HERPETOMONADS FROM THE ALIMENTARY TRACT OF CERTAIN DUNG-FLIES.

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(With Plate XIX and 4 Text-figures.)

Introduction.

In 1903 Léger pointed out that a flagellate apparently identical with *Herpetomonas muscae-domesticae*, Burnett, may occur in other species of flies frequenting the neighbourhood of houses. The species found to be infected were *Homalomyia scalaris*, F., *Pollenia rudis*, F. and *Theicomyza fusca*, Macq. Beyond certain slight variations of form, the parasites from the different hosts showed no noteworthy morphological deviation from the type species.

Roubaud (1908) has also recorded *H. muscae-domesticae* as occurring along with *Herpetomonas* (*Leptomonas*) *mesnili* from species of *Lucilia*, and with *Herpetomonas* (*Leptomonas*) *mirabilis* from *Pycnosoma putorium*.

The list of herpetomonads from insects steadily increases, and there is at present too great a tendency to regard each new herpetomonas "find" as a separate species peculiar to the host. Where the generic name of the host is used in forming the specific name of the parasite this custom is convenient enough, affording as it does a ready index of distribution: where the specific name is more fanciful, there is less to be said for it. In either case, until more of the known forms have had their life-cycles worked out, it must be admitted that the arrangement is artificial and should be looked on as tentative.

I have recently found flagellates resembling *Herpetomonas muscae-domesticae* in three dung-flies—*Scatophaga lutaria*, F., *Neuroctena anilis*,

Fallen, and *Homalomyia*, sp. (probably *H. corvina*, Verrall[1]). The material was sufficiently abundant to admit of my working out the life-cycle, and I found that the herpetomonads of these three flies were at each stage of their development morphologically indistinguishable from one another and from *H. muscae-domesticae*, so far as I have been able to study that species.

I regard *Musca domestica* and other non-biting flies frequenting similar feeding-grounds as subject to infection by a common flagellate. In view of the known polymorphism and great plasticity of herpetomonads, it seems more logical to refrain here from the multiplication of species, and to regard slight deviations from the *muscae-domesticae* type as no more than might be looked for in response to the slightly different environment[2]. One is tempted to doubt whether forms such as *Herpetomonas lesnei*, Léger, *Herpetomonas sarcophagae*, Prowazek, and *Herpetomonas* (*Leptomonas*) *drosophilae*, Chatton and Alilaire should really rank as distinct species[3], and whether *Herpetomonas* (*Leptomonas*) *mesnili*, Roubaud, and *Herpetomonas* (*Leptomonas*) *mirabilis*, Roubaud, are not slight variations of one peculiar form.

In studying the herpetomonads of flies it is necessary to keep in mind that one rarely finds more than one stage well represented in the gut of an individual insect. It follows therefore that without careful study of a sufficiently large number both of the adult flies and of their larvae, the account is too incomplete to be of much value except as a record of distribution.

So far as I am aware, Patton (1909) is the only observer who has succeeded in tracing out the life-cycle of a herpetomonad from a non-biting fly. I am glad to be able to substantiate his account from what I have seen in the three species of dung-flies that I examined. A rich material has enabled me to treat certain points in fuller detail.

Material and Methods.

The flies were caught during the summer months near a pond in the neighbourhood of Aberdeen, where they were usually found feeding on

[1] I am much obliged to Mr Percy Grimshaw for kindly identifying these flies.

[2] Alexieff (1909) makes similar observations on the variability of the flagellate parasites of amphibians.

[3] Chatton and Alilaire would of course object that their flagellate had but one flagellum and is therefore not a *Herpetomonas* in their sense of the name. I shall return to this point presently.

human excrement. The rate of infection was pretty high—about 60 %. The parasites were always confined to the alimentary canal. The larvae of *Scatophaga lutaria* and of *Homalomyia* from the same feeding-grounds were even more richly infected than the adult insects. In the autumn I took a large number of these larvae into the laboratory, and kept them under observation at a temperature of 60°—70° F., with a view to finding out what changes were undergone by the parasite during the insect's development. Most of the larvae pupated, and a number of flies came out after about three weeks.

I made attempts to cultivate the herpetomonads on agar-agar. The flagellates were taken from the gut of both kinds of larvae. They lived for two or three days on the new medium, but without multiplying, and in nearly every case assuming a rounded up or semi-encysted condition: the bacterial growth by that time had become so great that it was found impossible to isolate the flagellates in culture, and the attempt was abandoned.

The gut of the fly or larva was examined for parasites in the usual way—*i.e.* it was dissected out in a drop of normal salt solution and examined under the microscope. If the parasites were present their characteristic movements betrayed them at once, even under a low power. The gut was then divided into three portions, and teased out. For Giemsa preparations I tried fixation with osmic acid vapour and with vapour of 40 % formation, as well as the usual dry method. For adult flagellates I found the dry method exceedingly good, but for encysting and encysted stages the wet method gave much less deformation.

While admitting the great value and brilliance of the Romanowsky stains, I am of opinion that a more reliable cytological stain, such as iron-haematoxylin, should be used as a control wherever possible. Herpetomonads stained with iron-haematoxylin often present a very different appearance from those stained with Romanowsky stains (either after dry or wet fixation)—a circumstance that is at least suggestive in view of the interpretations placed on certain highly dubious structures described by authors from Romanowsky preparations.

For iron-haematoxylin preparations, I spread the gut contents in a thin film on a cover-glass, and dropped it on the hot fixative (Schaudinn's sublimate-alcohol) after the method recommended by Schaudinn. The stain did not always act very satisfactorily, but occasionally, when I got a good differentiation, the result was excellent and was an interesting commentary on the Giemsa preparations.

I place great importance on the study of the living organism. I tried neutral red and methylene blue as *intra vitam* stains, but did not find them helpful: with good lighting, all the essential structures are quite clear on the unstained flagellate.

Life-cycle of Parasite and Mode of Infection of Host.

The parasites were always found in the alimentary canal of the fly. Careful search failed to show any infection of the ova, nor were flagellates ever found in or near the ovaries. This points to the infection being "casual"—*i.e.* by accidental ingestion of the cysts with the food. The high percentage of infection is scarcely surprising, considering the feeding habits. The flies crowd thickly over the surface of the dejecta, abandoning it only when startled, and returning as soon as possible to continue their meal: their food must quickly get thoroughly contaminated with herpetomonad cysts from their droppings. In this connection it is interesting to note that the *Neuroctena*, which is the most heavily infected, is also the most sluggish, and rarely flies to any distance from its feeding-grounds: if not actually feeding, it may usually be found in the close vicinity on the leaves of low-growing shrubs. The flies examined were mostly caught in the months of June, July and September. As they were frequently seen *in coitû* upon their feeding grounds, I thought it not impossible that their larvae would also harbour the parasite. This proved to be the case. In the end of September the patches of excrement on which the flies had been caught were found to be a moving mass of infected dipterous larvae, mainly of *Scatophaga lutaria* and *Homalomyia*, sp. As was to be expected, the infection in the larvae, remaining as they do on their feeding-grounds throughout, was both more frequent and very much more intense than in the adult insects. As Patton has suggested, the degree of infection probably depends directly on the number of cysts ingested. Examination of larvae of all sizes showed me that infection may occur at any period of larval life, even very young larvae being richly infected. It is difficult to find the stages showing formation of the adult flagellate from the ingested cysts. When such stages occur they are found in the upper end of the mid-gut. Enormous multiplication of the flagellate takes place throughout the length of the mid-gut, so that in places the dark-brown gut contents are almost replaced by a seething mass of parasites. When the larva stops feeding previous to pupation, the flagellates begin to round up and collect in the hind-gut, where they encyst. The

majority are passed out with the faeces, but a few half-encysted forms may be found attached to the disintegrating gut-epithelium during the metamorphosis. Of a number of flies I examined on emerging from their pupal cases and before they had fed, only one was found to contain a few half-encysted flagellates in the hind-gut.

I think it likely therefore that the flies reinfect themselves when they begin to feed. *The infection of the adult fly, is probably not to any extent directly continuous with that of the larva, but is freshly acquired.* The cycle in the fly follows much the same course as in the larva. Pre-flagellate stages are infrequent, but when they occur are found in the mid-gut along with the adult flagellates. Rounding up stages occur in the intestine. By far the most common condition was that in which the rectum was full of enormous numbers of encysting and encysted forms. The cysts were found in the faeces. All the stages were never seen at one time in one host.

It will be seen from the above that the mode of infection and the behaviour of the parasite in the adult flies of *Scatophaga lutaria*, *Neuroctena anilis*, and *Homalomyia* agree with Patton's account of *H. muscae-domesticae* in *Musca domestica*. That author, however, while stating that infection is probably not hereditary, gives no information regarding larval infection, and one is left to conclude that only the adult flies harbour the parasite. Prowazek (1904) found *H. muscae-domesticae* in the ovaries of *Musca domestica*, and records larval infection.

I have examined a considerable number of larvae of *Musca domestica* and have never found a trace of infection—a great contrast to the condition of things in *Scatophaga* and *Homalomyia*, where the larvae are seldom quite free from the parasite.

DESCRIPTION OF PARASITE.

The morphology of *Herpetomonas muscae-domesticae* has received much attention from many skilled observers. The flagellates forming the subject of the present paper agree closely with the type-species, but in looking through a large material, I have noted certain points that seem to me worth recording.

A. *Stained material.*

Patton has divided the life-cycle of herpetomonads and allied forms into three periods—(1) pre-flagellate, (2) flagellate, and (3) post-flagellate. I propose to follow this arrangement.

1. *Pre-flagellate.* This stage was found occasionally in small groups and clusters in the mid-gut of the fly, and more rarely in the upper end of the mid-gut of the larva. Its comparative infrequency suggests that flagellation is a rapid process. I tried to induce freshly hatched-out flies to feed on infected food-material, in the hope of studying more closely the details of flagellation, but this attempt was unsuccessful. Pre-flagellate stages in Giemsa stained preparations are small round or oval bodies—$3\mu \times 2{\cdot}5\mu$ to $4\mu \times 3\mu$ (figs. 1—8)—the cytoplasm staining intensely blue, the circular nucleus a uniform deep red: the kineto-nucleus[1] is a small deeply-staining rod-shaped body, either immediately anterior to the nucleus or slightly to one side of it: numerous smaller chromatin-like granules are scattered through the cytoplasm. The future position of the flagellum is usually already indicated by a rose-pink area extending from the kineto-nucleus to one end of the cell. Occasionally the flagellar root can already be seen forming within this flagellar vacuole as a darker-staining strand. The whole cell is in some cases surrounded by a definite cyst membrane, staining deep pink, but more often this has already dissolved. The flagellum comes to the exterior as a delicate pink "brush," which rapidly takes on the more definite appearance of a stout flagellum, and proceeds to elongate. Division may take place even at this early stage (see fig. 8).

2. *Flagellate.* This was by far the most common stage in the larva, where flagellates were found in enormous numbers in the mid-gut; in the adult fly they were not so abundant, but when present, were always in the mid-gut.

The adult flagellates in the larva differ somewhat from those in the fly (cf. figs. 11 and 26). The flagellate in the fly (fig. 11) differs in no essential from *H. muscae-domesticae,* the appearance of which, in Giemsa preparations, is well-known. The average dimensions of a full-grown flagellate are $25\mu \times 2{\cdot}5\mu$ (not reckoning the flagellum). The body is roughly cigar-shaped, slightly blunt behind, and furnished anteriorly with a long (30μ), relatively thick flagellum. The flagellum arises from the neighbourhood of the kineto-nucleus, but its actual origin seems to be in a minute granule in front of that body: to this granule the term "blepharoplast" might be more consistently applied. The root of the flagellum within the body of the organism is about 4μ long and it is

[1] I propose to follow the nomenclature of Minchin (1908) which has since been adopted by others. The term "blepharoplast," if used at all, ought in this case to be reserved for the granule at the base of the flagellum.

sometimes markedly thickened. In Giemsa preparations there is often a small granule at the point of emergence of the flagellum: this does not appear in staining with iron-haematoxylin, and is perhaps due to a deposit of the Romanowsky stain. The rhizoplast and flagellum are very much thicker in Giemsa than in iron-haematoxylin preparations: with regard to the flagellum, the Giemsa certainly gives the proportions more as they are in life. The kineto-nucleus is a tongue-shaped or rod-shaped body, placed about 6μ from the anterior end. In Giemsa preparations it is very large and conspicuous, measuring as much as $2\mu \times \cdot 8\mu$: it stains a dark rich red, in the midst of which it is usually possible to make out a more deeply-staining central body. With iron-haematoxylin (figs. 24 and 25), the kineto-nucleus appears smaller, stains uniformly and tends to be circular or rod-shaped, rather than tongue-shaped. The tropho-nucleus lies posterior to the kineto-nucleus, about half-way along the body. It is oval or roughly circular, measuring about $3\mu \times 2\mu$. In Giemsa preparations it stains pinkish-red, and almost always appears to consist of a fine reticulum on which small chromatin granules are distributed. Iron-haematoxylin gives a very different picture: here the stain is mainly taken up by the central karyosome, and there is a well-marked nuclear membrane; sometimes there is a faint net-work suggested between the karyosome and the membrane, but of chromatin granules there is no hint. In Giemsa preparations the cytoplasm stains a clear blue, tinged with purplish in places, and fading into pink in the neighbourhood of the flagellum and kineto-nucleus. Occasionally vacuolated areas appear, and not infrequent, especially in the smaller flagellates, is a clear sinuous line visible between the kineto- and tropho-nuclei and descending thence into the posterior part of the cell. This line corresponds in position with the spiral "Doppelfaden" described by Prowazek in *H. muscae-domesticae*, but I never succeeded in staining it, and am quite at a loss as to its true nature. I have never seen any hint of a cytostome in these flagellates, and am not inclined to regard it as an "intestinal canal[1]." After Giemsa's stain the cytoplasm appears full of deeply-staining, chromatin-like granules, especially numerous in the region posterior to the tropho-nucleus. That these are not composed of chromatin, however, is well seen on staining with iron-haematoxylin, which the cytoplasm takes on very uniformly, showing a hint indeed of reticular structure but very rarely containing anything that could be called granules. It is probable that

[1] Cf. Léger (1902), *Compt. rend. Acad. Sc.*, CXXXIV. p. 781.

these bodies are of the nature of reserve food-stuff or of excreta. I was unable to detect the posterior diplosome of Prowazek.

The above is a description of the adult flagellate from the fly: it is well to compare with it the corresponding stage from the larva (fig. 26). Here individuals are smaller, the rhizoplast is much shorter, the kineto-nucleus is smaller, ·8μ, stains more evenly, and tends to be circular, the tropho-nucleus is placed further forward in the body, the granules in the cytoplasm are few in number, and restricted to the extreme posterior end.

The interesting experiments of Miss Porter (1909) leave no doubt that these granules in the cytoplasm of herpetomonads are an expression of the degree of metabolic activity, and must not be taken as an indication of sex. This author states that *H. jaculum* in a highly nutritive medium developed large numbers of refractive granules, and when deprived of food, proportionately few. In the case of the flagellates at present under discussion, the smaller size and clearer cytoplasm of the parasites from the larval gut may well be explained by their much denser crowding there and the relatively small food supply per individual.

Division is longitudinal. The rhizoplast usually divides first, and it can be seen that each half carries with it a basal granule (figs. 12 and 23). I have never found stages in which the new rhizoplast was growing up from the basal granule. I am therefore inclined to believe that to this extent there is splitting of the flagellum root. Beyond this point, however, I consider that the second flagellum is a new formation, and does not arise by splitting of the original flagellum[1]. Figs. 12 to 17 illustrate this very well. For some time the two flagella lie closely together, and this may last until the new flagellum has almost reached the length of the first, producing the deceptive appearance of a bi-flagellate organism. This is most frequently the case in the parasite in the fly. In the larva, for some obscure reason, the splitting of the flagellate body usually takes place at an earlier stage in the formation

[1] There is great diversity of opinion as to the mode of origin of the new flagellum in *H. muscae-domesticae* and allied forms. Prowazek (1904) describes the new flagellum in *H. muscae-domesticae* as growing up from the divided basal granule, which he figures at the point of emergence of the flagellum: from this later on in like manner a new rhizoplast grows down. Patton (1909), referring to the same flagellate, finds that longitudinal fission begins with a splitting of the flagellum root. Miss Porter (1909) states that as in *H. jaculum* she has "watched the flagellum of the living *H. muscae-domesticae* divide in two." My own observations on this parasite are in agreement with those of Berliner (1909) on *H. jaculum*.

of the second flagellum. Thus there are comparatively few bi-flagellates seen (fig. 28); much the most common appearance is that in figs. 18 and 19, where the daughter organisms are hanging end to end, the one with a long flagellum, and the other with no more than a short stump yet formed. During the splitting of the rhizoplast, the kineto-nucleus can be seen to undergo division. In the iron-haematoxylin preparations it frequently appears kidney-shaped or tri-lobed at this stage, but an attenuated dumb-bell shape is also seen. Within the kineto-nucleus in Giemsa preparations the central body can be seen dividing in dumb-bell fashion. I have never been able to make out any karyokinetic figures in the tropho-nucleus as stained with Giemsa: it stains a uniform dull red at this stage, or shows the same appearance as in the resting stage. With iron-haematoxylin, however, it can be seen that at a very early stage the karyosome becomes elongated in the direction of the long axis of the flagellate, and the nuclear boundaries become vague. Gradually the ends of the now dumb-bell shaped karyosome recede further and further from one another, until they are only connected by a very faintly-staining strand, which then breaks (figs. 21—24). I never saw any unequal division, but as the exceedingly rapid multiplication proceeds, it is not surprising that the daughter flagellates tend to get smaller and smaller in size. At a certain stage, possibly determined by "depression" following on long-continued multiplication, the organisms proceed to encyst.

3. *Post-flagellate.* The attachment of the herpetomonad flagellates to the epithelium of the hind-gut, their rounding up to form "gregarine" stages and finally cysts, are processes that have often been described. I have little new to add, though it was very common to find the rectum of *Neuroctena* simply swarming with encysting herpetomonads. I would point out, however, that here, as elsewhere, iron-haematoxylin gives a very different picture from Giemsa (cf. figs. 34 and 37 with figs. 39 and 40). The kineto-nucleus appears much smaller, and the nucleus, instead of staining diffusely or appearing to have all the chromatin concentrated around its margin, shows a distinct central karyosome surrounded by a clear space bounded by a nuclear membrane. The numerous "chromatoid" granules in the cytoplasm of Giemsa preparations are here practically absent. A word about the disappearance of the flagellum. In some species of *Herpetomonas* the flagellum is apparently "cast off" in the last stage of encystment: in others, the portion external to the cell fades away and is absorbed. In his account of *H. muscae-domesticae,* Prowazek (1904) speaks of the flagellum as

being withdrawn into the cell. Patton (1909), also referring to *H. muscae-domesticae*, says the flagellum degenerates and is shed. Rosenbusch (1910) describes the withdrawal of the flagellum into the cysts of *Crithidia muscae-domesticae*, Werner. In the flagellates from *Neuroctena* and *Homalomyia* the kineto-nucleus travels back, taking the end of the flagellum with it, till it comes to lie alongside or even behind the tropho-nucleus. In this way the greater part of the flagellum is actually drawn into the interior of the cell, where it is afterwards absorbed (see figs. 33 to 37). The position of the kineto-nucleus at these stages produces a crithidia- or even a trypanosome-like appearance, but there is, of course, no hint of an undulating membrane[1]. Frequently the connection between the kineto-nucleus and the end of the flagellum is lost, and the kineto-nucleus wanders into a different part of the cell.

On one occasion only did I see a glutinous cyst-wall formed, such as has been described by Prowazek and others (fig. 38). It appeared as a faint pink cloud round a large circular cyst, in the centre of which was the tropho-nucleus, surrounded by a number of small, deeply-staining granules.

The final stages of encystment were not often met with. They are small, oval or circular bodies ($3\mu \times 3{\cdot}5\mu$—$4\mu \times 2{\cdot}5\mu$), the cytoplasm staining a deep blue with Giemsa, and containing a dark-red tropho-nucleus and a kineto-nucleus: a reddish cyst membrane could sometimes be made out. Fig. 41*b* shows such a cyst stained with iron-haematoxylin.

Conjugation.

Curious flagellates were occasionally found in Giemsa preparations both from the larva and from the fly, where the tropho-nucleus had disappeared, leaving the cytoplasm staining rose-pink (figs. 29—31). Otherwise these forms seemed to be quite normal, though some of them were very small (fig. 30). I was interested to notice in one case (fig. 29) that division could apparently take place in the virtual absence of the tropho-nucleus. The kineto-nucleus was dividing, as was also the base of the flagellum; two minute, faintly-staining dots alone indicating the normal position of the divided tropho-nucleus.

Such individuals would be regarded by certain authors as male gametes. I have watched the living organisms for hours at a time, and have never seen any sign of conjugation. For my part, I consider these

[1] One cannot help being struck by the resemblance of some of these stages to the flagellate of *Crithidia muscae-domesticae* as figured by Werner.

forms as degenerate, and without any sex significance. I see that Berliner (1909) also interprets as degenerate similar flagellate individuals of *Herpetomonas jaculum*, Léger. I must admit, however, that on two occasions I met with appearances in my Giemsa-stained material that might be looked on as the beginning of conjugation of male and female individuals. Fig. 20 shows such a case. The large half-encysted individual, with pale-blue vacuolated protoplasm, the kineto-nucleus indistinguishable from other darkly-staining granules in the cell, and with the tropho-nucleus situated to one side and apparently extruding chromatin, might be the female, while the small flagellate form alongside, without tropho-nucleus, and with its cytoplasm staining pale-pink, might be the male gamete. On the other hand, the juxtaposition of these apparently sexual individuals might be mere chance. If conjugation be a regular occurrence in this group of flagellates, it is at least surprising that we have hitherto had so little convincing proof of the act itself. Authors who arbitrarily fix on "male" and "female" characters do not give sufficient consideration to the effects known to be produced in protozoa by periods of long continued multiplication[1]. Nuclear hypertrophy, nuclear absorption, the production of undersized individuals, and the suppression of cell-division, resulting in the formation of abnormally large individuals, are all well-known signs of degeneration in protozoa, and have to be reckoned with. It is true, of course, that conjugation may be resorted to as a means of restoring the karyoplasmic equilibrium, but I hold that in the case of the herpetomonads and their allies, we have insufficient proof of this. It should be remembered that encystment is another process that may be made use of by protozoa for self-regulation after periods of prolonged multiplication and consequent depression. This regulation may be effected by extrusion of chromatin

[1] In Hindle's recent very interesting work (1909) on *Trypanosoma dimorphon*, he tentatively distinguishes, on morphological grounds, male, female, and indifferent forms. It is interesting to notice that the "female" is formed from the "indifferent" trypanosome by a process strongly suggestive of the beginning of cell degeneration. The sluggish movements, "stumpy" form, very dense protoplasm containing chromatoid granules, the very large, densely-staining tropho-nucleus, and the frequent extrusion of chromatin from the nucleus, are all features that one is accustomed to associate with cell degeneration. Further, these forms do not occur during the earlier stages of the attack; that is to say, they appear only after the organism has been multiplying by division for some considerable time, and when we might reasonably expect to find some sign of "depression." Hindle himself states that these "female" forms may be found "in all stages of degeneration" in the blood of the rat, but he evidently considers that this would not occur if the conditions were favourable for conjugation: such conditions he thinks might be offered by an intermediate host.

within the cyst, or by the more elaborate processes of autogamy with their attendant karyoplasmic readjustments. The figures and descriptions given by authors (Patton, Berliner, 1909), though not conclusive, suggest that any sexual process may be looked for in the cyst, and is possibly an autogamy.

B. *Observations on the living organism.*

The study of stained material alone is often misleading, and should always be supplemented, where possible, by observation of the living organism. I cannot help thinking that, if this method had been resorted to in the first instance, *Herpetomonas* would now have been described as having a double flagellum[1].

In the case of the fly, one is often able to keep the flagellates under observation in their natural medium, for the wall of the gut is sufficiently transparent to admit of accurate observation of its contents. In the larva this is usually not possible, as there is a great abundance of dark-coloured semi-fluid material in the gut. It is very interesting to watch the flagellates attach themselves to the wall of the intestine, and collect there in groups and clusters during the early stages of the encysting process.

More often I simply teased out the gut-contents in a drop of normal salt solution, and sealed the coverslip well down with wax. The flagellates live under these conditions for as long as 36—40 hours. The adult flagellate moves with extreme rapidity, the body rigid and vibrating like a compass needle from a point about half-way down its length, under the ceaseless lashing of the stout flagellum[2]. Very conspicuous are a number of strongly refractive granules in the posterior end of the body. With good lighting the tropho- and kineto-nuclei and the rhizoplast can also be clearly seen. I have frequently watched the process of longitudinal division. The second flagellum can be seen as a short process springing up at the base of the old flagellum: it increases rapidly in length, and follows the movements of the old flagellum exactly, as though the two were enclosed within the same sheath, or else simply adherent to one another. In this condition they may remain for some time. The body of the animal now begins to split longitudinally, starting from the anterior end, and the two flagella

[1] Berliner (1909) and Porter (1907) have given accounts of the division of *Herpetomonas jaculum in vivo*.

[2] The movements of *Herpetomonas* have recently been well described by Porter (1909).

are torn apart: by now they are usually so nearly equal in length that their independent movements tend to separate the daughter flagellates quickly from one another. This is the condition of things in the parasite from the fly. In that from the larva, as I have said, longitudinal splitting of the body often takes place before the new flagellum has grown up very far (text-fig. 1). The consequence is that the two daughter flagellates tend to hang together by their ends until such time as the new flagellum has grown sufficiently long and strong to work against the old one and so effect the separation. It was only by studying these processes in the living organisms that I came to understand the apparent rarity of the final stages of division in the fly, and their great abundance in the larva.

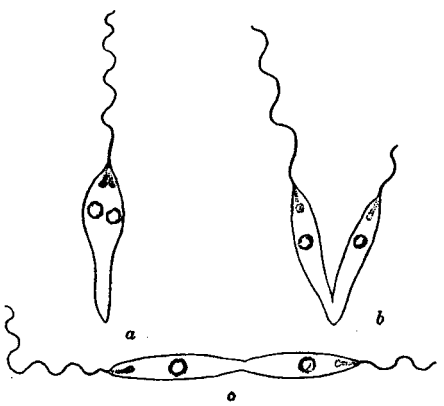

Fig. 1.

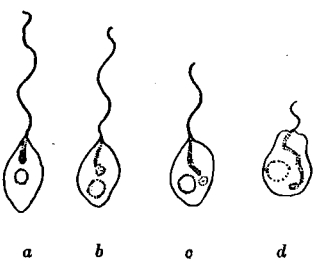

Fig. 2.

After two or three hours the movements of the flagellates have become very much slower, and it can be seen that the body is shorter and more rounded, and has lost much of its rigidity. The flagellum has also become shorter, partly through actual loss of length, but chiefly through being drawn into the body, where it can be traced as a refractive line running back to the kineto-nucleus, now situated near the anterior border of the tropho-nucleus (text-fig. 2). This withdrawal of the flagellum continues until a mere stump is left protruding, and the kineto-nucleus is generally lost to sight among the refractive granules in the hinder part of the now pear-shaped cell. All forward progression of the organism gradually ceases, but the flagellar stump continues to move jerkily to and fro for hours. I have seen division take place at this stage, the longitudinal split starting posteriorly and running

forward, and the flagellum going over to one of the daughter halves without a second flagellum being formed: the halves hung together for a long time and I did not see them finally separate. The organisms all died at this stage, and I never saw the encystment completed.

While the flagellum is being withdrawn, and the body is assuming its pear-shaped form, the cytoplasm becomes rather sticky, and I noticed a strong tendency for flagellates to adhere to one another side by side, either in groups or in pairs. This was often suggestive of the preliminaries for conjugation, but though watched for a long time, these chance groupings never went any further: the organisms either separated after a brief struggle, or else died. It is not infrequent for individuals in this condition to become bent on themselves, the adjacent portions of the body-wall then adhering to one another (see text-fig. 3, *a* and *b*) and producing an appearance not unlike the first stages in the rounding off

Fig. 3.

Fig. 4.

of *Trypanosoma vittatae* as observed by Robertson (1909). I never found, however, that they developed further. Another instance of the "stickiness" of the cytoplasm was sometimes shown, where the flagellum had got bent back along the body, and adhered firmly to it. Where the flagellum was still pretty long and active, its efforts to continue movement under these abnormal conditions resulted in the gradual lifting up from the cytoplasm of the body of a sort of *pseudo* undulating membrane (text-fig. 4, *a* and *b*). The movements of this structure were so exactly those of a true undulating membrane that it was difficult to believe one was not dealing with a small, blunt trypanosome: observation of the earlier stages of course explained the true nature of the case.

I starved several young larvae of *Homalomyia*, and on examining them found that the parasites had all collected in the hind-gut and were in process of rounding up.

Some of the flagellates that I tried to cultivate on agar-agar continued to live for three or four days, but showed no signs of multiplication. They all assumed the form of the flagellates under the sealed-down cover-slips—*i.e.* the body had become pear-shaped and the kineto-nucleus had travelled back into the body, drawing the flagellum with it.

HERPETOMONAS OR LEPTOMONAS?

I believe that the above account throws light on the value of certain so-called generic characters brought forward in the recent discussion on the classification of the herpetomonads and their allies.

It is necessary to recapitulate briefly. From his observations on the flagellate of *Musca domestica*, Prowazek was led (1904) to state that the genus *Herpetomonas*, Kent, possessed a double flagellum.

Patton (1909) showed clearly that this view was erroneous, the two flagella occurring only in individuals in course of longitudinal division. In support of this he mentioned that if a sufficient number of flies be examined, it will be found that, while in some of them almost all the flagellates have a double flagellum, in others the majority have a single flagellum. Further, in tracing out the life-history he showed that the pre- and post-flagellate stages have only one flagellum. Patton has described many other herpetomonads from various insect hosts, and these he finds fall in line with the flagellate of the fly. He therefore includes them all under *Herpetomonas*, that is, a trypanosomatid having a single flagellum, and with the kineto-nucleus placed some distance in front of the tropho-nucleus in the adult form. Donovan (1909) and Porter (1909) confirm Patton's statements.

Prowazek (1909) repeats his statements, and still holds to the view that *Herpetomonas, sensu stricto*, is a bi-flagellate. Still more confusion has recently been brought in by the repeated attempts of certain of the French school—notably Chatton, Alilaire, and Roubaud—to support Prowazek's view by splitting up the genus *Herpetomonas* into two. They would revive *Leptomonas*, Kent for such forms as have one flagellum and no rhizoplast, reserving *Herpetomonas* for those with a double flagellum and a rhizoplast.

These authors are very doubtful about the value of Patton's definitions of the genera *Crithidia* and *Herpetomonas*, contending that the presence of an undulating membrane and the relative positions of the kineto- and tropho-nuclei are features too variable to be depended on. Chatton and Alilaire (1908—1909) prefer to base their classification

on other characters "d'acquisition ancienne et paraissant actuellement soustraits a l'influence du milieu." As such they select the number of the flagella, and the presence or absence of a rhizoplast[1].

My observations on the herpetomonads in dung-flies are in accordance with those of Patton. The formation of the second flagellum is the immediate forerunner of longitudinal division: in the flagellate as it appears in the larva this is still more evident. The bi-flagellate appearance simply depends on the time at which the longitudinal split occurs,—in the parasite in the larva it is, as I have said, usual to find splitting at a very early stage, and bi-flagellates are therefore rare. The cause of this different behaviour in the fly and in the larva is obscure, but I regard it as an expression of the effect of a slightly different medium on the same organism.

Then as to the second "constant" *Herpetomonas* character—the presence of a long rhizoplast: I have showed that the flagellates in the fly are usually provided with a long, well-marked rhizoplast (the thickness, however, depending a good deal on the stain employed), while in the flagellates from the larva the root of the flagellum is very much shorter, and is often no thicker than the flagellum itself[2]. In certain Giemsa preparations the flagellar root does not stain definitely, but appears as a diffuse pinkish area: such cases seem comparable to Chatton's description and figures of *Leptomonas agilis*, but may merely be due to faulty staining (fig. 28).

If the classification of Chatton and Alilaire be accepted, then the only logical conclusion is that I am here dealing with two different parasites—a *Herpetomonas* from the fly, and a *Leptomonas* from the larva. I prefer to regard the parasite as the same throughout, and I consider that the mere fact that the rhizoplast may assume such different appearances under different conditions, does away with its value in classification.

[1] The authors do not explain how those characters are useful with regard to *Crithidia*, which is the genus particularly offensive to their logical sense.

[2] For my part, I am inclined to regard thickening of the flagellum root, *i.e.* "a well-marked rhizoplast," as the beginning of another division. Supposing that the splitting of the flagellum root is the first step in longitudinal fission, it may often happen that a second division is preparing before the first is completed.

The inconstancy of the rhizoplast is also indicated by the fact that two such careful observers as Berliner and Porter working independently on *H. jaculum* in 1909 are entirely disagreed as to whether a rhizoplast is present in this form or not.

SUMMARY.

1. *Musca domestica* and other non-biting flies frequenting similar feeding-grounds, are probably all liable to infection with a common flagellate. The great variability of this form is shown on comparing the flagellates in the larvae with those in the mature flies of *Homalomyia*, sp. and of *Scatophaga lutaria*.

2. Infection is casual—*i.e.* by the mouth. In the case of the dung-flies examined, the larvae ingest faecal matter infected with herpetomonad cysts: the cysts develop into flagellates in the mid-gut, where they multiply with great rapidity: towards the close of larval life, when the larva stops feeding, they round up in the hind-gut, and are for the most part passed out as cysts. A few survive the pupal stage in a half-encysted condition, but it is probable that the infection of the adult fly is usually freshly acquired. The cycle in the fly is similar to that in the larva, and is in agreement with Patton's account of *Herpetomonas muscae-domesticae*. The parasite was never found in the ovaries or ova. Patton's suggestion that the degree of infection depends directly on the number of cysts ingested, is borne out by the much higher rate of infection in the larvae than in the flies: this is not surprising when we remember the complete restriction of the feeding larva to the infected area.

3. It is important to use some reliable cytological stain such as iron-haematoxylin as a control to Romanowsky stains where possible, seeing that very different results are sometimes given by the two methods.

4. The apparent double flagellum is produced in the course of longitudinal division. The new flagellum grows up alongside the old, and is not merely split off from it: this is best seen in the flagellate from the larva, where the body of the organism usually divides at an earlier period than in the fly. Study of the living flagellate is necessary to a clear understanding of the process of division.

5. I have seen no conjugation in the living herpetomonads. Occasionally flagellates were met with in Giemsa preparations devoid of a tropho-nucleus. Such individuals might be regarded as male gametes; it is more likely that they are simply degenerate forms. Sufficient consideration is not given to the possibility of degeneration in richly-nourished, rapidly multiplying protozoa, such as the trypanosomes and their allies.

6. In encystment the flagellum is not cast off bodily, but is drawn down into the cell by the kineto-nucleus, which moves to a position either alongside of, or posterior to the tropho-nucleus. In this way apparent *Crithidia* or even trypanosome forms are produced, but there is no hint of an undulating membrane. Small blunt "trypanosomes" were also produced occasionally by adhesion of the flagellum to the body-wall in the "stickiness" resulting from confinement under a cover-glass: the efforts of the flagellum to free itself raised up an undulating membrane, and produced a very deceptive appearance.

7. The early stages of encystment could be induced by keeping the flagellates under waxed-down cover-slips, where they would continue to live for 30—40 hours. Similar results were got by transferring flagellates to agar-agar, or by subjecting the larval host to starvation for a day or two.

8. From what I have seen, I do not agree with Chatton and Alilaire's suggestion to divide the genus *Herpetomonas* into *Leptomonas* and *Herpetomonas* proper. I do not think that these authors are justified in regarding the double flagellum and the presence of a long rhizoplast as generic characters.

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EXPLANATION OF PLATE XIX.

All the figures were outlined with Zeiss' drawing-apparatus (Abbé), using Zeiss $\frac{1}{12}$" achromatic objective and ocular 4.

Figs. 1—20 and 26—38 are stained with Giemsa's stain, figs. 21—25 and 39—41 with iron-haematoxylin.

Figs. 1—3. Pre-flagellate stages of herpetomonad from dung-flies. Fig. 1 is from mid-gut of larva of *Homalomyia*, sp.?, figs. 2 and 3 from mid-gut of fly of *Homalomyia* and of *Neuroctena anilis* respectively. Giemsa.

Fig. 4 (*a* and *b*). Pre-flagellates from mid-gut of flies of *Scatophaga lutaria* and *Neuroctena*, showing flagellum forming. (*a*) is surrounded by a cyst-wall: in (*b*) this has disappeared. Giemsa.

Figs. 5—8. Further stages in formation of the flagellum. Giemsa.

Fig. 9. Small flagellate. Giemsa.

Fig. 10. Flagellate in which the body has not yet assumed the characteristic form. Giemsa.

Fig. 11. Adult flagellate from mid-gut of fly (*Homalomyia*) showing single flagellum and well-marked "rhizoplast." Giemsa.

Figs. 12—16. Stages in longitudinal division of flagellates from flies of *Homalomyia* and *Neuroctena*, showing splitting of rhizoplast, upgrowth of second flagellum, and division of nuclei. Giemsa.

Figs. 17—19. Further division stages from mid-gut of larva (*Homalomyia*) to illustrate precocious splitting of the body, with the resulting end-to-end arrangement of two individuals, one with a short and one with a long flagellum. Giemsa.

Note:—Owing to the colours employed in the original drawing, the nuclei in Fig. 17 appear light instead of dark.

Fig. 20. Sexual individuals in conjugation? From rectum of larva (*Scatophaga*). Giemsa.

Fig. 21. Flagellate from mid-gut of larva (*Homalomyia*) showing tropho-nucleus in process of division. Iron-haematoxylin.

Figs. 22—24. Further stages in division of flagellates from fly (*Neuroctena*). Note relatively small kineto-nucleus, well-marked tropho-nuclear karyosome, and absence of "chromatoid" granules in cytoplasm. Iron-haematoxylin. Cf. figs. 12—16.

Fig. 25. Adult flagellate from mid-gut of fly (*Neuroctena*). Iron-haematoxylin. Cf. fig. 11.

Fig. 26. Adult flagellate from mid-gut of larva (*Homalomyia*) showing small kineto-nucleus, short rhizoplast, and scarcity of "chromatoid" granules in cytoplasm. Giemsa. Cf. fig. 11.

Figs. 27 and 28. Early division stages in flagellates from larva (*Scatophaga*). In fig. 28 the rhizoplast is practically absent, the second flagellum has grown up almost to full length, and the tropho-nucleus shows a karyosome. Giemsa.

Figs. 29—31. Flagellate individuals without tropho-nucleus. Fig. 29 from mid-gut of fly (*Neuroctena*) and figs. 30 and 31 from hind-gut of larva (*Homalomyia*). In fig. 29 longitudinal division is taking place. Giemsa.

Figs. 32—37. Stages in process of rounding up and encystment in hind-gut of fly. Figs. 34—37 show gradual withdrawal of flagellum and migration of kineto-nucleus into posterior portion of cell. Giemsa.

Fig. 38. Large vacuolated cyst with thick, irregular cyst-wall. Giemsa.

Figs. 39 and 40. Encysting stages. Iron-haematoxylin. Cf. figs. 34—37.

Fig. 41. Post-flagellates from hind-gut of fly (*Homalomyia*). Iron-haematoxylin.

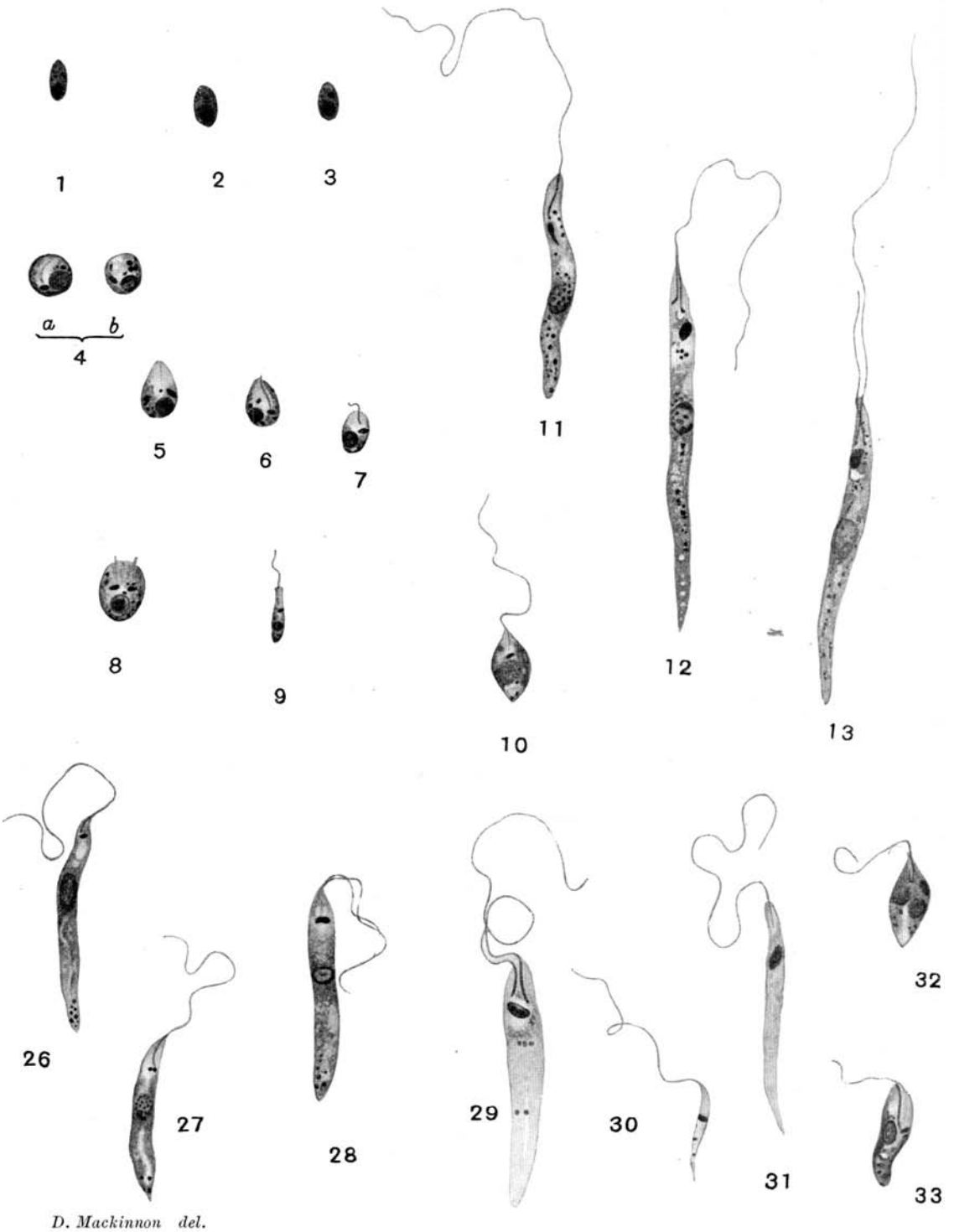

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PLATE XIX