THE KALA-AZAR TRANSMISSION PROBLEM.

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THE problem of how kala-azar spreads from man to man probably constitutes to-day the most important unsolved problem in tropical medicine. Two of the writers (R. K. and B. M. D. G.) have now had the opportunity of experimental work upon this question both in Assam and in Bengal for some five years: whilst the third (L. E. N.) has for two and a half years been upon special duty as kala-azar research worker at the Calcutta School of Tropical Medicine. It may therefore be not out of place to summarise the experimental work carried out at this School upon this problem during the years 1921 and 1922 : and to publish experimental results which, although they include but few positive findings, yet seem to the writers to raise points of interest. We have not discovered how kala-azar spreads from man to man : yet we consider that this problem can only be solved by co-ordinated and co-operative work under a unified direction and along certain lines of investigation. To quote Lt.-Colonel J. Cunningham's apt expression, many laboratories in India are to-day continuing to "peck" at the problem. We do not consider that such a policy of non-co-operation is likely to lead to fruitful results.

In dealing with this subject we do not desire to cover historical and much discussed findings : nor to re-open thread-bare controversies. Yet we consider that the problem of kala-azar transmission can only be solved if it be studied from a systematic and broad point of view.

I. *The Systematic Position of Leishmania donovani.*

If this be attempted then the first thing to settle is the true systematic position of the parasite of Indian kala-azar. Patton (1922) has now re-named this parasite *Herpetomonas donovani.* Whilst every worker upon the problem will agree with his suggestion that the parasite of Indian kala-azar is phylogenetically an insect herpetomonad, yet we desire to emphasise the fact that at present this parasite is only known as a parasite of man and as an (involuntary) inhabitant of the gut of the bed bug. The genus *Herpetomonas* occurs chiefly as parasites of the gut of invertebrates (chiefly insects) : secondarily in the latex of certain *Euphorbiaceæ*—where the infection with *H. davidi* is derived from the herpetomonads of hemiptera living upon these plants. Natural infections in vertebrate hosts have however been recorded, apart from those in experimental animals. Thus Dutton and Todd (1903) found a herpetomonad in the blood of Gambia mice : the Sergent Brothers (1907) an infection in a pigeon : the same authors with Lemaire and Senevet (1914) infection of a gecko : Fantham and Porter (1920) infection in a fish : and Laveran and Franchini (1921) infection in three out of seven dormice examined. A herpetomonad totally different from Leishmania has been described by Franchini (1913) as infecting man,—*Hæmocystozoon braziliense.* In view of the facts however that the true phylogeny of the parasite is unknown, that it has never yet been found in man in its herpetomonad form, that its mode of transmission is unknown, and that both Leishman's and Donovan's names are associated with its discovery, we are of opinion that —in spite of Lt.-Colonel W. F. Patton's great authority—it is better to adhere to the older nomenclature and to call the parasite *Leishmania donovani,* Ross (1903).

II. *Is kala-azar transmitted at all?*

At this point we wish to ask whether kala-azar is really transmitted from man to man or not. Wenyon (1914) has thrown out the interesting suggestion that kala-azar in man may be merely an end phase of insect herpetomoniasis : that the parasite may be a herpetomonad of some biting insect which abounds in the infected localities and its natural transmission be from insect to insect *via* a contaminative cycle : and that man may occasionally be bitten and accidentally infected by this insect. In this event kala-azar would not be transmitted from man to man at all, but each individual case in man would merely constitute an end phenomenon. Such a suggestion is supported by the large volume of experimental work carried out by several workers, but especially by Fantham and Porter and by Laveran and Franchini from 1913 to 1922. These workers claim to have repeatedly succeeded in artificially infecting vertebrates with the flagellates of different insects both associated with and unassociated with vertebrates; a kala-azar-like disease having resulted, and *Leishmania*-like forms being found in the liver and spleen. This work is so well known that it need not be referred to in further detail.

Were this suggestion to hold good all the experimental work in India on kala-azar during the last twenty years would represent merely wasted effort : and the reason for the difficulty experienced in solving the kala-azar transmission problem would be that such transmission does not occur. The importance of the suggestion is therefore very great. We consider however that three facts, if not others also, negative this view:—

(I) No special biting insect has been recorded as especially prevalent in connection with kala-azar epidemics or in kala-azar areas:

with the possible exception of *Conorhinus rubrofasciata* by Awati (1922). The special household and family incidence of kala-azar suggests its transmission from man to man rather than the special prevalence of some species of biting insects in the locality.

(II) The careful work by Hoare (1921) and that by Shortt (1923), also the attempts of Wenyon (1908), (1914), Chatton (1919) and Noller (1920) to produce experimental leishmaniasis in vertebrate hosts by injection of insect herpetomonads all gave completely negative results. Hoare's paper is a model of careful and painstaking work, and should carry much weight. He concludes as follows:—"The conclusions of Fantham (1915), and the more general conclusion that leishmaniases are 'arthropod-borne herpetomoniases' are very interesting from the theoretical point of view, and it is quite possible that later discoveries will prove this to be a fact, and not merely a hypothesis. The facts available at present, however, do not in my opinion permit one to assert that the natural herpetomonads in insects, especially in those not associated with vertebrates, may become pathogenic when introduced into the latter, as the author suggests." A study of the literature compels us to accept Hoare's conclusion as a true presentation of the case: and, although *L. donovani* may be phylogenetically an insect herpetomonad, yet, as we know it to-day, it is a natural parasite of man, and not of any other proved host.

(III) Knowles (1920) suggested that the successful treatment of cases by intravenous tartar emetic might act as a factor in stopping the spread of the disease. It is pleasant to be able to record that this idea has now passed from the realm of hypothesis into that of fact: and in a paper read before the annual meeting of the Assam Branch of the British Medical Association at Haflong in January 1923 Lt.-Colonel T. C. McCombie Young, I.M.S., Director of Public Health, Assam, brought forward very striking evidence of the value of successful treatment of cases as a factor in stopping the spread of kala-azar. In a discussion with the senior writer (R. K.) Colonel Young shewed the full statistics upon which his paper was based. Briefly the experience of the kala-azar staff in Assam in recent years is this; that if there are only 4 or 5 cases of kala-azar in a village and they are all simultaneously placed under treatment, the disease is stamped out and disappears from the area. The village has a clean bill of health for the ensuing 2 or 3 years. On the other hand if there are some 10 to 20 cases and only a few of them come under treatment, the spread of the disease continues and pursues its usual rate of progress. These facts are quite inconsistent with the view that Indian kala-azar is in reality an insect herpetomoniasis and that man is merely a secondary host of the parasite, and his infection an accidental and end phase. Were kala-azar in reality insect herpetomoniasis, the successful treatment of cases should not influence the spread of the disease.

We may conclude therefore that essentially *Leishmania donovani* is a parasite of a man, rather than of any insect: and that kala-azar is transmissible from man to man and spreads amongst the human rather than amongst the arthropod population of infected areas.

III. *Secondary Factors in the Production of kala-azar.*

During the last two years in Calcutta we have been increasingly impressed with the importance of secondary factors in inducing kala-azar. As pointed out by Napier (1922), although the fever and mode of onset of kala-azar may vary very widely, yet three definite clinical types of onset may be clearly recognised:—(a) commencing with an enteric-like fever; (b) resembling chronic malaria, but with increasing size of the spleen and liver; and (c) commencing as a dysentery, either of amœbic or of bacillary origin, more frequently the latter.

A large percentage of the cases of kala-azar in permanent residents in Calcutta who applied for treatment at the School during May, June and July 1922, gave a definite history of having been treated for enteric fever at one of the large Calcutta hospitals during the previous December or January. The fact that the Widal was usually negative in these cases suggested that the original diagnosis of enteric was mistaken, but in two such cases the B. typhosus had been recovered from the fæces and in a third case from the blood. In others the Widal reaction which was negative at 1 in 20 dilution had been positive to 1 in 100 only three months before.

On the other hand there are now several instances in the literature,—Patton (1914, p. 503, case 79), Mackie (1922, p. 330), Knowles (1920, pp. 141-142), where infection with *L. donovani* has been practically symptomless. Mackie (1922) raises the question of the possible existence of symptomless kala-azar "carriers." The present vogue, in Bengal at least, of half treating patients and stopping the antimony treatment as soon as the clinical symptoms are ameliorated is possibly turning loose throughout the Province hundreds of individuals who still harbour *L. donovani* in their tissues, but are comparatively free from symptoms. A further good example of symptomless *leishmaniasis* is Dr. Brahmachari's case of dermal leishmanoid. The patient remained perfectly well and free from symptoms for about two years between the date of being cured of kala-azar and the appearance of the skin lesions: yet it seems fair-

ly clear that the parasite must have been present in his tissues during this interval.

In brief it may be asked whether infection with *L. donovani* is always attended with symptoms. Is it possible that the true etiology of the onset of kala-azar is infection with *L. donovani* plus some secondary factor? We suggest that attention should be paid to this point. Is it possible that symptomless herpetomoniasis of man is widespread in the endemic areas : that a human carrier may carry the infection for months or years, may possibly be infective to others, yet shew no symptoms himself, and yet when his health is undermined by coincident enteric fever, severe malaria or amœbic or bacillary dysentery and the resistance of his tissues is weakened, that he may become suddenly the victim of true, and—in the untreated subject, usually fatal—kala-azar ? As will be seen later " transient leishmaniasis "—an infection with *L. donovani* which can be detected only by culture of liver puncture fluid,—is not uncommon in experimental kala-azar animals. Does anything of a similar kind occur in man ? Is kala-azar in man but an expression of a more widespread and usually symptomless herpetomoniasis of man in the infected areas ? The point is one of importance in connection with the transmission problem, since widespread infections which only give rise to kala-azar in a small proportion of infected individuals and then only at a later date when some secondary disease factor causes the appearance of symptoms, might be simultaneous and not consecutive. We do not desire to lay any stress on this unorthodox and possibly improbable view : yet the facts are there. Kala-azar appears to be too often the sequel of some other preliminary disease—enteric fever, relapsing malaria or dysentery—for this phenomenon to be the result of pure chance. We propose to study and culture the blood of near relatives and of household associates of kala-azar patients : and to analyse the results found in a later communication.

To summarise these introductory sections we may conclude that the name *L. donovani* should be adhered to for the parasite of Indian kala-azar—a parasite whose only known host at present is man : that kala-azar is essentially an infection of man rather than of any other host and is normally transmitted from man to man : and that the question of the existence of kala-azar carriers and of possibly symptomless human herpetomoniasis in the endemic areas should receive attention.

Taking these premises as granted and assuming, as has been universally held to be the case, that the beginning of an outbreak of kala-azar in any area is the arrival in that area of an infected patient ;—if any really *systematic* study of the problem before us is to be undertaken, then the first question to answer is :—

IV. *How does the Virus of kala-azar escape from the Infected Patient?*

Here we have many possible alternatives.

(A) *The sputum and nasal mucus.*—During 1922 at the School the sputa and nasal mucus of nine untreated kala-azar patients were carefully examined (*a*) in fresh preparations :(*b*) in Leishman stained films : and (*c*) in cultures upon N. N. N. medium. Nothing suggestive of *L. donovani* was found. Both epistaxis and broncho-pneumonia are common complications of kala-azar : yet there is no special reason to suppose that the parasite is eliminated from the patient *via* the sputum or *via* the nasal mucus.

(B) *The Urine.*—The catheter urines of six untreated male cases of kala-azar were examined by the same methods. Nothing suggestive of *L. donovani* was found. As shewn by Knowles (1920) albumin and urobilinogen are commonly found in the urine of kala-azar cases : yet the first is an expression of the interstitial fibrosis of the kidney which accompanies the ravages of the disease, and the second of the fine intercellular cirrhosis of the liver and its inability to deal with the decomposition products of hæmoglobin. There is nothing to especially indicate elimination of the virus *via* the urogenital system.

(C) *The Skin.*—The text-books appear to be unanimous in stating that *L. donovani* is found in the cutaneous lesions of kala-azar patients. Upon what basis of evidence this statement rests we have been unable to discover. Christophers (1904-1905) and other workers are stated to have seen *L. donovani* in skin lesions in kala-azar patients: but we have been unable to confirm such findings. Blister fluid from three untreated kala-azar cases was examined in 1922, but no parasites seen : streptococci being present in two of the three cases. The condition known as " dermal leishmanoid " will be considered later, but—despite Manson's suggestion to the contrary—we can see no special reason to incriminate the skin as the channel of elimination of the parasite. There are no skin lesions characteristic of the disease. Bengali Hindus, who are prone to contract kala-azar, are scrupulously cleaning in person and do not ordinarily suffer from *Pediculus* or *Acarus* infestation. Scabies—(as distinguished from septic folliculitis, with which it is often confused)—is relatively rare in Bengali patients. We have especially examined kala-azar patients with a view to discovering any skin lesions especially associated with the disease : and with the exception of " dermal leishmanoid,"—a condition which points rather to invasion of the peripheral blood than to elimination by the cutis vera—we have been unable to discover that kala-azar patients are

particularly prone to skin lesions of any type. There is nothing, in fact, which would lead one to conclude that the parasite is usually eliminated via the skin. "Antimony rashes," —whether due to antimony administration or not,—are not infrequently seen during the course of treatment, yet the roseola spots, when punctured, do not shew *L. donovani.* Unless any evidence of real value to the contrary be forthcoming, we cannot regard the skin as a channel of elimination of the virus.

(D) *The Fæces.*—Here the problem is more complicated. The special association of endemic and epidemic kala-azar with bad conservancy or with an entire absence of any conservancy methods whatever has been emphasised by many of the Assam workers in particular. Bad conservancy or none, general filth, and overcrowding and kala-azar go hand in hand. In Calcutta the community, above all others, which appears to be especially affected by kala-azar is the poorer Anglo-Indian one. Their water supply, which is the general piped supply of the city, is unimpeachable : but their kitchen and conservancy arrangements leave much to be desired. Colonel McCombie Young informs us that, of the staff of the special kala-azar hospitals on Assam, the only members who have contracted kala-azar are three sweepers. Critien (1910), Mackie (1914), and Knowles (1920) have described "cystic bodies" as occurring in the stools in kala-azar dysentery and simulating *L. donovani.*

In a recent article Marian-Perry (1922) records the finding of *L. donovani* in masses in the submucous tissue of the jejunum of an infected patient. The first illustration of his paper is rather unconvincing, whilst the second is extremely diagrammatic. It is unfortunate that this author gives no details as to the source of his material. Yet Lt.-Colonel F. P. Mackie, I.M.S., who has seen the original sections, assures us that the findings are beyond question *L. donovani*: and we must accept it as proved that in kala-azar the parasite is present in immense numbers in the submucous tissues of the jejunum and—presumably—in the bases of any intestinal ulcers which may occur.

In brief the evidence in favour of elimination of the parasite *via* the fæces is not inconsiderable. Accordingly during 1922 special attention was paid to this point, and Table I* shews the protozoal findings in 265 stools from 210 kala-azar patients and in 456 stools from 320 patients suffering from diseases other than kala-azar. (It may be remarked, in passing, that the diagnosis of kala-azar depended in every instance upon the finding of the specific parasite in either spleen puncture films or cultures or in peripheral blood films or cultures.)

A study of Table I shews that the findings in the stools of kala-azar patients are not strikingly different from those in the stools of patients suffering from other diseases,—except in one particular. Every stool here reported upon was examined in both saline and in 1 per cent. Iodine emulsion with a Zeiss 1|7th inch oil immersion objective and a Zeiss × 12.5 binocular eyepiece,—a combination which gives high magnification, stereoscopic vision and great detail and definition, and which the senior writer (R. K.) considers unequalled for examination of fresh fæcal films. In addition whenever objects were encountered of uncertain or doubtful character air dried and Leishman or hæmalum stained films of stool emulsion spread upon slides smeared with serum, and very frequently fæcal films fixed with Schaudinn's fixative and stained by Haidenheim's iron-hæmatoxylin process, were examined.

The results shewn in Table I may be taken as accurate within the ordinary limitations of routine laboratory examination. They were not pre-judged and were only put together from the laboratory records after a year's work upon the question. In only two particulars do the stools in kala-azar differ materially from those of non-kala-azar patients :—

(*a*) In the kala-azar stool, whether formed, semi-formed, or diarrhœic, yeasts are a most prominent feature :—57 per cent. as against 10 per cent. in non-kala-azar stools. So constant is this finding that if the stool be found to be loaded with yeasts and the patient to come from a kala-azar area the suspicion of possible kala-azar should be aroused. In non-kala-azar yeasts have been especially found in the stools of patients suffering from sprue, diabetes, intestinal tuberculosis, and what may be termed diarrhœa in association with pancreatic deficiency, as evidenced by the abundance of oil and fat globules and soap spheres in the stools. The fact that the stool in kala-azar tends to be laden with yeasts requires some explanation. As is well known in kala-azar the appetite tends to be voracious, and the digestive faculties to be low. This may possibly account for the unusual frequency of yeasts in kala-azar stools. At present we are studying the hydrogen-ion concentration in kala-azar and non-kala-azar stools, respectively, in order to ascertain whether there is any significant difference in the pH readings.

(*b*) In 13 instances in the stools from kala-azar patients "cystic bodies" were encountered: but *in every instance in a stool loaded with yeasts.* The forms seen do not shew a kinetonucleus clearly differentiated from a less deeply staining globular macronucleus : and although they simulate *Leishmania* often very closely, a careful study of these "cystic bodies" convinces us that they are only yeasts

* The Tables, giving all the experimental data, will be found in the Appendix to this paper.

of an aberrant type. We would here draw attention to and deplore the tendency of certain workers to consider atypical and doubtful—more especially mononucleate forms—as *Leishmania*. Mononucleate forms cannot be considered to be *Leishmania*: and are far more probably yeasts.

Further the finding of "doubtful bodies" in the spleens of experimental animals is not proof of *Leishmania* infection: since the most marked and prominent feature of *Leishmania* parasites is the deeply staining kinetonucleus. Even in the "torpedo forms" found in the human spleen and described by Knowles (1920), although macro-and micro-nuclei are so closely approximated that at first sight one might mistake them for a single nucleus, yet careful focussing will always shew both nuclei. In five years of careful study of the fæces in kala-azar the senior writer has never once come across undoubted forms of *L. donovani*. It may be urged that if *L. donovani* is eliminated from the patient *via* the fæces it may be in some sepsis-resistant and hitherto unrecognised form : yet one would surely expect in years of careful study of this question to have come across such novel forms, had they been present. The case for the elimination of the parasite *via* the fæces is still non-proven.

(E) *The Peripheral Blood.*—During 1922, 442 blood films from 140 proved cases of kala-azar in both out-patients and in-patients were examined at the School. Nineteen per cent. of cases and 12 per cent. of films shewed *L. donovani*. Up to 15 parasites per large hyaline mononuclear leucocyte, and either one or two parasites per infected polymorphonuclear leucocyte were encountered. These findings are based upon an examination of only 3.2 films per patient on an average. Had a larger number of films been examined per patient the percentage of positive results would probably have been increased. In Bengal, just as in Assam, *L. donovani* can be found in peripheral blood films from kala-azar cases if only the blood be examined often enough and with sufficient thoroughness.

During 1921 at Shillong the junior writer (B. M. D. G.) cultured the blood of 33 consecutive kala-azar patients on N. N. N. medium, using Row's technique. In 2 of these cases the cultures remained sterile and shewed no parasites : both were patients who had received a considerable amount of antimony treatment and were clinically cured. In the other 31 cases, all untreated, the cultures were in every instance positive. The peripheral blood culture work at Calcutta in 1922 is shewn in Table II. Of 19 untreated kala-azar cases, all gave positive cultures of the peripheral blood. With four other patients—suspected kala-azar—the cultures were negative. In two of them spleen puncture films and cultures were also negative : so that both were probably *not* kala-azar: whilst the other two were also probably some other disease. Peripheral blood culture is, in fact, so reliable a method of diagnosis when properly carried out and using Row's method of leaving the blood overnight in citrate saline in the cool incubator before sowing into the N. N. N. tubes : that we may place the reliability of the tests for kala-azar in the following order :—

(*i*) Culture of spleen puncture fluid on N. N. N.

(*ii*) Culture of the peripheral blood by Row's method.

(*iii*) Examination of spleen puncture films: no cultures taken.

(*iv*) Culture of liver puncture fluid on N. N. N.

(*v*) The aldehyde reaction.

(*vi*) Examination of liver puncture films : no cultures taken.

(*vii*) Examination of peripheral blood films : no cultures taken.

The evidence is now conclusive that the virus of kala-azar is always present in the peripheral blood of the untreated patient. The paucity of positive findings in peripheral blood films is simply an expression of the scantiness of the parasites.

During 1922 two cases of a new type of infection with *L. donovani* in man came to light: and as these cases tend to support the contention that the virus of kala-azar is always present in the patient's peripheral blood they may be considered in some detail.

V. *Dermal Leishmanoid.*

Several workers have recorded the finding of *L. donovani* in skin lesions in kala-azar patients. Such findings however in no way correspond to the entirely new condition of "dermal leishmanoid" first described by Brahmachari at the February 1922 meeting of the Medical Section of the Asiatic Society of Bengal. A second case of the same condition was reported by Dr. S. P. Bhattacharji, Assistant Professor of Tropical Medicine, Calcutta School of Tropical Medicine in March 1922. Dr. Brahmachari's case has already been reported in full,—Brahmachari (1922-1923). We are very much indebted to Dr. S. P. Bhattacharji for permission to give the history of his patient, which is as follows :—

The patient is a Hindu male adult who first came under treatment for kala-azar some four years ago. He received 32 intravenous injection of antimony salt, and was discharged clinically cured. Two years later he contracted syphilis followed by an extensive generalised secondary eruption of so severe a character that it simulated small-pox. Novarsenobillon was given and the condition cleared up. The patient's general health now remained good for another 1½ years : but by degrees

nodules, resembling those of nodular leprosy, began to appear on his ears, hands, arms, back, chest, and especially in the scrotum. Twenty further injections of potassium antimony tartrate and six of novarsenobillon were given : and the skin is now clear.

Details of the laboratory work in connection with both cases are given in Table III It will be seen that films from five nodules in connection with the two patients shewed numerous *L. donovani* and a rich growth of typical *Leishmania* flagellates was obtained from a nodule of the first patient. On the other hand peripheral blood films and cultures shewed no parasites and in the second patient, in whom the spleen was still palpable and could be punctured, spleen puncture films and cultures were negative. The virus was transmitted to a *M. rhesus* monkey, using the pocket flap technique, and not only did the monkey develop localised nodules full of *L. donovani*; he also developed a third nodule by auto-inoculation at the canthus of the eye. A photograph of the infected monkey is shewn in Plate A. Films from the monkey's nodules shewed numerous *L donovani* and N. N. N. cultures gave a rich growth of flagellates. The disease remained localised in the monkey and did not visceralise: peripheral blood films and cultures giving negative results : as also did films and cultures from liver puncture of the monkey on two occasions.

Neither patient at the time of examination shewed any clinical evidence whatever of kala-azar : except for the residual enlargement of the spleen in the second case. All attempts to demonstrate visceral infection by cultural methods failed : whereas the nodules and skin were full of *L. donovani*. The etiology of this new disease, " dermal leishmanoid," appears to be as follows :—A patient suffering from kala-azar is partially or incompletely treated. The parasites are circulating in his peripheral blood. He acquires a petechial rash from any cause : in Dr. Brahmachari's case a rash possibly due to antimony, in the second patient a secondary syphilide. The parasites settle in the petechial spots of the rash and grow in the endothelial cells of the skin and subcutaneous tissues, where they are more or less sheltered and protected from antimony circulating in the peripheral blood. By degrees the patient recovers completely from his kala-azar, of which disease neither clinical nor laboratory evidence can now be obtained. But the parasites continue to multiply undisturbed: and the final picture is that of a patient in good health with no evidence of visceral disease : but covered from head to foot with a skin disease, clinically simulating nodular leprosy, but actually due to *L. donovani*.

Both cases appear to support the contention that the virus of kala-azar is present in the peripheral blood and that this constitutes its real channel of elimination from the infected patient.

In passing it should be noted that when *L. donovani* causes a cutaneous lesion, as with these two patients, the lesion is entirely different from that produced by *L. tropica*. The granuloma tissue is less fibrotic and more cellular : there is no tendency to skin atrophy or ulceration : and much less fibrosis and thickening than in oriental sore. The second patient shewed some excoriation of the skin of the scrotum, owing to irritation of the lesions from the dhoti : but there was nowhere in either patient the slightest tendency to spontaneous ulceration of the skin. Both cases afford evidence to support the contention that *L. donovani* and *L. tropica*. are two entirely different parasites.

A study of these cases and of the facts given in Section IV brings us to our first temporary conclusion in connection with the transmission of kala-azar. We do not claim that all other possible channels of elimination of the parasite have been absolutely excluded : but we may take it as a working hypothesis that the virus of kala-azar normally escapes from the infected patient *via* the peripheral blood stream. And, in the absence of any considerable hæmorrhages from the skin or mucous membranes, this must mean *via* some biting and blood sucking insect. For the moment we may lay aside the question of what insect.

VI. *The Flagellate Phase.*

Once ingested by the insect concerned, *L. donovani* rapidly becomes converted into its herpetomonad form. And if the conditions of environment under which *L. donovani* passes into its flagellate form in culture media are similar to those in the biting insect, then the five conditions laid down by Rogers (1905) must be present: *viz.*:—

(1) A lowered temperature of between 18 and 28°C. (optimum 22°C.); (2) asepsis is essential and the environment must be sterile; (3) the presence of either blood or hæmoglobin is necessary ; (4) oxygen must be present ; and (5) a slightly acid environment is favourable to the development of the herpetomonad form.

The *L. donovani* flagellate having been produced we may next consider its reactions to different conditions and changes of environment.

(*a*) *Temperature.*—As shewn by Knowles (1920) a small proportion of *L. donovani* flagellates will withstand freezing for 3 days. In order to test this matter further the following experiments were carried out:—

(*a*) Tubes of rich and very active flagellate N. N. N. cultures were packed in ice and salt and placed in the refrigerating room at a temperature of minus 2°C. They were examined daily. At 24 hours a few were still actively motile : at 48 hours all were immobile, but many be-

came actively motile again after a few hours at room temperature. At 72 hours only a few regained sluggish motility when brought to room temperature. No post-flagellate forms were seen.

On the other hand, once the flagellate has been formed, it will withstand body temperature successfully for some time. A set of very active flagellate N. N. N. cultures was placed in the warm incubator at 37°C and their contents examined daily. At 24 hours the majority of the flagellates were still actively motile. A few had died. At 48 hours the cultures still shewed many active flagellates. At 72 hours most of the flagellates were dead : and only a few still shewed active motility. At 92 hours all flagellates were dead and many disintegrated. In fresh preparations and Giemsa stained films none of these immobile flagellates shewed any tendency to rounding up or to conversion into *Leishmania* forms. They simply died as flagellates. A few had lost their flagella however.

We may conclude from these experiments that, although a range of temperature of from 18 to 27°C is necessary for the production of the flagellate form : yet when once formed, the flagellates will to some extent withstand exposure either to freezing or to body temperature for a short period, 24 to 48 hours.

(*b*) *Sepsis.*—As noted by Cornwall (1916) and his colleagues sepsis kills the flagellate of *L. donovani* rather by exhaustion of nutriment from the culture, than by any direct toxic action upon the flagellate itself. It is the universal experience of workers on the disease that N. N. N. cultures in the cool incubator immediately die if the medium becomes contaminated.

Yet to a certain limited extent, the flagellates will withstand a septic environment. A tube which had been 24 hours in the 37°C incubator still shewed the majority of flagellates to be very actively motile. When this tube was re-examined at 48 hours it was found to be contaminated with staphylococcus albus : yet many actively motile flagellates were still seen. At the third day when the tube was heavily overgrown with staphylococcus a few motile flagellates were found. On the fourth day no parasites could be detected. At no time were rounded up *Leishmania* forms seen.

To test the influence of B. coli on the flagellate the following experiment was carried out:—

Tubes of rich and very active flagellate cultures upon N. N. N. were inoculated with B. coli and incubated :—(*a*) at 22°C : and (*b*) at 37°C. In the cool incubator a few flagellates were still sluggishly motile at 24 hours, but no flagellates at all were seen at 48 hours. In the warm incubator no flagellates were to be found after 24 hours.

We may conclude that, although sepsis ordinarily kills the *L. donovani* flagellate yet it does not do so immediately, even under the artificial conditions of the test tube. Under natural conditions it is possible that it may survive even longer. We are at present trying to device experiments by which the products of bacterial growth can be continuously removed by dialysation from contaminated cultures, in order to see whether under such conditions the flagellate may not survive.

(*c*) *Hæmoglobin.*—In order to test the behaviour of the flagellates in the absence of hæmoglobin the following experiment was carried out:—

N. N. N. medium was made up and sterile ascitic fluid added instead of rabbit blood. Tubes were inoculated from cultures full of actively motile *L. donovani* flagellates and incubated (*a*) at 22°C: (*b*) at 37°C. No growth took place : and it apears as if hæmoglobin is a necessary factor for the continued existence of the parasites.

(*d*) *Anærobiasis.*—It is stated by Rogers (1905) that if an active flagellate culture be placed under anærobic conditions multiplication of the parasites at once ceases : and they gradually die out. In order to test these conditions the following experiment was carried out:—

Active cultures on N. N. N. medium were placed in Buchner's tubes in the 22°C and 37°C incubators. At the end of 24 hours all were immobile at both temperatures, and at the end of 48 hours no flagellates could be found. They had all disintegrated.

(*e*) *Reaction of environment.*—As shewn in a separate paper by Napier (1923) a slight acidity of the culture medium is very favourable to the development of the flagellate form : and in N. N. N. medium acidified with $\frac{M}{10}$ HCl successful flagellate cultures may be obtained within 48 hours of inoculation of the tube with spleen juice. On the other hand on subculture back from such acid medium on to ordinary N. N. N. the subcultures usually fail. Napier has found experimentally that successful cultures can be obtained on N. N. N. medium at a range of pH of from 4.75 to 8.0. Above and below these limits the cultures do not take.

Now it has been shewn by Megaw and others that achlorhydria is very common amongst Indian patients : so common indeed that it ceases to be remarkable. Further investigation of this point is called for : yet there must be many individuals, both Indian and European, in whom from time to time the pH of the gastric contents is not at its normal level of 1 to 2: but at a pH of from 5 to 6 or even greater: a pH which would allow of survival of the flagellate for at least a short period of time.

We may, therefore, enquire what would be the fate of the herpetomonad form of *L. donovani* if accidentally ingested either once, or

upon repeated occasions, by an individual suffering from achlorhydria.

Table IV records the results of a few preliminary experiments with reference to this possibility. It will be seen that exposure to alkalies and admixture with fæcal emulsion kills the flagellates. Hence it is unlikely that the flagellates would survive long in the alkaline contents of the duodenum of a warm-blooded animal. The gastric environment however is different : the bacterial content of gastic juice is not as a rule high ; whilst the acidity may be temporarily reduced from many different causes. Man must become infected with kala-azar *via* either the subcutaneous or the oral route. Has the latter possibility received sufficient attention ?

Table V records the results of certain experiments with active flagellate cultures on warm-blooded animals: the flagellate cultures being administered (*a*) by injection : and (*b*) by feeding. The feeds were given either by tying a short piece of fine rubber tubing to the nozzle of a 5 c.c. Roux syringe and passing it down the œsophagus, a method which we have found especially suitable for small animals : or by laparotomy and injecting the cultures into the stomach with a hypodermic syringe fitted with a fine needle.

Few as are the experiments recorded in Table V it must be admitted that the results are completely disappointing. On ingestion by warm-blooded vertebrates the flagellates seem to be at once destroyed: the infection does not appear to visceralise and cultures from the spleen and liver give negative results. Further, after hypodermic injection, the blood and serum of warm-blooded animals, unless previously decomplemented, appear to at once destroy the flagellates. Pigeon No. IV, Table V (A), was selected as being a particularly small bird. 1¼ c.c. of very active flagellate culture crammed with herpetomonad forms of *L. donovani* was given by a clean intravenous injection into the wing vein. No *Leishmania* could be detected in films taken immediately, or in films taken half an hour and an hour later. Cultures from the viscera and heart blood upon N. N. N. remained negative. The injected dose of flagellates seemed to have been instantly destroyed without leaving the blood stream.

Further work upon the possible infectivity of the flagellate phase is perhaps indicated. Yet every worker upon the disease, with one exception, has recorded the difficulty of infecting experimental animals with the herpetomonad phase. With regard to Row's post- and super-post flagellates, and aflagellates and ⊙ forms, we have often seen such forms in fresh preparations and in stained films. These forms shew no cell envelope and differ entirely from the true post-flagellate, which, if it exists, should possess a definite cell membrane, as figured by Patton (1912), in figures 67 to 73 of his Plate. It may be remarked that aflagellate forms can be produced by adding distilled water to an N. N. N. culture and leaving it at room temperature. The flagellates lose their flagella, swell up and become rounded. Plasmolysis and chromatolysis supervene, however, and no definite cell envelope, such as a true post-flagellate form should possess, is seen.

Turning from warm-blooded animals we next studied the behaviour of very active N. N. N. flagellate cultures in cold-blooded animals. Our objects in doing so were two-fold: partly to test the hypothesis of a cold-blooded reservoir of kala-azar infection: mainly, however, in the hope that in cold-blooded animals we might find animal hosts more readily susceptible to experimental infection than are warm-blooded ones. Details of these experiments are given in Table VI, A and B.

Into the anterior lymph sac of each of 19 frogs was injected from 2 to 5 c.c. of very active flagellate culture : the water of condensation of from 3 to 6 rich N. N. N. cultures being used for each injection. The fate of the injected flagellates was then studied at intervals of from 28 minutes to 14 days. In general it may be said that the results were negative. Actively motile flagellates were still found in scanty numbers at the site of injection up to 1¾ hours after injection : but for the most part the injected flagellates round up, become aflagellate, die and are phagocytosed. The viscera (spleen, liver and heart blood) of 16 of these frogs shewed no *Leishmania* forms on most careful examination of both fresh preparations and stained films. N. N. N. cultures from the viscera gave no growth. The usual fate of the injected flagellates is to be rapidly destroyed.

In one instance, No. 1, scanty flagellates were seen 3¾ hours after injection in a film from the spleen : but the culture was negative. In another instance, No. 7, liver films taken ¾ths of an hour after injection, shewed a few rounded extracellular *Leishmania* forms. Frog No. 2, however, killed 1¾ hours after injection of 2 c.c. of very active flagellate culture into the anterior lymph sac, shewed unexpected findings. These are illustrated in Plate B.* At the site of injection both free *Leishmania* forms were seen : figs. 14, 15, 16 & 17 : and also active phagocytosis, fig. 18. In the liver, however, figs. 1 to 8 : and in the spleen, figs. 9 to 13, scanty *Leishmania* forms were seen: a few still flagellate, fig. 11 : many extracellular, figs. 2, 4, 7 and 12 : many intracellular, figs. 1, 3, 6, 8, 9, 10 and 13. As control non-inoculated frogs, the dozens of frogs used to provide material for and examined in connec-

* Plates B, C, D were originally in colour, but have been reproduced in black and white in publishing.

Trypanosoma rotatorium has occasionally been seen, but nothing resembling the forms met with in Plate B has ever been encountered in any control frog. We are certain that the forms encountered in a study of these slides are true *L. donovani.* Further they appear to be large and rather vacuolated forms which have come from flagellates which have only recently penetrated within the cells.

Exceptionally, then, *L. donovani* flagellates, when injected into the anterior lymph sac of a frog may survive in scanty numbers up to 1¾ hours after injection: whilst very exceptionally they may pass into the internal viscera, enter cells, and become *Leishmania*-like in form. Yet even though visceralisation may occur as a transient and immediate sequel, the infection is soon destroyed both at the site of inoculation and in the viscera. The virus does not survive, except for a period of a few hours, in the tissues of the frog.

In the case of the *Varamus flavescens,* Table VI. A, No. 9, one suspicious form was encountered in the spleen films. Accordingly the spleen was emulsified and injected intraperitoneally into a white rat (Table VI. A, No. 12). The rat died nearly five months later. A *Trichomonas* was found in stained films from the liver and spleen. Fresh preparations were at once examined under the dark ground, and actively motile *Trichomonas* encountered. The gut, which had hitherto remained unopened, was now opened and its contents examined. A heavy intestinal infection with *Trichomonas muris* was present.

This finding raises a point of interest. One cannot say whether the penetration of the *Trichomonas* from the gut to the liver and spleen occurred before or after death of the host. Wenyon (1920) has recorded the finding of *Trichomonas hominis* deeply embedded in the mucous membrane of the gut wall in sections: and several workers have suggested that occasionally *Mastigophora* parasitic in the intestine may gain access to the abdominal viscera through either an abraded or even an intact mucous membrane. If this be possible might not the herpetomonad form of *L. donovani,* if present in the stomach, possibly and occasionally gain access to the abdominal viscera ?

Details of the feeding experiments with frogs are shewn in Table VI. B. It will be seen that, in general, the flagellates are very rapidly destroyed in the gut, even more rapidly than are the mammalian R. B. Cs. which are present with them in the water of condensation of the cultures. A few actively motile *Leishmania* flagellates may, however, survive in the gut up to 48 hours at room temperature : and up to 3 days if the frog be kept at 22°C. This finding agan confirms the conclusion given above that the flagellate of *L. donovani* will survive for some time in the presence of a mild grade or sepsis : or, at least, in the septic environment of the frog's intestine. By way of controls the frogs utilised for class purposes may again be quoted : as the study of the intestinal protozoa of healthy frogs is frequently set in the practical classes. Of the natural intestinal *Mastigophora* of frogs *Trichomastix* and *Trichomonas* appear to be the most common: whilst a *Bodo* and an *Octomitus* are occasionally to be encountered. No herpetomonad has ever been encountered, and the forms met with in these frogs up to the 3rd day after feeding were undoubtedly the flagellate *L. donovani* originally ingested. *Copromonas* we have not yet encountered in the fresh intestinal contents of frogs.

To summarise our experimental findings in frogs we may state that the flagellate form of *L. donovani* when ingested by frogs may survive in scanty numbers in the gut for 2 to 3 days : but the majority of ingested parasites are destroyed : and there is no evidence of visceralisation. In the anterior lymph sac the majority of injected flagellates are rapidly destroyed by phagocytosis at the site of injection. Occasionally the infection may visceralise : but only as a transient phenomenon. There is little evidence that the frog, at least, among cold-blooded animals is more susceptible to infection than are warm-blooded hosts.

It is, indeed, easy enough to postulate "reservoirs" of the protozoa responsible for human diseases in animals and other hosts outside man. Yet such hypotheses require the fullest confirmation before they can be accepted. Taken as a whole, the human protozoa shew a great specificity for their human hosts. Thus apart from man *Entamœba histolytica* occurs as a parasite only in the experimentally infected kitten : the malarial parasites of man can be made to yield only transient and symptomless infections in the higher apes : whilst the questions of the identity or otherwise of *T. rhodesiense* and *T. brucei* and of the identity or otherwise of *Balantidium coli* of man with the similar species found in the pig must be regarded as still unsettled.

Leishmania-donovani, as we know it to-day, is essentially a parasite of man : and in its herpetomonad form at least both warm-blooded and cold-blooded animals appear to be singularly insusceptible to experimental infection. Whether man, however, is as insusceptible to infecton with the herpetomonad phase of the parasite is a matter open to question.

VII. *The "Thick Tail" Phase.*

During 1922 the "thick tail" phase of *L.* donovani, originally described by Cornwall (1916), was seen in two sets of bed bugs fed upon flagellate cultures : once in six bugs fed three days previously : also in a second series

of bugs fed from 5 to 9 days previously (*vide* Table VIII (A): experiments of 16th and 29th June). No subsequent encysted forms were however seen. In the fresh dissections annular forms were also seen, standing out prominently as translucent, thick rings in the dissection among thick tails and normal flagellates.

Not only this, but we have twice in 1922 encountered similar thick tail forms in *Herpetomonas muscæ domesticæ*. On the first occasion the fresh dissection of a very heavily infected midgut shewed at first thousands of natural herpetomonads: but an hour later hundreds of thick tails were present among them. In the second instance the infection was less heavy and thick tails only appeared about 2½ to 3 hours after the dissection had been put up. Plate C figures some of the thick tails found in a Schaudinn-fixed Iron-hæmatoxylin stained film taken four hours after dissection.

The evidence all goes to shew that the "thick tail" of *L. donovani* is merely a phase of degeneration and not some part of the extra-human cycle. In the above two cases where it was met with in *H. muscæ domesticæ* N. N. N. cultures were taken. No thick tails or cysts were seen in the cultures which subsequently shewed only normal flagellates. It should be noted that none of the preparations mentioned were vaselined: and perhaps partial desiccation may be of importance in the formation of the thick tail. The appearances seen are strongly reminiscent of the dying phases of *Trichomonas hominis* in a stale stool, so well described by Dobell and O'Connor (1921, p. 69), nd mistaken by Castellani (1905) for "Entamœba undulans." The only importance of the thick tail phase is that, as with pseudo-amœboid and dying *Trichomonas*, the appearances shew that the cell envelope of *L. donovani* is thin, and easily distorted or distended.

VIII. *Which is the infective phase of the parasite?*

If *L. donovani* be a herpetomonad then its life cycle should consist of (*a*) pre-flagellate, —the *Leishmania* form found in man, (*b*) flagellate or herpetomonad: and (*c*) post-flagellate forms. The case for a post-flagellate form, if we exclude Row's bodies, rests chiefly on Patton's historical work of 1912, and figures 67 to 73 of his Plate. The forms therein depicted may possibly be pre-flagellates which have passed through the gut unchanged: yet Patton emphasises the facts that they are larger than the pre-flagellates, and that all stages from flagellate to post-flagellate were seen.

The *Leishmania* form is far more infective to experimental animals than is the flagellate form: and, in this connection, we would like to draw attention to the existence of transient leishmaniasis in experimental animals. Table VII records the results in experimental animals in 1921 and 1922 of experiments in which spleen juice or post-mortem spleen emulsion was administered (*a*) by injection: and (*b*) by ingestion. Out of 77 animals injected with spleen juice only seven shewed positive results:—Pups Nos. 2 and 10: monkeys Nos. 1, 2, 16 and 21: and white mouse No. 7—all injected intraperitoneally. And in no less than 5 out of these 7 positive animals films from liver puncture, or in the case of the white mouse, from the spleen and liver on post mortem failed to shew parasites on very careful search: yet yielded rich cultures of flagellates on N. N. N. medium.

Pup No. 10 shewed the virus present on culturing liver puncture fluid on the 51st day after injection. No parasites could be detected in liver puncture films or in the peripheral blood. Sub-lethal doses of the venom of *Echis carinata* were now given. It was hoped that the cytolytic action of the venom would cause desquamation into the circulation of capillary endothelial cells containing *L. donovani*, and that parasites would be found in peripheral blood films. Both attempts, however, failed: 1½ months later the infection had cleared. The pup died of pneumonia 6½ months after injection: films and cultures from the viscera shewed no *Leishmania*.

Monkey No. 16 shewed similar findings: positive liver puncture cultures but negative findings in liver puncture films on the 50th day after injection. It became very ill with what was apparently beriberi three months after injection: and was chloroformed. There was no post-mortem evidence of infection, even on cultural test.

Monkey No. 21 was inoculated intraperitoneally on the 27th November. On 28th December, 16th January, 1923, and 23rd February cultures from liver puncture fluid gave richly positive growths of flagellates, but no parasites could be detected in the films. Another monkey used in connection with work on epidemic dropsy died of dysentery. Examination of the fresh colon contents shewed no protozoa present other than a *Trichomastix*. Cultures yielded an organism resembling the Flexner-Y bacilli of human dysentery. An emulsion of this isolated organism in pure culture was injected per rectum into the colon of Monkey No. 21: but the animal unfortunately has never developed dysentery. It remains today in good health and free from all symptoms, although it has a symptomless *Leishmania* infection.

We have indeed found it far more difficult to induce experimental kala-azar in animals in Calcutta than have the Shillong workers. Whether this is due to differences in climate or not, we do not know. The Shillong workers appear to find it relatively easy to establish

PLATE A.

Dermal Leishmanoid Monkey. Shewing the nodules on the eyebrows. Both were full of *L. donovani*. (The scalp is being retracted by the hand of an assistant in order to make the nodules more prominent). Reproduced by kind permission of the Editor, *Indian Journal of Medical Research.*

PLATE B.

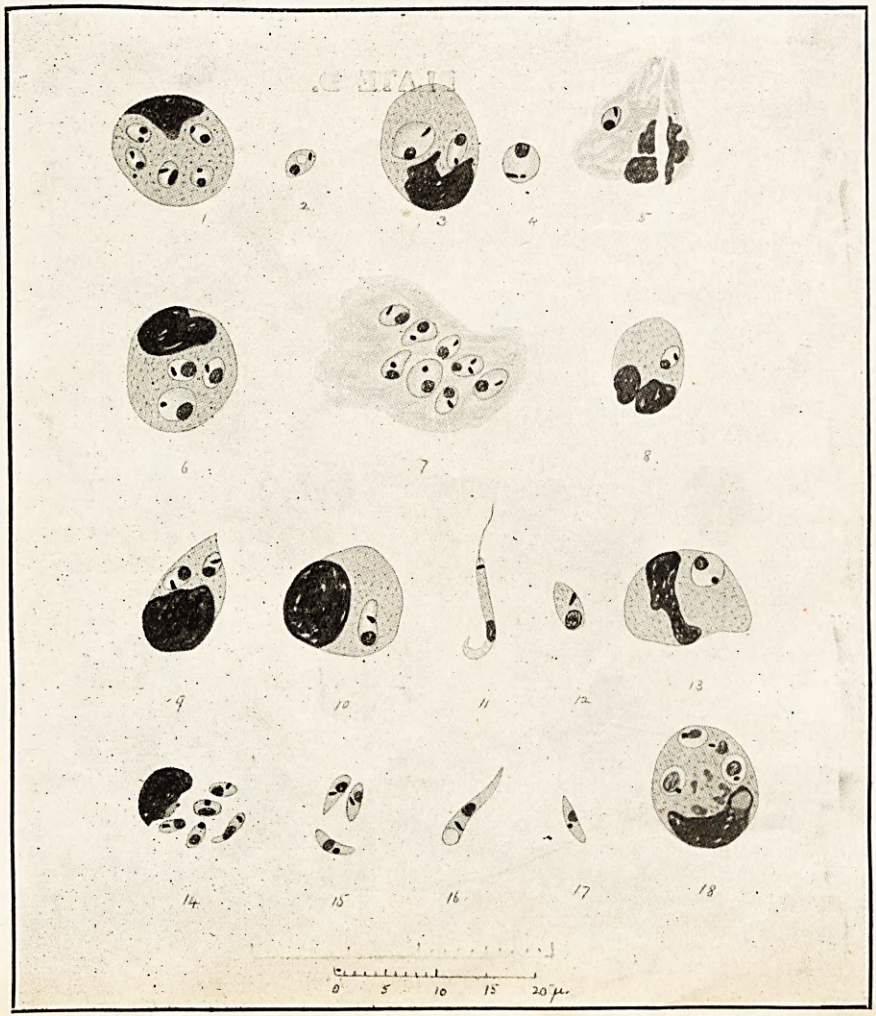
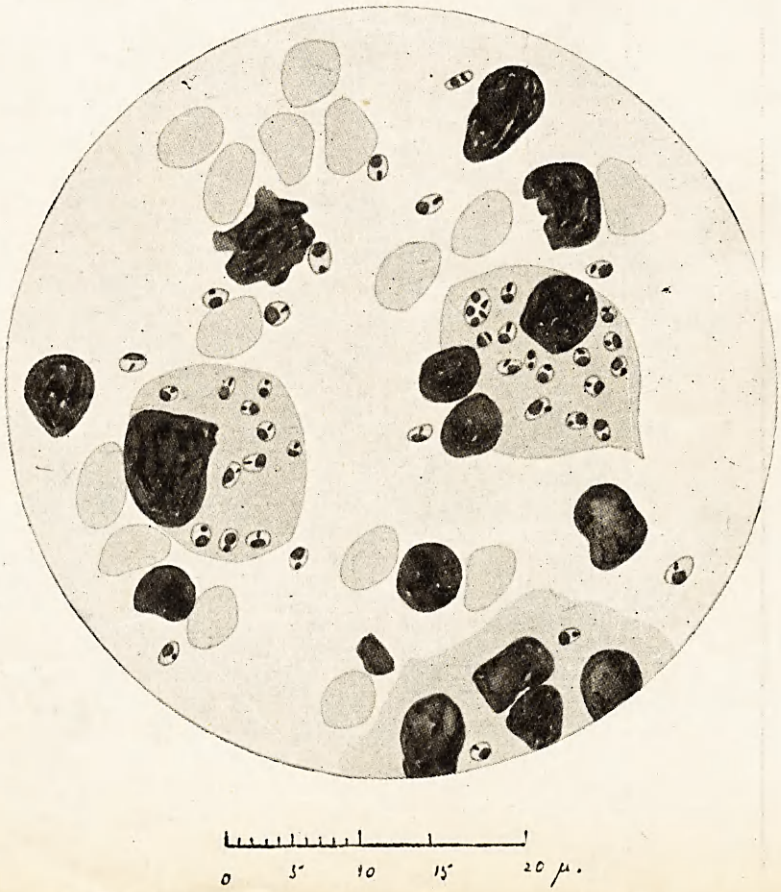


PLATE C.

Thick-tail phases of Herpetomonas muscæ domesticæ. Schaudinn fixation, Hædenheim's iron-hæmatoxylin staining.

PLATE D.



kala-azar in experimental animals. We have found it extremely difficult. What we do get, when a positive result occurs, is not kala-azar but a transient symptomless *Leishmania* infection, the existence of which can only be demonstrated by culture of liver puncture fluid on N. N. N. medium, or by the chance finding of very scanty parasites in smears from the organs.

A few attempts were made to transmit the infection from an infected monkey to an uninfected one. Details of these experiments are given in Table VIII. It will be seen that they all gave negative results, but their negative value is considerably reduced by the fact that we have found it extremely difficult to experimentally infect animals, even when large quantities of infected material are injected into them.

The few experiments with spleen juice on frogs and cold-blooded animals are shewn as items Nos. 3, 10, 11 (A) : and 9 (B), of Table VI. The results were completely negative.

IX. *The Need for an Animal Strain of the Virus.*

Our animal experiments in 1922 were directed especially towards one end : to try and secure a "fixed kala-azar animal virus" for experimental purposes.

It is difficult to over-rate the value of the human volunteer as an experimental laboratory animal. The final proof that malaria is transmitted from man to man by anopheline mosquitoes was only provided by experiments on human volunteers : as also was the infectivity of the blood in yellow fever. In connection with the problem of kala-azar transmission, experiments on the human volunteer are most urgently called for. After all, such experiment would be the crucial test, since man appears to be far more susceptible to infection than does any laboratory animal, and since the experimental infection, once acquired, could be cured by tartar emetic treatment.

Take the case of a set of bed bugs or other biting insects, which have been fed upon parasite-containing peripheral blood from kala-azar patients, some of which have shewn flagellate forms of *L. donovani* and which are now believed to be at the stage infective to man. How is the infectivity of such a batch of insects to be tested ? Eight or nine out of every ten experimental monkeys or dogs or other laboratory animals will fail to take infection with *L. donovani,* even after massive intraperitoneal injection. They will almost certainly fail to take the infection from infected insects shewing but scanty infection in their midgut. And here we may comment upon the only human experiment, which so far as we are aware, has been carried out in connection with the possibility of bed bug transmission. Mackie (1922) comments upon the intrepidity of Patton and Sundar Rao in allowing dozens of bed bugs, fed upon the blood of kala-azar patients and believed to be at the infective stage, to feed upon themselves. He asserts that this evidence alone is evidence strongly against bed bug transmission. We consider that such a reading of the experiment completely misinterprets the case. These "intrepid investigators" were so certain that *salivary* transmission does not occur in the bed bug that they allowed dozens of bugs, believed to be at the infective stage, to feed upon them, with completely negative results. They did not, so far as we know, conduct what they clearly would have regarded as the crucial but dangerous experiment : they did not crush such infective bugs upon abrasions on their skins, nor contaminate such abrasions with the fæces of infected bugs.

It is true that Patton (1922) has shewn that the virus of kala-azar will survive in the midgut of the infected bed bug for upwards of 41 days, as tested by culture of the contents of the gut on N. N. N. medium : but, as pointed out by Wenyon (1923) the parasites of rat trypanosomiasis, and *H. muscæ domesticæ* will also survive for long periods of time in the midgut of the bed bug : yet the bed bug has certainly no connection with the transmission of these infections.

In short, how is the infectivity of any insect possibly concerned to be tested, otherwise than by experiments upon the human and susceptible volunteer ?

If experiment upon the human volunteer be out of the question, at least upon any large scale, what alternative remains ? In our opinion, only one : to establish, if possible, a specialised and "fixed kala-azar virus" for some ordinary laboratory animal. Just as Pasteur once and for all time "fixed" the rabies virus for the rabbit, so, if the kala-azar virus could once be "fixed" in maximal, exalted, virulence for a given species of laboratory animal, it would then be possible, with regard to experimental laboratory work in the first instance at least, to altogether disregard man : to have at hand a standard virus with which to work : an animal readily susceptible to infection, and one suitable for an experimental study of the transmission of the disease.

It was to this end in particular that we directed our attention in 1921-22. Our results have, unfortunately, been almost entirely negative. Yet one strongly positive result remains to be recorded.

X. *Can Kala-azar be acquired by Ingestion?*

As we have shewn there are grounds for believing that the virus of kala-azar leaves the infected human host via his peripheral blood : *i.e.,* via some blood-sucking insect. Yet does it necessarily follow that man acquires the infection via the bite of such an insect ?

Table VII B shews the results in 1922 of feeding experimental animals with either

spleen juice, as obtained by spleen puncture of untreated patients or with post-mortem spleen emulsion. Of 20 such animals experimented upon, only one, Monkey No. 24, *M. rhesus,* yielded a positive result. This monkey on the 22nd May, 1922, was made to chew and swallow two large pieces of spleen, full of *L. donovani,* removed from an almost untreated kala-azar patient within a few hours of death. The meal was washed down by administering 30 c.c. of a thick emulsion of the same spleen by a stomach tube. The spleen was kept overnight on ice, and next day similar feeds and 30 c.c. by the stomach tube again administered. Cultures from the spleen on both dates gave a rich growth of flagellate culture. The monkey was marked by tattooing with Indian ink over the sternum between the two nipples for purposes of identification. During the course of routine examinations this monkey was liver punctured on the 11th July and 1st August subsequently.

Both films and cultures of its liver juice gave negative results. On the 25th November, seven months after feeding, this monkey—as identified by the tattoo marks—was brought into the laboratory in a dying condition by the animal attendant. It was extremely emaciated, had acute dysentery and was obviously dying. It was at once chloroformed. The post mortem revealed an extraordinary state of affairs. In the fresh mucus from the colon no protozoa were found other than a *Trichomastix,* which had probably nothing to do with the dysentery. Leishman-stained slides of this mucus shewed nothing suggestive of *Leishmania* parasites. (Being a Saturday afternoon the media rooms, etc., were closed and cultural and other examinations as to the cause of the dysentery were impossible.) There was no subcutaneous fat, and the animal was very emaciated. The spleen was at least four times the size of the spleen of a normal *M. rhesus* of similar size, and hard and fibrotic. Films from the spleen and liver shewed *L. donovani* in immense numbers: in fact in almost larger numbers than in any human infection with kala-azar. Plate D is from the splenic films of this monkey and shews the appearances in a single microscopic field. Innumerable parasites were also encountered in films from the bone marrow and heart blood, but none in films from the lungs. Cultures on N. N. N. from the internal viscera gave a rich growth of flagellates.

In brief, this monkey died from true, fulminant, overwhelming kala-azar, very similar to the acute form of the disease as met with in man, and accompanied by a terminal and fatal dysentery. The condition was entirely different from that of "transient and symptomless leishmaniasis" recorded above. One further fact, however, remains to be recorded: in giving the feeds and passing the stomach tube, several abrasions were caused on the monkey's lips and gums : and we cannot be certain whether the infection was acquired in reality via the gastric mucosa or via the abraded oral mucosa. On the other hand the extreme difficulty encountered in infecting animals via the skin by subcutaneous injections is evidence that the infection was here acquired by ingestion rather than by accidental contamination of abraded oral mucuous membrane.

As the post mortem was held late on a Saturday afternoon, it was difficult to conduct further experimental passages. N. N. N. media, which happened, luckily, to be at hand, was inoculated and gave a rich growth of flagellates. Such animals as were immediately available were collected, and the intraperitoneal passages shewn in Table VII (A) as Nos. 19, 20, and 21 made. Of these the first two unfortunately died from sepsis : (the media room was closed, and it was impossible to autoclave pestles and mortars). No. 21 shewed a subsequent septic sinus at the site of inoculation, but subsequently recovered, and gave positive liver puncture cultures on 28th December, January 16th, 23rd February and March 31st. An oral passage was attempted, (Table VII, B, No. 26) ; and this monkey subsequently shewed severe anæmia : but both liver puncture during life and complete post-mortem examination after it was chloroformed when obviously ill failed to shew infection.

Monkey No. 24 raises again the question of the possibility that kala-azar may be acquired by oral infection. Archibald (1914) has recorded experimental infection of monkeys via the oral route : whilst the case of Cornwall's white rat (1916, p. 706), which shewed transient *Leishmania* infection six weeks after feeding it on spleen juice, but was later still completely negative on post-mortem examination, again emphasise this possibility.

XI. *The Suspects.*

We have shewn that there is some *primâ facie* evidence that kala-azar is transmitted from man to man via some blood-sucking, localised and domestic insect. If so, which insect, in particular, is likely to be concerned ?

I & II. *Pediculi and Acari.*—These, we believe, can be excluded. Kala-azar in Calcutta is prevalent among the very cleanly Bengalis and the Anglo-Indian community. Neither are infested with these insects to any marked degree.

III. *Stegomyia.*—The localised and household distribution of the disease appear to exclude a flying host with a wide range of distribution.

IV. *Cimex hemiptera.*—The case for this much over-rated insect has been discussed ad nauseam during the past 20 years. We have no desire to re-open the discussion with reference to it. In our opinion two further pieces

of work remain to be carried out to determine whether the bed bug does or does not transmit kala-azar. The first is serial section cutting of bugs believed to be at the infective stage. This has been undertaken by Mr. C. M. Hutchinson, C.I.E., of Pusa, and may be safely left to his experienced hands and to his masterly protozoological technique. The second is some crucial experiment upon human volunteers who have had no possible association whatever with the disease, and this awaits the human volunteers concerned. If the bed bug transmits kala-azar, this statement is surely capable of proof. Hitherto we do not consider that proof has yet been furnished.

V. *Conorhinus.*—If, as we are informed, the Shillong workers have succeeded in infecting Conorhinus with *L. donovani*, and have found that the parasite passes in the midgut of *C. rubrofasciata* into its herpetomonad phase, then the case for this insect must be re-examined. A bite from an adult *Conorhinus* would be an event which could hardly pass unnoticed by a patient, and of which we can obtain no evidence on questioning patients. Yet, in its larval and nymphal stages, *Conorhinus* has all the habits and activities of a bed bug : it is a pure blood feeder, and its midgut might be suitable for the development of the *L. donovani* flagellate. *Conorhinus* is frequently met with in Calcutta and in Assam. Awati (1922) has noted that it is apparently absent from the district of Jorhat in Assam, a district which presents the peculiarity of being apparently absolutely free from kala-azar, although surrounded by other and infected districts.

In Table VIII (A) and (B) will be found the details of the experimental work during 1922 on *C. hemiptera* and *C. rubrofasciata*: these experiments call for little comment, as all results were negative.

VI. *Sandflies.*—The work of the Sergent brothers and their colleagues (1921) shews that oriental sore is almost certainly transmitted by *Phlebotomi*: and, although the exact cycle of development in the insect, and its exact mechanism of transmission remain to be worked out, the case for the sandfly in this connection may be taken as almost proved. We admit that argument from analogy is a dangerous proceeding, as every barrister will agree. Yet in Africa we have two human trypanosomes, very similar in morphology, yet causing sleeping sickness of two different clinical types, and transmitted by two different species of *Glossina*. It is tempting to infer that in India we have two *Leishmania* parasites, very similar in morphology, responsible for two different human diseases, the one transmitted by *P. papatasii*, the other possibly transmitted by some other species of *Phlebotomus*.

It will be recalled that Mackie (1915) found *P. minutus* in Assam, and found that some ten per cent of these insects were infected with a herpetomonad : but whether a natural herpetomonad of their own, or one which is identical with *L. donovani* remains open to doubt.

We have consulted Dr. N. Annandale, C.I.E., and Major J. A. Sinton, V.C., I.M.S., with regard to the distribution of the Indian species of *Phlebotomi*, and are very much indebted to both, and especially to Major Sinton, for full and very detailed information on this matter. Both *P. papatasii* and *P. minutus* appear to be almost universal in India, and to occur in all Provinces. Of the sandflies of Assam but little appears to be known. One species alone, *P. argentipes*, appears to have a distribution in India somewhat similar to that of kala-azar. It is a very common sandfly of Calcutta and Bengal : and has also been reported from Pusa and from Travancore : but not from elsewhere in India. This, however, may be simply from our want of knowledge on the subject.

The case for the sandfly at least deserves investigation. Unfortunately the insect is one of the most difficult to work with experimentally, and to rear and keep in the laboratory.

VII. *Fleas.*—The Mediterranean workers have always emphasised the possible rôle of fleas as vectors of kala-azar: but, despite the large volume of work upon these insects, the case for them cannot be taken as proved. Fleas are rather scanty in Bengal, as compared with the Western side of India: and, unless there be some special species prevalent on this side of India, as compared with Western India, there does not seem much evidence to incriminate any species of flea.

XII. *The Environment of kala-azar.*
Kala-azar in Calcutta City.

The conditions under which kala-azar develops in Assam, both in tea garden coolie lines and in jungly villages, are so well known that they need not be re-described. Kala-azar, however, is, if anything, even more common in non-epidemic form in Bengal, and quite prevalent in Calcutta itself. Any solution of the transmission problem, therefore, must be one which is equally applicable to the tea garden coolie lines in Assam, to the rural villages of Assam and Bengal, and to the Anglo-Indian quarter of Calcutta City. It may therefore be not out of place to consider conditions in the latter. In order to ascertain the facts with regard to Calcutta City the case sheets of nearly 1,000 patients who had been treated for kala-azar at the Out-patient clinic at the School (L. E. N.) during the last 18 months were examined. From them cases were selected where all details of the patient's residence and movements for at least six months or more prior to acquiring the disease were

fully known and recorded. The diagnosis of kala-azar in all these 478 cases were confirmed, in almost every instance, by spleen puncture or by peripheral blood findings, and in a few only, where spleen puncture was contra-indicated, by a strongly positive aldehyde test.

These 478 cases fall into two groups:—(*a*) Cases of kala-azar amongst temporary residents in Calcutta, *i.e.*, 238 persons who had contracted kala-azar outside Calcutta, and who had come to the city for treatment, and who had resided in Calcutta for from one week to three months before coming under observation. The distribution of these cases is shewn upon the spot map, Plate E I (*b*). Cases of kala-azar among permanent residents of Calcutta City, who had never resided out of Calcutta within six months or more of the onset of the first symptoms of the disease—240 cases. The distribution of these cases is shewn upon the spot map, Plate E II.

A comparison of maps E I and E II at once shews many features of interest. Wards 15, 16 and 17, which are the European wards, shew very little kala-azar; as also do wards 5 and 7, which are chiefly business and not residential areas. With these exceptions the temporarily resident cases, map E I, are fairly evenly distributed all over the city. In fact they live wherever they can secure accommodation. There is, however, a tendency for distance from the Hospital to affect the number of cases in any given ward, the largest number coming from ward 9, the ward adjacent to the one in which the Hospital and School are situated. This corresponds exactly with what might have been anticipated.

Turning to map E II, however, and to the distribution of kala-azar among permanent residents of Calcutta, matters are at once seen to be strikingly different. The effect of proximity to the Hospital largely disappears, although there is a fairly large number of cases in Wards 8 and 9. The cases from the north end of the city,—(Division I),—have dwindled from 71,—or 30 per cent. of the 238 imported cases,—to 15,—or only 6 per cent. of the 240 indigenous cases ; and there is a very marked density of cases in ward 14 and the adjacent wards.

On maps E I and E II are marked out for comparison two special areas—area A in North Calcutta, which includes Wards 1, 2, and 3 ; and area B, near the centre of the city, which consists of Ward 14 and adjacent portions of Wards 13, 15, 19 and 20. The actual boundaries of area B are Dhurrumtollah to the north, Free School Street to the west ; Royd Street, Elliot Road, Bijli Road and Beniapara Lane to the south ; and S. Road, Entally and Linton Street to the east. The square area and number of population in these two areas are much the same, although both are slightly greater in area A. The number of *imported* cases of kala-azar in each area is also about equal, 39 and 37 cases respectively.

Turning to the *indigenous* cases of kala-azar in Calcutta City, it is found that no less than 121, or more than half the 240 indigenous cases come from area B. Area A only shews two indigenous cases. The immediate conclusion in the face of these figures is that area B is the endemic focus of kala-azar in Calcutta; but that area A—although repeatedly exposed to fresh infection from without—remains relatively free from infection. It is necessary, however, to enquire into factors other than environment which might possibly influence these figures. The composition of the population in areas A and B differs very widely; in area A the inhabitants are mostly Hindus—including a considerable number of the more wealthy Hindus; in area B the population is a mixed one of Anglo-Indians, Indian Christians, and the poorer class of Hindus and Mahommedans.

The possible factors which have to be considered are :—

(*a*) The proximity of the hospital to certain districts or its easy accessibility from others.

(*b*) The presence of other hospitals, which might withdraw the patients of any one area.

(*c*) The prejudice of the class of people who predominate in any area against coming to hospital.

(*d*) A comparatively marked tendency to attend hospital on the part of any class who have a limited distribution in the city.

(*e*) The marked susceptibility of any class or classes to the disease on account of their habits of life.

(*f*) The particular racial susceptibility of any one class.

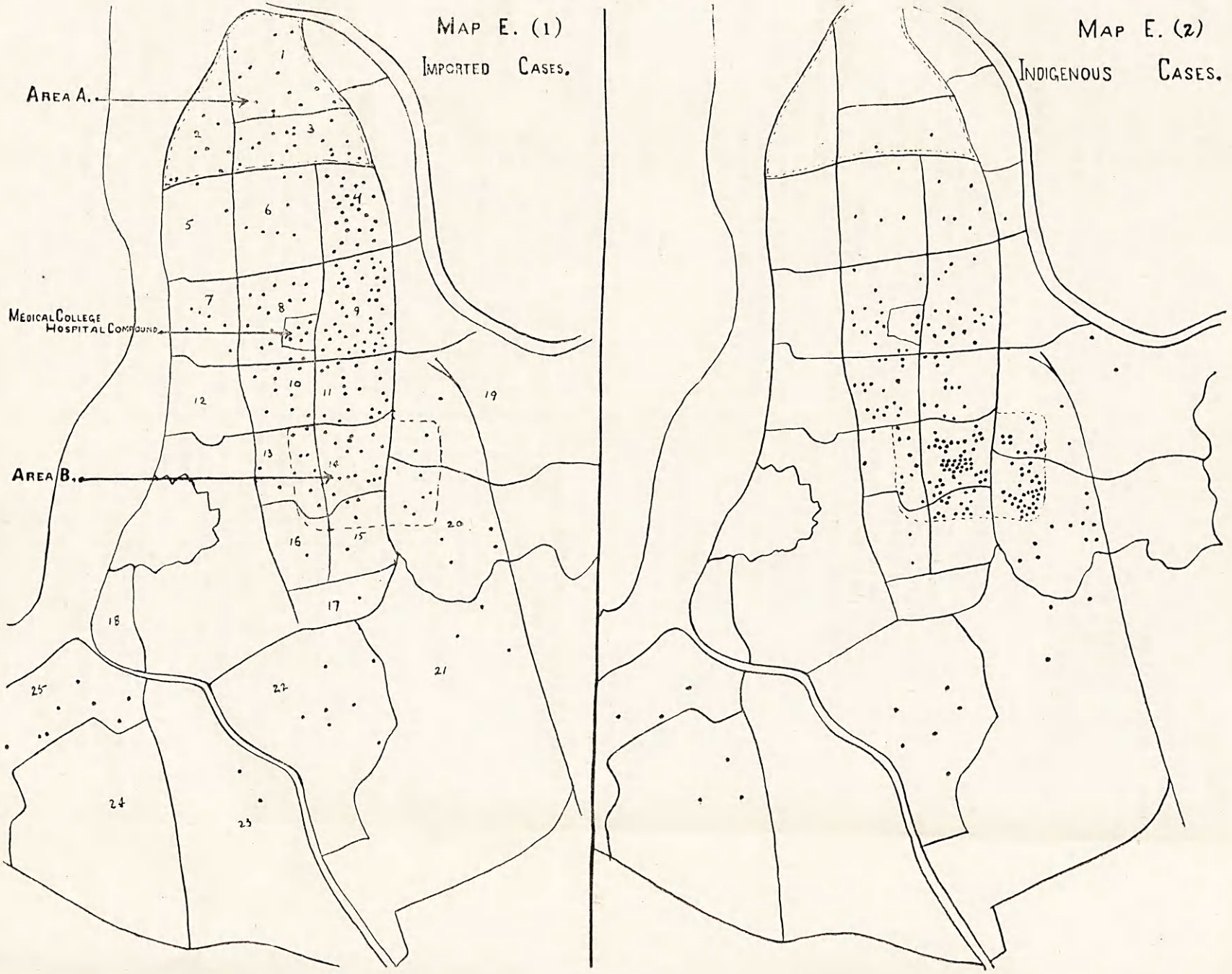
(*a*), (*b*) and (*c*) can be considered together. If these factors affected the figures, map E I should not shew the evenly distributed incidence which occurs in the imported cases.

Factor (*d*), although an apparent corollary to (*c*), cannot be dismissed so easily. The Anglo-Indian community is, of all others, the one which comes to hospital most readily for treatment. They constitute about 2 per cent. of the general population according to the census returns, but number 7 per cent. of the general medical out-patients at shewn on the rosters at the Medical College Hospital. In Ward 14, the centre of the "heavily infected area B," Anglo-Indians form 7.5 per cent. of the population. It might therefore be suggested that indigenous kala-azar is equally prevalent throughout Calcutta City, but that as Anglo-Indians are so numerous in Ward 14 and attend hospital so readily, this brings about the density of cases shewn in area B. The statistics for Ward 10 however dispose of this contention. In Ward 10 Anglo-Indians number 13.4 per cent. of the population, yet

THE KALA-AZAR TRANSMISSION PROBLEM.

By Major R KNOWLES, I.M.S., L. E. NAPIER. M.R.C.S., L.R.C.P., and Sub-Assistant Surgeon B. M. DAS GUPTA.

PLATE E.



Ward 10 does not shew the peculiarly heavy incidence shewn by Ward 14. Factor (*d*) is therefore not of much importance in determining the concentration of cases in area B. Indian Christians are also a class who are always ready to come to hospital, but are much more widely scattered throughout the city. If the Anglo-Indian and Indian Christian cases be removed from map E II, a very marked concentration of cases is still apparent in area B.

Factors (*e*) and (*f*) may be considered together. From the figures available it is easy to calculate the percentage of cases in Ward 14 which should be Anglo-Indians, if the incidence of the disease was equal in all communities in the ward. As follows:—In general medical out-patients at the Medical College Indians are to Anglo-Indians as 93 : 7. But examination of the registers shews that some 50 per cent. of these out-patients come from outside Calcutta City, and practically none of them are Anglo-Indians. The true proportion amongst general out-patients who are permanent residents in Calcutta City is therefore:—

	Indians.	Anglo-Indians.
General out-patients from Calcutta residents.	43	7
Proportion in general Calcutta population.	98	2
Proportion in population of Ward 14.	92·5	7·5

If the kala-azar incidence fell equally on the two communities in Ward 14, the proportion of cases attending the Hospital should be Indians : Anglo-Indians as $\frac{92{\cdot}5}{98} \times 43 ; \frac{7{\cdot}5}{2} \times 7$; or as 42.76 : 26.25 ; or as 67 per cent. to 33 per cent.; and, even after making all allowances for the large number of Anglo-Indians in Ward 14, this latter figure is still considerably below the actually recorded figure of 52 per cent. The figures for the whole of Calcutta (Napier 1922), demonstrate the same thing. Anglo-Indians constitute 13.7 per cent. of all kala-azar cases seen as against only 7 per cent. of general medical out-patients at the Medical College Hospital; and 25 per cent. of the indigenous kala-azar cases as against an estimated 14 per cent. of general medical out-patients who are permanent residents of Calcutta. Clearly there is an increased tendency among the Anglo-Indian community to contract the disease.

If so, then why ? Here is a fact whose close investigation may throw some light upon the transmission of the disease. Is this predominance among Anglo-Indians due to (*a*) racial susceptibility ; or (*b*) to their habits of life ? If (*a*) racial susceptibility were the cause one would expect to find that susceptibility even more marked among Europeans. This is not the case ; the disease is extremely rare amongst Europeans in Calcutta. On the other hand it shews a relative predominance also among the Indian Christians, whose habits of life are very similar to those of the poorer class of Anglo-Indians; (*b*) we do not propose to discuss until further information has been acquired; but in general it is interesting to note that area B and Ward 14—especially the heavily infected area just east of the Wellesley Street tank—correspond far more to rural conditions than do area A and northern Calcutta. In area A there has been much rebuilding, the streets are very narrow, the population is congested, and overcrowding general. There are few open spaces. In area B there has been little re-building, there are several open spaces, the prevailing type of house is the old fashioned but rather ruined stone house with large rooms and deep verandahs. Vegetation abounds. The affected Anglo-Indian population consists largely of families who have come down in the world, who find it hard to make ends meet, but who stick to quantities of family furniture with which their rooms are lumbered. Also the rooms in area B are probably darker and the atmospheric humidity higher than in area A.

Maps E I and E II appear to us to present evidence against the bed bug theory of transmission. The houses in northern Calcutta are infested with bed bugs ; the infectious material, as shewn in map E I, is constantly being imported into the area, yet there is an almost complete absence of the disease among the permanent residents. It has been suggested that the invasion of Calcutta by the large numbers of patients who come to the city for treatment might lead to a serious extension of the disease among the residents. These two maps seem to us to demonstrate very forcibly that this has not yet occurred.

Conclusions.—From a study of maps E I and E II we may conclude:—(1) That the conditions requisite for the transmission of kala-azar from one patient to another are to be found at their best (or worst), as far as Calcutta City is concerned, in area B—*i.e.*, Ward 14 and parts of the surrounding wards ; that these conditions exist to a less extent in wards 4, 6, 8, 9, 10 and 11 : but that they are almost totally absent from area A, *i.e.* Wards 1, 2, and 3. (2) Also that there is a slight but quite definite relative preponderance of the disease in Calcutta residents among the poorer Anglo-Indian and Indian Christian communities. If we can but secure the co-operation of the entomologists, which we so badly need, we now propose to carry out an entomological survey of area B—utilising area A as control ; and to commence general investigations into the living conditions of the populations of the two districts.

XIII. *Summary and Review.*

(1) We consider that the systematic position of the parasite of Indian kala-azar is so peculiar that it should be retained in the genus

Leishmania, Ross (1903), rather than in the genus *Herpetomonas.*

(2) *L. donovani* is primarily a parasite of man and is carried from man to man. There is considerable evidence to negative the view that *L. donovani* is primarily a herpetomonad of some insect, and that the infection of man is merely accidental : a hypothesis which would mean that kala-azar is not transmitted from man to man at all.

(3) Secondary factors, such as enteric fever, relapsing malaria and dysentery appear to play some part in determining the onset of kala-azar in some cases : and the question is raised as to whether symptomless infection of man with *L. donovani* may not exist, and whether it may not indeed be widespread in the endemic areas.

(4) We believe that the bulk of the evidence available points to the elimination of the parasite from the infected person via his peripheral blood stream, *i.e.,* via some blood-sucking insect.

(5) This conclusion is emphasised by the laboratory findings in connection with the new form of infection with *L. donovani* first described by Brahmachari as " dermal leishmanoid."

(6) Although *L. donovani* will only pass into its herpetomonad form under certain well-defined environmental conditions, yet when once formed, the herpetomonad form is somewhat resistant. It will, to some extent, withstand body temperature, a mild grade of sepsis, anærobiosis and a wide pH range of from 4.5 to 9.0. Conditions in the stomach of an individual with achlorhydria —a finding which is very common in Indians —might be such that the flagellate could survive for some hours.

(7) Warm-blooded animals being very difficult to infect experimentally with *L. donovani* flagellate cultures, the behaviour of the flagellates in cold-blooded animals, chiefly frogs, was studied. It was hoped that we might here find a type of animal readily susceptible to *Leishmania* infection. Despite some evidence of transient visceralisation after injection into the anterior lymph sac, our findings shew that the flagellates are rapidly destroyed when introduced into such animals, either by injection or by ingestion.

(8) The thick tail phase may occur with herpetomonads other than *L. donovani,* and is, we believe, a phase of degeneration of the flagellate, rather than any phase in the true life cycle.

(9) On injection of animals with kala-azar spleen emulsion there sets in not infrequently a transient and symptomless leishmaniasis. It can only be detected by culture of liver puncture fluid on N. N. N. medium.

(10) As matters at present stand there is no crucial test which can be applied to ascertain whether or not any batch of fed insects is really infective. Positive cultures on N. N. N. from the gut of such insects prove that the virus is still living in their gut, but this does not constitute proof of their infectivity to man. In the absence of human volunteers, who would constitute the most suitable experimental animals for such a test, it is desirable to try to establish a " kala-azar fixed virus " in some species of animal.

(11) Our attempts to do so have hitherto met with no success. In one instance however a *M. rhesus* appears to have contracted an overwhelming and fatal kala-azar infection by ingestion of spleen emulsion. Although we believe the peripheral blood to be the channel of elimination of the parasite, yet the possibility of infection by ingestion deserves consideration.

(12) The case for *Conorhinus* and for *Phlebotomus* as possible vectors deserves further study.

(13) Kala-azar being very widespread in Bengal, and very prevalent in Calcutta City, especially among the Anglo-Indian community and in Ward 14 and " Area B," any solution of the transmission problem must be one which will fit in with the conditions met with in the tea garden coolie lines in Assam, the rural villages of the endemic areas in Assam and Bengal, and in Calcutta City alike.

Generally speaking, those with most experience of kala-azar are in favour of one of two theories of transmission : one group of workers insist that kala-azar is spread by some blood-sucking insect: the Assam medical men however emphasise its close association with bad conservancy or with no conservancy at all, and are in favour of infection by ingestion. Is there any theory which will unite both points of view ? Is it possible that the parasite of kala-azar is picked up from the peripheral blood of infected persons by some blood-sucking insect whose gut happens, at the moment, to be sterile: that in its midgut *L. donovani* flagellates : and that the dead—or more probably the living—body of this insect now comes to contaminate water or food supplies : is ingested by an individual whose stomach is in a condition of achlorhydria : that, upon digestion of the insect, *L. donovani* flagellates are set free and survive for a sufficiently long period to enter the lymph stream and so disseminate into the viscera ? We do not desire in any way to lay emphasis on such a speculation, but it is one which might unite the opposing schools of thought. Further, kala-azar may be acquired perhaps, rather by repeated exposure to infection, than by a single inoculation or feed.

It is deplorable that the financial circumstances of India to-day render it impossible to set on foot a well organised kala-azar commission under unified direction. For almost

twenty years different laboratories in India have "pecked" at the problem. A continuance of such a policy is likely to lead nowhere. The co-ordinated services of the entomologist, the protozoologist, the clinician and the statistician are called for. Three main lines of enquiry appear to be called for : in the field a close and continuous study of the biting insects associated with infected and non-infected areas and houses respectively: in the laboratory a study of the environmental conditions under which the flagellate form of *L. donovani* passes, if it pass at all, into its post-flagellate *Leishmania* phase: and the attempt to establish a "kala-azar fixed virus," an animal strain which will render one independent of random and casual human clinical material, and provide material for an intensive study of the problem.

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APPENDIX.

Tables of Experimental Data.

TABLE I.

General Analysis of Protozoal Findings in 721 stools from 530 patients in hospital, 1922.

Kala-azar cases = 210 patients : 265 stools. Non-Kala-azar cases = 320 patients : 456 stools.

	Kala-azar Cases.		Non-Kala-azar Cases		Combined Total.	
	Number.	Percentage.	Number.	Percentage	Number.	Percentage.
Character of stool.—						
Formed	42		76		118	
Semi-formed	102		143		245	
Semi-fluid	25		41		66	
Fluid	63		117		180	
Dysenteric	18		70		88	
Not recorded	15		9		24	
Protozoal Findings.—						
E. coli: vegetative	10		22		32	
encysted	21		32		53	
E. histolytica: vegetative	8		51*		59	
encysted	17		33		53	
E. nana: vegetative	26		43		69	
encysted	14		22		36	
I. butschlii: vegetative	3		2		5	
encysted	3		9		12	
Giardia: vegetative	3		7		10	
encysted	25		34		59	
Trichomonas: vegetative	18		24		42	
Chilomastix: vegetative	18		25		43	
encysted	5		6		11	
Enteromonas: vegetative	2		6		8	
encysted	0		1		1	
Cercomonas	0		4		4	
Prowazekia	0		1		1	
Embadomonas (?)	1		1		2	
Isospora hominis	1		1		2	
Other Findings of interest.—						
No protozoa found	140	52·9%	251	55·0%	391	54·2%
Numerous yeasts	152	57·3%	46	10·1%	198	27·5%
Blastocystis	70	26·4%	105	23·0%	175	24·3%
"Cystic bodies" (*vide* text)	13		0		13	
Charcot Leyden crystals	11		46*		57	
Spirochætes	20		22		42	

* NOTE.—A number of admitted cases of amœbic dysentery is responsible for these high figures.

TABLE II.

PERIPHERAL BLOOD EXAMINATIONS IN KALA-AZAR, 1922.

Patients examined, 140. Positive, 27; or 19.3 per cent.
Films examined, 442. Positive 54; or 12.2 per cent.

ANALYSIS OF PARASITE FINDINGS.

1	in polymorphonuclear	occurred 39 times	39 parasites
2	„ „	„ 8 „	16 „
1	in hyaline	occurred 13 times	13 „
2	„ „	once ..	2 „
5	„ „	„ ..	5 „
8	„ „	„ ..	8 „
15	„ „	„ ..	15 „
1	parasite	occurred free ..	1 „
Cluster of 3 parasites		free occurred once	3 „
		Total ..	102 parasites

102 parasites in 442 films; or an average of one parasite per 4.3 films.

PERIPHERAL BLOOD CULTURES IN KALA-AZAR, 1922.

Peripheral blood of 23 untreated case cultured : 19 were positive ; (of whom one failed to show parasites in spleen puncture films, and two gave negative aldehyde tests) : 4 were negative ; all only clinically like kala-azar (two of these were also spleen punctured with negative results in both spleen films and cultures).

Peripheral blood of 7 partially treated cases cultured: 6 were negative (of whom three still gave positive spleen puncture films, and one negative spleen puncture films, but positive spleen puncture cultures) : 1 was positive.

Peripheral blood of 11 fully treated cases (receiving 2 gms. or more of antimony salt) cultured, 10 were negative ; and 1 still positive. [The positive case was a resistant one (E. G.), who still gave positive spleen puncture films.]

Total number of cases cultured; 41.

TABLE III.

Kala-azar Work 1922. *Experiments on two Dermal Leishmanoid Cases.*

(A). Dr. Brahmachari's Case.

Date.	Experiments.	Results.
9th February ...	Films from old lesion.	Full of L. donovani.
	Films from new nodule.	Numerous L. donovani.
	N. N. N. cultures from nodule.	Rich growth of L. D. flagellates, 12th day.
	Four peripheral blood films searched.	No parasites seen.
2nd March ...	Films from nodule...	Scanty L. donovani found.
	Rabbit inoculated on scarified cornea.	Failed to take.
	N. N. N. culture from nodule.	Rich flagellate growth. 10th day.
	Cultures of peripheral blood.	Remained negative and sterile.
	Cultures of blood oozing from cut nodule.	Rich flagellate growth. 11th day.
	Four peripheral blood films.	No parasites seen.

(B). Dr. Bhattacharji's Case.

Date.	Experiments.	Results.
28th February	Films from ear nodule.	Numerous L. donovani.
	Six films of peripheral blood.	No parasites seen.
1st March ..	Films from ear nodule.	Numerous L. donovani.
	N. N. N. cultures from nodule.	Rich growth of flagellates, 11th day.
	Rabbit inoculated on scarified cornea.	Failed to take. Opacity only. No L. donovani found.
	Male M. rhesus inoculated by flap method into both eye brows.	Both took : together with a third auto-inoculated nodule near the canthus : incubation period 18 days. (*vide* below).
9th March ...	Films from an ear nodule.	No parasites seen.
11th May ..	Films from chest nodule.	Numerous L. donovani seen.
	Patient spleen punctured.	No parasites seen in films ; N. N. N. cultures remained negative.
	Culture from nodule	Went septic.

(C). Further Experiments with the above "Dermal Leishmanoid" monkey.

1st March ..	Monkey inoculated	One nodule in each eyebrow, with third auto-inoculated nodule at canthus. 18th day.
22nd April ...	Films from both eyebrow nodule.	Numerous L. donovani in both.
	N. N. N. culture from nodules.	Rich growth of flagellates on 13th day.
	Liver punctured ...	Films and cultures negative.
	Monkey's peripheral blood.	Films and cultures negative.
5th May ...	Liver punctured ...	Films and cultures negative.
	Films from eyebrow nodules.	Now fail to show leishmania.
	Emulsion from nodule made and given intra-peritoneally to adult female M. rhesus.	Liver puncture, films and cultures from passage monkey shewed no L. donovani ; 20th June, 11th July, 1st August and 28th December.
23rd June ...	Collection of starved bed bugs fed on nodules.	No development ; nothing found on dissection.
24th June ...	.....	Monkey escaped and was last seen going over the roofs of northern Calcutta.

TABLE IV.

L. D. Flagellates. Experiments in vitro.

Date.	Experiments.	Results.
3rd May 1922 ...	Very active flagellate L. D. culture mixed with thin fæcal emulsion. Two tubes left overnight :— (*a*) at 22°C (*b*) at 37°C.	Next day—All dead. Stained films shew dead flagellates, chromolysis, plasmolysis. Also? scanty rounded forms still sluggishly motile.
9th June 1922 ..	Very active flagellate L. D. +++ culture taken and mixed :— (*a*) Culture+aa 0.3%. Pepsin one hour at 22°C =	Flagellates still active.
	(*b*) + aa 0·2% HCl. One hour at 22°C =	Flagellates still active.
	(*c*) + aa 0.2% HCl. One hour at 37°C =	Flagellates still active : some rounding up; marked clumping—rosettes.
	(*d*) + aa 0.3% Pepsin one hour at 37°C =	Flagellates still active : majority however are rounding up, aflagellate, and Leishmania-like in type.
10th June 1922	Very active flagellate L. D. +++ culture taken and mixed :— aa :— (*a*) + citrated frog's blood. One hour at 22°C =	Still actively motile.
	(*b*) + citrated rabbit blood. One hour at 22°C=	Still actively motile.
	(*c*) + citrated human blood. One hour at 22°C =	Still actively motile.
	(*d*) + citrated frog's blood. One hour at 37°C =	Dead, immobile. No rounded forms.
	(*e*) + citrated rabbit blood. One hour at 37°C =	Ditto.
	(*f*) + citrated human blood. One hour at 37°C =	Completely altered flagellates. No motility. Rounded Leishmania forms.
14th June 1922	Very active flagellate L. D. +++ culture taken and mixed :— (*a*) + aa 0.5% Na_2CO_3 —an hour at 22°C =	Flagellates collected in dead, immobile clusters. Here and there a few still sluggishly motile.
	(*b*) + aa 0.5% Na_2CO_3—an hour at 37°C =	
19th June 1922	Actively motile flagellate L. D. ++ culture desiccated for 3 days over quicklime in freezing chamber at minus 2°C.	Dead and rounded up flagellates seen.

TABLE V.

L. D. Flagellates. Animals 1922.

A. Injections only.

Serial No.	Date.	Experiments.	Date.	Results.
1	17th June ...	Pigeon. Contents of three +++ cultures I.V.		Blood films ½ an hour later = No L. D. seen. Halteridium +.
2	26th June ...	White mouse. Hypodermically +++ culture.	Died 27th June.	Films = bacteria. No L. D. seen. Cultures not taken.
3	29th June ...	White mouse. Three +++ cultures hypodermically.	Killed 1½ hours.	Films from site of injection and viscera= bacteria +. No L. D. seen? ante mortem septicæmia. No cultures.
4	6th June ...	Pigeon. 1½ c.c. of +++ culture I.V.	2 films at once= 2 films at ½ hour= killed 1 hour.	No L. D. seen. No L, D. seen. Visceral and heart blood films and cultures negative.
5	12th June ...	Whitey grey mouse. Hypodermically ½ c.c. of +++ 13 days old culture with many rounded forms of Row.	Killed 16th Nov.	Films and cultures negative.
6	14th June ...	White mouse. Hypodermically +++ culture 15 days old with many Row's forms.	Died 1st Jan. 23.	Films negative. Bacteria only. Cultures not taken.

L. D. Flagellates. Animals. 1922.

B. Feeds only.

1	9th June ...	White rat. Fed on 2 +++ cultures by cap pipette.	Killed at 3¾ hours.	Stomach = few rounded blastocystis-like forms. Small intestine = R.B.Cs. +. No L. D. found.
2	9th June ..	White rat. Fed on 2 +++ cultures.	Not traceable 16 Jan. 23.	
3	13th June ...	White rat. Fed on 5 +++ cultures.	Killed at one hour.	Stomach = R. B. Cs. yeasts. No L. D. seen. Small intestine = R. B. Cs. Rounded blastocystis-like forms.
4	6th June ...	White mouse. Fed on +++ culture.	Killed at ½ hour.	Stomach contents—R.B. Cs.+. No L.D. seen. Visceral films = nil.
5	9th June ...	Starved white rat for 2 days, 1 c.c. of +++ culture injected into stomach by laparotomy, under ether anæsthesia.	Killed 2¼ hours .	Stomach contents and visceral films = negative.
6	13th June ...	Starved white rat. 1 c.c. of +++ culture injected into stomach by laparotomy.	Killed at one hour.	Films of stomach and duodenal contents and viscera =nil. Cultures of viscera= negative.
7	14th June ...	Starved white mouse. 2 c.c. of +++ culture injected into stomach by laparotomy.	Killed at ½ hour.	Films of stomach and duodenal contents and of viscera = nil. Cultures of viscera = negative.

TABLE VI.

Kala-azar Cold-Blooded Animal Experiments, 1922.

A. Injections.

Serial No.	Date.	Experiments.	Date.	Results.
1	26th May··	3 c.c. of +++ flagellate culture into anterior lymph sac of a frog.	Killed 3¾ hours ··	Films : site of injections, heart blood, liver = nil. Films of spleen = scanty flagellates+. Cultures of all = negative.
2	25th May··	2 c.c. of +++ flagellate culture into anterior lymph sac of a frog.	Killed 1¾ hours ··	Motile L. D. flagellates at site of injection : free flagellate and intracellular L. D. forms in liver and spleen films. Cultures not taken.
3	2nd June ··	Big frog. 2 c.c. of spleen puncture fluid. L. D. +++ into anterior lymph sac.	Pithed. 9th June··	Films and cultures of viscera = no L.D. (Hæmogregarine present).
4	5th June ··	Small frog. 2 c.c. of +++ flagellate culture into anterior lymph sac.	Died 6th June ··	Films of local site and viscera = no L. D. found.
5	12th June··	Frog. +++ culture into anterior lymph sac.	Killed 2nd day ··	Films of viscera = no L. D. seen.
6	19th June··	Frog. 3 c.c. of +++ culture into anterior lymph sac.	Died 20th June ··	Films of viscera = no L. D. seen.
.	0.h June··	Frog. 1½ c.c. of +++ culture into anterior lymph sac.	Killed ¾s of an hour	Injection site = motile L. D. flagellates + few rounded Leishmania forms. Films of spleen and bone marrow = nil. Liver films = scanty extracellular leishmania forms.
8	24th June··	Frog. 2 c.c. of +++ culture into anterior lymph sac. (Frog then kept at 22°C).	Killed 3rd day ··	Viscera films = no L. D. found.
9	7th August	Varamus flavescens. 5 c.c. of +++ culture I. P.	Killed 22nd August	Viscera films negative. (One suspicious form in spleen films). * *Vide* below.
10	28th October.	Frog. 5 c.c. of p m. k.-a. spleen emulsion I. P.	Killed 11th November.	Viscera : films and cultures negative. (Hæmogregarine present).
11	28th November.	Turtle. 5 c.c. of p.m. k.-a. spleen emulsion subcutaneously.	Died 4th December,	Viscera : films negative. (Trypanosome present).
12	22nd August.*	White rat injected intraperitoneally with triturated spleen of above Varamus of 7th August.	Died 16th January, 1923.	No Leishmania found : films and cultures. Trichomonas muris found in gut, spleen and liver.
13	15th February, 1923.	Big frog. 5 c c. of very active L. D. flagellate culture : anterior lymph sac.	Pithed. 40 mins.··	Few motile L. D. at site of injection. Viscera films = nil.
14	··	Frog. 2 c.c. of very active L.D. flagellate culture : anterior lymph sac	Pithed. 1¼ hours··	No L. D. seen : site of injection and films of viscera.
15	··	Frog. 2 c.c. of very active L.D. flagellate culture : anterior lymph sac.	Pithed. 30 mins. ··	Numerous flagellate L. D. at site of injection : many active : many rounded up. Viscera : no L. D. seen. (Hæmogregarine +).
16	··	Frog. 2 c.c. of very active L.D. flagellate culture : anterior lymph sac.	Pithed. 28½ hours .	No L. D. seen : site of injection and viscera.
17	17th February.	Frog. 5 c.c. of very active L.D. flagellate culture : anterior lymph sac.	Pithed. 90 mins. ··	No L. D. seen : site of injection and viscera.
18	··	Frog. 5 c c. of very active L.D. flagellate culture : anterior lymph sac.	Pithed. 2 hours ··	No L. D. seen : site of injection and viscera.
19	··	Frog. 5 c.c of very active L.D. flagellate culture : anterior lymph sac.	Pithed. 2½ hours··	No L. D. seen : site of injection and viscera. Doubtful ? Leishmania (intracellular) forms seen in spleen films.
20	22nd February.	(4 *very* active flagellate cultures used). Frog. 3¼ c.c. of very active L. D. flagellate culture : anterior lymph sac.	Pithed. 2 hours ··	No L. D. seen : site of injection and viscera.
21	··	Frog. 3½ c.c. of very active L. D. flagellate culture : anterior lymph sac.	Pithed. 2½ hours··	Scanty L. D. flagellates at site of injection : a few still motile. Viscera nil.

TABLE VI.

Kala-azar Cold-Blooded Animal Experiments, 1922.

B. Feeds.

Serial No.	Date.	Experiments.	Date.	Results.
1	31st May	Frog : orally 4 c.c. of + + + + culture.	Killed ¾ ths of an hour.	Stomach=mammalian R. B. Cs.+ motile L. D. Sections of gut wall=? flagellates. Films of viscera=negative.
2	5th June	Frog : orally + + + culture given.	Killed 24 hours.	Stomach contents = mammalian R. B. Cs.+actively motile flagellate L. D. Films of viscera=negative.
3	17th June	Frog : orally + + + culture given.	Killed 48 hours.	Stomach = scanty motile L. D. flagellates +. Films of viscera=negative.
4	21st June	Frog : + + + culture injected into stomach by laparotomy.	Killed 1½ hours.	Stomach=mammalian R. B. Cs. +scanty motile L. D. flagellates. (Hæmorrhage also into stomach at injection site).
	3rd July	Four frogs fed on + + + culture.		
5		(*a*) Kept at room temperature.	Killed at 2 hours.	Stomach=actively motile L. D. flagellates +.
6		(*b*) Kept at 22°C.	Killed at 24 hours.	Stomach=actively motile L. D. flagellates +.
7		(*c*) Kept at 22°C.	Killed on 3rd day.	Stomach=few motile L. D. flagellates +.
8		(*d*) Kept at 22°C.	Killed on 3rd day.	Stomach=few motile L. D. flagellates +. Films of viscera from all four negative.
9	28th Oct.	Frog : 3 c.c. thick emulsion of p.m. k.-a. spleen. L. D. + given orally.	Killed 4th Nov.	Films and cultures of viscera negative.
10	12th Feb. 1923.	Big frog. 2¾ c.c. very active L. D. flagellate culture : stomach tube.	Pithed 85 mins.	Stomach=no L. D. Seen. Octomitus +. Lower gut=scanty L. D. flagellates+: several still very active: many dead. Active flagellate (? Leishmania) seen in fresh spleen and liver preparations: none in stained smears.
11		Big frog : 3 c.c. of very active L. D. flagellate culture by stomach tube.	Pithed 95 mins.	No L. D. seen in stomach or gut. Trichomonas, Trichomastix, Opalina and Entamœba seen. Viscera : no L. D. seen.
12		Big frog : 3¼ c.c. of very active L. D. flagellate culture by stomach tube.	Pithed 135 mins.	No L. D. seen in stomach, gut or viscera: fresh preparations and films.
13		Big frog. 3½ c.c. of very active L. D. flagellate culture by stomach tube.	Pithed 3½ hours.	No L. D. seen in stomach, gut or viscera: fresh preparations and fiims.
14	13th Feb.	Big frog. 2½ c.c. of very active L. D. flagellate culture by stomach tube.	Pithed 20 mins.	Scanty L. D. flagellates: some still motile in stomach and gut. In viscera nil.
15		Big frog. 3¼ c.c. of very active L. D. flagellate culture by stomach tube.	Pithed 36 mins.	Scanty motile L. D. flagellates in stomach. In viscera nil.
16		Big frog. 3 c.c. of very active L. D. flagellate culture by stomach tube.	Pithed 38 mins.	Scanty motile L. D. flagellates in stomach. In viscera nil.
17		Big frog. 2½ c.c. of very active L. D. flagellate culture by stomach tube.	Pithed 49 mins.	Scanty motile L. D. flagellates in stomach. In viscera nil.

TABLE VIIA.

Kala-azar Spleen Juice, etc. Animals, 1921-1922.

A. Injections only.

Serial No.	Date.	Details of Experiment.	Date.	Results.	
			Monkeys.		
1	23-12-20	M. rhesus : male. 2 c.c. of washed corpuscles from patient with scanty parasites in peripheral blood : intraperitoneally.	26-1-21	Weight increased. Blood culture negative.	
			9-2-21	Blood culture negative.	
			14-3-21	Weight increasing. Spleen enlarged. Spleen puncture, films +. L.D.	
			22-5-21	Spleen puncture : films negative, culture +. L.D.	±
			10-7-21	Spleen and liver punctured. Cultures negative.	
			18-1-22	Animal alive and well.	
			26-2-21	Blood culture negative.	
2	15-1-21	M. rhesus : female. 5 c.c. ++ p.m. spleen emulsion : intravenously.	16-3-21	Spleen enlarged. Spleen puncture, films negative.	
			30-3-21	Spleen and liver enlarged. Spleen and liver punctured, films and cultures negative.	
			20-4-21	Spleen puncture : films negative : cultures positive. + L. D.	
			21-8-21	Liver puncture : cultures positive, + L.D.	±
			20-10-21	Liver puncture : films and cultures negative.	
3	15-1-21	M. rhesus : female. 10 c.c. of ++ p.m. spleen emulsion intraperitoneally.	10-2-21	Liver puncture : films and cultures negative.	—
			14-3-21	Liver puncture : films and cultures negative.	
			6-4-21	Liver puncture : films and cultures negative.	
			17-6-21	Liver puncture : films and cultures negative.	
4	15-1-21	M. rhesus : male. 2 c.c. of ++ p.m. filtered spleen emulsion subcutaneously into forehead.	10-2-21 14-3-21 1-4-21	No signs of local or general disease.	—
			17-6-21	Died of tuberculosis.	
5	15-1-21	M. rhesus : female. 10 c.c. of ++ p.m. spleen emulsion, intraperitoneally.	17-1-21	Died of peritonitis.	—
6	16-2-21	M. rhesus. 10 c.c. washed corpuscles from case with parasites in peripheral blood.	1-5-21	Spleen and liver punctured. Films and cultures negative.	—
			27-5-21	Died suddenly. A hydatid cyst of the lung had burst into the bronchi and choked him.	
7	17-1-21	M. rhesus : male. 10 c.c. of filtered ++ p.m. spleen emulsion : intraperitoneal.	14-3-21	Liver puncture, films and cultures negative.	—
	7-4-21	10 c.c. of ++ p.m. spleen emulsion : intraperitoneal and intravenous.	17-6-21	Liver puncture : films and cultures negative.	
8	17-1-21	M. rhesus : male. 2½ c.c. ++ spleen emulsion subcutaneously.	11-4-21	Liver puncture, films and cultures negative.	—
	11-4-21	10 c.c. of ++ p.m. spleen emulsion : intraperitoneal and intravenous.	12-4-21 Died.	T.B. foci in lungs. Films from viscera, no L. D.	
9	7-4-21	M. rhesus : male. 10 c.c. of ++ p.m. spleen emulsion : intraperitoneal and intravenous.	24-5-21	Died after six weeks of generalised tuberculosis.	—

TABLE VIIA—(*contd.*)

Serial No.	Date.	Details of Experiment.	Date.	Results.	
		Monkeys.—(contd.)			
10	5-21	M. rhesus. 5 c.c. of washed corpuscles from an "infected" monkey = No. 1, above, intraperitoneal and subcutaneous.	8-21	Liver puncture, films and cultures negative.	—
11	5-21	'Hunuman" monkey. 5 c.c. washed corpuscles from k.-a. case, intraperitoneal and intravenous.	8-21 *et seq.*	Liver puncture : films and cultures negative.	—
12	5-21	M. rhesus. 5 c.c. of ++ p.m. spleen emulsion, intraperitoneal and intravenous.	8-21 *et seq.*	Liver puncture : films and cultures negative.	—
13	5-21	"Hunuman" monkey. 5 c.c. of ++ p.m. spleen emulsion : intraperitoneal and intravenous.	9-21	Liver puncture : films and cultures negative.	—
14	6-21	M. rhesus. 10 c.c. of ++ p.m. spleen emulsion, intraperitoneal.	9-21	Liver puncture : films and cultures negative.	—
	20-9-21	Re-inoculated with fresh spleen emulsion intraperitoneally.	20-9-21	Died immediately after injection, ? anaphylactic shock.	
15	22-5-22	M. rhesus, adult female. ++ p.m. spleen emulsion : 20 c.c. intraperitoneal : 10 c.c. into liver : 2 c.c. each eyebrow.	11-7-22 1-8-22 28-12-22	Liver puncture : films and cultures negative.	±
			6-1-23	Killed. Viscera films and cultures negative.	
16	22-5-22	M. rhesus, adult male. ++ p.m. spleen emulsion. 20 c.c. intraperitoneal, 10 c.c. into liver, 2 c.c. each testis, 1½ c.c. each eyebrow.	11-7-22	Liver puncture : films negative : cultures +. L. D.	—
			28-7-22	Blood films and aldehyde test negative.	
			29-7-22	Blood films negative.	
			29-7-22	Blood films after Echis venom, negative.	
			3-8-22	Blood films after Echis venom, negative.	
			28-8-22	Very emaciated. Chloroformed. Films and cultures from viscera negative. Cause of death, ? beriberi.	
17	28-10-22 25-12-22	M. rhesus, adult female. ++ p.m. spleen emulsion. 20 c.c. intraperitoneal, 10 c.c. into liver. ++ p.m. spleen emulsion. 20 c.c. intraperitoneal, 4 c.c. into liver.	30-12-22	Liver puncture, films and cultures negative.	—
18	27-11-22	M. rhesus, young female. ++ p.m. spleen emulsion. 20 c.c. intraperitoneal, 4 c.c. into liver.	4-12-22	Chloroformed when ill. Pyæmia found.	—
19	25-11-22	M. rhesus, adult female. ++ p.m. spleen emulsion from Monkey No. 24, Table VIB. 30 c.c. intraperitoneal, 4 c.c. into liver.	26-11-22	Died, septic peritonitis. Films and cultures not taken.	—
20	25-11-22	M. rhesus, adult female. ++ p.m. spleen emulsion from Monkey No. 24, Table VIB. 30 c.c. intraperitoneal, 4 c.c. into liver.	4-12-22	Chloroformed when very ill. Pyæmia present. Films negative, no cultures taken.	—
21	25-11-22	M. rhesus, young female. ++ p.m. spleen emulsion from same monkey. 25 c.c. intraperitoneal, 4 c.c. into liver.	28-12-22 16-1-23 23-2-23 31-3-23	Liver puncture, films negative, cultures. +. L. D.	

TABLE VIIA.—(*concld.*)

Serial No.	Date.	Details of Experiments.	Date.	Result.	
		Monkeys—(contd.)			
22	27-11-22	M. rhesus, adult female. ++ p.m. spleen emulsion. 25 c.c. intraperitoneal, 5 c.c. into liver, 5 c.c. subcutaneous.	28-11-22	Died, septic peritonitis. Films and cultures not taken.	—
23	4-12-22	M. rhesus, adult female. ++ p.m. spleen emulsion, 17 c.c. intraperitoneal.	30-12-22 4-1-23	Liver puncture. Films and cultures negative. Died ? from beriberi. Films and cultures from viscera negative.	—
		Dogs.			
1	17-8-21	"Kala," black female puppy. 4 c.c. washed corpuscles from case with parasites in peripheral blood into peritoneum.	21-9-21	Died in an emaciated state. No Leishmania in films or cultures. Liver films shewed mononucleate forms, probably cryptococcus, n. sp.	—
2	17-8-21	"Azar," brown male puppy. 4 c.c. washed corpuscles intraperitoneally as in No. 1.	8-12-21	Killed in emaciated state. Liver films = scanty L.D. +.	+
3	5-9-21	"Leishman," black and white male puppy. ++ p.m. spleen emulsion 5 c.c. intraperitoneal.	2-12-21	Died of piroplasmosis. No Leishmania in films before or viscera films after death.	—
4	5-9-21	"Donovan," black and white male puppy. 5 c.c. emulsion of spleen, liver and bone marrow from Pup No. 1. on p.m. intraperitoneally.	9-12-21	Died suddenly. Spleen and liver enlarged. Films negative.	—
5	8-12-21	"Anti," red brown male puppy. 5 c.c. of liver and spleen emulsion from Pup No. 2, intraperitoneally.	6-1-22	Killed "in extremis." Films and cultures of liver, spleen, bone marrow negative.	—
6	9-12-21	"Mony," white male puppy. 5 c.c. of liver and spleen emulsion from Pup No. 4 intraperitoneally.	5-5-22	Liver puncture, films and cultures negative. Films shew mononucleate forms. ? Cryptococcus.	—
			1-12-22	Killed. Films and cultures from viscera **negative.**	
7	6-1-22	"Leonard," liver and white male puppy. 5 c.c. liver and spleen emulsion from Pup No. 5, intraperitoneally.	31-3-22	Died. No L.D. found in films from organs, but a heavy endomyces infection in the bone marrow.	—
8	17-1-22	"Rogers," brown and white female puppy. 10 c.c. ++ p.m. spleen emulsion intraperitoneal, 2 c.c. subcutaneously in abdomen and thigh.	-4-22	Died in wasted condition. Films and cultures negative. Endomyces infection present in liver.	—
9	17-1-22	"Fran," liver and white male puppy. Same injections as No. 8, + 1 c.c intravenously.	5-5-22	Liver puncture, films and cultures negative. Ulcer over site of subcutaneous injection formed, but healed.	—
			10-7-22	Died. Films from viscera all negative.	
10	22-5-22	"Chini," white female puppy. ++ p.m. spleen emulsion. 20 c.c. intraperitoneal, 15 c.c. into liver.	12-7-22	Liver puncture, films negative, cultures +. L.D.	±
			28-7-22	Peripheral blood films negative, aldehyde test negative.	
			29-7-22 3-8-22	Peripheral blood films after injections of Echis venom negative.	
			29-8-22	Liver puncture, films and cultures negative.	
11	28-10-22	"Sergent," adult female dog. ++ p.m. spleen emulsion, 20 c.c. intraperitoneal, 4 c.c. into liver.	16-1-23	Chloroformed when in good condition. Films and cultures of viscera negative.	—
		White Rats.			
1	17-3-21	Spleen pulp into scrotal sac.	10 days	Killed. Viscera films negative.	—
2	17-3-21	Ditto.	3 weeks	Ditto.	—
3	17-3-21	Ditto.	5 „	Ditto.	—
4	17-3-21	Ditto.	8 „	Ditto.	—
5	19-3-21	Spleen pulp into peritoneum.	10 days	Ditto.	—
6	19-3-21	Spleen pulp intraperitoneal and subcutaneous.	8 weeks	Ditto.	—
7	21-3-21	Spleen pulp into scrotal sac.	10 days	Ditto.	—

TABLE VIIA—(*contd.*)

Serial No.	Date.	Details of Experiments.	Date.	Result.	
			White Rats—(contd.)		
8	21-3-21	Spleen pulp into peritoneum.	14 days	Suspicious forms in liver smear, but culture negative. ? Cryptococcus.	—
9	21-3-21	Spleen pulp, intraperitoneal and subcutaneous.	5 weeks	Killed. Films from viscera negative.	—
10	22-3-21	Spleen pulp into scrotum.	3 weeks	Died. Films from viscera negative.	—
11	22-3-21	Ditto.	5 „	Killed. Viscera films negative.	—
12	23-3-21	Ditto.	2 months	Ditto.	—
13	23-3-21	Ditto.	2 „	Ditto.	—
14	31-3-21	Spleen pulp into peritoneum.	2 months	Ditto.	—
15	7-4-21	Spleen pulp into scrotum.	3 weeks	Ditto.	—
16	7-4-21	Spleen pulp into peritoneum.	6 „	Died. Ditto.	—
17	17-4-21	Spleen pulp into scrotum.	4½ „	Died. Ditto.	—
18	5-5-21	Spleen pulp into scrotum.	4 weeks	Killed. Ditto.	—
19	22-5-22	++ p.m. spleen emulsion. 5 c.c. into peritoneum, 1 c.c. each testis.	3 months	Killed. Films and cultures from viscera negative.	—
20	22-5-22	++ p.m. spleen emulsion. 5 c.c. into peritoneum, 1 c.c. each testis.	3 months	Killed. Films and cultures from viscera negative.	—
			White Mice (Japanese).		
1	7-4-21	Spleen pulp into peritoneum.	10 days	Killed. Films from viscera negative.	—
2	17-4-21	Spleen pulp intraperitoneal and subcutaneous.	10 „	Ditto.	—
3	17-4-21	Spleen pulp intraperitoneal and subcutaneous.	3 weeks	Ditto.	—
4	17-4-21	Spleen pulp intraperitoneal.	6 weeks	Died. Films from viscera negative.	—
5	5-5-21	Ditto.	2 days	Died of sepsis.	—
6	6-5-21	Ditto.	4 weeks	Killed. Films from viscera negative.	—
7	22-5-22	++ p.m. spleen emulsion. 2½ c.c. intraperitoneal.	16-11-22	Killed. Films negative. Cultures : = L.D. +.	
8	22-5-22	++ p.m. spleen emulsion. 2 c.c. intraperitoneal.	26-5-22	Died of sepsis. Films negative.	—
9	22-5-22	++ p.m. spleen emulsion. 2 c.c. intraperitoneal.	26-5-22	Died. Sepsis.	—
10	1-6-22	++ spleen puncture fluid subcutaneously.	28-8-22	Killed. Films and cultures of viscera negative.	—
11	3-6-22	++ spleen puncture fluid subcutaneously.	1-9-22	Died. Viscera films shew bacteria only. No cultures taken.	—
12	6-6-22	++ spleen puncture juice from 3 patients. 2 c.c. subcutaneously.	19-9-22	Killed. Viscera films and cultures negative.	—
13	13-6-22	½ c.c. ++ spleen juice subcutaneously.	15-1-23	Killed. Films and cultures negative.	—
14	13-6-22	½ c.c. ++ spleen juice from two patients subcutaneously.	15-1-23	Killed. Films and cultures negative.	—
			Miscellaneous.		
1	22-5-22	Pigeon. 5 c.c. of ++ p.m. spleen emulsion intravenously.	22-5-22	Films at ¼, ½, and 1 and 2 hours = no parasites seen.	—
			3-7-22	Killed. Films and cultures of viscera negative. Halteridium +.	—
2	22-5-22	Cock. ++ p.m. spleen emulsion, 8 c.c. intravenously and comb infiltrated.	11-7-22	Killed. Viscera films and cultures negative. No local lesion.	—
3	28-10-22	Duck. ++ p.m. spleen emulsion. 10 c.c. intraperitoneally.	29-10-22	Died. Septic peritonitis.	—
4	28-10-22	Adult female cat. ++ p.m. spleen emulsion. 20 c.c. intraperitoneal, 10 c.c. into liver.	4-1-23	Died. Coccidiosis. Viscera films and cultures negative.	—
5	5-4-21	Three Flying Foxes each injected intraperitoneally with ++ p.m. spleen emulsion.	7-5-21	(*a*) Died. Films from viscera negative.	—
6			12-5-21	(*b*) Died. Films from viscera negative.	—
7			12-5-21	(*c*) Blood culture negative. Animal escaped.	—
8	24-3-21	Two Flying Foxes each injected intraperitoneally with spleen puncture fluid rich in L.D. bodies.	10-4-21	(*a*) Died. Viscera films and cultures negative.	—
9			21-4-21	(*b*) Died. Viscera films and cultures negative.	—

TABLE VIIB.

Feeding Experiments.

Serial No.	Date.	Detail of Experiment.	Date.	Result.	
		Monkeys.			
24	22-5-22 23-5-22	Adult female M. rhesus. On both days chewed and swallowed two large pieces of fresh p.m. k.a. spleen = L.D. ++. Each meal washed down with 30 c.c. thick emulsion of same spleen by stomach tube.	11-7-22 1-8-22 25-11-22	Liver puncture. Films and cultures negative. Liver puncture. Films and cultures negative. Dying of acute dysentery. Chloroformed. Spleen enormous and fibrotic. L.D. ++ in spleen, liver, bone marrow, kidney and heart blood films. Cultures = L.D. ++.	+
25	28-10-22	M. rhesus, adult female. Fed on large pieces of fresh p.m. spleen, L.D. ++, and 20 c.c. emulsion of same by stomach tube.	30-12 22	Liver puncture : films and cultures negative.	–
26	25-11-22	M. rhesus, adult male. ++ p.m. spleen of Monkey No. 24 above Swallowed large pieces, and 10 c.c. of emulsion by stomach tube.	28-12-22 13-1-23 20-1-23	Liver puncture. Films and cultures negative. Severe anæmia. Peripheral films negative. Very ill. Chloroformed. Viscera films and cultures negative.	–
27	27-11-22	M. rhesus, adult female. ++ p.m. spleen emulsion. 50 c.c. by stomach tube. (Some went down the trachea).	3-12-22	Died. Pneumonia plus septicæmia. Viscera films negative. No cultures. Degenerating L. donovani in stomach ?	—
28	27-11 22	M. rhesus, adult male. ++ p.m. spleen emulsion. 15 c.c. by stomach tube. Some got into trachea : choking fit.	28-11-22	Died : aspiration pneumonia. Films negative. No cultures ? Degenerating L.D. in stomach.	–
29	4-12-22	M. rhesus, adult male. ++ p.m. spleen. Swallowed and chewed pieces. 30 c.c. emulsion by stomach tube.	28-12-22 3-2-23 27-3-23	Liver puncture, films and cultures negative. Liver puncture, films and cultures negative. Died of mesenteric T.B. Films and cultures negative.	–
		Dogs.			
12	22-5-22 23-5-22	Pup. Swallowed very large pieces of fresh p.m. kala-azar spleen with L.D. ++.	12-7-22 1-8-22	Liver puncture, films and cultures negative.	–
13	28-10-22	Adult female white dog. Swallowed bits of p.m. ++ spleen and 20 c.c. emulsion by stomach tube.	29-12-22	Killed. Films and cultures from viscera and heart blood negative.	–
		White Rats.			
21	18-5-22	Fed by capillary pipette on spleen puncture juice, L.D. ++.	28-8-22	Killed. Films and cultures negative.	–
22	25-5-22	Fed by capillary pipette on spleen puncture juice, L.D. ++.	4-11-22	Died. Films shew bacteria only. No cultures.	–
23	22-5-22 23-5-22	Both days. Fed on ++ p.m. spleen emulsion by fine catheter.	16-1-23	Killed. Films and cultures negative.	–
24	31-5-22	Fed on ++ spleen puncture fluid by catheter attached to syringe.	21-6-22	Died. Films and cultures negative. Encysted tapeworm in spleen.	–
25	2-6-22	Fed on ++ spleen puncture fluid by catheter attached to syringe.	28-8-22	Killed. Films and cultures negative.	–
26	28-10-22	p.m. ++ spleen emulsion. 4 c.c. by catheter.	11-11-22	Found dead. Films shew bacteria only. No cultures.	–
		Miscellaneous.			
10	22-5-22 23-5-22	Half grown cat fed on large pieces of fresh p.m. kala-azar spleen, L.D. ++.	2652	Died. Films shew bacteria only. No cultures taken.	–
11	22-5-22 23-5-22	Hawk. Fed on both days on bits of same spleen.	19-7-22	Died. Films and cultures negative.	–
12	28-10-22	Adult female cat. 20 c.c. of fresh ++ p.m. spleen emulsion by stomach tube.	1-1-23	Killed. Films and cultures negative.	–

TABLE VIII.

Transmission Experiments.

(*A*). *With Bed Bugs, Cimex rotundatus.*

Date.	Nature of feed.	Results.	Animal Test.	Results.
April 1921 ...	About 40 fed on infected Monkey No. 1 of Table VII. Bugs fed at different stages of their existence: some only once some repeatedly.	Subsequently starved for period varying from 4 days to a month and then fed on.	(a) Young female M. rhesus. Monkey I. (b) Male "hunuman" monkey. Monkey II.	Liver puncture of both at 2, 4 and 6 months. Films and cultures negative.
March-April 1921	Spleen puncture fluid, etc., from kala-azar cases injected into ligatured scrotum of white rats so as to form bullæ under the skin: *vide* Table VII. (*a*) White rats. 25 bed bugs of different ages fed on injected sites.	Kept for varying intervals and then fed on.	Monkey III. Young male M. rhesus.	Liver puncture after 3 months, films and cultures negative. Killed at 6 months. Films shewed mononucleate forms? Cryptococcus. Cultures negative.
	Kala-Azar Work, 1922. *Bed Bug Experiments.*			
16th June 1922 ...	30 bugs fed on flagellate culture plus citrated blood in feeding tubes. Kept at 22°C.	2 shewed flagellates next day. 6 dissected, 3rd day. Thick tails seen.	Mouse 1. Received 15 crushed bugs, 3rd day. Mouse 2. Fed on 8 of these bugs, 3rd day.	Killed 16th January 1923. Films and cultures negative. Died 15th January 1923. Films negative (bacteria). No cultures taken.
21st June 1922 ...	Collection of bugs fed on flagellate culture plus citrated blood in tubes. Kept at room temperature.	Infected by fungi. Died.		
29th June 1922 ...	Collection of bugs fed on flagellate culture plus defibrinated rabbit blood in tubes. Kept at 22°C.	Dissections positive, flagellates and thick tails and ring forms. 5th to 9th days.	Mouse 3. Received 3 crushed bugs hypodermically. Mouse 4, 6 bugs rubbed into scarified skin. Mouse 5. Fed on 6 of these bugs. Mouse 6. Fed on 6 of these bugs.	Died 10th August 1922 Negative. Died 4th August 1922. Negative. Killed 14th October 1922. Negative. Died 15th January 1923. Films negative Bacteria +. No cultures.
7th July 1922 ...	12 starved bugs fed on k.-a. case with possitive peripheral blood. Kept in cool incubator.	Negative.		
25th July 1922 ...	36 bugs fed on flagellate culture plus defibrinated rabbit blood. Kept at room temperature.	Died from fungus infection.		
1st August 1922...	6 bugs found in bedding of k.-a. case in hospital; dissected.	Nil.		
9th August 1922	Large collection of starved bugs fed on untreated k.-a. case with positive peripheral blood. Kept at 27°C.	Many dissected over next 9 days; nil.		
18th August 1922 22nd August 1922	Large collection of starved bugs fed on (a) k.-a. spleen puncture fluid plus patient's blood; (*b*) on flagellate culture plus defibrinated rabbit blood. Mixed by accident. Lot (*a*) kept at room temperature. Lot (*b*) kept at 27°C.	No development found in first five dissected.	Mouse 7. Received 5 crushed bugs hypodermically. Mouse 8. 5 of these bugs rubbed into scarified skin. Mouse 9. Fed on 5 of these bugs.	Died 14th November 1922. Negative. Killed 5th December 1922. Films and cultures negative. Died 1st January 1923. Films negative. Bacteria only. Cultures not taken.

TABLE VIII.B.

Conorhinus and other Feeds.

Date.	Nature of feed.	Results.
1st February ...	Eight leeches fed on blood of k.-a. case with 24 parasites in 7 films.	Leeches kept at 22°C; dissected, up to 14th day. No parasite seen.
1st April ..	Adult female Conorhinus fed well on k.-a. case with positive peripheral blood. Kept at room temperature.	Dissected, 3rd day. R. B. Cs. in gut but no parasites seen.
25th April ...	Two newly hatched Conorhinus fed on k.-a. case with positive peripheral blood. Kept at room temperature.	Both dissected. 7th day. Results nil.
2nd May ..	Newly hatched Conorhinus fed on k.a. case with positive peripheral blood. Kept at 22·C.	Dissected. 8th day. Results nil.
5th May ...	Young Conorhinus nymph fed on k.-a. case with positive peripheral blood. Kept at 22°C.	Dissected 6th day Results nil.
9th May ...	Large, adult Conorhinus took full meal from k.a. case with positive peripheral blood. Kept at 22°C.	Dissected, 10th day. Remains of blood meal still in gut but no parasites found.
18th September	Collection of Conorhini and Stegomyia fed on k.-a. spleen emulsion plus patient's defibrinated blood in feeding tubes. Lot (*a*). Kept at room temperature. Lot (*b*). Kept at 22°C.	Subsequent examinations by Dr. Strickland; reports nothing found on dissection.
13th November	30 Conorhini fed on spleen juice plus patient's defibrinated blood. (*a*) kept at room temperature, (*b*) kept at 22°C.	Results nil.
16th November	12 Conorhini fed on peripheral blood of k.-a. case with parasites in films.	Results nil.
21st November	Collection of Conorhini fed on patient with positive peripheral blood.	Results nil.
27th November	Collection of Conorhini fed on fresh p.m. k.-a. spleen emulsion in bug feeding tubes.	Results nil.

TABLE VIIIC.

Miscellaneous Experiments.

Date.	Possible medium of transmission.	Source of infection.	Animals to which transmission was attempted.	Results.
April to August 1921 ..	Fæcal, urinary, etc., contamination possibility. Also flying blood-sucking insects.	No. 1 infected monkey. (Table VIIA), kept louse-free.	Female M. rhesus kept in same cage for 4 months. Kept louse-free.	Liver puncture at 4 and 6 months. Films and cultures negative.
May to August 1921 ...	Fæcal, urinary, etc., blood-sucking insects, lice.	No. 2 infected monkey. (Table VIIA), louse-infected.	Young male M. rhesus, also louse-infected, kept in same cage for 3 months.	Died wasted. Films from viscera negative.
May to August 1921 ...	Fæcal, plus flying blood-sucking insects.	No. 2 infected monkey, louse-infected.	Male M. rhesus kept in adjacent cage for 3 months.	Liver punctures, at 3 and 6 months. Films and cultures negative.

Current Topics.

Treatment of Cases as a Prophylactic Measure in Kala-azar.

A PAPER read at the annual 1923 meeting of the Assam Branch of the British Medical Association on the work of the special kala-azar staff in Assam by Lieutenant-Colonel T. C. McCombie Young, I.M.S., is one which will command general interest. As the paper is published in full elsewhere,—(Proceedings of the Assam Branch, B. M. A., Jan., 1923)—we here give only a precis of it. After dealing with the well-known history of the disease in Assam Colonel Young went on to comment upon the introduction of the tartar emetic treatment into India, the credit for which undoubtedly belongs to Sir Leonard Rogers. Clinically his results were confirmed by Muir at Khulna and by Dodds Price at Nowgong. The introduction of the method in Assam is undoubtedly due however to the Public Health Department of the Province, which created the special kala-azar hospital at the Shillong Pasteur Institute in 1917 for the study of the disease and its treatment. Knowles' dictum that a total course of 200 c.c. of a one per cent. solution of potassium antimony tartrate intravenously "seemed to be sufficient to sterilize the patient from infection" has been very adversely criticised : and it is now clear that many cases require a much more prolonged treatment, whilst in many of the so-called "antimony-fast" cases the want of success may apparently be due to hesitation in commencing the treatment, and to a lack of boldness in pushing the dosage at its commencement. It is now clear that Knowles was wrong : yet the circumstances of the time may be recalled : the treatment was on experimental trial, Knowles' reports of 1918 and 1920 were of a preliminary character only, the Assam Administration was waiting for the reports, and the dictum was based upon the fact that in 31 patients thus treated in Shillong, and in a good hill climate, after a course of 200 c.c. of the one per cent. solution, the spleens were still large enough to puncture, and yielded negative findings in both films and in N.N.N. cultures.