Studies on Parasitic Protozoa.

III.

(a) Notes on the Flagellate Embadomonas.

(b) The Multiplication Cysts of a Trichomastigine.

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With Plate 9.

(a) The Flagellate Embadomonas.

Introductory.

In 1911, I recorded from the intestine of trichopterous larvæ, a slipper-shaped flagellate with a relatively enormous cytostome. I named the organism *Embadomonas*, and pointed out that it showed affinities with *Chilomastix* Alexeieff, 1911 (Syn. *Macrostoma*, Alex., *Tetramitus* Alex.), from which it differed in its general form, and in the number of the flagella. The species from trichoptera I called *E. agilis*.

I have since found *Embadomonas* in far greater quantity in the intestine of the crane-fly larva (Mackinnon, 1912). Here there are two distinct species; one is morphologically indistinguishable from *E. agilis*, but the other and more abundant is a much larger, more robust form in which the details of structure, rather obscure in *E. agilis*, can be studied with ease. As I have also found dividing and encysted stages, I am now in a position to supplement my former description. I do so the more readily that I deplore the hasty and incomplete accounts of "new" protozoa of which the literature is already only too largely composed. Sometimes these "descriptions" are not illustrated by a single drawing: sometimes a figure of some selected stage is given, so little typical that it helps other observers scarcely at all in their work of identification. The classification of the flagellate protozoa is admittedly full of anomalies and confusions. The best way to set about clearing away some of these is to work out carefully, and as fully as may be, the structure and life-history of every "new" form described, the account to be accompanied in every case by an adequate and representative series of drawings. In this way comparison between different forms becomes at least possible, and in time a better understanding may lead to a more natural grouping. The present tendency, however, seems to be in the opposite direction. It is the fashion to start with a theory, and then go out in search of organisms wherewith to illustrate it; the mind and eye thus prejudiced are aware of and record only those cases that do what is expected of them, by conforming to the views of the particular school to which the so-called scientist belongs. The "centriole question" with its attendant absurdities and exaggerations is a case in point.

The Genus Embadomonas, Mackinnon, 1911.

Diagnosis.—This genus contains small slipper-shaped flagellates, characterised by a very large cytostome bordered by prominent lips, which are more or less siderophilous, and two flagella, not so long as the body, one acting as an organ of locomotion, and the other lying in the cytostome; the spherical nucleus is placed at the anterior end of the body; the two basal granules, from which rise the flagella, lie at the anterior border of the cytostome. There is a definite periplast, which prevents deformation of the body. The anterior part of the body shows a well-marked torsion. The cysts are relatively small, and are ovoid in form.

As "species characters" may be used: (1) The form of the body; (2) the nature of the periplast; (3) the degree of development of the cytostome and its lips; (4) the size of the cysts.

Embadomonas agilis, Mackinnon, 1911 (Pl. 9, fig. 21–26).

Exceedingly slender, slipper-shaped body, with the anterior end bent back, and the posterior end sharply pointed. There is a delicate periplast. The cytostome is large, but its borders only feebly siderophilous. The flagellum within the cytostome is exceedingly delicate and inconspicuous: it cannot be made out at all in any but the largest individuals.[1] The nucleus is spherical, with a central group of chromatin granules, and some peripheral chromatin; the nuclear membrane is often very indefinite.

Dimensions of flagellates: $4\,\mu \times 1{\cdot}5\,\mu$ to $11\,\mu \times 3\,\mu$.

Dimensions of cysts: $3{\cdot}5\,\mu \times 3\,\mu$ to $4\,\mu \times 3\,\mu$.

Habitat.—Intestine of trichopterous larvæ and larvæ of Tipula.

Embadomonas alexeieffi. Mackinnon, 1912 (Pl. 9, figs. 1–20).

Of much heavier, more "robust" build than the preceding species, the anterior end only slightly bent back, if at all—the posterior end nearly always rounded and blunt.[2] The periplast is relatively thick and well-developed. The cytostome is large, with prominent lips, which are very markedly siderophilous. The two flagella are well

[1] I overlooked the presence of this second flagellum until quite recently.

[2] The form of E. agilis may be compared to a lady's slipper with a high heel, that of E. alexeieffi to a clumsy sabot.

developed; the one lying in the cytostome often stains more deeply than the other. The spherical nucleus has an exceedingly definite outline; the chromatin is very variously disposed, but most common is the arrangement with a central group of granules and the peripheral chromatin mainly concentrated into a crescent-shaped mass at one side of the nuclear border.

Dimensions of flagellates: $7\mu \times 5\mu$ to $16\mu \times 9\mu$.

Dimensions of cysts: $5\mu \times 4\mu$ to $6\mu \times 5\mu$.

Habitat.—Intestine of larvæ of Tipula.

Division.

I have been able to find only a few individuals of Embadomonas (E. alexeieffi) in division, though I have searched through many preparations crowded with the organism. Few though these are, I think they are representative enough to show the general process of division. This is of some interest since we know practically nothing of the division in the allied flagellates, Chilomastix and Fanapepea.

The nucleus elongates (Pl. 9, figs. 4 and 5), and forms a spindle at the poles of which lie the basal granules supporting the flagella. The chromatin is disposed over the spindle in more or less definite granules, which travel to the poles as the spindle elongates, without first forming an equatorial plate, as far as I have been able to discover (Pl. 9, figs. 6, 7, and 8). The spindle thins and disappears (Pl. 9, fig. 9). Fig. 10, pl. 9, shows daughter nuclei concentrating, one at each side of the broadened organism, while the last trace of the spindle lies between them. The daughter nuclei now seem to go through a stage in which they appear as clear vesicles, with the chromatin in peripheral blocks and strands. Later, they take on the more usual form, with a central mass of granules and a peripheral layer.

A very marked constriction now appears midway between the nuclei (Pl. 9, figs. 11–15), and the two halves separate, hanging end to end till the uniting isthmus breaks. Flagellates recently emerged from division may have the posterior end drawn out into a point.

The fate of the cytostome and the flagella is very variable. In some rare cases the cytostome seems to widen out laterally, and to be divided between the daughter individuals by the new constriction that separates them one from another (Pl. 9, fig. 11). But as a rule (Pl. 9, figs. 6–10) it disappears, and apparently is reformed in each daughter individual. This second condition of things is said by Alexeieff to occur in Chilomastix caulleryi, and Kuczynski figures the same thing for Chilomastix intestinalis.

One flagellum may pass with its basal granule to each end of the spindle (Pl. 9, fig. 5); sometimes the basal granule divides there almost at once (Pl. 9, fig. 8), and the second flagellum grows out very early; at other times the formation of the second flagellum may not have taken place even at quite a late stage (Pl. 9, fig. 13).

On two or three occasions I found individuals, such as that shown on Pl. 9, fig. 2, in which there were two nuclei. These nuclei did not occupy the opposite sides of the organism, as one would expect if they were the product of a division, but lay pressed close together at the anterior end. They were each below the normal size for an organism of the size in which they occurred. Probably such individuals should be regarded as "freak" forms, but the possibility of a sexual process should be borne in mind.

Encystment.

The cysts of Embadomonas are very abundant in the larvæ of Tipula. The encysting individual ceases to feed and loses its flagella, its cytoplasm shrinks away from the pellicula, which surrounds the cyst like a loose coat and persists for a long time (Pl. 9, fig. 17). The cytoplasm becomes more compact and dark-staining, especially around the margins, and there it forms a thick and definite cyst wall, "à double contour" (Pl. 9, fig. 18). The cyst is from the first ovoid, but this shape becomes more marked in the later stages. Within the cyst the borders of the cytostome remain as dark-staining, loop-shaped strands. The nuclear membrane disintegrates and the chromatin escapes in groups of granules (Pl. 9, figs. 19 and 20). No multiplication seems to take place within the cyst, though probably there is some important readjustment of the nuclear substances. In this encysted state the organism is probably transferred from host to host through contaminated food. Very small individuals can sometimes be seen, measuring about 3·5 μ to 4 μ in length, with a vesicular nucleus, relatively very large cytostome, and only one long flagellum visible (Pl. 9, fig. 16). It is possible that these are individuals recently emerged from the cysts.

The Affinities of Embadomonas.

Embadomonas is certainly allied to Chilomastix Alexeieff, and to Fanapepea Prowazek.[1] In Chilomastix there are three anteriorly directed flagella, and the fourth lies in the cytostome: in Fanapepea there are only two anterior flagella, and the third in the cytostome. The number of the flagella and something in the shape of the organism seems to have impressed observers such as Prowazek, Wenyon, Alexeieff and Kuczynski with the idea that there must be a close affinity between these organisms and the trichomonads. In recent classifications (Doflein, 1911, and Alexeieff, 1914), this seems to be taken for granted; Kuczynski (1914) includes Chilomastix intestinalis in a study on the trichomonads; Prowazek goes so far as to declare that alongside his Fanapepea he "once" (!) found a species of Trichomonas with a short undulating membrane "die phylogenetisch zu der folgenden neuen Flagellatenart (Fanapepea intestinalis) führt." He gives one small and unconvincing figure of this

[1] It is not easy to make out, from Prowazek's very poor figures, in how far the genera Chilomastix and Fanapepea really differ. Alexeieff (1914) suggests that these are different names for the same organism, but if that be so, then Prowazek must have overlooked one of the anterior flagella.

new Trichomonas, which is like nothing in the world so much as Chilomastix, and which excuses Alexeieff's scepticism with regard to Fanapepea. And then he goes off into the favourite phylogenetic speculations: "Diese Flagellatenform deutet daraufhin dass die undulierende Membran der Trichomonaden phylogenetisch anders abzuleiten ist als die undulierende Membran der Trypanosomen, die nur eine Art Periplast-lamelle darstellt. . . . Die undulierende Membran der Trichomonaden stand dagegen ursprünglich als ein Strudel- und Lippen-organell direkt im Dienste der Nahrungsaufnahme, und trat erst, etc." All of which may be true, but there is literally nothing to prove it.

Now I think that, even if Chilomastix and its allies be related in some degree to the trichomonads, the resemblance is very largely superficial, and that if Embadomonas, with its two flagella, had been the genus first examined the closeness of the relationship would not have been so strongly insisted on.[1] For consider: The axostyle is a characteristic possession of the trichomonads; there is no trace of such a structure in Chilomastix or Embadomonas, nor in Fanapepea, for the matter of that. There is a cytostome in both kinds of flagellate, it is true, but in the trichomonads it is a comparatively small and insignificant, and sometimes a transient structure, while in the chilomastigines it is half as long as the body at least, extremely definite and constant, and bordered by prominent lips. The trichomonads are characteristically "naked" and extremely plastic[2]: the chilomastigines have a well-developed periplast "Der Körper ist von einer Pellicula bedeckt," Prowazek. The nucleus of the chilomastigines is not at all like that of trichomonads. Nor are the division phenomena very similar in the two groups, so far, at least, as I know them. The cysts of Chilomastix and Embadomonas resemble one another in essential points,

[1] For one might almost as plausibly derive Chilomastix through Embadomonas from a Bodo!

[2] Kuczynski (1914) mentions an exceedingly delicate periplast in trichomonads, demonstrable by special methods.

but are not like the cysts of trichomonads, so far as these have been described.

In fact, in the trichomonads and chilomastigines, we are dealing with two very highly specialised groups of flagellates, both of which must have diverged very considerably from some much simpler type, and neither of which is in the least likely to be the ancestor of the other.

As observations on the chilomastigines seem to be increasing, and as the references are scattered through the literature, I give a list of the species hitherto described.

Chilomastix Alexeieff (1911), (Macrostoma Alex. (1909), Tetramitus Alex.) with one flagellum in the cytostome, and three others directed forwards.

Chilomastix caulleryi (Alexeieff, 1909) from the intestine of the frog tadpole.

C. mesnili[1] (Wenyon), 1910, from the intestine of man.

C. bocis Brumpt, from Box salpa.

C. gallinarum Martin and Robertson (1911) from the rectal cœca of the fowl.

C. intestinalis Kuczynski (1914) from the intestine of the guinea-pig.

Fanapepea v. Prowazek (1911) with one flagellum in the cytostome, and two others directed forwards.

F. intestinalis v. Prowazek (1911) from the intestine of a baboon, and from human fæces.

Embadomonas, Mackinnon (1911) with one flagellum in the cytostome, and one other directed forwards.

E. agilis, Mackinnon (1911) from intestine of larval trichoptera and Tipula.

[1] Alexeieff (1914) thinks that these forms, C. caulleryi and C. mesnili, may be identical. This author strongly advances the opinion, which I share, that in the case of intestinal flagellates, at any rate, the "parasitic specificity" is by no means so close as used to be supposed. A number of protozoa, which are saprophytes rather than parasites in the strict sense, are capable of existing in the food-canal of widely different sorts of animals, and do so if the animals have access to the same contaminated food-supply. This fact should be kept in mind when new species are described.

E. alexeieffi, Mackinnon (1912) from intestine of larval Tipula.

(b) Multiplication Cysts of a Trichomastigine.

It is almost certain that delicate intestinal flagellates must usually be transferred from host to host protected from desiccation by a cyst. The information concerning the cysts of trichomonads is scanty, and much of it has been rendered suspect since Alexeieff (1912) showed that the so-called cysts of Trichomonas intestinalis have nothing to do with any flagellate, but are stages in the life-history of the organism that he calls Blastocystis enterocola.

Dobell (1909) figured and described two undivided protection cysts of Trichomonas ranarum, but his material was admittedly very scanty. Since then authors have again and again casually remarked that they have seen the cysts of one or another species, but they give no details and no figures.

Martin and Robertson (1911) found the cysts of the trichomonads of the fowl, while Kuczynski (1914), who has recently made an exhaustive study of a number of trichomonads, seems to have had no difficulty in obtaining the cysts, though he also refrains from giving any helpful pictures. He, indeed, goes on to quote Escomel (1913), who, speaking of his cultures of a trichomonad, says: "Le parasite qui a pris la forme ovale, s'enkyste. En cet état il peut vivre longtemps avant de se diviser. Lorsque les conditions du milieu sont favorables, la division s'opère et les jeunes Trichomonas s'échappent du kyste rompu."

In 1911 I described and figured what I considered to be the multiplication cysts of Trichomastix trichopterorum, Alexeieff (1914) has found very similar cysts in the intestine of Box salpa, and he ascribes them to Trichomastix salpæ, Alexeieff. His figures, inadequate though they are, show that he is dealing with division cysts comparable with those I described from trichoptera.

The published descriptions, then, being insufficient for purposes of identification, I think it helpful to give here a series of figures (Pl. 9, figs. 27–36) showing the division in the cysts of the trichomastigines from Tipula. In a general way these resemble very closely the cysts of Trichomastix trichopterorum Mackinnon, but I have obtained a much more complete series. There are two trichomastigines in Tipula, one a typical Trichomastix, which I could not distinguish from T. trichopterorum, and the other a form with an additional flagellum, Tetratrichomastix parisii Mackinnon. I am unable to say to which of these the cysts belong.

The flagellate rounds itself off, and the flagella show a strong tendency to adhere to the sides of the body[1] (Pl. 9, fig. 27). The cysts are almost spherical when fully formed, with a diametrical measurement of 4 to 5 μ. They have a relatively thick wall. Within the cyst the flagella may persist as dark coiled lines lying superficially in the cytoplasm. The cytoplasm is otherwise remarkably clear, and free from granules. The axostyle disappears. The basal granules separate, and between them extends a centrodesmosis (Pl. 9, fig. 28) on which the nucleus comes to lie. The nuclear membrane has disappeared, and the chromatin breaks up into four large masses, approximately spherical in form ; the basal granules move to opposite poles of the cyst, and the nucleus lies suspended between them (Pl. 9, figs. 29–31). The four chromosomes, if one may call them so, apparently divide, though I have not found a stage showing the process. The next stage available shows two daughter-nuclear masses, each containing four smaller chromatin clumps (Pl. 9, figs. 32 and 33); these move to opposite poles. The centrodesmosis draws out longer and longer, so that the two nuclear masses approach one another once more and lie superficially at one side of the cyst. Below them extends the loop of the centrodesmosis (Pl. 9, fig. 34), which breaks at last into two, each half forming the axostyle of one of the two

[1] Cf. Martin and Robertson's "swathed forms" of Trichomastix gallinarum.

new daughter-flagellates, as in the free-swimming stage.[1] Some of the flagella of the mother organism seem to persist through the entire process—others are re-grown (Pl. 9, figs. 34–36). In late stages the cyst tends to lose its spherical form, while the cytoplasm begins to divide into two, and the nuclei show the adult flagellate structure. There is no trace of any sexual process.

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Mackinnon, D. L.—"Some more Protist Parasites from Trichoptera," 'Parasitology' (1911), vol. iv, pp. 28–38, 1 pl. and 8 text-figs.

——— "Protists parasitic in the Larva of the Crane-fly, Tipula sp." (Preliminary note), ibid. (1912), vol. v, pp. 175-189, 27 text-figs.

[1] A great deal of discussion has centered round the division phenomena of the trichomonads, and the accounts of the formation of the axostyle are very contradictory. I sometimes wonder whether the slender, dark-staining line that forms the axostyle of Trichomastix trichopterorum, T. salpæ, Tetratrichomastix parisii, etc., is strictly homologous with the stout, non-siderophilous, supporting rod of Trichomonas eberthi, Trichomastix gallinarum, etc. The homology is always assumed, but supposing it not really to exist, then the discrepancies between the accounts of the behaviour of "axostyles" in division might be explained to some extent.

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Wenyon, C. M.—"A new flagellate (Macrostoma mesnili, n. sp.) from the Human Intestine," 'Parasitology' iii, pp. 210–216, 1 pl., 2 text-figs.

EXPLANATION OF PLATE 9,

Illustrating Miss D. L. Mackinnon's paper on "(*a*) The Flagellate Embadomonas; (*b*) Multiplication Cysts of a Trichomastigine."

[Figures drawn to scale (× 3900 ca.) under Zeiss comp. oc. 12 and 2 mm. apochromat. The stain employed was Heidenhain's iron-hæmatoxylin, after fixation with sublimate-alcohol.]

Figs. 1–20.—Embadomonas alexeieffi.

Fig. 1.—Typical flagellate, with large cytostome (*c.*), anteriorly placed nucleus (*n.*), two flagella (*f.*), two basal granules (*b.g.*), and numerous ingested bacteria (*b.b.*).

Fig. 2.—Large individual with pointed extremity and two half-sized nuclei.

Figs. 3 *a*, *b*, *c*.—Three types of nucleus. In 3*c* the basal granules are further from the cytostome margin than usual; this gives a false appearance of the flagella springing from the nucleus.

Fig. 4.—Rounded-off individual with elongated nucleus. The beginning of division?

Fig. 5.—The division spindle forming, with the basal granules at the poles. The new flagella have already been re-grown; the cytostome persists.

Fig. 6.—A stage comparable with fig. 5, but with both the flagella at one end of the spindle; the cytostome has completely disappeared.

Fig. 7.—The division spindle extending right across the rounded-off organism. The cytostome has disappeared and one new flagella has grown out.

Fig. 8.—A slightly later stage than fig. 7. The chromatin is concentrating at the poles; one of the basal granules has divided; the new flagella have both grown.

Fig. 9.—The drawn-out spindle shows a constriction in the middle.

Fig. 10.—The spindle is drawn out in the middle almost to breaking point; the nuclei are forming at each end; the cytostome of one of the daughter-individuals is appearing.

Fig. 11.—The cytoplasm begins to divide by a median constriction; the nuclei takes on the adult form. In this case the cytostome seems to be dividing in two. The new flagella have not yet been re-grown.

Fig. 12.—A slightly later stage than 11, in which the cytostomes are complete, and there is the full complement of flagella.

Fig. 13.—An individual with two nuclei recently emerged from a division, but with one large cytostome only, and no flagella.

Fig. 14.—The daughter flagellates separating.

Fig. 15.—Only a narrow strand of cytoplasm connects the separating individuals. In one of these the cytostome is only just beginning to appear even at this late stage.

Fig. 16.—A very small individual, with relatively large cytostome and one long flagellum. Young form recently emerged from cyst?

Fig. 17.—Encysting individual. The cytoplasm has shrunk away from the periplast (*p.*); the chromatin is escaping from the nucleus.

Fig. 18.—The cyst-wall is formed; the periplast (*p.*) still invests the whole as a loose envelope. The border of the cytostome and one of the flagella can be seen inside the cyst.

Figs. 19 and 20.—Slightly later stages, in which the nuclear membrane has disappeared, and the chromatin has escaped in clumps into the cytoplasm.

Figs. 21–26.—Embadomonas agilis.

Figs. 21, 22, 23.—Cysts of E. agilis.

Figs. 24, 25, 26.—Flagellate individuals. In the very small one, fig. 25, only one flagella is visible.

Figs. 27–36.—Encystment of a Trichomastigine.

Fig. 27.—Trichomastix rounding itself off. The flagella show a tendency to wrap round and adhere to the body of the organism.

Fig. 28.—The flagellate completely rounded off, but the cyst-wall not yet formed; the axostyle is disappearing; the basal granules have separated; between them lies a centrodesmosis.

Fig. 29.—The cysts formed. Within can be seen the nucleus and a complex interweaving of dark strands—probably the persisting flagella.

Fig. 30.—The nucleus, suspended on the centrodesmosis, divides into four large masses. The cyst-wall has not yet been formed in this case.

Fig. 31.—A stage comparable with fig. 30, except that the cyst-wall is complete.

Figs. 32 and 33.—The nucleus has divided into two, each containing four smaller chromatin masses. The centrodesmosis persists.

Figs. 34, 35, and 36.—The nuclei move nearer and nearer one another and seem to lie at one side of the cyst. The drawn-out centrodesmosis forms the axostyles of the new individuals. Fresh flagella are seen growing out from the basal granules. In fig. 36 the cytoplasm is beginning to divide.