SOME FLAGELLATE FORMS FOUND IN THE INTESTINAL TRACTS OF DIPTERA AND OTHER GENERA

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1. Introduction.

During our researches in 1903 concerning the life-history of Piroplasmata found in the blood-corpuscles of man and some of the lower animals, we were led to examine microscopically the contents of the intestinal tracts of large numbers of the various genera of flies obtainable in this portion of the United Provinces. As a result of these investigations one conclusion arrived at was, that but little was known concerning the parasites, especially the flagellate varieties, which find a habitat and develop in the intestines of the diptera, and that a knowledge of these forms was of primary importance to our work. Accordingly a prolonged study of the contents of the intestines of the commonest forms of diptera was first undertaken and concurrently our inquiry included other species of flies as they were met with.

Our original manuscript had to undergo modifications in several respects, after the receipt of a copy of Prowazek's communication in February, 1905.

The account of the anatomy of the common house fly was withdrawn as unnecessary, and the nomenclature more especially has been altered so as to fall in with that utilised by Schaudinn and Prowazek.

The methods of staining utilised in this research have been

* This paper was originally submitted to the Editor of the 'Indian Medical Gazette' on July 25th, 1905, but as it had not been published, owing to want of space, up to April 22nd, 1906, a request was made for its return, with a view to curtailing the contents. As this procedure was found to be impracticable, it has been determined to publish the paper privately, as the facts brought forward are already two years old.

Romanowsky with its modifications by Giemsa and Leishmann, and another less frequently made use of, namely Benda's later sulphate of iron hæmatoxylin in conjunction with acid-fuchsin, has in some instances been employed.

The descriptions of the flagellate and other forms of organisms found in diptera and other genera have been divided into those met with under two conditions, viz. :

(i) In the plains of the United Provinces, India ;

(ii) In the Himalayas at an elevation of 7500 feet above sea level (Muktesar) ;

and have been separately described.

In addition, a detailed description of the numerous flagellate organisms discovered in the body cavity of one fly have been added, together with a brief account of the results obtained after feeding the common house fly on mouldy wheat under varying conditions. The sample of mouldy wheat utilised in the experiments was taken from a quantity, during the rainy season of 1904, which was condemned as food, as it was believed to be the cause of diarrhœa and ill-health in the persons who made use of the same as food.

2. Microscopical Examination of the Intestinal Contents of the "Musca domestica" in stained Specimens, with Reference to the Presence of the "Herpetomonas muscæ domesticæ" (Burnett) and other Flagellate Forms.

(i) *In the Plains.*

Herpetomonas muscæ domesticæ (Burnett).

In the contents of the intestinal tract of the first four hundred house flies submitted to microscopical examination, flagellated organisms in appreciable numbers were only observed in three instances in the plains during the cold season of the year—that is, less than 1 per cent. of the flies examined.

A further prolonged daily examination of the same species, between the middle of November and the end of March of the following year, brought to light the fact that out of four hundred and twelve different specimens of flies prepared and stained by Romanowsky's method, microscopical examination revealed but two specimens in which flagellates were present—the first on November 16th, the second nearly four months later, on March 9th. These figures give a percentage of but 0·48, as against 0·75 per cent. in the previous research.

There appear to be two distinct varieties of the *Herpetomonas* in the common house fly, a large and a small form, exhibiting identical structures, but varying only in the size to which they attain, the former about 15 μ to 45 μ in length by 1·5 μ to 2·0 μ in width, the latter 6 μ to 10 μ in length by 0·75 μ to 1·2 μ in breadth. These flagellates consist of two parts, the body and the flagellum, their respective ratios being in the generality of cases as 1 : 2. The length of the body varies considerably in the various parasites encountered, being any length between 6 μ and 45 μ, and in a few instances even more. The body of the larger form resembles in shape the blade of an amputating knife, the posterior extremity tapering to a point or being slightly rounded. In every specimen of these flagellates one observes some instances of organisms which have lost their endoplasm, so that only the sheath or periplast remains, stained a faint pink in colour, and from such specimens many details regarding the structure of the organism can with care be made out. The body cavity is replete with protoplasm, interspersed in which are numerous micro-granules, many of which appear to be bipolar in form and stain blue but in some instances a dark red or violet colour. These forms are frequently observed to be more numerously deposited in the posterior half of the parasite. Two nuclei are present in the body-cavity, one situated about the centre and the other at the anterior extremity; the latter is in direct connection with the flagellate apparatus. A vacuole lying just anterior to the flagellate nucleus can be readily discerned, while in the majority of stained parasites it is not so distinct or cannot be recognised. Further, in some unstained specimens circular areas are present, the largest, for the most part single, lying just anterior to the central nucleus, but similar areas may be met with, though smaller, in the posterior half of the body-cavity of some organisms. Again, especially in the long form of *Herpetomonas*, a fairly broad track, one third to one fourth the width of the organism, may in some specimens be readily seen. Anteriorly, it appears to be in direct connection with the vacuole, while it pursues an undulating course to the posterior end of the organism. The protoplasm in this sinuous path does not stain. The twisted filaments, which run posteriorly from the blepharoplast, frequently proceed to their terminations along the same tortuous route. Assuming the nomenclature of Prowazek, the nutritive nucleus, which is for the most part oval in form, although sometimes circular, exhibits a granular structure which stains, lying over a reticulated ground-work, while a karyosome or irregular-shaped body may be frequently observed within the nucleus.

The flagellate nucleus or blepharoplast, which is smaller and more compact than the former, stains deeply, is most irregular in form, oval, circular, kidney, or trefoil shaped.

The structure of the blepharoplast can also be made out in specimens which have been subjected to pressure, and the karyosome may then be recognised.

The flagellate apparatus of the *Herpetomonas* is a marked feature; it stains a deep or purplish-red with Romanowsky and the modifications thereof. In structure it may be likened to a long Australian stock-whip with two lashes intimately blended, and a small leather loop which encircles the wrist when in use. The lashes represent the *flagella*, the knots connecting the stock the *diplosomes*, the rigid whip-stock the rhizoplast, the posterior end of which, running back towards the blepharoplast, is connected to that nucleus by the loop which encircles the wrist. In some specimens the latter may be observed as two fine pink lines, the intermediate protoplasm, if present, not taking the stain. In some few instances, a small button-like formation as described by Prowazek lying on one side of the blepharoplast has been observed, but in the great majority of organisms examined no such body could be discovered. It would appear, however, that the double filament which proceeds to the further end of the organism, finally terminating in a bipolar granule, is connected anteriorly with the ends of the two rhizoplasts (*vide* Plate IV, A 9), and that the filament or filaments when running to the posterior extremity of the organism usually lie over or under the nutritive nucleus; but instances have been observed where the filaments appeared to be in touch with a bipolar granule occupying a position in the nutritive nucleus.

The forms assumed during the developmental stages, particularly of the long form of *Herpetomonas*, in the intestinal tracts of the Diptera, appear to be largely influenced in each case respectively by the degree of pressure the individual members of such colonies are subjected to. As the greatest development occurs in the areas of least resistance, it follows that the forms of some of the parasites are markedly curved and irregular in outline (*vide* Plate I, C).

Coexistent with the *Herpetomonas m. d.*, in the intestinal canal of some Diptera, were observed other forms of flagellate organisms which assume what may be termed flag, rhomboidal, diagonal, and irregular-shaped forms.

(*a*) *Flag form.*—These organisms present a body and flagellate apparatus, the ratio of the length of the former to that of the latter in mature organisms being as 1 to about 4 (11·48 μ to 45·92 μ), total length 57 to 60 μ. Each body-cavity contains two nuclei, (*a*) a nutritive, large, open, and granular, in the centre of which a karyosome may occasionally be made out. The position of this nucleus varies considerably in different parasites, but a favourite site is in the posterior extremity of the body-cavity. (*b*) The second nucleus or blepharoplast is in direct communication with the flagellum, which is double, the two being united as in *Herpetomonas m. d.*, by a fine membrane. It exhibits in its different stages of development numerous forms, which always stain a dark red and are sharply defined when stained by Romanowsky.

The body-cavity of the organism is filled more or less with protoplasm, which may be evenly distributed throughout, but also collects in irregular patches, so that unstained areas or marked band forms are not uncommonly met with. The body of the organism is surrounded by a fine limiting membrane or periplast, which stains red and is prolonged visibly for some distance along the flagellate apparatus. In a few instances indications of this delicate structure have been observed in the course of the flagella somewhat remote from the body of the organism, so that it may ultimately become blended with the flagella. As in the case of *Herpetomonas m.d.*, this form of organism is furnished with rhizoplasts, diplosomes and fine filaments connecting the former with the blepharoplast. In addition, a distinct thread can be made out, sometimes single, sometimes double, running posteriorly from the flagellate nucleus in the direction of the nutritive nucleus. Instead, however, of proceeding in a direct line to its destination, it proceeds to the posterior limit of the body-cavity, and then curves round to where the large nucleus is centrally placed in the organism. In a few instances the filament appears to occupy the centre of an unstained path, which runs antero-posteriorly, similar to that which was previously noted in the long form of *Herpetomonas*. Occasionally, also, when such an organism is undergoing division, a broad, protoplasmic band may be observed in direct continuation with the posterior extremity of the blepharoplast. This band gradually tapers off until it terminates in a fine point, which lies in direct contact with the enlarged nutritive nucleus.

Division in this form of organism is longitudinal. The two nuclei respectively divide, and, as may be observed in Plate IV, B 2, the nutritive nucleus subdivides into eight chromosomes. Further allusion will be made to some peculiarities met with in regard to this form later in this paper.

(*b*) Another organism which may be a distinct species from the last mentioned one, the so-called rhomboidal form, differs principally from the flag-shaped parasite in that it lacks the well-marked extension of the periplast, which covers the flagella, and the flagellate apparatus proceeds from one acute angle of the organism instead of issuing at a right angle to the body of the parasite.

(*c*) Another parasite which may also be a modification of the flag-shaped flagellate (Plate II, C 5) we have designated the "poppy capsule" form. Its peculiarities are the shape of the body, containing the large nutritive nucleus, situated posteriorly, which stains a bright red, the flagellate nucleus and rhizoplasts being situated in the interior of the cavity, formed by a fine sheath or periplast, which extends, on the one side, from the large nucleus and becomes lost on the flagellum, and on the other is directed from the base of the so-called "capsule," and becomes continuous and blended with the flagellum on the opposite side. Several involution forms of this organism have also been observed.

(*d*) Probably a new species of flagellate (Plate II, C 1–3), $31{\cdot}5\mu$ in length, made up of a globular body at its posterior extremity and a cylindrical tube, terminating in a long flagellum. The globular body, $4{\cdot}5\,\mu$ in diameter, is somewhat similar in form to a Malpighian tuft of the kidney, the capsule represented by a fine, limiting membrane, which stains red. The cavity of the capsule is nearly filled by what resembles agglomerated drupes (*etærio*) stained a bright red colour. From the anterior surface of this body a cylindrical but tapering tube proceeds, which finally terminates in a long flagellum. The former measures 10 to $12\,\mu$ in length, and stains a deep blue colour, while the flagellum, which averages 15 to $16\,\mu$ in length, takes a bright red stain.

In Plate II, C 1 is represented an involution form of the same organism, in which the large agglomerated nucleus can be distinctly recognised.

(ii) *In the Hills* (*Himalayas*).

The winged insects which abound during the hot season at the above elevation principally belong to the genus Diptera (Muscidæ, Tabanidæ, and Hippoboscidæ), although there are but few of the latter. Muscidæ are present in great force, the *Musca domestica* or a closely allied species predominating, as might be expected. It has not yet been accurately determined whether the percentage of the latter species whose body-cavities contain flagellate forms at this elevation exceeds those found in the plains.

Table I.

Showing the different species and number of flagellates found in the gastro-intestinal tracts of specimens of Muscæ domesticæ *during the several months of the year at an elevation of 7500 feet, together with the shade temperature, rainfall, number of flies in which parasites were absent and present, and the percentage of those containing parasites.*

Month.	Shade temperature.		Total Rain-fall, Ins.	*Herpetomonas.*		Flagellate terminating in a knob.	Hill *Herpetomonas.*	Circular form of flagellate parasite.	Parasites in flies examined.		Total flies examined.	Percentage of parasites present.	Remarks.
	Min. deg.F.	Max. deg.F.		*Muscæ domesticæ.*	*Sarcophagæ.*				Absent —	Present +			
May . .	55·5	79·2	2·37	15	4	0	2	3	75	17	92	18·47	
June . .	60·5	81·0	1·50										
July . .	59·5	73·8	8·00	14	2	2	2	1	86	16	102	15·68	
August .	59·0	73·8	9·28	11	4	0	0	0	85	11	96	11·46	
September	55·5	74·6	4·90	6	2	1	1	1	85	8	93	8·60	
October .	46·8	68·4	0·00	1	1	0	0	0	98	2	100	2·00	
November	45·0	66·6	0·00	0	0	0	0	0	33	0	33	0·00	
December	24·3	59·0	1·04	0	0	0	0	0	0	0	0	0·00	No flies observed.
January .	21·0	59·0	1·47	0	0	0	0	0	0	0	0	0·00	No flies observed.
February .	23·9	53·0	7·42	0	0	0	0	0	0	0	0	0·00	No flies observed.
March .	35·3	64·0	1·41	0	0	0	0	0	0	0	0	0·00	No flies observed.
April .	38·0	80·8	0·49	0	0	0	0	0	90	0	90	0·00	
May . .	48·2	84·0	0·63	5	0	0	1	1	88	5	93	5·38	
June . .	49·2	81·2	6·84	23	0	0	0	0	67	23	90	29·56	

From the above table it will be observed that the flagellates found in the common house fly at an elevation of 7500 feet were most numerous during the hottest season of the year, when a minimum of 49·2° F. and a maximum temperature of 81·2° F. were registered. The percentage of flies in which the organisms were present gradually decreased between June and October, until during the month of November none were discovered. Further, during the cold weather very few, if any, flies at all were met with.

A short description of the flagellate forms discovered in the various genera and species examined in the hills may now be recorded.

Muscidæ.

Muscæ domesticæ (Plate V, figs. 1–10).

(i) A new species of *Herpetomonas,* somewhat resembling the

H. sarcophagæ, n. sp. (Prowazek). The mean length of the body in a large number of specimens is 10·07 μ, while the flagellum attains to a slightly greater length—12·6 μ. The chief points of difference observed in the vast numbers examined between this form and the above mentioned are: The position of the blepharoplast, which is nearly always situated close to the nutritive nucleus, in some instances in close apposition with it or lying posterior to that body. The rhizoplast is long and passes up a kind of canal or unstained space, which may occupy from two fifths to one half of the length of the anterior position of the body of the organism. The nutritive nucleus is large, open, and stains a bright red, while the flagellate nucleus stains a dark purple and measures, in its long axis, one sixth of the length of the body of the parasite. The body-cavity contains large micro-granules, which differ in number in different organisms.

(ii) The organism (Plate V, figs. 11–17) appears to be the same form of flagellate as was described in the flies met with in the plains.

In the mature form the flagellum passes along the whole length of the body to join the blepharoplast, posteriorly situated. The anterior extremity of the flagellum terminates in an enlargement or knob. The length of the body varies from 7 μ to 10 μ; that of the flagellum is about 9 μ in the developing organisms, while in the mature forms the flagellum is shorter than that of the body.

(iii) A new species of flagellate (Plate V, fig. 18), body and flagellum equal in length 8·2 μ. The latter terminates abruptly on joining the body of the organism. The blepharoplast is situated in the centre of the body-cavity, anterior to but in apposition to the nutritive nucleus. Two small red dots are discernible—one situated at the anterior extremity between the flagellate nucleus and flagellum and the other near the posterior extremity of the parasite.

(iv) *Rhomboid form* (Plate V, fig. 19).—The ratio of the body-length to that of the flagellum is as 1 to 4.

(v) *Flag form* (Plate V, fig. 20).—Ratio of length of body to flagellum about 1 to 4.

(vi) *Herpetomonas m. d.* (Burnett) (Plate V, figs. 21–25).—The five examples depicted have all been classed under the above species, large and small forms, although the length of body to flagellum does not exactly conform to the ratio of 1 to 2 found in the same species in the plains. Fig. 21 represents the gregarine form of Leger, Fig. 22 the long thin variety, 32·8 μ to 45 μ, or as 1 to 1·36; Fig. 23 an example of the minute *Herpetomonas*; Fig. 24 a short variety, length 22·96 μ to 37·72 μ, or a ratio of 1 to 1·69; and Fig. 25, the long and broad form, exhibiting the filament running from the blepharoplast, under the nutritive nucleus to a micro-granule at the posterior extremity of the parasite. The measurements of this latter form are, length of body 31 μ, that of the flagellum 42 μ, a ratio of 1 to 1·35.

(vii) *Flagellate, n. s.p.* (Fig. 26).—This form, undergoing division into three, presents three nutritive nuclei and but one large circular flagellate nucleus; it is differentiated from the others mentioned above by the extreme length of its flagellum—body 14·76 μ, flagellum 37·72 μ, a ratio of 1 to 2·55.

(viii) *Circular form* (Fig. 27).—This parasite coincides with the flagellate (Plate III, E 6 and 7) found in the plains. Length of body 8·30 μ, flagellum 44·82 μ, ratio as 1 to 5·4.

Tabanidæ.

(ix) *Filaria embryos* (Plate V, figs. 28, 29).—Contained in the body-cavity of a fly, *Tabanus tropicus*. Body length 98·4 to 114·8 μ, breadth 1·64 to 1·72 μ. The cephalic extremity rounded off, distinct indications of a sheath, caudal extremity gradually tapers from one eighth of the length of the parasite and ends in a sharp point. Marked granular aggregation about junction of middle with the posterior third of the body. A V-shaped organ is visible in both specimens, and one, Fig. 29, exhibits a tail spot.

3. Description of the various Flagellate Organisms encountered in the Intestinal Contents of a single Dipteran in the Plains.

The fly in question, resembling the *Musca domestica* but covered in parts by a whitish bloom, was caught whilst feeding on the exposed sore of a patient who at first was diagnosed as the subject of Delhi sore, but in the tissues of which no pyriform bodies or circular forms of immature bodies could be discovered. The fly was killed and stained specimens were prepared within a short period of its being caught. There was, therefore, no time for any development of parasites, which might have been imbibed by the fly just previous to its capture. It is also somewhat unlikely that it had had access to the sore on a previous occcasion when the wound was being dressed. In the excreta of this variety of Diptera two resting forms were discovered in stained specimens, but the species of parasite to which they belonged could not be determined. The microscopical examination of the contents of the intestines revealed the presence of numerous species of flagellate organisms, two of which were found in considerable numbers, and may be first described :

(i) One resembling the *Herpetomonas m.d.* in general outline and structure, but with a shorter and broader body, 10 to 14 μ in length; the central nucleus larger and more open, the flagellate nucleus also larger, and the flagellum shorter than in *H. m. d.* Further, the nucleus, which in the latter form when undergoing reduction divides into eight, in this variety formed sixteen. On the appearance of Prowazek's monograph it was considered that this parasite must coincide with *H. sarcophagæ, n. sp.*, of that observer, described as possessing a larger nucleus, richer in chromatin, nucleus with sixteen chromosomes, and when agglomerating the flagellate draws out longitudinally, and the cellular body possesses a long caudal process. The stumpy appearance of the flagellate, greater breadth, and shortness of the flagellum compared with the length of the body may point to the fact that this is a modification or a new species. In addition, the variable position of the blepharoplast with regard to the nutritive nucleus and the shortening of the flagellum (retraction) before its division are marked features in this species.

(ii) This species of flagellate in its mature form presents characteristics which immediately differentiate it from any of those previously obverved (Plate III, G 2, and Plate IV, D 2–12). Its length varies between 7·65 and 10 μ ; it possesses two nuclei, one the nutritive, free and granular, which stains a bright red with Romanowsky and its modifications, and a blepharoplast which is smaller, compact, and takes a much deeper colour—dark red or purple. In addition there is a well-marked flagellum, which is connected with its nucleus by a fine filament, which runs the whole length of the body-cavity and terminates in every instance in a round knob. The position of the flagellum which protrudes from the mature organism is short in comparison with those of most other flagellates, and is not equal to the length of their bodies. The nutritive nucleus in immature individuals is centrally placed and later occupies a position at the posterior extremity of the organism. The flagellate nucleus, which is at first placed close to but anterior to the blepharoplast, gradually alters its position until it finally becomes situated at the most remote or posterior portion of the organism. The flagellum, terminating in a knob, is at first coiled up in the body-cavity, for the most part occupying the anterior half, but later, when the flagellate nucleus alters its position to the posterior extremity of the organism, it becomes untwisted and protrudes until its full length is attained.

One form (Plate IV, D 11) is particularly noticeable, for the body of the organism appears to lie directly upon its flagellum, which has become external, free, and markedly undulating. When division of the nucleus takes place (Plate IV, D 12), the flagellum becomes partially or wholly retracted and coiled up in the body-cavity. The movements of this form of organism are also characteristic, for it progresses in the same way as a flat-fish swims, undulating movements of the body, passing in a horizontal direction from before backwards. Up to the present it has not been possible to discover any differentiation of the flagellate apparatus, with regard to the presence of rhizoplasts and diplosomes. When agglomerating in rosette form, sixteen (16) individuals have been noted, corresponding with the number of divisions or chromosomes which the nutritive nucleus forms. The flagella in such rosettes are centrally placed, as is observed in *Herpetomonas sarcophagæ* and *Herpetomonas m. d.* It remains to be proved to what species this parasite belongs, perhaps a sub-species of *Herpetomonas*, but certainly not a trypanosoma following the true type ; or it may belong to an entirely new species. It will be interesting to discover whether its mode of agglomerating can be accounted for as in the previously mentioned ones on the supposition of Schaudinn "that the body-axis primarily is bent back on itself, so that the morphological anterior end lies next to the posterior end." The immature forms of this parasite are found in spheroid colonies or aggregations (Plate III, F, fig. 4), in which individual cells lie in a superficial layer, in a gelatinous matrix, and in some instances the mass would appear to be surrounded by a very fine limiting membrane. Further, the primary aggregation appears to show indications of secondary colonies, so that some seven or eight subdivisions or more may be made out. These subdivisions probably form the subsidiary rosettes made up of some sixteen units, which agglomerate with their flagella centrally placed, before finally drawing out longitudinally. In Plate III, F, fig. 5, is depicted a small detached portion of a colony from such a one as is represented in Fig. 4, but in which the individual immature forms are more developed. This form of parasite may turn out to have pathological interest, for the flagellate of Delhi sore remains to be developed from the pyriform bodies found, the organism recognised and the particular species named.

Certain other flagellate parasites were also discovered in addition to the foregoing, the most interesting of which have been depicted in the illustrations accompanying this paper. A short description of the chief characteristics of these several forms may now be recorded.

(iii) Small circular or oval bodies (H 1–5) possessing two nuclei, one a nutritive, large, the elements of which are more or less scattered, and the other flagellate, which is small, circular, and close. In this form the flagellum, instead of extricating itself from the membrane at the nearest point, bisects more or less the cell, and protrudes at a point in the opposite circumference.

(iv) A bipolar flagellated organism (Plate III, I 13) 25 μ in length, presenting two small nuclei in the body portion and a third, situated towards the termination of the more developed flagellum.

(v) The immature stage of a new form of parasite (Plate III, I 14) oval body, 10 μ in length. Exhibits nutritive nucleus undergoing division, a large flagellate nucleus, a flagellum which appears to be connected with its nucleus by a fine thread, and which, after sweeping round the limiting membrane of the cell, becomes much more marked as it passes between the two nuclei and terminates externally in a well-marked, rounded extremity (Knob). This form presents some characters in common with the new form above described (Plate IV, D 1–13).

(vi) A pear-shaped flagellate (Plate III, I 15), exhibiting a nutritive nucleus undergoing division, situated at the posterior extremity of the cell, a flagellate nucleus, well marked, centrally placed at the periphery, from which a thick flagellum passes, but not terminating in a bulbous extremity. Length of flagellum and body of organism about equal, viz. 10·71 μ.

(vii) A flagellate organism (I 16) presents a body in which the two nuclei are centrally placed, the flagellate nucleus anterior to the nutritive, which shows signs of division, while three dark dots are visible posteriorly. Numerous bipolar micro-granules are visible in the body-cavity. Length 19 μ.

(viii) A ribbon form of organism (I 17), 26·5 μ in length, by 2·46 μ in breadth, observed in some numbers. Two oval-shaped nuclei, somewhat indistinct, are centrally placed, while the extremities terminate in dark-stained and pointed lobes.

(ix) A trypanoplasma (I 18), exhibiting two well-marked flagella and undulating membrane, two nuclei, the nutritive undergoing division, the blepharoplast in connection with the anterior and most developed flagellum. A third small nucleus is visible in the posterior portion of the body-cavity, from which the undulating membrane appears to take its origin.

(x) A form of *Herpetomonas* (I 19), exhibiting more or less rounded extremities. Body-cavity containing large number of micro-granules, a nutritive and a flagellate nucleus in close apposition, surrounded by a light-coloured area. There appears to be but a single flagellum. The organisms figured as K 7 appear to be somewhat similar forms of parasites to the above. These possess attenuated posterior extremities, by which the two are united. This may point to the fact that in the rosette formation these organisms agglomerate after the mode of T. Lewisi, Evansi, and Brucei. It is more likely, however, that division has taken place longitudinally, and that the two units are still connected by their posterior extremities.

(xi) A single example of *Herpetomonas m. d.* (K 1), to which is attached a gregarine-like parasite. This latter organism is stained a dark blue with Romanowsky. The body-cavity of the host appears to be devoid of protoplasm, which has been sucked dry by the parasite; consequently only the elements staining red are depicted.

(xii) A spheroid colony (K 2) of four bodies, which are surrounded by a very fine limiting membrane. The chromatin is situated for the most part at one extremity, and occupies in each instance respectively a half or a third of the total length of the immature parasite. In the anterior two fifths of the body-cavity of one organism, 10·7μ in length, may be observed a fine thread passing posteriorly in a direct line to a minute blepharoplast, while the chromatin occupies the posterior two fifths. The central fifth of the body-cavity appears to contain neither protoplasm nor chromatin, as shown by their staining properties. These forms resemble somewhat the gregarine stage of the *Herpetomonas m. d.*, but most probably are the immature stage of the organism depicted in Plate III, K 3, which, it will be observed, are laden with chromatic material. The organism presents two flagella, together with two distinct flagellate nuclei and a nutritive nucleus undergoing division, all being massed together at the anterior end of the organism.

(xiii) Another kind of parasite (K 4), much smaller than the last mentioned but also containing much chromatic material, was discovered lying amongst a number of what were taken to be examples of *H. Sarcoph., n. sp.*

(xiv) Two other forms of organism (K 5) exhibit (i) absence of flagellum and blepharoplast and what appears to be reduction into four of the nutritive nucleus; (ii) absence of flagellum, reduction of single blepharoplast, reduction into two of each nutritive nucleus.

(xv) *H. Sarcophagæ* (K 6), copulation. Early stage before fertilisation, but apparent absence of blepharoplasts.

(xvi) Second reduction divisions of the blepharoplasts (etheogenesis); *vide* separation of the flagellate nuclei (K 8).

(xvii) An undescribed form of trypanoplasma (K 9), broad and rounded at one extremity and tapering more or less at the other, length of body 16·5 μ. At the former end a large, well-marked, clear space or vacuole is seen, across which the flagellum passes to join the blepharoplast, while at the latter a clear channel can be observed along which a second flagellum passes to join the second but smaller blepharoplast. It may be noted that the periplast on one side of this flagellum is continued a greater distance than on the other. In the centre of the body-cavity a single nutritive nucleus, which is enlarged before reduction is situated; this must be common to both the flagellate apparatuses. The protoplasm of the body-cavity is stained blue after Romanowsky, and numerous bipolar micro-granules are scattered about through its substance; in addition three such bodies may be made out, directly connected with the nutritive nucleus (*vide* enlargement of trypanoplasma, Plate, IV, D).

4. Organisms observed in the "Stomyx Calcitrans" and the "Culex" Mosquito.

During the course of our observations numerous organisms representing several genera have been discovered in the body-tissues of the common house fly, and of different species other than the *Musca domestica*. For instance, a cell with a double contour indistinguishable from the *Cryptococcus farciminosus Rivoltæ* in *Stomyx calcitrans* and *Musca domestica*, *Herpetomonas m. d.* (Burnett) in the *Culex* mosquito; a spirillum in the larva, pupa, and mature *Culex*, flagellated organisms, generally in pairs, in the larva of *Culex*. The two latter forms may be further referred to.

Spirilla forms (Plate V, fig. 30).—These are commonly found in the body-cavities of the larva of *Culex* mosquito. The organisms vary in length according to the number of individual spiral units they respectively possess, 9·84 μ, 13·12 μ, and 16·40 μ, each unit measuring exactly 3·28 μ in length.

Flagellates in larva of Culex (Plate V, figs. 31–37).—These forms are totally distinct from those observed in Diptera. The mature parasite exhibits a body in form somewhat spindle-shaped, length 8·20 to 8·5 μ, and from 4·01 to 4·90 μ in breadth. A large, open, nutritive nucleus is situated in the posterior half of the body-cavity, lying at a short distance is the blepharoplast from which the flagellated apparatus runs anteriorly. The flagellum protrudes from the anterior extremity from 4·92 μ to 6·56 μ. The flagellum generally leaves the body of the parasite a little to one side of the median line, and the periplast may be observed prolonged and forming a sheath for a short distance on one side of it. In addition, the body-cavity contains a large number of circular and highly refractile bodies, which in the mature form of the parasite are collected in its posterior half; they number between twenty and thirty. In the majority of mature forms one of the refractile bodies remains in the anterior half of the organism, and in several instances was observed to occupy a position close to but on one side of the extreme anterior extremity of the flagellate. No distinct rhizoplast could be differentiated, and the same holds good for the diplosome, although in two instances a minute red-stained dot has been observed in the position it should occupy. The circular bodies or immature forms (Fig. 31) of this flagellate, corresponding to the circular, oval, or pyriform bodies of Trypanosomidæ and Herpetomonidæ, etc., measure 2·46 μ to 3·28 μ in diameter, and exhibit nutritive nuclei and blepharoplasts. The more developed forms of the parasite present, in addition to the two nuclei above mentioned, numerous refractile bodies, circular in form, which are scattered through the protoplasm, and later a flagellate apparatus which at first is contained in the body-cavity, but which during further development extends and protrudes from the anterior portion of the cell. Figs. 34 to 36 exhibit groups of parasites usually consisting of four units, undergoing division.

5. Experiments in the Plains.

Bearing in mind that corn and rice in a mouldy condition, when partaken of as food by man, have been supposed to act as the carriers of "causal agents" in certain forms of disease, experiments were initiated with a view to determine whether Diptera fed upon such material would develop organisms in their intestinal tracts as a result of such food, and if so, what species of organism would be developed. With this end in view, a number of house flies were obtained; one fourth were destroyed and the contents of their intestines submitted to careful microscopical examination in stained specimens. As no flagellate organisms were discovered in any of these Diptera, the remaining number were fed, one fourth on dry, mouldy samples of wheat of a greenish-grey colour, while another fourth were given corn from the same source, but previously moistened with sterilised distilled water, and the remaining flies were kept as controls to those undergoing the test experiments.

In addition, a further set of experiments with additional flies were conducted with samples of the same mouldy wheat as utilised in the feeding experiments. Small quantities of corn were placed in sterilised test-tubes moistened with sterile distilled water, and certain tubes were exposed to a temperature of 27° C., while others were exposed to the temperature of the air, which in the cold season fell to a minimum of 4° C.

We may now detail the results of the above observations.

(i) *Feeding* Musca domestica *on Mouldy Wheat.*

Flies examined after a period of forty-eight hours.—Stained specimens of the intestinal contents obtained from the proventricle and neighbourhood of the Malpighian tubes exhibited enormous numbers of circular and pyriform bodies (Plate III, A), singly and in colonies, varying considerable in size, but all presenting certain characteristics, viz. the protoplasm stained a lighter or darker tint of blue, and in addition the presence of two nuclei—one a large, somewhat circular, open and granular in structure, taking a bright-red stain, and occupying respectively different positions in the individual cells, sometimes centrally placed, but in the majority near to or bordering on the periphery of the cell; while the second nucleus or blepharoplast was represented by a line or two lines, stained a deep red colour. These showed either no differentiation in structure or presented the characteristics of a bipolar body or bodies, while in other instances the straight body resolved itself under a high power into three circular bodies, lying in a straight line. In addition, some of the pyriform and circular cells contained bipolar micro-granules, which stained either blue or a faint violet colour, and less frequently red. These granules at this stage were not constantly present; in fact, they were only exceptionally noted.

The intestinal contents of one fly only, after a period of forty-eight hours' feeding on mouldy corn, exhibited but a single species of flagellate organism (Plate III, E). These cells are somewhat oval or heart-shaped in form, and are mainly remarkable for the large size of their nutritive nuclei, which stain a bright red colour, their round-shaped blepharoplasts (surrounded by a circular area which does not stain), which are always double, and for the amount of protoplasm the cells contain. This latter material is interspersed with one or more vacuoles and a varying number of micro-granules. The double flagella which proceed from the blepharoplasts are four times the length of the cell itself, and exhibit diplosomes and rhizoplasts, as observed in the long flagellate *Herpetomonas m. d.* The large cell (E 7), the length of which is 18·36 μ by 15·3 μ in breadth, is a good example of the above species undergoing division before it has attained its mature form and developed its flagellate apparatus.

After a period of forty-eight to seventy-two hours.—Certain modifications in the structure of the bodies noted up to forty-eight hours were observed. Both nutritive and flagellate nuclei altered their position from the direct periphery of the cell and became more centrally placed, the protoplasm became more distinct, and micro-granules could be generally observed, but the most striking changes were the development of one or two flagella according to whether there was a single or double blepharoplast present in each cell. In a minority of instances the nutritive nucleus either gave indication of the change or had already undergone division into two (Plate III, B 4).

Other forms of parasites were also discovered and noted; these were spindle- and flask-shaped, the former probably immature *Herpetomonas m. d.*, while the latter belonged to an unrecognised species. *After a period of seventy-two hours* the contents of the proventricle of flies examined contained innumerable examples of parasites of various species, presenting different stages in their development, ranging from the pear-shaped and circular bodies, which were either furnished with budding or more developed flagella to organisms which appeared to be fully developed. These latter consisted for the most part of the rhomboidal, circular, and flag forms, interspersed amongst them being examples of the *Herpetomonas m. d.* In addition at this stage (*a*) sphæroid agglomerations of immature parasites were met with, which for the most part showed the flagella centrally placed (Plate III, D 2), although at the periphery of the mass several may be observed to present free flagella. (*b*) Thick rosettes of the spindle-shaped *Herpetomonas* which were present in fields of which (D 3) represents one fifteenth part of one mass, present in the specimen. These forms agglomerate with the flagella centrally placed, thus differing from *T. Evansi*, *T. Lewisi* and *T. Equiperdum*, which agglutinate by their posterior extremities, and from the *H. butschlii* (S. Kent), which also forms rosettes with their posterior extremities centrally placed and their flagella free at the periphery. The question arises whether this latter form of parasite belongs to the same class as the *Herpetomonas m. d.* (Burnett). It would appear to occupy a position in closer relation to the *Trypanosomidæ*.

(ii) *Feeding* Musca domestica *on Damp Mouldy Wheat.*

These Diptera for the most part succumbed within a period of twenty-four to forty-eight hours, and in one instance only were parasites observed in the intestinal contents. These belonged to the species *Herpetomonas m. d.*

(iii) *Mouldy Wheat kept in Sterilised Distilled Water at 27° C. for some Days and Examined microscopically at Intervals.*

Within twenty-four hours in specimens of the fluid stained by the above-mentioned methods, numbers of bacilli, diplococci, and yeast-cells developed. In addition, but after forty-eight hours, scattered through the mass of bacteria were cells which presented a circular, oval, or irregularly oval, form, varying in diameter from 6 μ to 16 μ. In the young and circular forms the protoplasm stained a dark blue, the nutritive nucleus large, open, and frequently containing a karyosome in the centre. The nucleus measured from one third to two fifths the diameter of the cell, while a rod-shaped blepharoplast or flagellate nucleus could always be observed lying parallel or at an angle to the periphery of the cell. In addition micro-granules and a few bipolar bodies were scattered through the contents of this immature form of organism. Previous to the formation of vacuoles in some of the larger cells one observed the nutritive nucleus breaking up into large granules and becoming more or less scattered, while the blepharoplast just previous to or later undergoes division. Very occasionally a second flagellate nucleus would appear at the opposite side of the mother-cell, and division of the nutritive nucleus then took place previous to the complete division into two separate organisms. At a later date of incubation the most striking feature in the larger form or irregular-shaped cells was the presence of one large or frequently multiple vacuoles scattered through the protoplasm, together with the formation of light-coloured areas which stained faintly blue. When this change was noticeable seldom or never could a stained nutritive nucleus be recognised, although the flagellate nucleus would in such instances be as distinct as those observed after a period of only forty-eight hours' incubation. This immature form of flagellate is exactly similar in form and characteristics to those depicted in Plate III, E 1–4, as found in the intestinal contents of one of the house flies seventy-two hours after being fed upon dry mouldy wheat, but with this great difference, that no flagellate apparatus had been developed, or any indication of such growths had been discovered in the incubated specimen of wheat at 27° C. Further, it would appear that the development of the flagellate apparatus had been prevented by a temperature somewhere between that of the body of the fly and that of the incubator, 27° C.

(iv) *Mouldy Wheat kept in Sterilised Distilled Water at about 4° C.*

Cultures were kept exposed at the temperature of the air during the cold season of the year. Of the numerous fresh and stained specimens examined flagellate forms were frequently noted, the flagellum appearing after a period of twenty-four to forty-eight hours, but generally after the expiration of the latter period.

In no instance were flagellate protozoa discovered in the contents of the intestinal tracts of Diptera confined as controls to the above experiments, and kept without any food, or when sugar was given, during the period of observation.

In drawing conclusions from the feeding experiments on Diptera, it must be remembered, as bearing upon these results, that young rats (*m. d.*) harbour in enormous numbers the *T. Lewisi*, which at a later date, in full-grown animals of the same species, frequently disappear from the circulation; although examination of such mature animals' blood may be conducted daily for periods of a month or more, no mature or involution forms of the trypanosoma may be discovered. Sometimes when inoculated with *T. Evansi*, an animal will succumb with large numbers of flagellate forms in its circulation, some of which will be examples of the *T. Lewisi*, so that although the latter organism could not be found before inoculation with the *T. Evansi*, the immature forms of *Lewisi* must have been present, lying dormant, and ready to spring into active existence directly favourable conditions appeared for their development. So with regard to the results of the experiments with Diptera, it must be borne in mind that in some instances the *Herpetomonas m. d.* and other species of flagellate organisms were developed in the experimental flies concurrently with those which may have been taken in with the corn. The former were certainly lying dormant in the intestinal tract of flies under experiment, and directly the hosts received certain food derived from the mouldy wheat developmental changes proceeded. Until the exact time which has to elapse between infection and the maturation of these flagellate forms in the body of the hosts has been experimentally determined, we can only state that Diptera may take in the involution forms of certain flagellates, and that these bodies can pass through the various stages and attain a certain degree of maturity during a period of seventy-two hours. The above experiments were, however, carefully carried out, and controls kept in each instance. We are, therefore, justified in believing that the majority, if not all the forms described other than the *Herpetomonas m. d.* were derived from the mouldy wheat supplied to the flies as food.

Are any of the Flagellates observed in "Musca Domestica" traceable to a Doubly Flagellated Trypanoplasma?

Schaudinn expressed his opinion that the entire body of the flagellate *Herpetomonas m. d.* can be traced to a doubly flagellated trypanoplasma, and Prowazek states that "both forms are to be traced back to bipolar trypanoplasma with two flagella, only in the course of phylogenesis sometimes one pole or the other has been suppressed." Let us see how far this surmise holds good for the different forms of parasites we have encountered in our inquiry in this country.

(*a*) *Herpetomonas m. d.*—Out of the thousands of parasites we have observed one (Plate IV, A 10) appears to be reverting to the trypanoplasma form, for a second flagellum has developed at the posterior extremity of the body, in direct connection with a bipolar granule which appears to have become considerably hypertrophied. There is, however, no attempt or indication of a fold in the organism.

(*b*) *Herpetomonas sarcophagæ* (Prowazek), *n. sp.*—In Plate IV, A 11 may be observed an example of this form of parasite undergoing division, together with the development of a second flagellum, intimately connected with a bipolar body, which latter is either part of the smaller of the two nutritive nuclei or is superimposed on it. This instance is the nearest approach we have encountered to a bipolar flagellate in this species of *Herpetomonas*.

(*c*) *Flag form of Herpetomonas, n. sp.*—In this form of parasite, which appears to be but a modification of the long *Herpetomonas m. d.* (Plate IV, B 5), the anterior two thirds of the individual parasite is curved upon itself, and the posterior third folded up to join the point of the arch formed by the other two portions. Division is usually longitudinal, but less common modes are observed:

(i) When a horn-like second prolongation takes place on the same side of the parasite as the former, from the extremity of which a second flagellum develops, with enlargement of the first.

(ii) When the flag form appears to become reduplicated, end to end, and the second flagellum develops in the longitudinal axis at the opposite extremity of the parasite to the first formed one.

(*d*) *Rhomboidal or diagonal-shaped* Herpetomonas.—From several instances observed in the intestinal contents of flies fed upon mouldy wheat, it would appear that this variety of parasite originated from a treble folding of the *Herpetomonas m. d.* according to the form it assumes (Plate III, D 5). For in a few instances—

(i) The fold or folds can be distinctly made out (Plate IV, B 6).

(ii) The position of the nutritive nucleus coincides in the folded variety with that occupied by it in the ordinary long form of *Herpetomonas*.

(iii) The ratio of the length of the flagellum to that of the body of the long variety of *Herpetomonas* is fairly constant as 2 to 1, whereas that of the rhomboidal form is as 4 to 1. If, however, the latter variety be considered as exhibiting a treble fold, and its contour be measured, the ratio will be found to be again as 2 to 1.

(iv) In the rhomboidal form of the parasite the fine filament, which proceeds posteriorly from the blepharoplast, instead of running directly to its destination by a short route, through or near to the nutritive nucleus, generally proceeds at once to the posterior limit of the parasite and then curves round into the body of the parasite, following the exact course which would be followed in the long *Herpetomonas m. d.* artificially folded three times.

In the four forms of parasites above mentioned we can make out the following facts in support of Schaudinn's and Prowazek's theories :

(i) *Herpetomonas m. d.*—One example of a second flagellum, forming near the posterior extremity of the parasite, but no indication of a fold.

(ii) *Herpetomonas sarcophagæ.*—A second flagellum which has developed from one half of the nutritive nucleus, which has undergone division.

(iii) *Flag form, n. sp.*—Reduplication of the parasite, end to end, with the formation of a second flagellum in the longitudinal axis of the parasite, with an indication of a fold midway between the extremities, so that the morphological anterior end might lie next to the posterior end. This newly-described form thus bears out Schaudinn's opinion, for in addition to being a doubly flagellated organism at times, it also exhibits indications of a fold midway between the two opposite flagella.

(iv) *Rhomboidal form, n. sp.*—Treble folding of the long form of *Herpetomonas m. d.*, but no indication that this parasite reduplicates itself horizontally and develops bipolar flagella.

Remarks with regard to Certain Points of Interest bearing on the Etiology of Beri-beri.

Such a number of eminent observers have investigated beri-beri during the past twenty years that it is with some diffidence that we would offer certain suggestions with regard to the possible etiology of the disease.

Much has been written with regard to the *rôle* played by *rice* in the etiology of beri-beri. It is stated that the inhabitants of Lower Bengal, preparing their rice in a particular manner, never become the subjects of the malady; while in the Malay Peninsula, neighbouring islands, and some other parts of the East, other methods of preparation of rice are resorted to, and the disease is endemic. In studying the literature of beri-beri symptoms are recorded which, when taken together, lead us to detect a certain resemblance to some of those exhibited in diseases whose causal agents are respectively protozoa (flagellates), and the pathological changes produced are more or less due to the biological chemical products (toxins) of such organisms. Among the various factors bearing on the etiology, and noted as occurring in the course of beri-beri, the following may be mentioned:

(i) Beri-beri is said to specially affect the working classes, professional and leisured classes and merchants being practically exempt. (Hunter and Koch.)

(ii) The rapid increase of cases in the month of May, with a further rise in July, and the maintenance of the high level in August and September. (Hunter and Koch.)

In Japan the prevalence of beri-beri in summer was considered to be due to the fact that only rice, tea, and hot water were partaken of, together with fish three times a month. The prevalence of the disease in the depths of winter in one island north of Japan was attributed to the natives having to subsist almost entirely on rice. (Takaki.)

(iii) Great mortality in Hong-Kong—average in males 49·5 per cent., in females 35·4 per cent. (Hunter and Koch.)

(iv) Further, with regard to symptoms, anæmia, emaciation, muscular paresis, and paralysis may be all present.

Pathological Changes observed Post Mortem in Beri-beri.

The following changes are all, in various degrees, found in cases of trypanosomiasis:

"A clear yellowish, jelly-like substance round the base of the heart and coronary arteries.

"Epicardial and subendocardial petechial hæmorrhages occasionally present.

"Amount of granular and fatty degeneration of the cardiac muscle varies in each individual case.

"Intestinal changes affecting the mucous membrane of the small intestines.

"Membranes of the brain and spinal cord may be thickened, in patches, or generalised.

"Cerebro-spinal fluid in excess.

"Gelatinous material found involving the lumbar portion of the cord." (Hunter and Koch.)

All the above symptoms are not incompatible with the effect produced by the generalisation of a toxin in the human system. We would draw attention to the fact that the incidence of beri-beri appears to virtually coincide with the duration of the rainy season, and this synchronises with the period of the year during which forms of trypanosomiasis in India make their appearance and fresh cases are continually occurring.

Further, on reference to the table on page 7 it will be seen that the months of the year May to September inclusive, during which several species of flagellates are found to be present in numbers in certain Diptera in India, are almost identical with those during which beri-beri cases increase and the disease maintains its high level of incidence. It does not require a great stretch of imagination to presume that flagellates, as a class, may generally increase and multiply in Nature during the same months under favourable conditions. We would therefore primarily insist upon the search for protozoa in rice, to the entire exclusion of other forms of micro-organisms. Specimens of rice, husked and unhusked, should be examined under all circumstances, but notably rice of inferior and cheap quality, and also that obtainable just prior to and during the rainy season, which has been lying in godowns in bulk. The portion of grain which has been in contact with the damp ground should in the first instance be examined, and any samples which are undergoing or have undergone change should be submitted, with other specimens, to examination, microscopically in stained specimens, after the method of Romanowsky or one of the modifications thereof, viz. Giemsa, Nocht, and Leishmann. Cultivations should be made and flies fed on samples of dry grain, and the same after soaking in sterilised distilled water at the temperature of the air, and in an incubator not exceeding 25°–27° C.

At the same time no opportunity should be allowed to escape of examining the blood and tissues, especially in early cases of beri-beri. The spleen, lymphatic glands, bone-marrow and cerebro-spinal fluid demand special attention in every such research. The objects to be kept in view are the discovery of a protozoon or protozoa:

(*a*) In the tissues, etc.;

(*b*) In the rice taken as food in endemic areas; and

(*c*) Should a protozoon be discovered does it coincide with any of the forms which will probably be found if a careful examination of rice under various conditions be undertaken?

Instead of the practical experience of the Japanese with regard to the eradication of beri-beri from their navy (by the substitution of a European for the aboriginal diet of the country, with the exclusion of rice to a very great extent from the daily ration), being in direct opposition to the theory that rice may be the *fons et origo* of beri-beri, or at least an important factor in bringing the disease about, it would appear to be a most powerful argument in its favour. Therefore the question as to whether *rice* can harbour under any conditions certain protozoa or other organisms to which beri-beri may be traceable deserves careful attention, and investigation upon such lines should be undertaken and not allowed to drop until some definite conclusion has been arrived at.

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DESCRIPTION OF PLATE I.

A.—Figs. 1, 5–9. The large elongated forms of *Herpetomonas muscæ domesticæ* in various stages. Figs. 2, 3, 4. Examples of the small variety of *Herpetomonas*.

B.—Fig. 1. Parasite exhibiting two nutritive nuclei and flagellate nucleus undergoing division. Fig. 2. Ditto. Fig. 3. Small form of *Herpetomonas m. d.* undergoing division. Figs. 4–7 included under C.

C.—Outlines of several parasites, curved and irregular, due to pressure during developmental stages, either *en masse* or in small numbers when development has taken place between muscular fasciculi. Fig. 1, a large parasite, exhibiting nutritive nucleus, undergoing division into eight parts; nucleus surrounded by micro-granules which stained red in colour after Romanowsky.

D. E. F.—Showing large and small varieties of *Herpetomonas* in the various stages before and after longitudinal division. After division, but before separation of the two parasites, a thin fine membrane joins the two forms together. F, figs. 2, 3. In the early stage, after separation, some parasites present appearance of undulating membrane, only half body-cavity occupied by protoplasm.

E.—Fig. 4. Parasite attacked by spermatozoon of fly. Anterior portion has penetrated periplast and is buried in the vacuole.

D.—Fig. 5. Spermatozoa of Diptera in one showing what appear to be flagellate and nutritive nuclei (the latter after division), together with micro-granules at the posterior extremity of the body-cavity.

D.—Fig. 9. Example of *Herpetomonas m. d.* exhibiting a second flagellate apparatus developing at the posterior extremity (*vide* Plate IV, A, fig. 10).

PLATE I.

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DESCRIPTION OF PLATE II.

A.—Various forms of flag-shaped and rhomboidal flagellated organisms, showing the different stages of development and division observed in stained specimens of the contents of the intestine of one example of Diptera (*musca domestica*).

B.—Immature form of spermatozoon of host (*musca domestica*).

C.—Figs. 1, 2. Encysted forms of new species of flagellated protozoon.

Fig. 3. Flagellated organism, one end terminating in a globular body, resembling somewhat a Malpighian tuft in the cortical portion of the kidney (mammalian) surrounded by a limiting membrane or capsule.

Fig. 4. Undetermined organism.

Fig. 5. Poppy capsule form, with large nutritive nucleus at the posterior extremity, while a fine limiting membrane, which proceeds from the apex and base of the so-called body, is continuous and merges into the flagellum, in the cavity of which membrane is observed the blepharoplast or flagellate nucleus.

PLATE II.

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DESCRIPTION OF PLATE III.

A to E inclusive, forms observed in Diptera after experimental feeding on mouldy wheat.

A.—Circular, oval, pear-shaped, and irregular bodies, each exhibiting a large, somewhat circular, chromatin body (nutritive nucleus) and one or more straight or curved chromatin bodies (blepharoplast), or flagellate nucleus. After forty-eight hours. × 1000.

B.—Further development of various shaped bodies, showing a flagellum or flagella, proceeding from a single or a double blepharoplast. In most instances it may be observed that the flagellate nucleus is more or less remote from the nutritive nucleus in the body of each cell respectively. After seventy-two hours. × 1000.

C.—Fig. 1. Rhomboidal form. Fig. 2. A small group selected from a large mass of immature forms. Fig. 3. Gregarine form. Fig. 4. A few selected different forms of parasites lying in contact, the nutritive nucleus in the majority undergoing proliferation. × 1250.

D.—Fig. 1. See under F, fig. 14. Fig. 2. A circular agglomeration or sphæroid of *Herpetomonas*, flagella centrally situated. Fig. 3. A thick rosette, representing one fifteenth portion of the total mass present in the specimen, agglomerating with the flagella centrally situated. ×

Fig. 4. Long form of *Herpetomonas* showing large number of micro-granules in the protoplasm of the anterior and posterior portions of the body-cavity.

Fig. 5. Diagonal or rhomboidal form of parasite which clearly exhibits its formation from a treble folding of the long variety of *Herpetomonas*.

E.—Figs. 1–5. An oval form of organism, mainly remarkable for its large nutritive nucleus and small round flagellate nucleus, which is always double. The protoplasm always stains a dark blue and exhibits vacuoles. Fig. 6. A parasite which, already provided with a double flagellate nucleus, has a further blepharoplast, which has undergone division, attached to the limiting membrane. Fig. 7. Parasite which has undergone complete division without the development of one or more flagella. The protoplasm of the original cell has not stained, while the new portion has stained deeply. Fig. 8. Spermatozoon of fly; one flagellum has divided into two, the second exhibiting a ribbon-like development.

F to K inclusive, forms observed in a fly (Diptera) caught feeding upon an open sore in the human subject.

F.—Fig. 1. Small collection of immature, pear-shaped bodies, consisting of about eight elements. × 1000.

Fig. 2. Agglomeration of new form of flagellate organisms consisting of sixteen parasites. Flagella centrally situated. Flagellate nucleus situated at posterior extremity; flagellum runs whole length of body-cavity, terminating in an enlargement. × 1000.

Fig. 3. Agglomeration of *H. sarcophagæ*, *n. sp.*, consisting of sixteen parasites. Flagella centrally situated. ×

Fig. 4. A sphæroid colony, immature forms of F 2, new species. A globular aggregate, in which individual cells form a superficial layer in a gelatinous matrix. Fig. 5. Small detached portion of another colony, more developed elements.

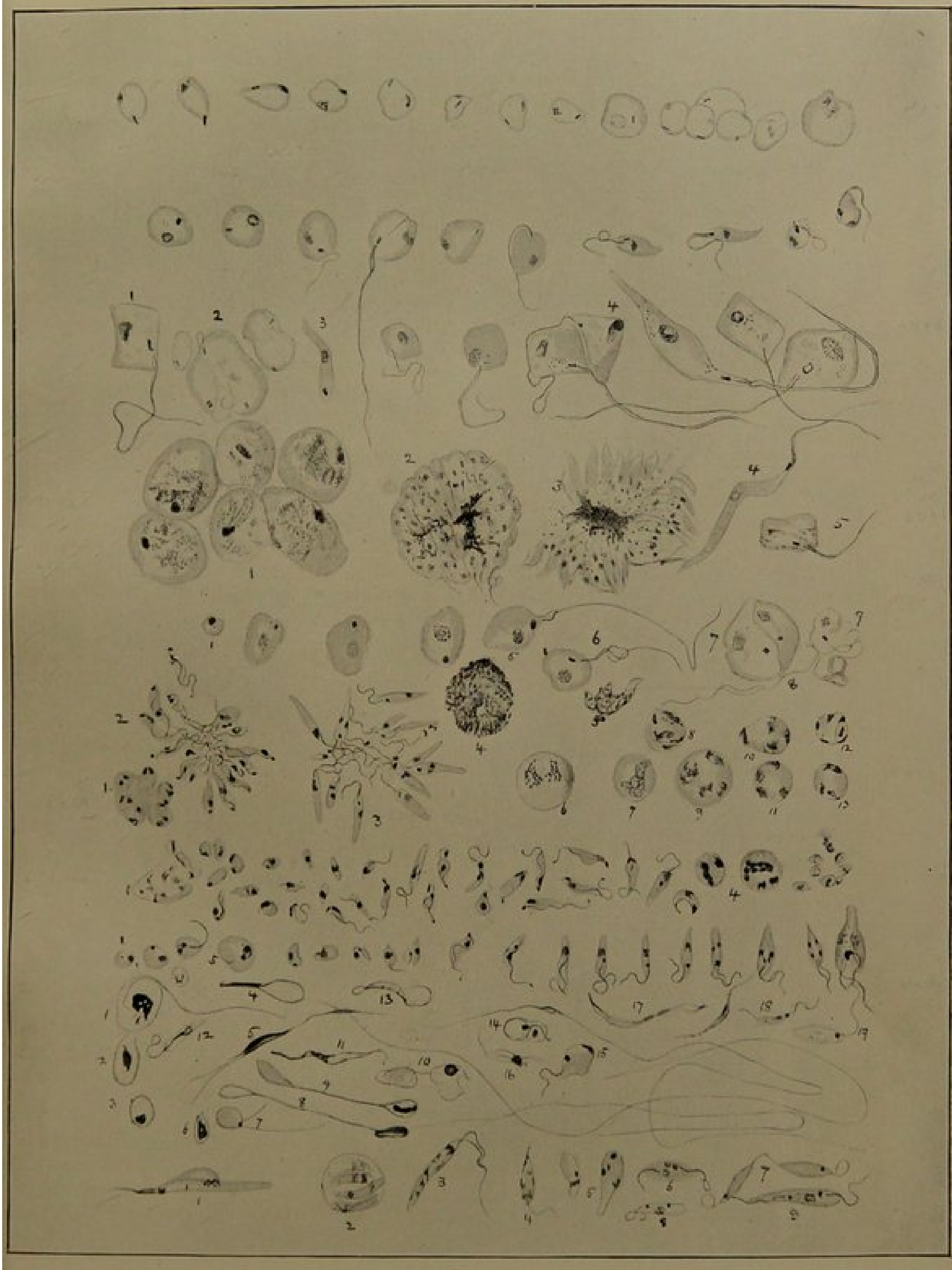
Figs. 6–13. Pathogenesis of *H. sarcophagæ*, various stages.

Fig. 14, D *i*. Group of six depicted from a mass consisting of 22 units in the stained specimen. These appear to be examples of what Prowazek describes under Parthenogenesis as the hybrid stage. Tightening off of the blepharoplast with the protoplasm in two, and formation of a membrane.

G.—Fig. 1. Collection of some sixteen pear-shaped bodies in a gelatinous matrix.

Fig. 2. Different stages of immature parasites undergoing development.

PLATE III.



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principally those of n. sp. of flagellate of F, fig. 2, whose flagellum terminates in a knob. There are several examples of *H. sarcophagæ* depicted.

Fig. 3. A pear-shaped body exhibiting a flagellum at the anterior extremity which runs up to the flagellate nucleus, a looped flagellum proceeding from the opposite extremity.

Fig. 4. Two small sphæroidal colonies, encapsuled, probably *H. sarcophagæ*, the smaller one containing some five and the larger about thirteen pear-shaped bodies presenting respectively well-marked chromatin bodies.

H.—Figs. 1–5. Small circular and oval flagellate forms, new species.

Figs. 6–12. Various forms of F 2, new species of flagellate.

Figs. 13–20. Various stages in the development of *H. sarcophagæ* ?.

I.—Figs. 1–11. Various stages in the development of the spermatozoon of *musca domestica* found in such large numbers scattered through the microscopical preparations of intestinal contents of flies.

Fig. 12. A *Herpetomonas* (sarcoph.) presenting a second flagellum coming off from a bipolar micro-granule situated posteriorly to the nutritive nucleus.

Fig. 13. Trypanoplasma form with two flagella, presenting two small nuclei in the body-cavity and one situated in the more developed flagellum.

Fig. 14. *New species?* Oval body, immature stage, containing nutritive and flagellate nuclei, flagellum coiled in the body-cavity for most part. Small portion projecting, terminating in a knob. Much larger than F, fig. 2.

Fig. 15. *New species?*. Another form, mature, absence of terminal flagellate knob.

Fig. 16. Flagellate organism, undulating membrane, together with nutritive and flagellate nuclei, *new species?*.

Fig. 17. Ribbon form of organism observed in some numbers, two indistinct oval nuclei in body-cavity, terminal extremities, one presenting a vacuole.

Fig. 18. *New species.* Trypanoplasma presenting two flagella, undulating membrane, nutritive nucleus undergoing division, and a large blepharoplast at one extremity of body-cavity and a small one at the opposite end.

Fig. 19. Form of *Herpetomonas* containing large number of micro-granules exhibiting nutritive and flagellate nuclei lying together (*vide* K, fig. 7).

K.—Fig. 1. *Herpetomonas m. d.*, with parasite attached, parasite stains deep blue. The host has lost all signs of protoplasm in body-cavity.

Figs. 2, 3. Four immature gregarine forms contained.

Fig. 4. Same form, but much more pointed.

Fig. 5, *a* and *b*. Examples of resting forms of *Herpetomonas sarcophagæ*.

Fig. 6. Coagulation of *Herpetomonas sarcophagæ*.

Fig. 7. Two parasites attached together by their posterior extremities (*vide* I, 19).

Fig. 8. *Herpetomonas sarcophagæ*, exhibiting large open nutritive nucleus and two blepharoplasts, one posteriorly.

Fig. 9. Trypanoplasma, exhibiting circular clear space (vacuole), through which rhizoplast passes to blepharoplast; a second flagellum occupies a clear canal, running to a second flagellate nucleus, one nutritive nucleus common to both undergoing division.

DESCRIPTION OF PLATE IV.

Diagrammatic enlargements of some of the various forms depicted in the previousplates.

A.—Figs. 1–8. *Herpetomonas m. d.*, showing the different forms assumed by the nutritive and flagellate nuclei, rhizoplasts, and filaments during different stages of development. 5. Anterior extremity exhibits an oval unstained cavity, through which the rhizoplasts and connecting links with the flagellate nucleus appear to occupy.

Fig. 9. Artificial dislocation of the blepharoplast (*a*) and nutritive nucleus (*b*), showing the fine connecting links between the circular flagellate nucleus and the rhizoplasts, also the continuation of the filament posteriorly, although the nutritive nucleus has been removed.

Fig. 10. Parasite undergoing division in which anterior extremity is involved, and both nuclei, while in addition a flagellum has developed, at the posterior extremity, which is connected by a fine filament with a much enlarged tripolar body. Trypanoplasma, *vide* Plate I ?.

Fig. 11. *Herpetomonas sarcophagæ ?, n. sp.* (Prowazek) undergoing division, together with the formation of an additional flagellum, intimately attached to an interior body in connection with the smaller of the two nutritive nuclei.

Fig. 12. Illustrating two additional peculiar forms of flagellate nuclei in *Herpetomonas* undergoing reduction, together with the apparent connecting links between the rhizoplasts and blepharoplast.

B.—Fig. 1. Flag form showing filament passing straight to nutritive nucleus.

Fig. 2. Filament passing from blepharoplast to the nutritive nucleus, round the extremity of the organism, and again joins the flagellate nucleus. The nutritive nucleus presents eight well-marked divisions (chromosomes).

Fig. 3. Bicornua form of parasite, showing stage previous to development of the second flagellate apparatus, in the newly-formed cornu.

Fig. 4. Flag form showing filament passing the extremity of organism before proceeding to the nutritive nucleus.

Fig. 5. Mode of development of flag form, one curve laterally and a fold between the two, of the long form of *Herpetomonas m. d.*

Fig. 6. Mode of development of the diagonal or rhomboidal form of parasite, from a treble folding of the long form of *Herpetomonas m. d.*

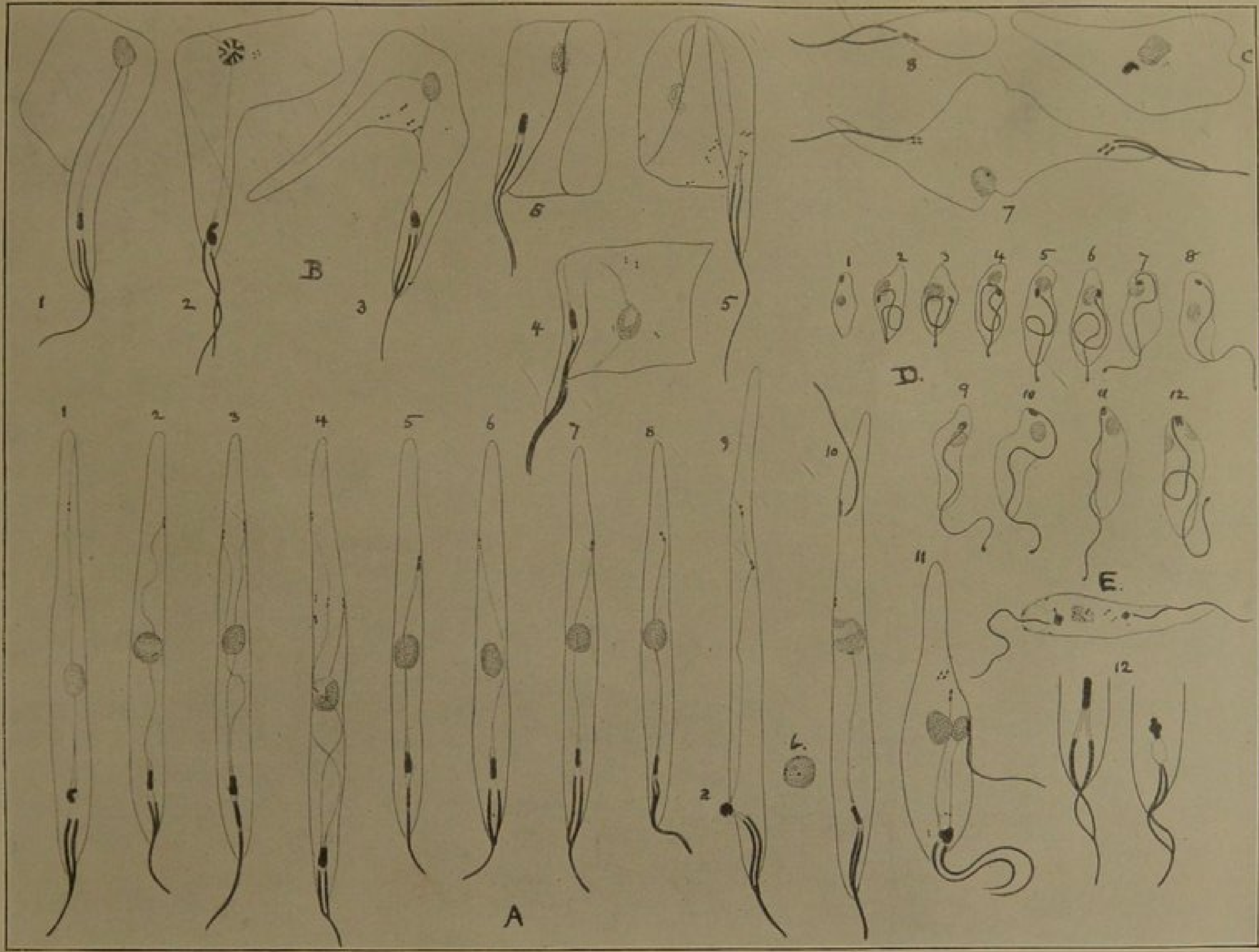
Fig. 7. Division by development of a second cornu and flagellum in a horizontal direction, segmentation of nutritive nucleus.

Fig. 8. Small pear-shaped parasite.

C.—Fig. 1. Appears to be an instance of copulation of two like forms. *Herpetomonas sarcophagæ* ?, accompanied by an early stage of reproduction in both forms of nuclei simultaneously.

D.—Figs. 1–12. 1. Exhibits no sign of flagellate apparatus, and probably belongs to a different species. 2–12. Certain stages observed in the new species of flagellate. Primarily the flagellum is situated within the body cavity of the organism, together with the terminal knob of the flagellum. 12. Partial retraction of the flagellum when division of one or other nucleus takes place.

E.—Trypanoplasma, bipolar flagella, new species.



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DESCRIPTION OF PLATE V.

Figs. 1–10.—*Herpetomonas, n. sp.*, discovered in the intestinal contents of *musca domestica* at 7500 feet elevation.

Figs. 11–17.—*Flagellate, n. sp.* (*vide* Plate IV, D 2–12). Flagellum running whole length of parasite, and free end terminating in a rounded enlargement.

Fig. 18.—*Flagellate, n. sp.* Length of body and flagellum equal.

Fig. 19.—*Rhomboidal form* of flagellate.

Fig. 20.—*Flag form* of flagellate.

Fig. 21–25.—*Herpetomonas m. d.*, showing various forms and stages of development found in various diptera.

Fig. 26.—*Flagellate, n. sp.*, undergoing division into three. Three nutritive nuclei, but one flagellate nucleus and two rhizoplasts. Flagellum 3·33 times length of body.

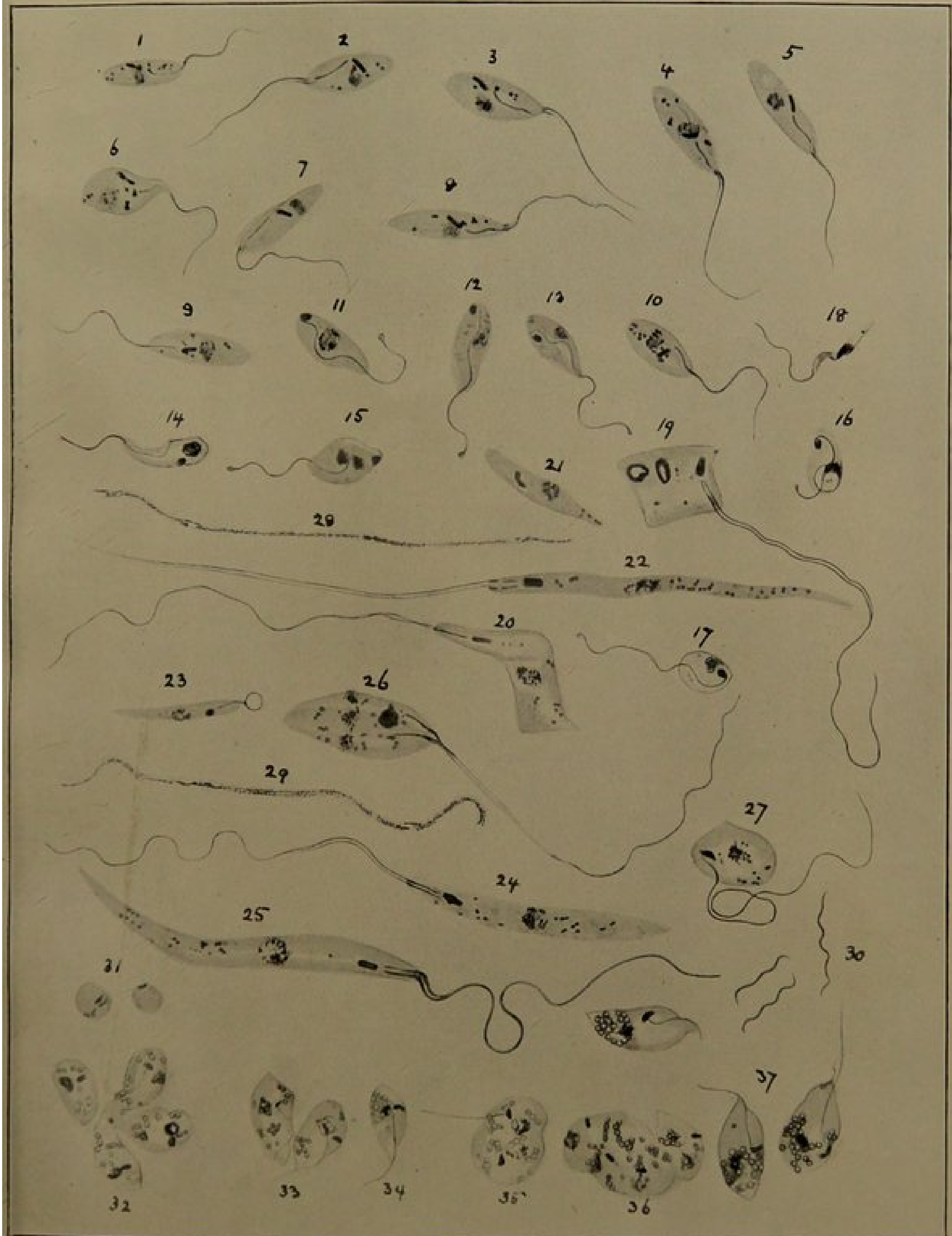
Fig. 27.—*Circular form* of flagellate. Length of flagellum four times diameter of the body.

Figs. 28, 29.—*Filaria embryos*, discovered in the intestinal contents of *Tabanus tropicus*. Fig. 29 exhibits the sheath at the cephalic extremity, a marked V-shaped organ and granular aggregation.

Fig. 30.—*Spirillum* discovered in the intestinal contents of *Culex* larva, pupa, and mature mosquito in the plains.

Figs. 31–37.—Flagellate forms met with in *Culex* larvæ in the plains. Fig. 31. The immature or circular bodies. Fig. 32. Four young forms, one exhibiting a flagellate apparatus. Fig. 33. Two young parasites, refractile bodies scattered through the body-cavities. Fig. 34. Young mature forms, the refractile bodies collecting at the posterior extremity. Fig. 35. Parasite undergoing division into two. Fig. 36. Division into four parasites. Fig. 37. Three mature flagellates exhibiting prolongation of periplast on to flagellum, and one refractile body situated at the anterior extremity of the organism.

PLATE V.



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