Herpetomonas lygaei.

By

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(With Plate I and 2 Textfigures.)

In an earlier paper (1) I drew attention to the close resemblance between a species of *Herpetomonas* and the Leishman-Donovan body. Although throughout its life cycle this *Herpetomonas* is much larger than the corresponding stages of the Leishman-Donovan body, its striking similarity to that parasite can leave no doubt that the study of these insect flagellates will not only decide the exact biological position of the human parasite, but will also throw important light on the, as yet unknown, stages in its development. Since writing the above paper I have been fortunate enough to discover another *Herpetomonas* in the intestinal tract of the Lygaeid bug, *Lygaeus militaris* Fabr., which as will be seen later is almost identical with the Leishman-Donovan body and in the present paper I propose describing in detail its structure and life cycle.

Material and methods.

Lygaeus militaris, the type species of the genus, is pale sanguineous in colour, its pronotum has a black central anterior margin to which are connected two long arcuated fasciae reaching to the posterior margins near the lateral angles. There is a small black spot near the apex of the clavus and a transverse black

fascia on the corium. The wing membranes are pale brownish ochraceous with a spot at the base and two spots sometimes coalescing abont the middle line.

Mr. Distant (2) gives the distribution of this bug in India as follows: Pnnjab, Hardwar, Bangalore and Mysore. Curiously enough it has not been recorded from Madras where it is very plentiful, and Mr. Maxwell Lefroy, Entomologist to the Government of India, who kindly identified it, informs me it is one of the commonest bugs in India. It is also found in Burma, Malay Archipelago, Anstralia, Sonth Africa and the Sondan where it is known as the Dura plant bug. In Madras it is almost exclusively fonnd on *Calotropis gigantea*, feeding on the juice of its pods, young leaves and seeds: it is interesting to note that the distribution of this plant as given by Hocker (3) corresponds almost exactly with that of the bng. *Lygaeus militaris* is found in Madras throughout the year but is very abundant from April to September; the adult female lays its eggs, usually thirty-four in number, at the root of the plant and in six days the nymphs hatch out. They shed their skins five times and after about five weeks arrive at maturity.

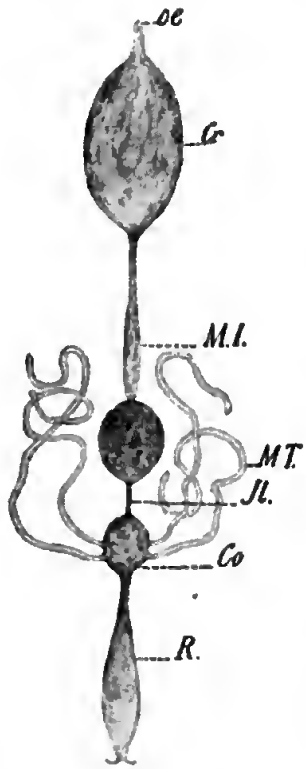

Fig. A.
Alimentary canal of *Lygæus militaris* Fabr.
Oe = Ösophagus; *Cr* = Crop; *MI* = Mid-intestine; *MT* = Malpighian tubes; *Il* = Ileum; *Co* = Colon; *R* = Rectum.

The alimentary tract of this bug (Fig. A) consists of a short narrow oesophagus (*Oe*) opening into a large sacculated crop (*Cr*), which when distended with the white juice of *Calotropis gigantea* bulges well into the lower segments of the abdomen. The crop opens into the long mid-intestine (*MI*), which is narrow at first but gradually becomes wider ending abruptly in a large dilatation, which always contains a yellow fluid; this dilated portion probably corresponds to the pyloric ampnlla of other insects. Dr. Sharp (4) rightly states, "that in the Rhynchota it is difficult to assign any of the parts posterior to the crop to the divisions nsual in other insects and it is said the distinction of parts histologically is as vague as it is anatomically". The small intestine consists of a short narrow ilenm (*Il*)

which opens into the dilated colon (*Co*), into which four long malpighian tubes (*MT*) are inserted. *Lygaeus* is oneof the insect forms in which the nrinary tubes, instead of opening into the jnnction of the mid-and hind-intestine open into the large intestine. The colon is continuous with the short straight rectnm which ends externally at the anus.

On examining in the fresh condition the contents of the crop of a specimen of *Lygaeus militaris* I found it to be swarming with a flagellate which on staining with Giemsa's stain I recognised as a species of *Herpetomonas*. In all the specimens subsequently axamined in addition to the flagellates in the crop many were seen massed in bundles in the colon, and below the openings of the malpighian tubes innumerable flagellates were attached by their flagellar ends to the intestinal epithelium, their posterior poles lashing from side to side; still further down the epithelium was covered with small ronnd and oval bodies (Fig. B) which, though smaller, are similar to bodies I have seen in the rectums of flies (*Musca domestica* and *Sarcophaga sp.?*) infected with *Herpetomonas muscae domesticae* and *Herpetomonas sarcophagae?* respectively.

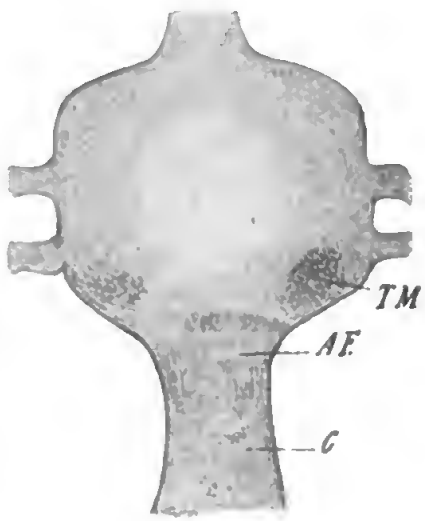

Fig. B.
Colon and rectum of *Lygæus militaris* showing parasites as seen in the fresh condition (somewhat diagrammatic). *TM* = Tanghed masses of flagellates; *AF* = Attached flagellates; *C* = Cysts.

These forms represent the encysted stages of the flagellates. Prowazek (5) in his description of *Herpetomonas muscae domesticae* describes the formation of the cyst of this flagellate, he however does not mention the occnrrence of the cysts in masses in the rectum of the fly. In all the species of *Herpetomonas* I have had the opportnnity of stndying, the encysted stages were readily found in the rectnms of their insect hosts.

From the above observations it was clear that the flagellates of *Lygaeus militaris* after remaining for an indefinite period in the npper part of the alimentary canal pass down and collect in the large intestine where they finally encyst. The cysts as well as nnmerous living flagellates are passed

ont in the fluid faeces and the former are readily fonnd in stained smears of dried faeces.

A large number of adult bugs were collected and placed in glass jars with the leaves and pods of *Calotropis gigantea*; when a number of eggs were laid they were transferred to a clean bottle in order later to study the method of infection. The adult specimens, male and female, were examined at regular intervals and it was found that they were all infected, some more than others, and in the large intestines of the majority parasites exhibiting all the various stages of encystment were abundant. A number of young nymphs were placed in the jars with the adults while many more were kept quite separate; it was thus possible to settle the exact method of infection.

On dissecting out the digestive tract, the crop was isolated and placed in a small drop of saline solution on a clean slide; its contents were then expressed with two needles and the fluid on being well mixed was made into a thin film with a bit of slide. It was rapidly dried, fixed in absolute alcohol and stained either with Romanowsky's or Giemsa's stain. The contents of the colon with the free flagellates were similarly made into a thin film. That part of the large intestine containing the attached flagellates and cysts on being isolated was teased into smaller pieces and each was smeared out with the edge of a slide and stained as above. The fluid faeces was drawn into a fine pipette, blown on to a clean slide and made into thin films, the dry faeces was scraped of the pods and leaves, mixed with a little normal saline solution and lightly smeared out. These methods were fonnd to give the best results.

Structure and life cycle of the parasite.

The discovery of the encysted stage of the parasite in the faeces of the bug led to a search being made for the cysts in the crop contents, and after examining a number of stained preparations I found this stage in large numbers in about half the films. My previous studies of the life cycles of several species of *Herpetomonas* and *Crithidia* and more especially of the development of the Leishman-Donovan body in *Cimex rotundatus* Sign. have been of great help in working out the life cycle of this *Herpetomonas*. As in all the above parasites the life cycle invariably begins with the ingestion of the non-flagellate stage by its host, I will begin my description of the parasite from this point.

The cysts are always free in the crop contents and are exactly similar to those found iu the faeces. They are either oval, round or pear shaped bodies (Plate I Fig. 1) measuring from 3,5 μ to 4 μ in length and from 2 μ to 2,5 μ in breadth. Their protoplasm stains a delicate blne with Romanowsky's stain and light pink with Giemsa's and when highly magnified is seen to consist of a delicate reticnlum which in parts is finely vacuolated. The parasites are clearly outlined by a coudensed layer of protoplasm, the periplast, which stains deep blue with Romanowsky's staiu bnt pink with Giemsa's. The uuclens, nearly always at the rounder end of the parasite, is a compact mass staining deep pink and measnring about 1 μ iu diameter; it may be circular or more commonly kidney-shaped with the concavity directed towards the centre of the cell. When highly magnified it is seen to contain four chromosomes, this number however is not always constant as very ofteu only two can be distiuctly seen; many of the nnclei show a central pale area, while the margin stains much darker probably owing to the chromosomes occnpying the periphery. In specimens deeply stained by Giemsa's stain and subsequently decolorised the recticnlated nnclens appears to be surrounded by a distinct membrane (Plate I Fig. 1). The blepharoplast, about one third the size of the nuclens, is almost always circular in shape and stains deep magenta with Romanowsky's and Giemsa's stains. It is as a rule sitnated about the centre of the parasite but may be displaced to either side. Owing to its small size and great avidity for the staiu it is not possible in ordinary specimens to demonstrate any inner strncture bnt in rnptured cells it is seeu to contain (under a magnification of 2700 diameters) a small chromatic dot; in some specimens it had the appearance of a small ring. There was no other structnre visible in the cysts.

A general enlargement of a cyst is the first stage towards development; it increases in length and breadth, its protoplasm retaining its original staining characters there being no increased vacuolation. At the same time the nnclens enlarges, its network becomes looser and stains less deeply while the chromosomes increasing in number pass to each side. It now has a bilobed appearance, its central portion staining lightly indicating the line of cleavage and when division is completed the danghter nuclei separate and pass to the sides (Plate I Fig. 2). Iu such a cell the blepharoplast appears thickened and elongated later, however, the ends become rounded and it looks like a diplococcus. On the division and separation of the nnclei and blepharoplasts the posterior end of the

parasite becomes indented and a pale area begins to develop down the centre and the parasite finally splits longitudinally into two. In the majority of these pairs the posterior or nuclear ends pass in opposite directions so that the anterior ends remain in close opposition (Plate I Fig. 3). There appears to be a slight variation in this method of division, the two nuclei on separating sometimes pass to the opposite ends of the cell, the blepharoplasts remaining just beyond the centre of the parasite and division now takes place through a shorter diameter. Each parasite immediately begins to divide again in exactly the same manner as described above (Plate I Fig. 3) and the resulting four bodies remain attached by their anterior ends (Plate I Fig. 4). This appearance is very characteristic of the parasite and is the commonest method of development; a small percentage of parasites however instead of dividing again develop into flagellates. I will first describe the changes undergone by the groups of four parasites.

On examining a stained preparation of the crop contents of a bng, which contains many of these forms, in nearly every group one or more of the parasites has already flagellated, the remainder showing varying gradations in size; some without developing further have divided again while others have commenced to elongate preparatory to division and flagellation. These gronps of flagellated and non-flagellated forms are firmly connected together by their anterior ends, and in the fresh condition the whole group is drawn along by the flagellate (Plate I Fig. 9 and 10). Althongh I have had the opportunity of studying at least a dozen species of *Herpetomonas*, this is the only one I have seen that exhibits this curions method of mnltiplication. On stndying groups of four parasites many of them will be seen to have simnltaneously increased in size (Plate I Fig. 5) their anterior ends become elongated and pointed while the posterior ends are still rounded, their nuclei are considerably enlarged, stain a lighter pink and contain from eight to ten circular chromosomes; the blepharoplasts are also enlarged and are almost round. The protoplasm of these cells stains a deeper blne with Romanowsky's stain, is more vesicular in structure and in parts has a fine granular appearance. Each parasite is distinctly ontlined by a periplast. In still older specimens the posterior ends lose the rounded shape and become more and more elongated and as growth proceeds the cells become spindle-shaped (Plate I Fig. 6); their nuclei and blepharoplasts now occupy a central position lying as a rule close to each other. On staining the cells deeply

with Giemsa's stain the protoplasm of the posterior ends appears markedly vesicular, stains deep blue and contains small pink staining strands (Plate I Fig. 6); the anterior ends stain uniformally pink and often contain a pale area near the blepharoplast. If a number of these deeply stained spindle-shaped cells are examined under a high magnification, in one or more a small pink rod is seen lying in the pale area between the blepharoplast and the anterior end of the parasite (Plate I Fig. 6), in older specimens it is seen as a short straight pink filament arising from a point close to the blepharoplast but in no way connected directly with it (Plate I Fig. 6). This pink staining filament is the flagellum and in its earliest stage it is always seen to arise from an achromatic area in close proximity to the blepharoplast. In still later specimens it extends towards the anterior end of the parasite finally projecting free of it almost exactly at its centre and once free grows into a long wavy flagellum (Plate I Fig. 6 and 10). It is important to note that the flagellum under a high magnification consists of a single thick filament and not of a number bound together. As the parasites arrive at maturity the blepharoplasts pass down towards the anterior ends and the individuals of the group soon separate and swim away.

In those groups in which the parasites have not all developed at the same time one may have become an adult flagellate and attached around its anterior ends are the younger parasites in various stages of development (Plate I Fig. 9). Frequently one or more of the young cells is unchanged while the remainder have enlarged, become spindle-shaped and divided preparatory to flagellation, or as is more commonly the case one of the original cells has divided, half becoming an adult flagellate, another has divided again and the third has become spindle-shaped and divided while the fourth remains unchanged (Plate I Fig. 9). In this way a group consisting of five, seven or nine cells in varying stages of development is formed. As the remaining cells develop and flagellate the groups break up and if watched for some time in the fresh condition the adult flagellates can be seen freeing themselves and swimming away. In the films of crop contents there are always some pairs of spindle-shaped cells attached by their anterior ends, these parasites can be traced back to cysts which have only divided once; they are exactly similar to the spindle-shaped cells described above (Plate I Fig. 6).

A typical adult flagellate (Plate I Fig. 11) measures from 20 μ to 25 μ in length and from 4 μ to 5 μ in breadth, its posterior

end is markedly pointed, its body cylindrical and its anterior end blunt. Its protoplasm which stains dark blue is vesicular in structure, the posterior portion containing a number of pink staining granules; the anterior end stains more pink than blne and usnally contains one unstained area between the nuclens and the blepharoplast and another between the blepharoplast and the anterior end. The whole parasite is outlined by a delicate periplast which is best seen when deeply stained by Giemsa's stain. The nncleus circular in shape lies almost at its centre, it stains light pink and is surrounded by a well marked membrane; its chromosomes, from eight to twelve in nnmber are arranged radially along its margin, some are more conspicnons than others and appear as short rods. I have not been able to satisfy myself that the nncleus has a karyosome, as in some specimens its central portion is clear, while in others it is occupied by what looks like a chromosome. The blepharoplast, more rod shaped than round, stalns almost black and measures about 1,5 μ in length, it is always situated about 4 μ from the anterior end and in many parasites has a douhle appearance snggesting commencing division. The flagellum, about 40 μ in length consists of a single stout filament which arises from the achromatic space just anterior to the blepharoplast and passes out of the anterior end. The intracellnlar portion does not differ in structnre from the remainder and it has no basal granule in connection with it.

On examining a flagellate which is about to divide longitudinally the nucleus appears mnch enlarged, stains less deeply and is full of large circnlar chromosomes (Plate I Fig. 12); the blepharoplast is also thickened and elongated. In suitable specimens a small pink filament is seen lying in the achromatic space close to the root of the flagellnm from which it is entirely separate; this strncture represents the early development of the second flagellum and appears to be formed in the same way as the original one. In parasites more advanced towards division it is seen growing out towards the anterior end and on becoming free is intimately associated with the first flagellum. In a still fnrther stage the blepharoplast splits transversely and the two halves separate; at the same time the nncleus elongates and divides as described above and on a clear line forming between the roots of the flagella the anterior ends of the parasites separate first and later division extends to the posterior ends (Plate I Fig. 13). It is quite common in stained preparations to observe all the stages of longitudinal division from the formation of the second flagellum up to the complete separation of the two

parasites. I have never observed any of these flagellates dividing unequally, nor do they appear to divide more than once as the majority are all about the same size and extremely thin forms were never seen.

It will be remembered as I pointed out the flagellates collect in large numbers in the pyloric ampulla and colon and that in the latter situation they are seen coiled up together in tangles; on rupturing in the fresh condition a colon containing these entangled flagellates they will be seen to free themselves and are therefore in no way connected with each other. Still further down the large intestine innumerable parasites are found attached to the intestinal epithelium in rows by their flagellar ends, their posterior poles lashing from side to side suggesting the movements of cilia; between these forms and the cysts are parasites of all sizes which are entirely without flagella. All these cells represent the various changes undergone preparatory to encystment which I will now describe.

On examining a stained preparation of the attached flagellates in addition to the adult forms described above a large number consist of short stout parasites, some with long and others short flagella (Plate I Fig. 17 and 18); their protoplasm stains deep blue with Giemsa's stain, is full of large and small vacuoles, and their posterior ends contain a number of light pink granules. These pink staining dots are best seen in compressed parasites as they are often obscured by the voluminous protoplasm. I have observed these chromatoid granules in the encysting stages of several species of *Herpetomonas* and *Crithidia*; in *Herpetomonas muscae domesticae* in particular they are very large. The nuclei of the encysting forms of the flagellate of *Lygaeus militaris* stain light pink and contain a large number of circular chromosomes; their blepharoplasts are also considerably enlarged and lie transversely to the long diameters of the cells about 5 μ from the anterior ends (Plate I Fig. 17). The flagella are often seen arising from a large vacuole close to the blepharoplasts, and in many of the cells they are quite short only measuring 10 μ, the greater portion having broken off (Plate I Fig. 18). The nuclei and blepharoplasts in most of these parasites are seen in all stages of division, the flagella are thickened and are often seen splitting longitudinally. The anterior ends of the parasites become indented and the blepharoplasts and nuclei on dividing separate, the flagellum at the same time splitting into two (Plate I Fig. 19). The cells divide further, the line of separation passing through the groups of granules at the posterior ends, some

of which remain in each cell, and two shorter parasites are thus produced. When the flagellates are separating the two flagella may often be seen attached throughout the greater part of their length or by their ends alone in which case they exhibit a shredded appearance (Plate I Fig. 19). At this stage it is not uncommon to find that the flagella have entirely broken off and are only represented by a few strands lying in the anterior ends of the parasites (Plate I Fig. 20). All these appearances indicate that as the flagellum degenerates it is not drawn up into the body of the parasite but the extracellular portion first breaks off and the remainder is absorbed. The parasites resulting from this first division measure from 10 μ to 12 μ in length and from 4,5 μ to 5 μ in breadth; their nuclei are situated abont the centre and the blepharoplasts close up to them. These parasites soon begin to divide again, the nuclei and blepharoplasts enlarging and dividing transversely and on the cells splitting two shorter forms are produced (Plate I Fig. 20 and 21). These in their turn divide longitudinally (Plate I Fig. 22) and the resulting four bodies are seen lying with their anterior ends in close contact (Plate I Fig. 24). These groups represent the final stages of encystment and are found massed together in the rectum being very loosely attached to the epithelium; they are passed out in the faeces and retain their staining characters even when it has become dry. The cysts in the rectum only differ from those I have described from the crops in that their blepharoplasts are more rod-shaped than circular; in the cysts from the faeces the blepharoplasts are always circular (Plate I Fig. 25). This then concludes the life cycle of the parasite; I am not able at present to say how long it takes to complete its development.

The method of infection.

There is at present considerable confusion regarding the method by which these flagellates are transmitted from one insect to another, so that careful observations on this point are of some importance. Since the researches of the late Dr. Schaudinn (6) on the evolution of *Trypanosoma noctuae* in *Culex pipiens* and those of Prowazek (5) on *Herpetomonas muscae domesticae* in the house fly it is generally believed that these insect flagellates in addition to other methods of transmission actually penetrate the ova of their host and infect the second generation. I have directed special attention to this point in the case of the flagellate of *Lygaeus militaris* and although I

examined a number of infected bugs the flagellates were never found in any other sitnation but the alimentary tract. Twenty female bugs which were snbsequently fonnd to be infected were allowed to ovnlate and the eggs were transferred to a clean bottle; a large nnmber of these eggs were examined by smearing them out on clean slides and staining the films deeply with Giemsa's stain, but in none of the slides could I find any parasites. Some of the eggs were kept for six days and when the nymphs hatched out they were separated and were examined at regular intervals; their alimentary tracts were dissected out, and examined in the fresh condition for flagellates, after which they were smeared out and stained with Giemsa's stain. These nymphs had descended from infected bugs and if the infection were inherited, they should have been themselves infected but I was nnable to find any parasites in their alimentary tracts. On placing some of the remaining nymphs in the bottle in which the adult bngs had previonsly been kept I found that after a fortnight a large percentage contained flagellates in their crops. If the nymphs and the adults of *Lygaeus militaris* are watched it is soon seen that while feeding on the juice of *Calotropis gigantea* they suck up the faeces which others have deposited on the leaves and pods of the plant, and I frequently observed a bug actually inserting its proboscis into fresh fluid faeces. All these observations prove that the infection is contaminative and not hereditary.

The biological position of the parasite.

In 1881 Saville Kent (7) created the genus *Herpetomonas* for the flagellate described by Burnett and others as *Bodo muscae domesticae* from the alimentary tract of *Musca domestica*. Later he provisionally included in this genus the parasite found by Lewis in the blood of rats in India naming it *Herpetomonas Lewisi* and this name was retained by Bütschli and others. Recent work particnlarly that of Laveran and Mesnil (8), has however shown there is a marked divergence in the morphology of the two parasites and has led to Lewis' parasite being placed in the genus *Trypanosoma*, the flagellate of the house fly remaining as the type species of the genus *Herpetomonas*. This genus contains a large number of flagellates, the majority as far as is known insect parasites, which in their adult stages are characterised by the complete absence of an undulating membrane, the single flagellum being attached to the anterior end of the parasite by a short intra-cellular

portion. The blepharoplast is always anterior to the nucleus usually midway between it and the anterior end. The type species *Herpetomonas muscae domesticae* according to Prowazek (5) however has a double flagellum and Lühe (9) restricts this genus to all snch flagellates.

I have had the opportnnity of studying the flagellate of the house fly and am unable to confirm Prowazek's view of its flagellar apparatus; in order to settle this point I carried out a number of feeding experiments in the hot weather of 1907 when flies were abundant in Madras and as a result was able to study the early development of the parasite in the midgut of the fly. Plate I Fig. 14 represents two young forms in one of which the flagellum, a single filament, has just been extruded and is not yet completely uncoiled; in the other parasite it is still seen lying in the 'flagellar vacnole' which is approaching the surface of the cell. Plate I Fig. 15 is a young flagellate of *Herpetomonas muscae domesticae* and it will be seen that it has a single flagellum, division not having begun. The majority of adult flagellates have the appearance of a donble flagellnm as fignred by Prowazek but this can only represent the commencing division of the flagellate. Léger (10) is also of this opinion and figures the adult flagellate of *Herpetomonas muscae domesticae* with a single flagellum. The flagellate of *Lygaeus militaris* is therefore undonbtedly a species of *Herpetomonas* and so far as I am aware differs in many respects from the known species. I therefore propose naming it *Herpetomonas lygaei.*

Comparison of the parasite with that of Kala Azar.

The close similarity between *Herpetomoas lygaei* and the Leishman-Donovan body necessitates a detailed comparison between the structnre and life cycles of the two parasites. As is well known the parasite of Kala Azar occurs in man only in its non-flagellate stage which is one of active multiplication and in no sense a resting stage. It is an oval body (Plate I Fig. 7) measuring from 3,8 μ to 4 μ in its longest diameter and from 2,8 μ to 3 μ in its shortest, its protoplasm stains a delicate blue with Romanowsky's stain and appears to be more condensed towards the sides. When stained deeply with Giemsa's stain its protoplasm stains light pink and the body of the parasite is seen to be outlined by a delicate pink staining periplast. The nucleus is a compact round, oval or kidney shaped mass lying to one side, it stains light pink and when examined

with a high magnification two and sometimes four chromosomes are seen lying in its reticnlum. Opposite the nucleus and usually lying at the periphery of the cell is the rod-shaped blepharoplast which stains deep magenta and does not appear to have any inner structnre. Christophers (11) has described a fine filament of a chromatic nature extending from the nucleus to the blepharoplast. Major Donovan, I. M. S., showed me some specimens stained with a modification of Jenner's stain, where this filament was clearly seen; I am unable to offer any explanation as to its nature. The above is the usual appearance of the parasite whether seen in films of splenic or peripheral blood. In man the parasite multiplies either by simple longitndinal fission when the nuclens and blepharoplast elongate and divide or by multiple segmentation (Plate I Fig. 14) as described by Laveran (12), Mesnil (12) and Christophers (11); in this case one of the parasites enlarges the nnclens and blepharoplast dividing a number of times and from four to six or more parasites are formed. From the description of the corresponding stages of *Herpetomonas lygaei* it will be seen that it is distinctly smaller than the Leishman-Donovan body and this is especially noticeable in the nuclens and blepharoplast; I have never seen the pink staining filament mentioned above in this parasite. *Herpetomonas lygaei* also divides by simple longitudinal fission, the two resnlting parasites invariably dividing a second time prodncing characteristic groups of four parasites; multiple segmentation of one cell into four or more is never seen in this parasite.

In my (13) researches on the development of the Leishman-Donovan body in *Cimex rotundatus* I have shewn that on the second day the parasite enlarges, its protoplasm becomes vacnolated and the nnclens and blepharoplast begin to shew the earliest changes towards division. At this point one of two changes may take place the parasite may either flagellate or may increase still more in size, the nnclens and blepharoplast dividing a number of times; 'flagellar vacnoles' then develop near the blepharoplasts and later the flagella pass out and the cell divides into from four to eight flagellates. The formation of the flagellum is very characteristic (Plate I Fig. 8), it first appears as a small pink body lying in a vacnole close to the blepharoplast and later on enlarging passes to the periphery of the cell when it is extruded. In the case of *Herpetomonas lygaei* the four cells resulting from the second division elongate (Plate I Fig. 5), their nuclei and blepharoplasts at the same time enlarging; when the cells have become spindle-shaped the flagellum develops

in the achromatic area close to the blepharoplast and appears as a fine pink filament. The large, round or oval blue staining flagellates so characteristic of the development of the Leishman-Donovan body are never seen nor is there any true rosette formation. The commonest methods of development of the four parasites are shewn in Plate I Fig. 6, 9 and 10.

The adult flagellate of the Leishman-Donovan body as seen in *Cimex rotundatus* measures from 12 μ to 20 μ in length and from 4 μ to 5 μ in breadth, its posterior end is pointed while the anterior end is rounded; the nucleus lies about the centre of the cell and the blepharoplast about 1,5 μ from the anterior end. The flagellum, a long thin filament, measures from 16 μ to 24 μ and is often seen arising from a chromatic dot just anterior to the blepharoplast. The adult flagellate of *Herpetomonas lygaei* is longer and stouter than that of the Leishman-Donovan body and its blepharoplast usually lies abont 4 μ from its anterior end. The flagellate of the Leishman-Donovan body after remaining five days in the midgut of *Cimex rotundatus* begins to divide irregularly into more than one smaller form, this fact together with the observations on the encysting stages of *Herpetomonas lygaei* suggest that the parasite of Kala Azar again passes back to its non-flagellate stage in the bug most probably in its pharynx when it could readily be introduced into man by the bug when feeding.

Concluding remarks.

Herpetomonas lygaei is a true parasite of *Lygaeus militaris* passing its complete cycle in the alimentary tract of the bug; there is no evidence to shew that the infection is hereditary but all the observations point to the bug ingesting the parasite when obtaining its food. A careful stndy of the flagellates in the fresh and stained preparations before encystment commences has failed to shew any sexual dimorphism, differences in size merely representing forms before and after longitudinal division. The so-called male, female and indifferent cells could not be recognised nor were any of the parasites seen conjugating. From the description of the non-flagellate and flagellate stages of *Herpetomonas lygaei* it will be seen that it is almost identical with the Leishman-Donovan body, I therefore propose adopting the suggestion made by Rogers (14), that the parasite of Kala Azar belongs to the genus *Herpetomonas*, naming it *Herpetomonas donovani* (Laveran and Mesnil).

At present our knowledge of these flagellates of the genus *Herpetomonas* is almost entirely limited to the adult forms very little attention having been paid to their non-flagellate stages and in the majority of the described species this stage is not even known. It will be remembered that Prowazek (5) begins his account of *Herpetomonas muscae domesticae* with the adult flagellate and describes in detail its structure and method of encystment, but makes no mention of the early development of the parasite in the midgut of the fly; so that in the type species of *Herpetomonas* the non-flagellate stage similar to the Leishman-Donovan body is not sufficiently recognised. In order to simplify the study of these flagellates of the genus *Herpetomonas* I have found it convenient to divide their life cycles into three stages, preflagellate, flagellate and postflagellate. In their preflagellate stages they are round or oval bodies with a large nucleus and round or rod-shaped blepharoplast; they multiply by simple longitudinal division as in the case of *Herpetomonas lygaei* or in addition by multiple segmentation as in *Herpetomonas donovani* and the *Herpetomonas* of *Culex pipiens*, which I (1) have recently described. In the species I have studied this stage is passed in the midguts of the larvae, nymphs or adults of their respective insect hosts; in the case of *Herpetomonas donovani* and the parasite of Delhi boil it is passed in man.

The flagellate stage is characterised by the formation of a flagellum and the division and multiplication of the resulting flagellates. In *Herpetomonas donovani* and in the *Herpetomonas* of *Culex pipiens* in addition to this change many of the cells develop into rosettes by the consecutive division of their nuclei and blepharoplasts and on the formation of flagella from eight to twenty or more flagellates are produced. The formation of the flagellum in at least two of the species, *Herpetomonas donovani* and *Herpetomonas muscae domesticae*, is preceeded by the development of what appears to be a vacuole close to the blepharoplast, later the flagellum is seen lying in the vacuole and when the vacuole ruptures it is extruded. In the *Herpetomonas* of *Culex pipiens* and in *Herpetomonas lygaei* the flagellum first appears as a fine pink-staining filament in the achromatic space just anterior to the blepharoplast. None of these flagellates that I have studied have a double flagellum. The flagellate stages of the majority are found in the mid- and hind-guts of the nymphs or adult insects, that of *Herpetomonas donovani* in the mit-gut of *Cimex rotundatus*; the flagellate stage of the parasite of Delhi boil is not yet known.

The postflagellate stages are passed in the rectums of their adult insect hosts. The flagellates after becoming attached to the intestinal epithelium divide more than once and at the same time the free portion of the flagellum becomes detached while the intracellular portion is absorbed. The parasites then become encysted and are attached loosely in masses to the rectal epithelium. The cysts are passed out in the faeces of the insects and are again ingested by the larvae, nymphs or adults as the case may be. In blood-sucking insects infected with these flagellates the cysts are not ingested by the adults as they feed exclusively on blood, but are swallowed by their larvae or nymphs. The presence of harmless flagellates in mosquitoes, fleas and tsetse flies is thus readily explained.

I wish to record the occurrence of a small *Coccidium* in the alimentary tract of *Lygaeus militaris*, as far as I am aware these *Sporozoa* have not previously been recorded from the *Rhynchota*. In conclusion I have to thank Major Donovan, I. M. S., for painting the figures on the plate accompaying this paper.

Madras, December 1907.

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Explanation of plate.

Herpetomonas lygaei (Patton).

Fig. 1. Two oval cysts from the crop of *Lygaeus militaris*, note the nuclear membrane in the upper parasite.

Fig. 2. Parasite also from the crop showing commencing division, the blepharoplast is about to divide.

Fig. 3. Two parasites the result of simple longitudinal division, the nuclei and blepharoplasts in each parasite are about to divide again; the chromosomes have divided and passed to the sides and a pale line is seen passing through each nucleus.

Fig. 4. Four cells, the result of simple longitudinal division, in making the film they have become separated.

Fig. 5. Four parasites shewing commencing elongation the anterior ends are becoming pointed, the protoplasm has increased in volume and is more vesicular.

Fig. 6. Four spindle-shaped cells flagellating, the nuclei are commencing to divide, the blepharoplasts are situated close up to the nuclei shewing that each cell is preparing to divide longitudinally; the flagella are seen in all stages of development; note the characteristic pink staining anterior ends of the parasites.

Fig. 9. Group of seven parasites which have originated from four cells similar to those in Fig. 5. One has divided and half has become an adult flagellate, another has elongated and then divided, the third has divided again while the fourth is as yet unchanged. This is the commonest method of development of the groups of four cells.

Fig. 10. Four elongated cells similar to those shewn in Fig. 6, one shows the early formation of the flagellum. The large cell has a very distinct vacuole just anterior to the blepharoplast.

Fig. 11. An adult flagellate; note the position of the blepharoplast about 4 μ from the anterior end.

Fig. 12. An adult flagellate shewing commencing longitudinal division, the new flagellum having developed from a point close to the root of the original one.

Fig. 13. An adult flagellate shewing a further stage of longitudinal division, note the parasites beginning to separate at their anterior ends.

Fig. 17. Stout flagellate just prior to encystment; note the pink staining granules in the anterior end.

Fig. 18. Further stage of a similar parasite; most of the flagellum has broken off, the blepharoplast is close up to the nucleus as division is about to begin.

Fig. 19. First division before encystment, note the flagellum has split and a shredded portion joins the two halves; the chromatoid granules are well marked at the posterior end.

Fig. 20. The degeneration of the flagellum, only a few intracellular strands being left.

Fig. 21. Longitudinal division of a such a cell; the parasites are separating at their anterior ends.

Fig. 22. Longitudinal division of two similar cells; each has a well marked vacuole near the blepharoplast.

Fig. 23. Two smaller cells about to divide again, note the large nuclei and blepharoplasts.

Fig. 24. Group of four cysts attached by their anterior ends; note the large rod-shaped blepharoplasts which are very characteristic of this stage.

Fig. 25. Three cysts from the dried faeces of *Lygaeus militaris*, note the round blepharoplasts and the periplasts surrounding the parasites.

Herpetomonas donovani (Lav. and Mesn.).

Fig. 7. The two cells on the right are from a splenic blood smear and that on the left is from the peripheral blood of a patient suffering from severe diarrhoea, note this parasite shows four chromosomes in its nucleus.

Fig. 8. Large blue staining cell from the midgut of *Cymex rotundatus* (Signoret), the second day after ingestion; note the pink mass between the blepharoplast and the margin of the cell, it is the developing flagellum.

Fig. 14. Eight parasites, the result of multiple segmentation of two cells, taken from intra vitam splenic puncture; the cells are lying in the detached protoplasm of an endothelial cell.

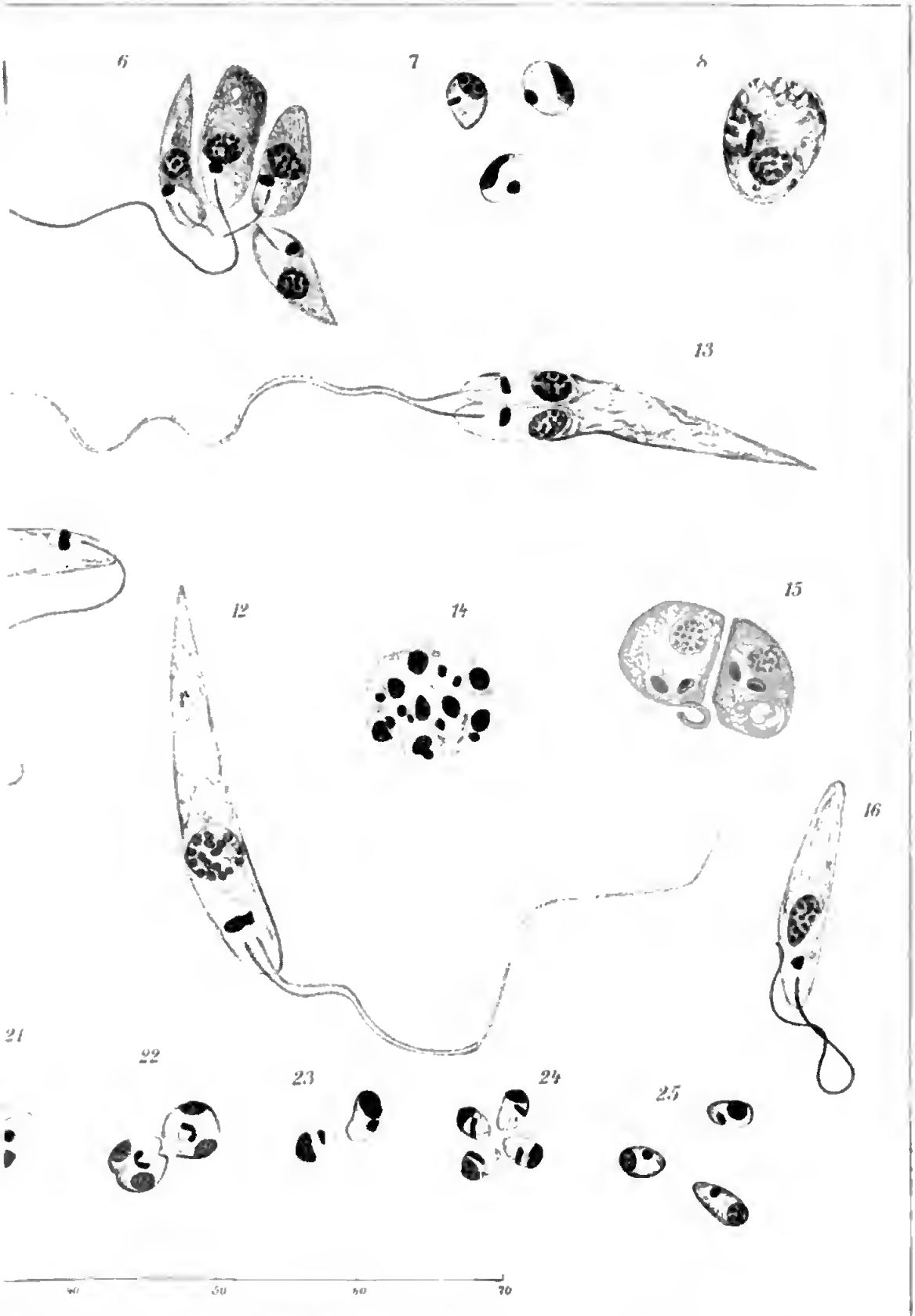
Herpetomonas muscae domesticae (Burnett).

Fig. 15. Two young cells shewing the method of formation of the flagellum; in the cell on the left the flagellum a single stout filament, is just extruded and is uncoiling; in the parasite on the right the flagellum is on the point of being extruded. Note the two blue staining bodies in the cells, probably diplosomes (?).

Fig. 16. A young stout flagellate, note the large blepharoplast and the single flagellum.

All the figures were drawn through a camera lucida with a Zeiss' No. 2 apochromatic objective, N. A. 1,40 and a Zeiss' compensating ocular No. 12 and are therefore magnified 2700 diameters.

W S Patton gez



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