blood changes concurrent with or subsequent to anti-rabic inoculations which are explainable on the theory of inoculation of foreign albumins.

A.—The probable non-existence of a rabies toxin.

(1) *Extracellular toxin.*

If such did exist we should expect to have the symptoms of rabies in animals rapidly induced by the injection of fresh rabies virus in massive doses—an intoxication as distinguished from an infection. But no such phenomenon can be definitely said to occur. The injection of 1 cc. of a thick emulsion of fresh rabies virus (fixed)—an emulsion of about 10 per cent.—will not produce any immediate symptoms of specific intoxication in a rabbit or other animal even when inoculated subdurally. Nor will it even produce rabies any quicker than the inoculation of 0·2 cc. of a 0·2 per cent. emulsion—a very much smaller quantity. In a similar way relatively enormous doses of fixed virus may be inoculated subcutaneously or intraperitoneally (*vide* detailed description and references, pp. 23, 24) without producing any symptom of intoxication.

Further, we found (p. 17) that a prolonged attempt at immunisation with nine-day cord alone failed to produce any immunity, a fact which would seem to indicate that if the function of the rabies toxin was to produce a degree of immunity to the rabies virus then the former was not present in this case. As far as we understand the arguments brought forward, the existence of such a toxin is largely based upon the necessity of explaining the rapid immunising power of inoculations of fixed rabies virus. One view which is current with regard to this point is that the toxin introduced with the material inoculated is rapidly absorbed and produces its effect upon the central nervous system, whereby the latter is rendered unassailable by the rabies organism, whether that of the original infection or of the subsequent injected material. Once this is accomplished, antibody production to the organism results in the ordinary way. But if we have no

sufficient evidence of the existence of a toxin we have no right to invoke its aid in explanation of the immunity brought about.

(2) *Intracellular Toxin.*

The non-existence of an endotoxin in fixed virus is more difficult to prove. In the case of a dead bacterial emulsion, say of B. typhosus, inoculated subcutaneously we obtain, if the dose is sufficient, evidence of distinct toxic effects. The symptoms of typhoid fever cannot be explained except upon the basis of a certain amount of intoxication; and as we are unable to obtain a typhoid toxin apart from the bodies of the organism itself, we speak of a typhoid endotoxin as distinguished from an extracellular and separable toxin. Again, we have evidence of the production of a definite immunity to typhoid fever by the inoculation of dead emulsions of the organism.

Now how does the case stand with the rabies virus?

(1) We have no convincing evidence to bring forward in favour of the production of toxic effects by the subcutaneous inoculation of fixed virus.

(2) The symptoms of the actual disease together with the fatal issue might quite easily be ascribed to purely mechanical interference with the functions of the cells of the central nervous system—such for instance as might be produced by the rapid sporulation of a protozoon or sudden increase in numbers of an organism which found itself in a situation suitable for multiplication. Moreover the undoubted relation between distance of the infected wound from the central nervous system and the length of the incubation period would seem to militate against the idea of any toxic action and favour the mechanical view. In fact we have here a purely physical relation between length of course and time of development of symptoms.

(3) There remains still the argument from a consideration of the immunity produced by the use of dead emulsions. In the case of rabies there is little or no evidence for the production of any immunity from the use of dead virus. The evidence in fact is entirely in the other direction, namely, that dead virus has no immunising power. This would point to the existence of some material difference between living and dead virus in the case of the rabies organism which does not exist in the case of the Bacillus typhosus. Immunity by means of dead typhoid emulsion is explained partly as immunity to endotoxin—the endotoxin having been unaffected by the mode of preparation of the vaccine—partly as due to the production of anti-microbic stuffs. But apparently we get no such immunity in the case of dead rabies emulsion. Have we then any right under these circumstances to conclude in the case of rabies that there exists an endotoxin like that present in the Bacillus typhosus? We think not.

How then are we to explain the immunity obtained by inoculation of a

rabies virus against rabies infection? We venture to put forward the following tentative explanation as a possible one. We conceive that the explanation may lie in a difference between street virus and fixed virus. Many authors, amongst whom are Marx[1] and Nitsch,[2] are now inclined to support the view that fixed virus is comparatively innocuous for man. May it not be the case then that by continued subpassage through the rabbit the original street virus has been rendered practically harmless for man although more virulent for the rabbit? Man may be "atreptic"[3] towards fixed virus. We have a certain analogy too in the case of the small-pox virus which by continued subpassage through the calf is rendered more and more virulent for this animal but much less so for man. No one has suggested, so far as we know, that the immunity obtained to small-pox by the use of a small-pox vaccine is due in any special way to a preliminary immunisation by small-pox toxin.

We imagine then that anti-bodies may be produced to living harmless fixed virus just as they would to any non-infective foreign albuminoid and that these anti-bodies have at the same time the power of counteracting the infective action of street virus. The effectiveness of the method might depend essentially on the innocuousness of living fixed virus, whereby comparatively large quantities can be safely introduced subcutaneously in the human being, with the result that large quantities of rabies anti-bodies are rapidly produced *locally*, pass into the circulation, and so counteract the infection by street virus.

To sum up, then, we see no sufficient grounds for the acceptance of the view of the existence of a rabies toxin, and therefore we reject that theory of immunity which builds upon its presence. We have also incidentally commented upon the fact that there is no case for immunising power in dead rabies virus. We can therefore now more confidently state the corollary already given in a tentative way to the proposition on page 16, *viz.*, that:—*Slow desiccation kills the rabies virus and the* immunising *power of a given portion of rabies cord is a function of the unkilled remnant of rabies virus contained in it.*

B.—Toxicity of non-rabic foreign nerve material.

In the examples we have cited where massive doses of rabies virus were used without producing any toxic effect we are in almost all cases compelled to

[1] Thus Marx (Art. "*Lyssa*," *Handb. f. Path. Mikroorg.* Kolle & Wassermann, p. 1284) says Fixed virus is, in purely subcutaneous injection, quite harmless for man and apparently much less infective for animals than street virus.

[2] Nitsch, *vide* Extr. *Bull de l' Inst. Past.*, 1905, p. 300.

[3] Ehrlich, Second Harben lecture "On the Atreptic Function," *Journ. of the Roy. Inst. Pub. Health*, July 1907.

utilise as the medium by which the virus is conveyed foreign nerve material. It follows then that in many animals, foreign nerve material, administered as we administered it, is comparatively non-toxic. This is not so however in all cases. Delezenne,[1] Delille,[2] and Marie[3] have drawn attention to the toxic action of foreign nerve material. Marie in a criticism says with regard to normal nerve substance that it is far from being innocuous:—"Nous ferons remarquer que les filtrats de substance normale sont au de là d'une certaine dose loin d'être inoffensifs." Delille explains his want of success in obtaining in certain cases a neurotoxic serum as due to the toxicity of the cerebral material of the dog for certain animals. "La trop grande toxicité de la substance cérébrale du chien pour ces animaux" (sheep, rabbit, guinea pig) "a d'ailleurs été vraisemblablement la seule cause de nos insuccès." We have confirmed these observations.

What, then, is the relation of man towards the nerve material derived from the rabbit? We have the analogy of a foreign albumin and its effect upon man and animals in the administration of horse serum,[4] as in the use of diphtheria anti-toxin—"Phenomenon of Arthus." The serum here is invariably horse serum. The American writers Rosenau and Anderson[5] have shown that in a certain number of cases toxic symptoms and even death have been produced in man by this serum. The toxic symptoms would seem to be due to the serum itself and not to the anti-toxin contained in it. Numerous other works have been written on the subject of the 'serum disease,' all of which go to show that man and animals are liable to a toxæmia from the introduction of foreign serum. A condition of hypersensibility is set up by the first injection and the second aggravates this condition with production of actual evidence of toxic action. If foreign serum stuffs can set up constitutional disturbances we should expect that foreign nerve material might be very likely to do the same. Further, although most of the work hitherto published on this subject has reference to foreign serum, it has also been shown[6] that a condition of anaphylaxia can be set up by appropriate injection of a proteid such as ordinary egg albumin—a result quite in accordance with our views of the toxic action of foreign nerve albumin. The symptoms, too, which are described as typical of this condition in animals are in the first two stages very strikingly similar to those which we have sometimes observed in man in the course of anti-rabic treatment.

[1] *Ann. Past.*, 1900, p. 686.
[2] „ „ 1906, p. 838.
[3] *Bull de l' Inst., Past.*, 1904, p. 392.
[4] Arthus. *Compt. Rend. de Soc. de Biol.* t. LV, pp. 817-820.
[5] Hypersusceptibility. *Journ. Amer. Med. Assoc.*, 1906, p. 1,009.
[6] Vaughan and Wheeler, *Journ. Inf. Dis.*, 1907, No. 3, p. 476.

C.—Comparison of the effects of treatment by the methods of Pasteur, Höyges and others and conclusions to be drawn therefrom.

In Höyges' system of treatment the amount of nerve material injected is very much less than in the Pasteurian* whilst the amount of living rabies virus is at least as great. Now, in our experience of Höyges' method we have found an almost total absence of the concurrent symptoms which we have already referred to, *viz.*, local inflammation, local irritation, joint pains, anorexia, headache, fever or urticarias. Höyges† himself states that he has never experienced as a result of his treatment any of those 'accidents paralytiques' which have been observed by all who make use of the Pasteurian mode of treatment. Remlinger remarks that these 'accidents paralytiques' would seem to have been rather more frequent where Puscariu's treatment,[1] in which rabies material which has been heated instead of dried, is used. In both the Pasteurian and Puscariu's[2] methods considerable quantities of nerve substances are injected. Remlinger's explanation of the difference of effects in the different systems is this, that in Höyges' method there must be less rabies toxin injected than in the other two methods. This conclusion, as will now be evident from our statements, is one with which we do not agree. Remlinger's argument may be set forth in logical fashion thus—

Premiss 1.—There is a rabies toxin.

Premiss 2.—Höyges' method of treatment is never followed by '*accidents paralytiques*' whilst the Pasteurian and Puscariu's methods are occasionally so followed.

Conclusion.—These latter methods then must involve the inoculation of larger quantities of rabies toxin than the former.

Our argument may be set forth:—

Premiss 1.—There is no sufficient evidence for the existence of a rabies toxin, whilst there is abundant evidence for the toxic action of foreign nerve material.

* Höyges; Pasteurian (Kasauli) 1 : 72; this proportion is for the light schemata.

† No cases out of 25,872 persons inoculated.

[1] Remlinger, *Anns. de l'Inst. Past.* No. 10, 1905, p. 643. A note states that MM. Puscariu and Lebell have modified the type of their inoculation, eliminating the emulsions heated to 40,° 35° and 30° C. and diminished the duration of the treatment. Since they did so they have had no more of these accidents.

[2] Remlinger *loc. cit.*

Premiss 2.—Höyges' method of treatment necessitates the introduction into the system of much less foreign nerve material than either Pasteur's or Puscariu's methods.

Conclusion.—The administration of an excess of foreign material and not of an excess of rabies toxin is the most likely explanation of the noxious effects of those systems of treatment in which the accidents to which we refer are found to occur.

D.—Existence of blood changes in man concurrent with or subsequent to anti-rabic inoculations which may be explained on the theory of inoculation of foreign albumins.

We had intended in this connection to make a detailed comparison of the results following anti-rabic immunisation according to the Pasteurian and Höyges' methods. But except for the clinical observations which we have just recorded we have not been able to carry out this intention in full. We have however made certain observations preliminary to this investigation which are worth recording here. The blood of certain patients complaining of local and general symptoms (itching, joint pains, eruptions, etc.) during anti-rabic treatment was taken and compared with the blood of untreated persons (Tables X, XI, and XII).

The two chief blood changes to which we devoted attention were the alkalinity and salinity. A small quantity of blood was taken from a patient and allowed to clot. The same was done with untreated persons at the same time. The serum which separated was used for the test. As our technique in both these investigations is our own we give a description of it here.

Technique of estimation of alkalinity of small quantities of blood serum.

The principle consists in determining the amount of N/100 H_2SO_4 which is able to neutralise the alkalinity of 50 cmm. of test serum. The indicator used was neutral red. Small strips of paper were dipped in a weak stock solution of neutral red and allowed to dry. Successive additions of N/100 acid were made to the serum by means of one of Wright's pipettes graduated in 5 cmm. up to 50 cmm. and thereafter indicating 450 cmm. and 500 cmm. After each successive addition of acid a very narrow strip of neutral red paper was dipped into the mixture to determine the reaction and the strips were retained for final comparison with one another. As little fluid as possible was taken up by the strips so as to remove the less from the field of action. With practice only

three or four were necessary in making an estimation. We give an example of the method.

TABLE X.

Test serum.	N/100 H_2SO_4.	Result.
50 cmm.	50 cmm.	+
	75 „	+
	100 „	+
	110 „	+
	120 „	+
	130 „	+
	140 „	—

+ = alkaline.

— = acid.

The method is sufficiently delicate to distinguish at least between N/40 and N/42.

In the above example we place the neutral point somewhere between the addition of 120 cmm. and 130 cmm.—say 125 cmm.=N/40.

In our comparison of the alkalinity of the blood of persons treated with that of persons untreated, we may utilise the conception of an alkalinity index. To obtain this figure we compare the alkalinity of the former to that of the latter taken as unity. Thus if the blood serum of a patient requires 110 cmm. of N/100 H_2SO_4 to neutralise 50 cmm. as compared with 125 cmm. N/100 H_2SO_4 in an untreated person, the alkalinity index of the patient would be $\frac{110}{125}$=0·88.

The amount of N/100 H_2SO_4 required to neutralise the serum of untreated persons (average of 7 observations) was 132·14 cmm.=N/37·8. This amount was taken as our normal in the following observations:—

TABLE XI.

Patients' names.	Day of treatment.	Alkalinity of serum.	Alkalinity index.	Normal alkalinity.
Capt. M. . .	11th . . .	N/40	0·94	132·14 cmm. of N/100 H_2SO_4 are required to neutralize 50 cmm. of normal serum =N/37·8.
Lt. K. . . .	,, . . .	N/40	0·94	
Nana . . .	12th . . .	N/41·66	0·91	
Shanker . . .	,, . . .	N/41·66	0·91	
Miss T. . . .	,, . . .	N/42·66	0·91	
Moti Khan . .	11th . . .	N/38·4	0·98	
Pte. T. . . .	,, . . .	N/41·66	0·91	
Multan Singh . .	13th . . .	N/45·45	0·83	

The observations are few in number, but such as they are they indicated that the alkalinity of the blood in these persons was distinctly diminished.

Technique of estimation of salinity of small quantities of blood serum.

The chlorides of the serum are estimated as sodium chloride with the help of a standard solution of silver nitrate. This standard solution is one of 0·2906 per cent., of which 1 cc=·001 grm. NaCl.

The indicator is absorbent paper which has been dipped in 5 per cent. potassium chromate and dried. Paper was used, because it enabled us conveniently to get indicator in sufficiently small quantities. A small strip of this paper is added to 50 cmm. of the test serum contained in a watch-glass; then successive additions of the standard silver nitrate solution are made to the serum until a permanent faint pink colour is manifest. A Wright's graduated pipette is used here also.

We might on the same principle as in the case of the alkalinity give here a salinity index. The normal salinity as determined by this method from nine observations was 0·819 per cent. The following table gives some of the results obtained by estimation in persons undergoing anti-rabic treatment and who complained of some of the symptoms which we have previously detailed :—

TABLE XII.

Patients' names.	Day of treatment.	Salinity.	Normal salinity.
Nana	12th	0·780 per cent.	0·819 per cent.
Shanker . . .	12th	0·770 „	
Miss T.. . . .	12th	0·760 „	
Pte. T.	11th	0·770 „	
Moti Khan . . .	12th . . .	0·760 „	
L/C. R.. . . .	12th	0·675 „	
Multan Singh . . .	12th	0·777 „	

The observations here made on alkalinity and salinity are few in number and only form part of a scheme for a much wider investigation. The technique, being a very simple one, seems to us worth recording.

Since this paper was written we have had an opportunity to do some further systematic work with the help of Captain McCay, I.M.S., on the effect of anti-rabic treatment upon patients. Captain McCay used Wright's method[1] of estimation of the salinity of the blood and confirmed the results which we obtained as regards diminution of the salt content of the serum. Another important point is brought out in this investigation with regard to the effect of inoculations in lowering blood pressure. In this case the patients tested were not in any way selected. In the series Europeans and Indians are both represented, as are also cases treated by the Pasteurian (light and intensive) method and Höyges' method (light and intensive).

An inspection of the tables and charts appended shows at once a marked difference between patients undergoing Pasteurian treatment as compared with those treated by Höyges' method. In the former case the blood pressure rapidly falls and there is also rapid diminution of the concentration of salt in the serum. In the latter case the blood pressure is scarcely lowered at all and indeed may show an actual rise, whilst the salt concentration of the serum does not fall to anything like the same extent. The great lassitude which patients undergoing the Pasteurian form of treatment almost all feel may possibly be ascribed in part to their low blood pressure. There was no fall of weight in patients undergoing either the one or other form of treatment. We have already stated our views on

[1] Wright and Kilner, *Lancet*, 1904, p. 921.
„ and Ross „ 1905, p. 1,164.
McCay, *Lancet*, 1907, p. 1,483.

the subject of the causes of the marked differences which exhibit themselves in patients treated by the one or other method, *viz.*, that they are due to the presence of an excessive amount of foreign nerve material in the Pasteurian system of inoculations. That the symptoms following injection are not more frequent or more pronounced may be due to the fact that the inoculations are made daily. Recent work[1] on anaphylaxia would seem to support this view. A very interesting further point brought out was that in patients (only three observations) undergoing intensive Pasteurian* treatment the blood pressure did not fall to any great extent but remained more or less level. In one of these cases, however, a very marked fall occurred on the day of the first appearance of symptoms of rabies—Case 13, Table XIII ; Case I, fig. VI. In these intensive cases (Pasteurian) there was, however, the same marked fall in the salt content of the blood as in those treated by the light method. We desire to record here our great indebtedness to Captain McCay for the observations which he made. The following tables and the curves record the results obtained:—Column IV, Tables XIII, and XIV, shows the salt concentration as determined in three observations, in the same individual, one before treatment, one in the middle of treatment (12th or 13th day), and one towards the end of treatment. Column V shows the blood pressure values. The blood pressure fall or maintenance, as the case may be, is also shown graphically—figs. IV-VII.

[1] Gay and Southard *Journ. of Med. Res.* 1907, No. 2. p. 143.

* When we use this term Pasteurian it has reference only to the method of treatment by desiccated cords. The entire system of anti-rabic treatment as at present practised is entirely due to the genius of Pasteur although it has undergone in some cases modification.

TABLE XIII.

Observations on patients undergoing anti-rabic treatment, Pasteurian method.

I	II	III	IV	V	VI
No.	Caste.	Salt content equivalent of blood in NaCl per cent.	Salt concentration of serum in NaCl per cent.	Blood pressure.	Blood examination.
1	H. . .	$\frac{N}{50}$ = ·1167 . .	1·75	...	
"	" . .	$\frac{N}{43·75}$ = ·133 . .	1·06	...	Hb. = 68% r. b. c. = 4,800,000. w. b. c. = 7200.
"	" . .	$\frac{N}{40}$ = ·146 .	1·02	...	Hb. = 68% r. b. c. = 5,600,000. w. b. c. = 5600.
2	" . .	$\frac{N}{47·5}$ = ·123 . .	1·23	97 mm.	
"	" . .	$\frac{N}{50}$ = ·1169 . .	·935	84 "	
3	Sikh .	$\frac{N}{60}$ = ·097 . .	·974	...	
"	"	$\frac{N}{50}$ = ·1169 . .	·93	100 mm.	Hb. = 68% r. b. c. = 5,400,000. w. b. c. = 6900.
"	" .	$\frac{N}{50}$ = ·1169 . .	·87	110 "	Hb. = 92% r. b. c. = 5,600,000. w. b. c. = 9000.
4	H. .	$\frac{N}{50}$ = ·1169 . .	·93	...	
"	" .	$\frac{N}{50}$ = ·1169 . .	·877	104 mm.	Hb. = 78% r. b. c. = 6,200,000. w. b. c. =
"	" .	$\frac{N}{45}$ = ·129 . .	1·03	110 "	Hb. = 84% r. b. c. = 5,230,000. w. b. c. = 6700.
5	" .	$\frac{N}{57·5}$ = ·1016 . .	·932	...	
"	" .	$\frac{N}{50}$ = ·116 . .	·846	...	Hb. = 76% r. b. c. = 4,400,000. w. b. c. = 7500.
"	" .	$\frac{N}{47·5}$ = ·123 . .	·861	...	Hb. = 78% r. b. c. = 4,300,000. w b. c. = 6800.

H = Hindu. M = Mahommedan.

Observations on patients undergoing anti-rabic treatment, Pasteurian method—contd.

I	II	III	IV	V	VI
No.	Caste.	Salt content equivalent of blood in NaCl per cent.	Salt concentration of serum in NaCl per cent.	Blood pressure.	Blood examination.
6	Sikh .	$\frac{N}{40}$ = ·146 . .	1·096	108 mm.	
„	„ .	$\frac{N}{47·5}$ = ·123 . .	·89	96 „	Hb. = 84% r. b. c. = 5,900,000. w. b. c. = 8100.
„	„ .	$\frac{N}{47·5}$ = ·123 . .	·922	108 „	Hb. = 82% r. b. c. = 5,700,000. w. b. c. = 9000.
7	„ .	$\frac{N}{67·5}$ = ·086 . .	·866	107 „	Hb. = 78% r. b. c. = 5,600,000. w. b. c. = 5200.
„	„ .	$\frac{N}{50}$ = ·116 . .	·928	93 „	
„	„ .	$\frac{N}{40}$ = ·146 . .	·876	105 „	Hb. = 76% r. b. c. = 4,400,000. w. b. c. = 6400.
8	H. .	$\frac{N}{45}$ = ·129 . .	1·161	103 „	Hb. = 74% r. b. c. = 4,000,000. w. b. c. = 5800.
„	„ .	$\frac{N}{45}$ = ·129 . .	·910	81 „	
„	„ .	$\frac{N}{75}$ = ·079 . .	·79	99 „	Hb. = 80% r. b. c. = 5,400,000. w. b. c. = 7800.
9	„ .	$\frac{N}{67·5}$ = ·086 . .	1·116	...	Hb. = 88% r. b. c. = 6,800,000. w. b. c. = 7800.
„	„ .	$\frac{N}{65}$ = ·09 . .	1·08	...	
„	„ .	$\frac{N}{70}$ = ·0835 . .	·75	...	Hb. = 89% r. b. c. = 6,700,000. w. b. c. = 9500.
10	„ .	$\frac{N}{67·5}$ = ·0866 . .	1·038	...	Hb. = 72% r. b. c. = 5,200,000. w. b. c. = 7100.
„	„ .	$\frac{N}{57·5}$ = ·1169 . .	·935	...	Hb. = 80% r. b. c. = 6,400,000. w. b. c. = 8900.

H = Hindu. M = Mahommedan.

Observations on patients undergoing anti-rabic treatment, Pasteurian method—concld.

I	II	III	IV	V	VI
No.	Caste.	Salt content equivalent of blood in NaCl per cent.	Salt concentration of serum in NaCl per cent	Blood pressure.	Blood examinaiton.
11	H. .	$\frac{N}{55}$ = ·1062 . .	1·484	...	Hb. = 88% r. b. c. = 5,050,000. w. b. c. = 9300.
„	„ .	$\frac{N}{52·5}$ = ·1113 . .	·834	...	Hb. = 84% r. b. c. = 4,800,000. w. b. c. = 7800.
12	„ .	$\frac{N}{50}$ = ·116 . .	·928	...	Hb. = 74% r. b. c. = 4,200,000. w. b. c. = 10930.
„	„ .	$\frac{N}{50}$ = ·116 . .	·812	...	Hb. = 82% r. b. c. = 5,620,000. w. b. c. = 9000.
13*	M. .	$\frac{N}{52·5}$ = ·1114 .	1·002	105 mm.	Hb. = 80% r. b. c. = 5,800,000. w. b. c. = 11000.
„	„ .	$\frac{N}{50}$ = ·116 . .	·928	105 „	
„	„ .	$\frac{N}{40}$ = ·146 . .	·803	110 „	Hb. = 80% r. b. c. = 6,200,000. w. b. c. = 10500.
„	„ .	$\frac{N}{45}$ = ·129 . .	·806	108 „	

* This case was treated by the intensive method, but rabies developed on the 19th day of treatment.

TABLE XIV.

Observations on patients undergoing anti-rabic treatment, Hoyges' method.

I	II	III	IV	V	VI
No.	Caste.	Salt content equivalent of blood in NaCl per cent.	Salt concentration of serum in NaCl per cent.	Blood pressure.	Blood examination.
1	Sikh .	$\frac{N}{47·5}$ = ·123 . .	·953	104 mm.	
„	„	$\frac{N}{47·5}$ = ·123 . .	·8961	106 „	

H=Hindu. M=Mahommedan.

Observations on patients undergoing anti-rabic treatment, Höyges' method—concld.

I	II	III	IV	V	VI
No.	Caste.	Salt content equivalent of blood in NaCl per cent.	Salt concentration of serum of NaCl per cent.	Blood pressure.	Blood examination.
1	Sikh .	$\frac{N}{37·5}$ = ·156 . .	·936	108 mm.	
2	H. .	$\frac{N}{50}$ = ·116 . .	1·287	107 „	
„	„ .	$\frac{N}{52·5}$ = ·113 . .	1·244	104 „	
3 *Intensive.*	M. .	$\frac{N}{42·5}$ = ·1375 . .	·961	112 „	Hb. = 80%. r. b. c. = 5,600,000. w. b. c. = 8750.
„		$\frac{N}{60}$ = ·097 . .	·97	110 „	
„	„ .	$\frac{N}{60}$ = ·097 . .	·925	108 „	Hb. = 86%. r. b. c. = 5,600,000. w. b. c. = 8750.
4	H. .	$\frac{N}{40}$ = ·146 . .	·876	103 „	
„	„ .	$\frac{N}{47·5}$ = ·123 . .	·984	105 „	
„	„ .	$\frac{N}{50}$ = ·1169 . .	·935	102 „	
5	H. .	$\frac{N}{65}$ = ·97 . .	·67	...	
„	„ .	$\frac{N}{70}$ = ·083 . .	·75	...	
„	„ .	$\frac{N}{70}$ = ·083 . .	·91	...	

H = Hindu. M = Mahommedan.

VI.—Selection of rabbits necessary to the upkeep of the "fixity" of rabies virus.

We may consider in this paper an interesting point which we have observed amongst the "passage" rabbits. Pasteur in his classical researches found that with successive passages of rabies virus in dogs and monkeys a degeneration of the virus of a progressive nature occurred. In the case of rabbits, on the contrary, the strength of the virus—originally derived from the dog—continued to increase with passage until a point was reached at which no further increase of strength was attained, as judged by the length of the incubation period. The virus then became Pasteur's 'fixed virus,' producing symptoms of rabies in a rabbit on the 6th or 7th day after subdural inoculation. This is true to a remarkable degree, but we have found on going into the matter closely that even with continuous subpassage in rabbits a considerable amount of degeneration of virus can take place. We found, however, that the virus can be kept constantly up to strength by a careful selection of the rabbits used to afford the material for subpassage. By using those rabbits only which show marked symptoms of paresis on the 6th day it is quite possible to obtain a virus which will invariably give rise to paresis on the 6th day. Indeed it is quite possible to determine the oncoming of a certain amount of paresis by the 4th day in the great majority of instances—the evidence for this being a tremor of the head, distinct, though often of extremely short duration. We were at first lead to investigate this matter by going closely into the periods of incubation of rabies in the rabbits, which for some years past had continued to be inoculated with a virus which certainly was originally a 'fixed' virus. We found, however, quite a marked difference in the length of the incubation periods, which varied according to the material used for inoculation. Thus rabbits inoculated from a given piece of rabies medulla gave an incubation period of six days, seven days, eight days or even nine days, as the case might be when no special attention was paid to the date on which the rabbit affording the material first showed symptoms. But by careful attention to taking only material for subpassage derived from rabbits which had developed definite symptoms of paresis by the 6th day, we succeeded in eliminating almost entirely those which after inoculation did not develop first symptoms until the 7th, 8th, and 9th days. Prof. Höyges[1] mentions how he used this very method of selection in order to attain more rapidly to a condition of 'fixed' virus. What he found useful for the rapid attainment of this condition we have found necessary for the upkeep. The point is an important one as it is on the criterion of 'fixity' of the virus that the standard character of the method of dosage used in anti-rabic inoculation is based. The following tables show how this method of selection affected the constancy of the incubation period.

[1] *Ann. Past.*, 1888, p. 134.

From a state of affairs in which only 24·5 per cent. showed paresis on the 6th day we reach a point in seven months in which 95 per cent. developed paresis on the 6th day. Thus—

TABLE XV.

During *February 1906*—163 rabbits inoculated:—

24·5	per cent.	developed	paresis	on the	6th day.
62·5	„	„	„	„	7th day.
10·4	„	„	„	„	8th day.
2·4	„	„	„	„	9th day.

During *March 1906*—191 rabbits inoculated:—

59·7	per cent.	developed	paresis	on the	6th day.
38·7	„	„	„	„	7th day.
9·4	„	„	„	„	8th day.
1·05	„	„	„	„	9th day.

During *May 1906*—235 rabbits inoculated:—

87·6	per cent.	developed	paresis	on the	6th day.
8·05	„	„	„	„	7th day.
3·4	„	„	„	„	8th day.
0·4	„	„	„	„	9th day.

During *August 1906*—210 rabbits inoculated:—

95	per cent.	developed	paresis	on the	6th day.
1·9	„	„	„	„	7th day.
1·9	„	„	„	„	8th day.
0·4	„	„	„	„	9th day.

Whatever be the explanation of the matter the fact remains, that by careful attention to this point—that the rabbit supplying medulla for subpassage should show symptoms of paresis by the 6th day—it is possible to attain the result that all or nearly all the rabbits used will show paresis by that day. Moreover this point must continue to be attended to, even after the virus has become "fixed."

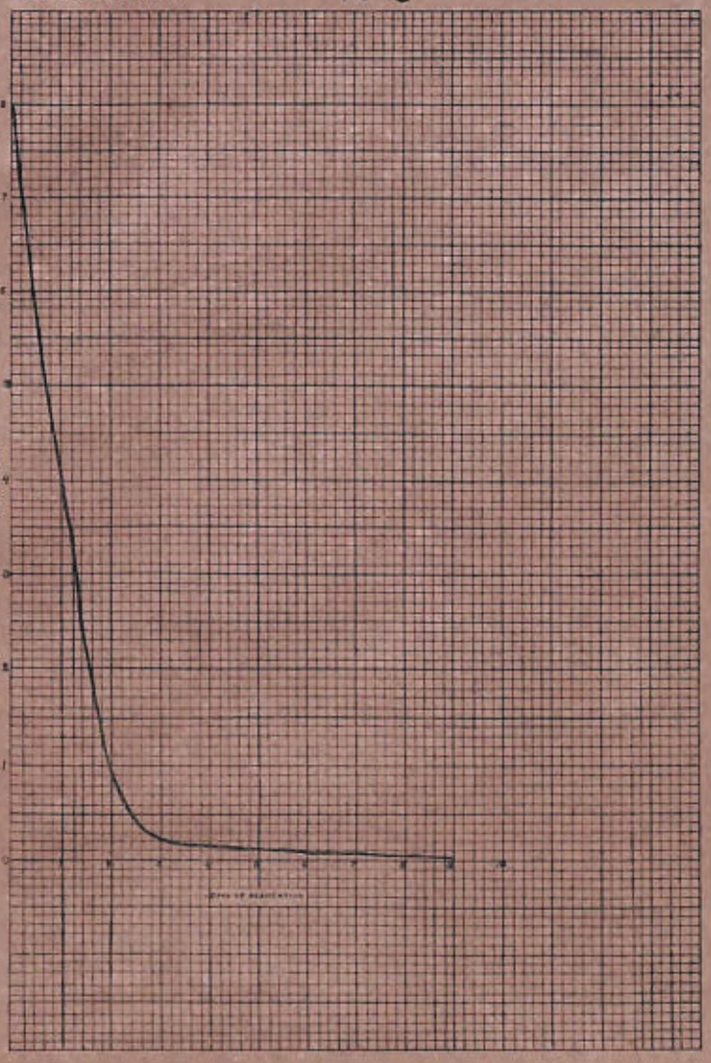
CONCLUSION.

We have now reached the conclusion of the record of those investigations upon rabies which we have been conducting during the past 18 months. It is unnecessary further to draw the threads of a somewhat lengthy argument together; we may, however, summarise our conclusions.

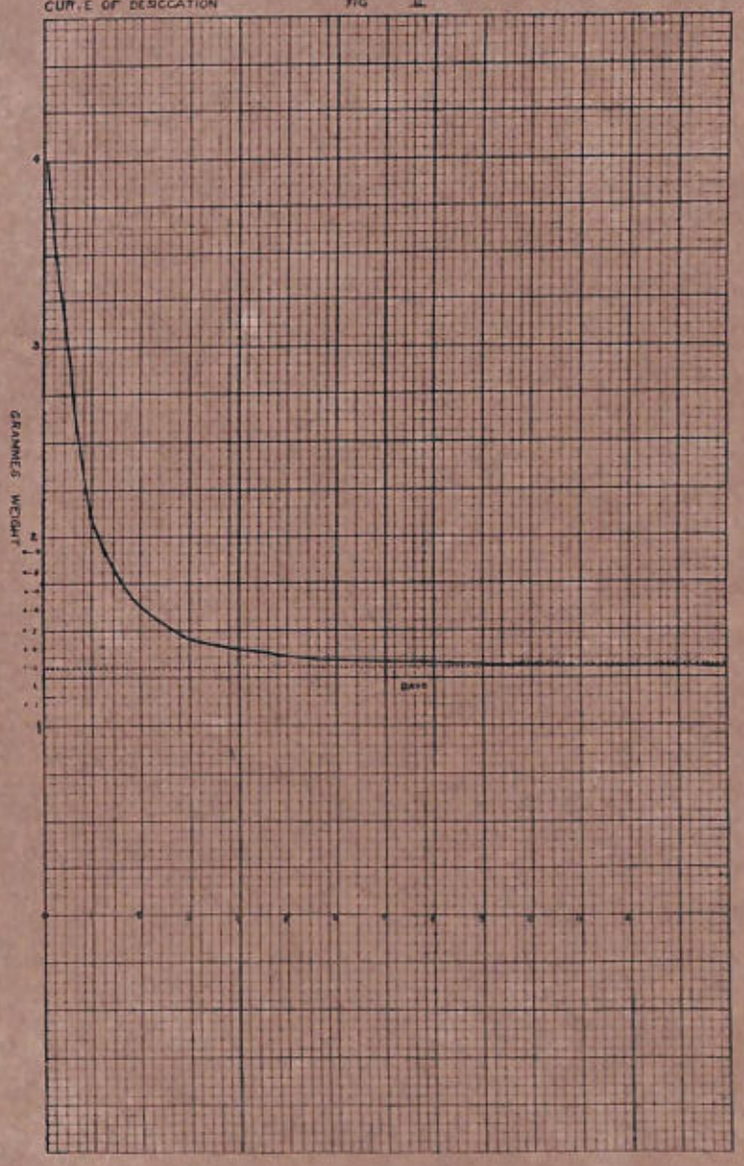
1. There is evidence of a direct proportion between infectivity and duration of desiccation of rabies nerve material.
2. There is no evidence for the existence of a rabies toxin.
3. The quantity of living fixed virus and the duration of time of administration are the only points which need be considered in making out a scheme of anti-rabic treatment.

4. There is great advantage in using fresh material in anti-rabic immunisation (as in Höyges' or Ferrans' methods) over dried or heated material, because the former method involves the introduction of less injurious foreign nerve substance and is more accurate as regards dosage than the latter.

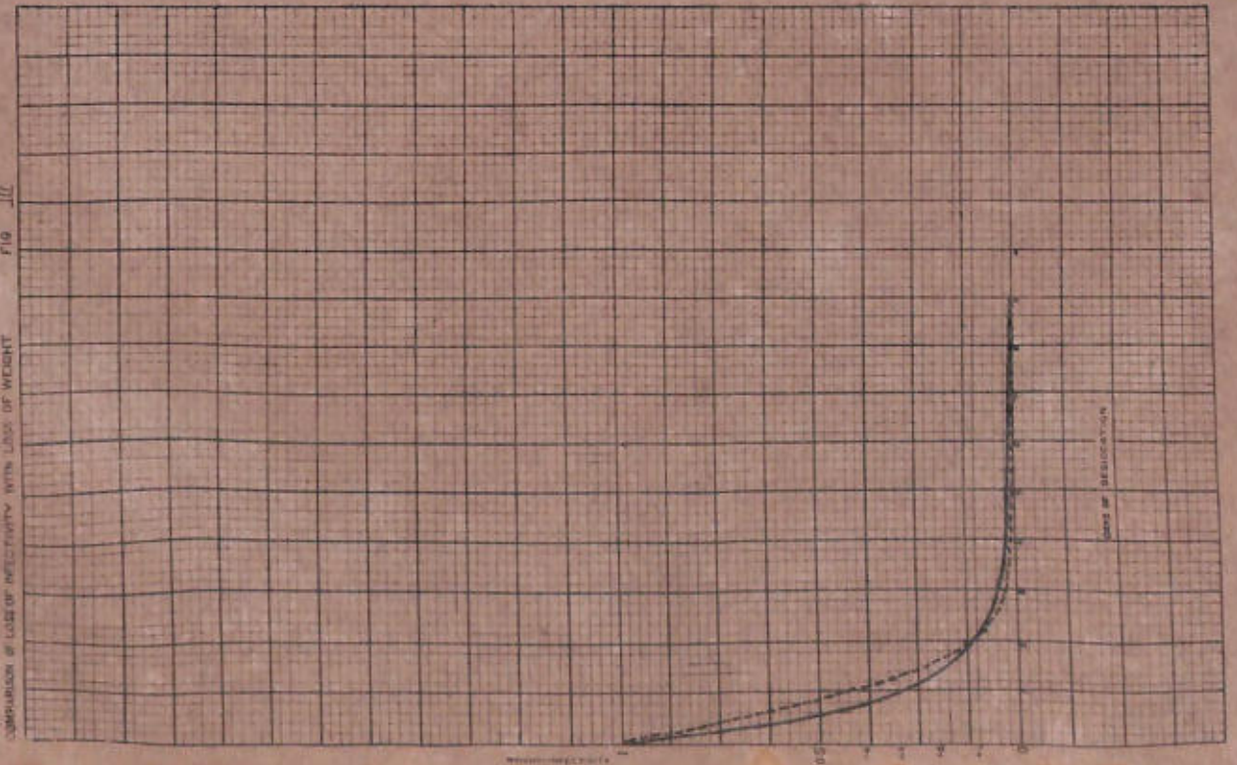
CURVE OF INFECTIVITY FIG I



CURVE OF DESICCATION FIG II

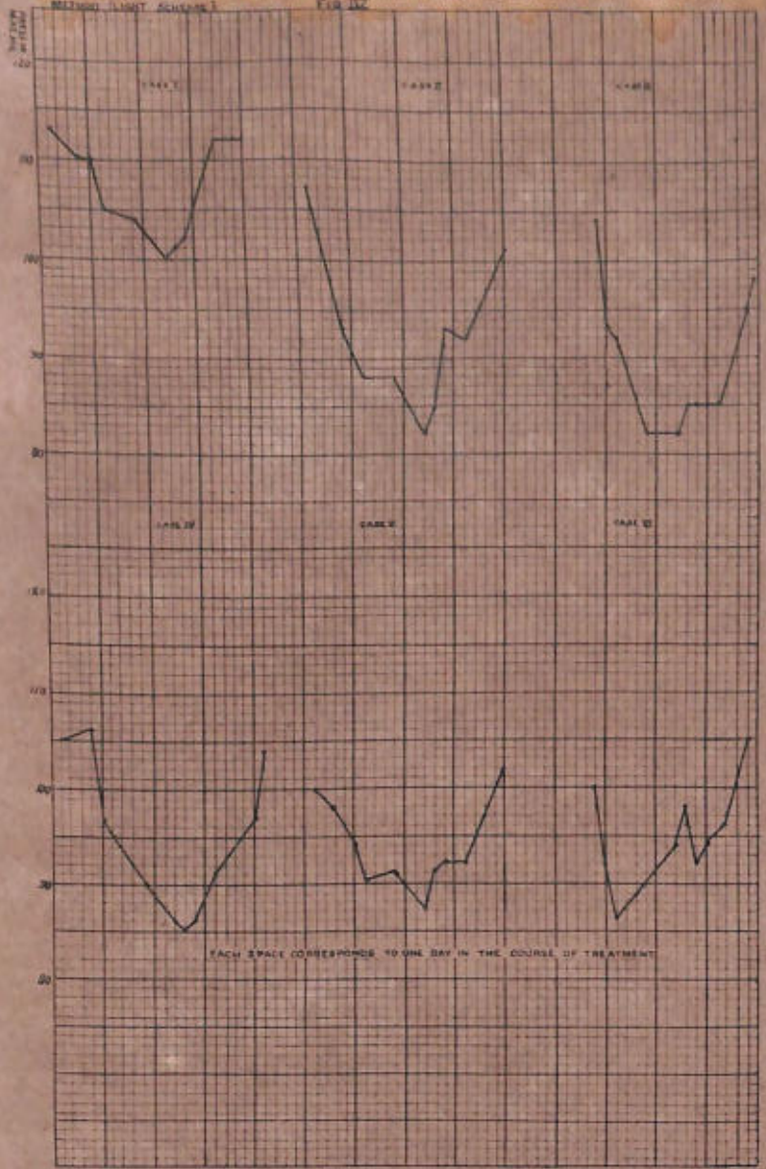


COMPARISON OF LOSS OF INFECTIVITY WITH LOSS OF WEIGHT FIG III



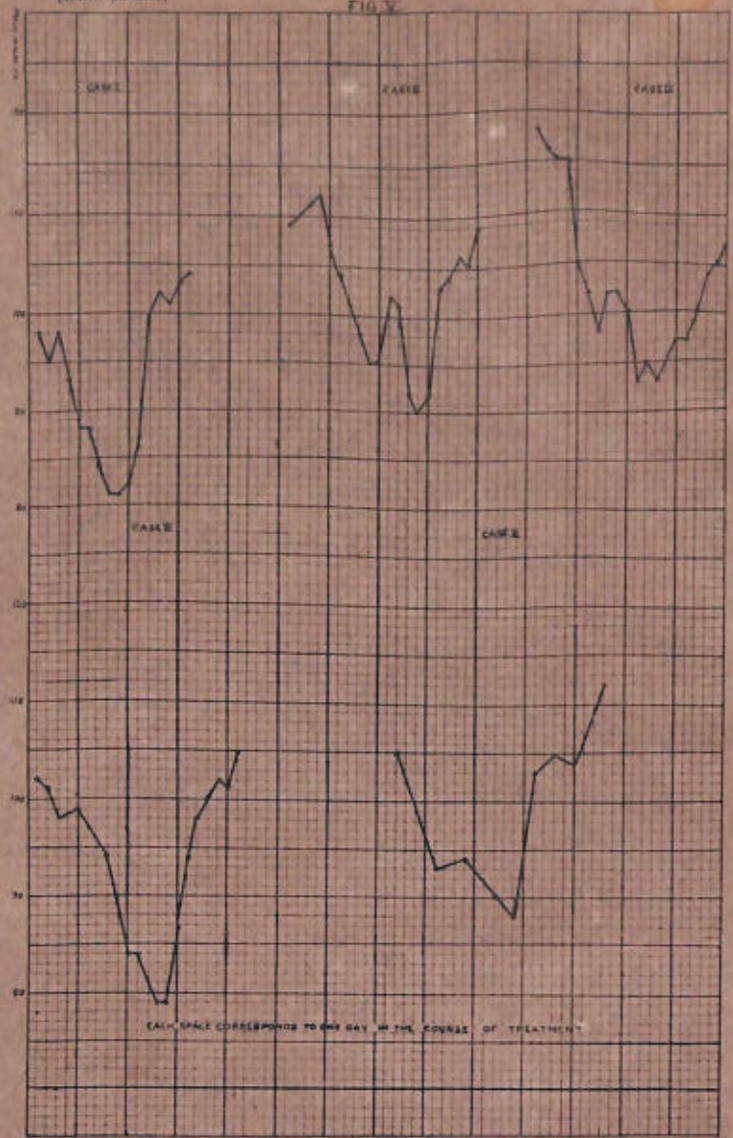
CHARTS OF BLOOD PRESSURE OF BRITISH SOLDIERS DURING ANTI RABIC TREATMENT BY THE PASTEURIAN METHOD (LIGHT SCHEME)

FIG IV



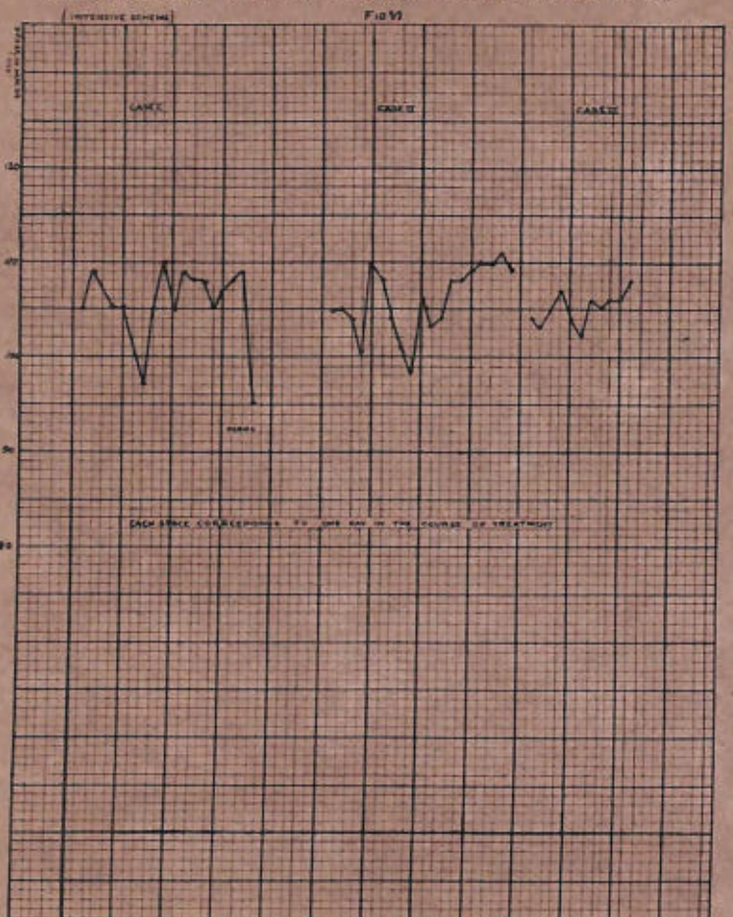
CHARTS OF BLOOD PRESSURE OF PATIENTS DURING ANTIRABIC TREATMENT BY THE PASTEURIAN METHOD (LIGHT SCHEME)

FIG V



CHARTS OF BLOOD PRESSURE OF PATIENTS TREATED ANTIRABICALLY BY THE PASTEURIAN METHOD (INTENSIVE SCHEME)

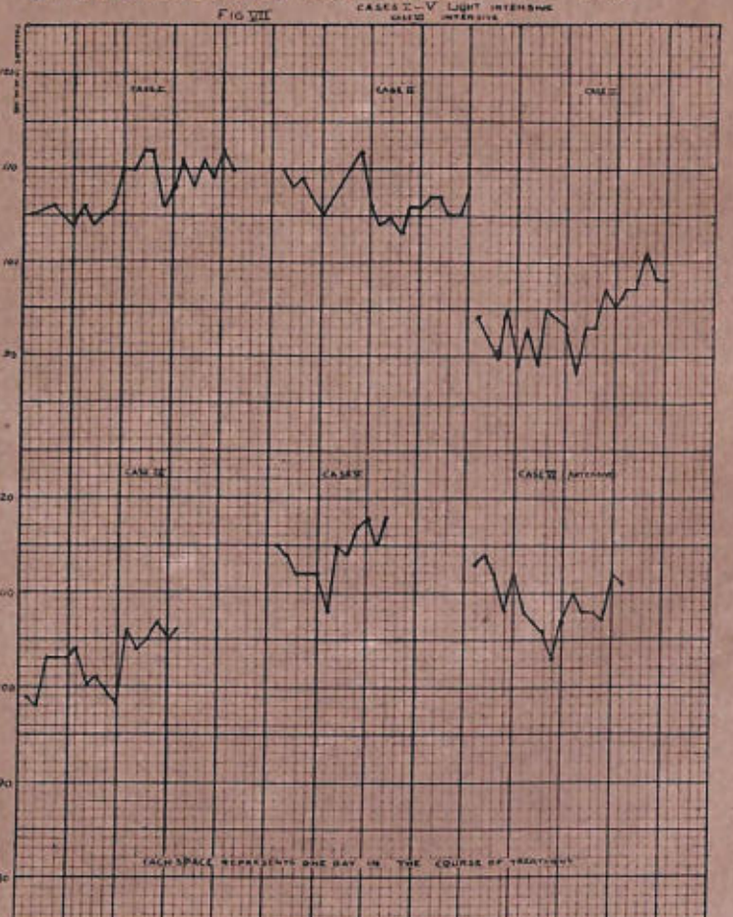
FIG VI



CHARTS OF BLOOD PRESSURE OF PATIENTS TREATED ANTIRABICALLY BY HOYLES METHOD

FIG VII

CASES I – V LIGHT INTENSIVE
CASES VI INTENSIVE



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ISSUED UNDER THE AUTHORITY OF THE GOVERNMENT OF INDIA BY THE SANITARY COMMISSIONER WITH THE GOVERNMENT OF INDIA, SIMLA.

CALCUTTA

SUPERINTENDENT OF GOVERNMENT PRINTING, INDIA

1907

Price Annas 12 or 1s. 2d.