On some more Gregarine parasites of Indian Earthworms.

By

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(With Plates 11—15.)

Introductory.

GHOSH (1923), BHATIA (1924), and BHATIA and CHATTERJEE (1925) have previously described a number of monocystid parasites from various earthworms found in India. A complete list of the hosts examined and the parasites described from them is given in the paper mentioned last.

The junior author of the present paper has had the opportunity of examining a number of other earthworms. He made preliminary observations on a species of *Nematocystis* found in *Eutyphoeus incommodus* (BEDDARD), while working at the Central Research Institute, Kasauli, during the summer vacation of 1922. On thorough examination of the preparations then made by him, we have come to the conclusion, that the parasite belongs to a new species. He has further had the opportunity of examining the contents of the seminal vesicles of *Pheretima posthuma* (L. VAILL.), *Pheretima suctoria* MICH., *Pheretima elongata* (E. PERR.) *Erythraeodrilus kempi* (STEPH.), and *Megascolex trilobatus* (STEPH.), while working at the Royal Institute of Science, Bombay, and has made a series of preparations

from these worms. He wishes to thank Col. Harvey, I.M.S., Director Central Research Institute Kasauli, for permission to work there, and to Prof. P. R. Awati, I.E.S., Professor of Zoology, Royal Institute of Science, Bombay, under whom he has had the pleasure to work for the last two years, for the encouragement and guidance given by him.

Our best thanks are also due to Dr. J. Stephenson of the Edinburgh University, for specific identification of the worms submitted to him.

We have now gone over all the preparations together and the results of our observations are recorded in the following pages. The microphotographs in Plates 13—15 have been prepared at the Zoological Laboratory of the Government College, Lahore.

The genus Monocystis.

Monocystis matthaii nov. spec.

Diagnosis. *Monocystis* attaining a size up to 238 μ. The trophozoites and cysts are so large that in the infected animal they are seen with the naked eye as rounded projections of the size of pin heads, studding the median seminal vesicles. The form of the trophozoite is variable, being most commonly spherical, sometimes ovoid or kidney-shaped. Nucleus is spherical, with a single spherical karyosome, placed eccentrically. Cysts are ellipsoidal, reaching up to 468 μ in their long diameter.

Host. *Megascolex trilobatus* (Steph.) from Bombay. The organism characterised above was found abundantly in the seminal vesicles of *Megascolex trilobatus* (Steph.), collected at Bombay, during the months of July, August, September and October. An interesting observation was made that the parasite is mostly confined to the median seminal vesicles of the host. The lateral seminal vesicles contained them but rarely and very few at a time.

The hosts secured from Lal Bagh (a place near Parel in Bombay) were highly infected, sometimes the entire seminal vesicles being studded over by the parasites, but the same hosts obtained from Charni Road garden (another part of Bombay) has never been found to be infected by the parasite. Hesse (1909) has recorded a similar observation in the case of *M. hirsuta* occurring in the seminal vesicles of *Lumbricus castaneus*, Sav., collected at Montessaux, but not in the same host taken at Grenoble. The present authors have previously remarked on the occurrence of parasites being confined

to hosts in particular localities, in the case of certain cephaline gregarines (1924).

Trophozoites. The usual form of the trophozoite is spherical. As in other species of Monocystis, the parasite can assume somewhat different shapes caused by the movements of the endoplasm. It is then seen to be ovoid or kidney shaped, or even top-like. These different forms are clearly seen in our fixed and stained preparations. In its most common form which is spherical, the trophozoite recalls the form of *M. villosa* HESSE, but it shows more active movements and consequent changes of form. The dimension of the trophozoite, nucleus and karyosome are also markedly different. Moreover there are no hairs covering the parasite, which is a characteristic feature of *M. villosa*. The epicyte is thin and does not show any meridional striations. The sarcocyte is hyaline and little developed. The myocyte fibres are not distinguishable.

The endoplasm appears very dark in life. It is finely granular and the alveoli are compactly arranged and contain large grains of reserve material.

The nucleus is placed in different parts of the body. It is spherical and possesses one large spherical karyosome, which is generally a homogeneous, compact mass. The karyosome is rarely seen to be vacuolated, as described for other species. The karyosome is eccentric in position. In addition numerous tiny chromatin grains are sometimes seen to be scattered over a fine reticulum of linin, though most often all the chromatin appears to be condensed in the karyosome. The dimensions of a number of trophozoites, and their nuclei and karyosomes are given below:

Length of trophozoite.	Width of trophozoite.	Diameter of Nucleus.	Diameter of Karyosome.
63 μ	Spherical	14 μ	3,5 μ
126 μ	„	31,5 μ	8,7 μ
133 μ	108,5 μ	31,2 μ	9,6 μ
189 μ	Spherical	38,5 μ	10,5 μ
231 μ	„	42 μ	10,5 μ
231 μ	203 μ	35 μ	10,5 μ
238 μ	Spherical	35 μ	10,5 μ

Association and encystment. Association usually takes place between equal-sized individuals, though the sporonts are sometimes unequal in size also. As the sporents come together, they contract greatly, sometimes so much so, that the dimensions of the two taken together are only slightly greater than a single free trophozoite. In some cases the nucleus in each gametocyte is found to

have divided into daughter nuclei, before the associants have secreted a cyst-wall round themselves. Cysts of different sizes are met with. The largest reach 468 μ in their longer diameter. The cysts are ellipsoidal and contain spores of the usual form and structure. The average size of the spores is 14,5 μ by 7,6 μ and each spore contains 8 sporozoites.

We have come across cases of solitary encystment also in this species. More rarely we have encountered cysts containing three individuals. Such abnormal cysts have been previously found by Cuénot (1901) in *Diplocystis*, by Berndt (1902) in *Gr. cuneata*, by Woodcock (1906) in *Cystobia irregularis*, by Cunningham (1907) and Robinson (1910) in *Kalpidorhynchus*, and by Bastin (1919) in *Monocystis agilis*. Contrary to the opinion expressed by Berndt and Woodcock, Bastin has been able to support Cuénot's view regarding the possiblity of development taking in these cysts, as he found that the associants proceeded to the stage of gamete formation. In one of our preparations, we find that all the three associants inside a cyst contain fully developed spores, and are thus able to give further support to the view of Cuénot and Bastin.

The genus Nematocystis.

Eight species of this genus have been described so far. *Monocystis magna* Schmidt was referred to this genus by Hesse and three other species described by him in 1909. Cognetti de Martiis has described two (1918 and 1921), Berlin a new variety (1924) and Bhatia and Chatterjee two more species and occurrence of two others in new hosts (1925). One new species is described below.

Nematocystis stephensoni nov. spec.

Diagnosis: Monocystid of an elongated, cylindrical form, short and swollen or elongated and thin. Body highly deformable, presenting a number of bulgings and constrictions, with blunt extremities. Size up to 1260 μ in length and 100 μ in thickness. Epicyte shows fine longitudinal striations. Nucleus elliptical with a single central karyosome, which consists of a central deeply staining portion surrounded by a vacuolated layer.

Host: *Eutyphoeus incommodus* (Beddard) found at Kasauli.

These parasites were discovered by one of us, while working at the Central Research Institute, Kasauli, during July and August 1922. A large number of specimens of *Eutyphaeus incommodus*

Bedd. were examined, and about 40 per cent of the worms were found to be infected with this species of Nematocystis. No other monocystid was encountered in the seminal vesicles of this host, and thus the study of different stages was not complicated by any mixed infection. The parasite is abundantly found in an infected host and has been studied in all stages of development. Generally, in smears obtained from one worm, there is a preponderance of parasites in a particular stage of development.

In the living condition the parasites are quite opaque and are capable of contracting and elongating so rapidly, as to produce constrictions and bulgings, giving the parasites the same appearance as has been described for *N. magna* by Hesse and for *N. plurikaryosomata* by Bhatia and Chatterjee. The opacity of the granular cytoplasm is so great that the nucleus appears to be only faintly indicated.

In the earliest stages of development, the young trophozoite is contained within a blastophore and surrounded by a group of spermatic cells. It is then seen as a small rounded speck in which the cytoplasm and nucleus are clearly recognisable. As it grows, it becomes free, and is seen to lie in the contents of the seminal vesicle, not attached to any cell. During its growth, it increases both in length and width, but the increase in length is proportionally much greater, so that it comes to assume a vermiform appearance. As in other species of *Nematocystis*, the parasite looks like a nematode worm, both in its form and mode of progression. The largest sized individuals measure 1260 μ in length and 100 μ in width. The grown up trophozoite differs from the young in its cytoplasm being more compactly granular and the karyosome of its nucleus being more varied in form. The adult trophozoite differs from that of *N. vermicularis* by the absence of hairs or any epimeritic denticulations or other ornamentations.

In the stained specimens, the cytoplasm of the trophozoite exhibits the usual structure. The ectocyte is bounded externally by a fine cuticle. The entocyte is excavated by alveoli and a large number of ellipsoidal paramylon grains are lodged in this layer. The nucleus is generally situated about the middle of the body, but is often found to occur near one or the other extremity. This is due to its being tossed about by the movements of the cytoplasm. Its elongated axis generally lies in a direction parallel to the long axis of the body. The shape and structure of the nucleus is very characteristic and puite different from that of the other species.

The nucleus is of an elongated, regularly ellipsoidal form, and consists of (i) a fairly well defined nuclear membrane, (ii) a broad zone of peripheral achromatin, in which the network is not distinguishable, and over which fine chromatin grains are dispersed, and (iii) a large central, spherical or oval karyosome, which is like an entire nucleus in structure and appears very much like a nucleus within a nucleus (cf. Doflein 1916, p. 22). A clear whitish area surrounds the karyosome like a halo. The nucleus thus appears to consist of two concentric rings, and a deeply staining homogeneous mass in the centre (Fig. 12). The inner ring and the central homogenous mass together constitute the karyosome. That this is so, is borne out by the fact that in some preparations, the ring surrounding the central dark mass, appears to be only slightly less dark than the central mass. The vacuolar structure of this outer portion of the karyosome is well seen in preparations stained with Iron Haematoxylin or Iron Haematoxylin and eosin, but the central mass is not vacuolated. The structure of the karyosome is thus different from that described by Hesse in the nuclei of *N. magna* and *N. lumbricoides* (Hesse, 1909, pp. 150 & 155), in which cases, both zones of the karyosome are described as vacuolated. In our specimens, the central mass is not vacuolated and chromatin is found in the form of discrete particles over the outer vacuolated zone of the karyosome.

The central deeply staining homogeneous mass is variable in form. In young forms it may be spherical, somewhat crescent-shaped, or irregular in form, with a large amount of chromatin extruded into the outer vacuolated zone. In some cases chromatin masses are seen to form small bulgings from the central mass. In the larger specimens the central homogeneous mass is distinctly oval.

The nucleus measures 38,5 μ in diameter and the karyosome 15,7 μ in the largest specimens. In one specimen, the nucleus was found to contain two karyosome (Fig. 13), but the structure of these is different from that given above.

The dimensions of a number of trophozoites with their nuclei and karyosomes are given below:

Length of trophozoite.	Width of trophozoite.	Diameter of Nucleus.	Diameter of Karyosome.
35 μ	24 μ	7 μ	3,5 μ
56 μ	35 μ	17,5 μ	8,7 μ
105 μ	42 μ	17,5 μ	12,2 μ
182 μ	56 μ	24,5 μ	14 μ
238 μ	42 μ	35,5 μ	14 μ
1120 μ	20 μ	38,5 μ	15,7 μ

Development. Different stages in the life-history of this parasite have been found and studied, but it is unnecessary to describe them in full, as the development is on the usual lines. The gametocytes are equal in size. They become closely applied and pressed and flattened against each other. The cuticle covering the surfaces of contact becomes absorbed and the cytoplasm becomes continuous. The cysts containing them are slightly oval and measured 153 μ by 100 μ in the smallest to 314 μ by 246 μ in the largest ones. These cysts are visible to the naked eye, and appear quite white and opaque in the living condition. They have thick cyst walls and could not be stained properly in the smears by the ordinary methods. But the structure of the interior can well be studied in sections. In each gametocyte the nucleus divides repeatedly. The karyosome in the nucleus breaks up and the chromatin appears in the form of a small number of rod-shaped chromosomes. The nuclear membrane disappears and a spindle makes its appearance with a centrosome at either end. At this stage the chromosomes are seen lying in an equatorial plate (Fig. 14). This karyokinetic division of the nucleus is repeated and gives rise to a large number of daughter nuclei. The daughter nuclei travel to the surface of the gametocyte. The cytoplasm then breaks up into as many small masses as there are nuclei. These gametes appear as protuberances from the surface of the gametocyte. The gametes are similar and those from the two gametocytes cannot be distinguished. These gametes are seen to unite in pairs (Fig. 15). The cytoplasm becomes united first, so that for a time small masses of protoplasm containing two nuclei are visible. On the fusion of these nuclei taking place, zygotes are formed. The zygotes measure about 8 μ. Each zygote soon becomes oval and is surrounded by a thick sporocyst. In each spore, the single nucleus divides by three repeated divisions into 8 nuclei, and 8 sporozoites are formed. These 8 nuclei are seen to be scattered, and so in some of the resulting sporozoites, the nucleus would be near one end. The spores are spindle-shaped and the ends are not drawn out. The spores vary considerably in form, some of them being slightly longer and narrower, while others are somewhat shorter and broader. The average size of the spores is 15,5 μ by 8,1 μ.

The genus Stomatophora.

This genus was established by Drzewecki (1908) to include *Monocystis coronata* Hesse, who attributed to it the possession of a mouth and a peristome, as also an anal opening. Hesse (1909)

pointed out that the alleged mouth and peristome were parts of a sucker-like organ and there was no anal opening. He described another species also, and defined the genus as follows: "Corps ovoïde ou spherique. Pôle antérieur muni d'une ventouse pétaloide à côtes radiées. Sporocystes biconiques à pôles semblables non appendiculés". Bhatia (1924) has described a third species and as the result of a comparative study of all the three species shown that the organisms are characterised by the possession at the anterior end of the body, of a sucker like epimeritic organ with a central mucron and radiating meridional ribs, but the occurrence of a petaloid crown surrounding it is found only in *S. coronata.* According to him the definition of the genus as quoted above from Hesse ought to be modified by the omission of the word "pétaloide", as even in *S. coronata,* the crown of petals surrounding the sucker is independent of the sucker itself. To the genus thus defined may be referred a new species which is described below.

Stomatophora bulbifera nov. spec.

Diagnosis. Monocystid with an elongated body, with a broad and round anterior end and a narrow and pointed posterior end. Size up to 119 μ. The body is not marked with any furrows or epicytal striations. The sucker at the anterior end is an inverted balloon-shaped depression, with a central mucron and radial epicytal striations. Nucleus oval with a large spherical karyosome.

Host: *Pheretima elongata* (E. Perr.) from Bombay.

In examining the smears of the contents of the seminal vesicles of *Pheretima elongata* (E. Perr.) from Bombay, we have come across a number of specimens which belong to a new species of *Stomatophora.* The form of the trophozoite is elongated cylindro-conical, with the anterior end broad and rounded, and the posterior end narrower and pointed. The greatest width of the organism is at a distance of one-third the length of the body from the anterior end. The smallest sized individuals measure 56 μ by 24,5 μ and the largest reach a length of 119 μ and a width of 52,5 μ.

At the anterior end of the body is a distinct depression resembling an inverted balloon in its form (Figs. 18, 19). The epicyte covering the surface of the body is carried inwards so as to line this depression. In specimens stained with Ehrlich's haematoxylin, there are visible some deeply stained denticulations situated near the mouth of the depression, and epicytal striations are seen to run backwards, diverging like the spokes of an umbrella. The

denticulations thus evidently correspond to the ends of the striations. In the bottom of the depression is a deeply stained mass, which from its position and relationship corresponds to the mucron. It consists of a dense entocyte covered over by sarcocyte and epicyte. By careful focussing the striations are found to be continued over the dark mass. Thus the structure resembles closely that of *S. simplex* or *S. coronata*, except that the floor is not raised like the bottom of a bottle, and the mucron from which the ribs run out is not brought close to the opening of the apparatus. Consequently when the organism is seen from the anterior face (Fig. 20), the opening of the depression is seen as a distinct circle without a central dark spot which is found in the other species. In this aspect the anterior end appears as a flattened plate with a circular area corresponding to the mouth of the sucker. By virtue of the anterior depression, the organism invites comparison with the new genera *Choanocystis* and *Craterocystis* established by Cognetti (1918 and 1921). These genera appear to be closely related to *Stomatophora*. *Choanocystis* is provided anteriorly with a cup-like collar, from the bottom of which arises a retractile tentacle in the only species known so far. The external surface of the collar is capable of being raised into lobes, and there is no epicytal ornamentation. In *Craterocystis* the anterior pole is provided with a cup-like concavity, with well with developed myoneme fibrils, and the convex area is provided with epicytal striations. In both cases the subrotund form of the adult trophozoite is mentioned as a generic character. In our opinion the form of the trophozoite may turn out to be of specific value only, when more species of these genera are discovered later on. It is not difficult to refer our form to *Stomatophora*, as it differs in several respects from both *Choanocystis* and *Craterocystis*.

Over the body the epicyte is thin, and the sarcocyte forms a thick, hyalin and transparent layer. The myocyte layer is not distinguishable. The endoplasm is alveolar, the alveoli being wide and thin-walled. Paramylon grains are numerous, minute and spherical, and are seen as deeply stained particles adhering to the walls of the alveoli.

The nucleus is oval and placed with its long axis parallel to the long axis of the body. The nuclear membrane is thick and deeply stained. There is a single, large and spherical karyosome. The dense mass surrounding the karyosome is seen to be contracted off from the nuclear membrane, leaving some alveolar spaces, and chromatin grains are dispersed over this achromatin mass. The

measurements of a number of individual swith dimensions of the nucleus and the karyosome are noted below:

Length of trophozoite.	Width of trophozoite.	Length & width of sucker.	Nucleus.	Karyosome.
56 μ	24,5 μ	7 μ × 7 μ	10,5 μ × 7 μ	3,5 μ
84 μ	42 μ	10,5 μ × 14 μ	14 μ × 7 μ	3,5 μ
84 μ	49 μ	10,5 μ × 17,5 μ	14 μ × 7,9 μ	3,5 μ
112 μ	49 μ	10,5 μ × 14 μ	14 μ × 8,7 μ	5,8 μ
119 μ	52,5 μ	12,2 μ × 17,5 μ	15,7 μ × 11,3 μ	5,2 μ

The genus Rhynchocystis.

Five species of this genus are known before. Description of two new species is given below, one of which is of special interest as showing a kind of schizygy not noticed before among Monocystids. A key to the identification of all the species is added at the end.

Rhynchocystis mamillata nov. spec.

Diagnosis: *Rhynchocystis* with an elongated pear-shaped body; the anterior end is broader and provided with an epimerite consisting of a nipple-shaped mucron surrounded at its base by a ring in which the sarcocyte is well developed. Trophozoites attain a maximum size of 126 μ. Movements of the trophozoites are very slow. Body is not covered over with hair. Nucleus is oval and situated in the posterior half of the body.

Host: *Pheretima elongata* (E. Perr.) from Bombay.

The parasite is found in abundance, along with a number of other species, in the seminal vesicles of *Pheretima elongata.*

In its general form, the parasite shows some resemblance with *R. cognettii*, but it is more constant in form, and does not exhibit the changes of form shown by the latter species. In this respect as also in the complete absence of hair covering the body it differs from *R. pilosa.*

The trophozoite moves very slowly. The endoplasm with the contained granules is moved about in the interior of the body from pole to pole, but these movements do not affect the external form of the body. The body is elongated and pear-shaped, with a much wider anterior end and a narrower posterior end. The anterior end presents a strikingly differentiated region, which recalls the epimerite of *R. cognettii*, but the epimerite in this species is more volumious than in the latter. The epimerite is in the form of a conical or short cylindro-conical trunk. The central portion or mucron is broad and nipple-shaped and never pointed at the end.

It consists of granular endoplasm covered over by a thin layer of epicyte, over which there are no striations. The mucron is surrounded at its base by a ring-like differentiated region, which is a zone of sarcocyte and does not show any endoplasmic granules.

The body is never covered by any hair. The surface is often covered by sperms of the host, which are short and thick, and sticking close together to the epimeritic region form a thick investment round it (Fig. 21).

The ectoplasm is not thick, but the epicyte is fairly well developed. The endoplasm is granular, but granules of reserve material are fine and hardly distiguishable.

The size of the trophozoite varies. The youngest specimens we have come across measure 49 μ in length. The full grown trophozoite possessing an epimerite reaches a maximum size of 126 μ, the epimerite in these largest individuals measuring 12,2 μ by 7,5 μ.

The measurements of a few individuals with the dimensions of the nucleus and the karyosome are given below:

Length of trophozoite.	Width of trophozoite.	Size of Nucleus.	Diameter of Karyosome.
126 μ	49 μ	15,9 $\mu \times$ 8,8 μ	5,8 μ
120 μ	52,5 μ	15,7 $\mu \times$ 11,3 μ	5,2 μ
77 μ	31,5 μ	14,8 $\mu \times$ 8 μ	4,3 μ
49 μ	38,5 μ	14 $\mu \times$ 8,7 μ	4,3 μ

From the above measurements it will be seen that the relative dimensions of the tropozoite, nucleus, and karyosome differ markedly from those of *Rhynchocystis awatii* which is found in the same host. The size of the nucleus is larger than in the corresponding sized trophozoites of *R. awatii*.

The nucleus generally lies in the posterior half of the body, rarely in the middle or the anterior half. It is distinctly oval and contains a single spherical or slightly oval karyosome which is placed eccentrically. The karyosome is a homogeneous mass and is not vacuolated. The nuclear membrane is distinct and encloses a feebly developed network of achromatin with chromatin grains scattered on it.

The cysts and spores have not been identified.

Rhynchocystis awatii nov. spec.

Diagnosis. Monocystid with an elongated cylindrical body measuring up to 400 μ in length and in shorter and thicker indididuals up to 50 μ in width. The anterior end is generally provided

with a cylindro-conical epimerite. Epicyte is ornamented with fine longitudinal striations, which are more distinct and spaced out over the epimeritic region. Sarcocyte is poorly developed and free from granules. Nucleus is oval, with a large eccentric karyosome, and is generally placed about the middle of the body.

Host: *Pheretima elongata* (E. Perr.) from Bombay.

This would seem to be a rare parasite of the seminal vesicles of *Pheretima elongata*. We have found them only in a few hosts and only a few specimens at a time. Occasionally the trophozoites are found to possess a distinct epimerite. An epimerite is not found in all the individuals, but those few that are found to possess it, show it as a distinct structure. In preparations stained with Ehrlich's haematoxylin, the epimerite is in the form of a club-shaped trunk, dilated at its distal extremity and narrower at its base. We have never seen any trophozoite attached by its epimerite to the host cells, and presumably the structure falls off in most of the individuals.

On two occasions individuals have been met showing syzygies. The microphotograph of these pairs is reproduced in Plate 11—15, Fig. 34. In this case it will be noticed that the primite has lost its epimerite, but the satellite is attached by its epimerite to the posterior end of the primite. The primite is elongated and vermiform, with its anterior extremity pointed and the posterior extremity rounded and receiving like a socket the enlarged ball-like anterior end of the epimerite of the satellite. The posterior extremity of the satellite is also pointed. So far as we are aware, such an end to end syzygy has not been noticed before among Monocystids. In similar cases of syzygies among the Polycystids however, it is the protomerite of the satellite by which the latter is attached to the posterior end of the primite, the epimerite having been previously lost. The structure however of this organ of attachment in *R. awatii* seems to indicate that it is an epimerite. Another case of syzygy is also figured (Fig. 24), but in that case there is no epimerite.

In the free individuals, the form of the trophozoite varies considerably. Some specimens are so short and thick, while others are distinctly elongated and vermiform (Fig. 23). The ectocyte is thin. The entocyte is finely granular, less dense and sometimes excavated by small alveoli. The nucleus lies more often in the middle of the body, though in some cases it is found to be situated near the anterior end. It is oval in form, with its long axis lying along the length of the parasite. The nucleus usually measures

17,5 μ in its longer and 10,5 μ in its shorter diameter. It contains a single large karyosome, which is always eccentrically placed, and is not seen to be vacuolated. There is a distinct nuclear membrane, and the karyosome is surrounded by chromatin grains scattered over a linin network.

The following are the measurements expressed in microns, of a number of individuals:

		Nucleus		
Length of trophozoite.	Width of trophozoite.	Longer diameter.	Shorter diameter.	Karyosome.
392	21	15,1	10,5	5,5
281	35	—	—	—
196	35	14,0	10,5	3,5
168	35	19,2	10,5	3,5
126	49	17,5	10,5	3,5

The following table will serve as a key to the identification of various species of *Rhynochocystis*:

1. Body elongated, anterior extremity swollen into a bowl-ike head. Up to 2 mm. Mucron hyalin. Longitudinal epicytal striations over the whole body. Nucleus in the swollen head. *R. porrecta* (SCHMIDT).

2. Body elongated, cylindrical, hair covering the whole body; epimerite consisting of dense and homogeneous conical mucron surrounded by a considerable thickness of sarcocyte. Up to 0,5 mm. Epicytal striations present and most marked over the epimeritic region. Nucleus in the anterior portion. *R. pilosa* (CUÉNOT).

3. Body pear-shaped, up to 116 μ, with permanent anterior proboscis. Longitudinal epicytal striations over the proboscis and the body. Nucleus rounded, situated in the posterior region of the body. *R. hessei* COGN. DE MARTIIS.

4. Body pear-shaped, up to 144 μ with a proboscis as long as the body. *R. piriformis* BERLIN.

5. Form variable, pear-shaped, spherical or gregariniform, up to 129 μ. Epimerite metabolic, consisting of a conical or hemispherical mucron, surrounded by a crown of sarcocyte. Hairs on the mucron and sometimes at the posterior end. Nucleus rounded, position of the nucleus varies, but it is never in the epimeritic region. *R. cognettii* BHATIA & CHATTERJEE.

6. Elongated pear-shaped body, up to 126 μ; anterior end broader with a nipple-shaped mucron surrounded by a ring in

which sarcocyte is well developed. Nucleus oval, in posterior half of the body. *R. mamillata* Bhatia & Setna.

7. Elongated, cylindrical body, up to 400 μ, with cylindro-conical epimerite. Longitudinal epicytal striations more distinct and spaced out over the epimerite. Nucleus oval, generally situated about the middle of the body. *R. awatii* Bhatia & Setna.

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Explanation of Plates.

Illustrating paper entitled "On some more Gregarine parasites of Indian Earthworms". By Mr. B. L. Bhatia and Mr. Sam B. Setna.

Plates 11—12.

All figures are drawn from permanent preparations fixed with Bouin's fluid or Schaudinn's alcohol, and stained with iron alum haematoxylin, or Ehrlich's haematoxylin, or Delafield's haematoxylin counterstained with eosin. All figures are free hand sketches drawn under Leitz microscope.

Figs. 1—4. *Monocystis matthaii* nov. spec.

From preparations stained with Ehrlich's haematoxylin. Figs. 2 to 4 as seen under Leitz microscope provided with 4 mm ocjective and × 4 ocular.

Fig. 1. A young trophozoite, surrounded by well developed sperms.

Fig. 2. A nearly spherical trophozoite, the chromatin in the nucleus concentrated in the single eccentrically placed karyosome.

Fig. 3. A trophozoite resembling a top in form. Karyosome is eccentric and chromatin grains are scattered.

Fig. 4. Cyst showing two individuals in association.

Figs. 5—16. *Nematocystis stephensoni* nov. spec.

Figs. 5, 10—16 are from preparations stained with iron haematoxylin, figs. 6—9, from preparations stained with Delafield's haematoxylin and eosin. All are drawn as seen under Leitz microscope provided with 2 mm apochromatic objective.

Fig. 5. A young trophozoite inside a blastophore.

Figs. 6—9. Different forms of the young stages of the free trophozoites.

Fig. 10. A full grown trophozoite showing a constriction and bulgings, and exhibiting characteristic structure of the nucleus.

Fig. 11. Another trophozoite.

Fig. 12. Nucleus of fig. 11 more highly magnified, showing characteristic "nucleus within nucleus" type of structure. The large karyosome is slightly eccentric, and consists of a dark central mass, and a peripheral vacuolated region over which chromatin particles are thickly dispersed.

Fig. 13. A rare form of trophozoite, with nucleus showing two karyosomes.

Fig. 14. Transverse section of an association cyst. Nuclei in one are showing mitotic division.

Fig. 15. Transverse section through a cyst showing zygotes. The gamete nuclei are clearly seen in most of the zygotes. The cystal residue in the centre contains the remains of a number of nuclei.

Fig. 16. Transverse section through a cyst showing sporoblasts.

Fig. 17. Sections of spores, showing a single nucleus in one and eight nuclei in another.

Figs. 18—20. *Stomatophora bulbifera* nov. spec.

Figures are from preparations stained with EHRLICH's Haematoxylin. Figs. 18 and 20 are drawn as seen under LEITZ microscope with 4 mm objective and × 4 ocular. Fig. 19 as under 2 mm apochromatic objective and × 4 ocular.

Fig. 18. A well developed trophozoite showing the characteristic inverted baloon-shaped depression at the anterior end.

Fig. 19. Anterior end of the same, more highly magnified.

Fig. 20. A fully developed trophozoite, showing the sucker as seen from the anterior aspect.

Figs. 21—22. *Rhynchocystis mamillata* nov. spec.

From preparations stained with EHRLICH's haematoxylin, as seen under LEITZ microscope with 4 mm objective and × 4 ocular.

Fig. 21. An adult trophozoite with sperms sticking round the mucron.

Fig. 22. A typical trophozoite showing the nipple-shaped mucron with a ring of sarcocyte round its base. The oval nucleus is seen in the posterior portion.

Figs. 23—24. *Rhynchocystis awatii* nov. spec.

From preparations stained with EHRLICH's haematoxylin, as seen under LEITZ microscope with 4 mm objective and × 4 ocular.

Fig. 23. Two trophozoites attached in an end to end syzygy. The satellite shows the well developed cylindro-conical epimerite marked with epicytal striations.

Fig. 24. Another syzygy, but neither trophozoite showing an epimerite.

Plate 13—15.

The microphotographs in this plate were taken under LEITZ's microscope provided with apochromatic objectives, and with LEITZ's micro-photographic outfit.

Fig. 25. *Monocystis matthaii.* An adult trophozoite. 4 mm objective, × 2 ocular.

Fig. 26. The same as in Fig. 25, more hightly magnified, showing nuclear structure, 2 mm objective, × 4 ocular.

Fig. 27. *Monocystis matthaii.* Three individuals in a cyst.

Fig. 28. *Nematocystis stephensoni.* An adult trophozoite showing a constriction. The nucleus is situated in the posterior half, and shows a characteristic "nucleus within nucleus" type of structure. 4 mm objective, × 4 ocular.

Fig. 29. Nucleus of *N. stephensoni* more highly magnified to show details of structure. 2 mm objective, × 4 ocular.

Fig. 30. *Stomatophora bulbifera.* An adult trophozoite showing the characteristic inverted balloon shaped depression at the anterior end.

Fig. 31. *Stomatophora bulbifera.* A fully developed trophozoite showing the sucker seen from the anterior aspect.

Fig. 32. *Rhynchocystis mamillata.* An adult trophozoite with sperms sticking round the mucron.

Fig. 33. *Rhynchocystis mamillata.* A typical trophozoite with nipple-shaped mucron with a ring of sarcocyte round its base.

Fig. 34. *Rhynchocystis awatii.* Two adult trophozoites in an end to end syzygy. The nucleus in one lies about the middle, in the other near the anterior extremity. 4 mm objective, × 4 ocular.

Fig. 35. The same as in Fig. 34 more highly magnified to show the structure of the epimerite. 2 mm objective, × 4 ocular.

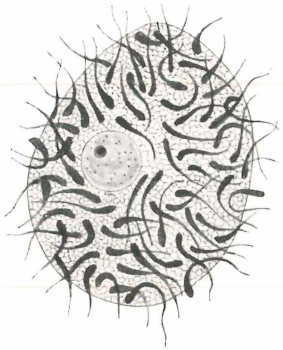


Fig. 1.

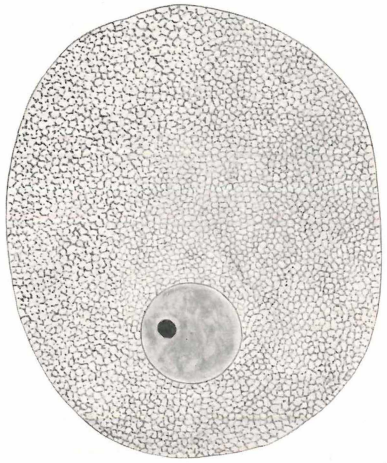
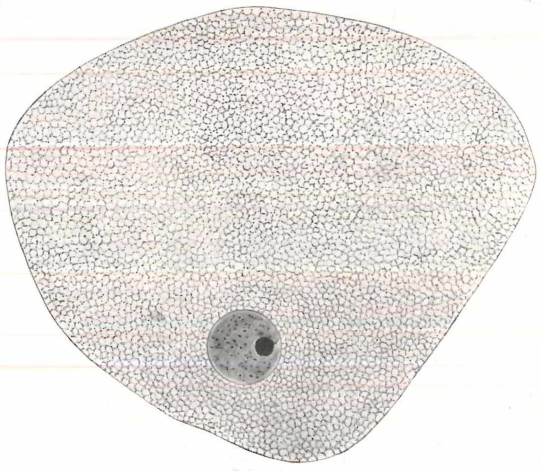


Fig. 2.



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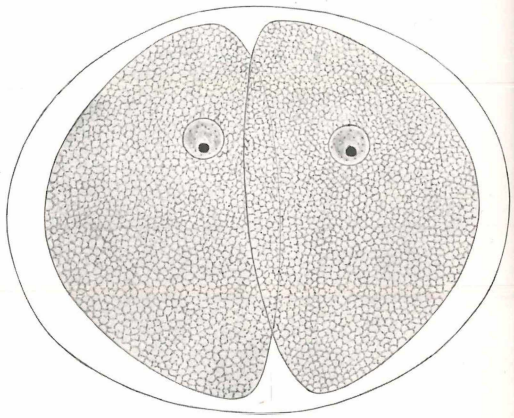


Fig. 4.

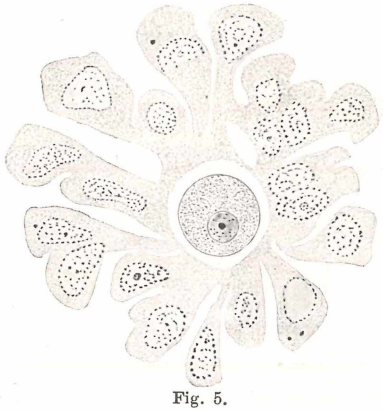
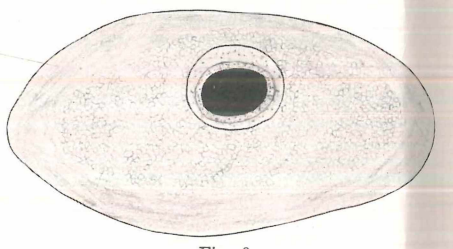


Fig. 5.



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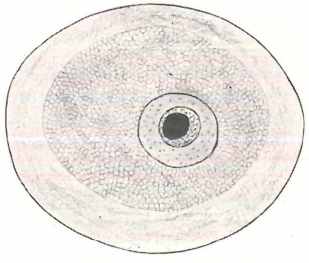

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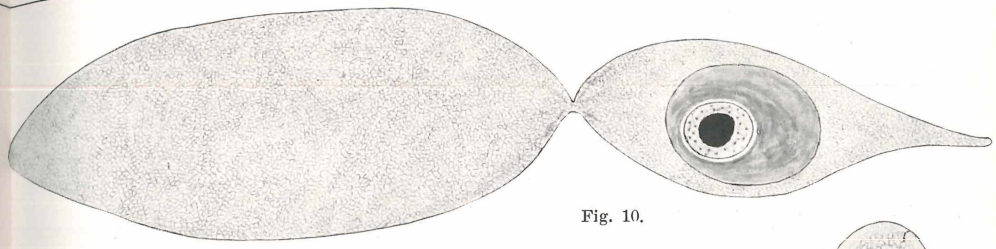

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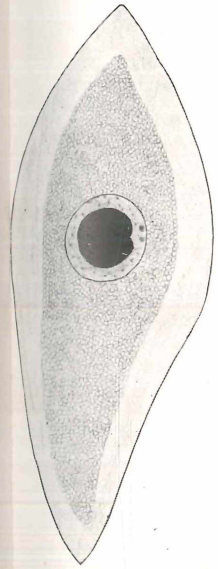

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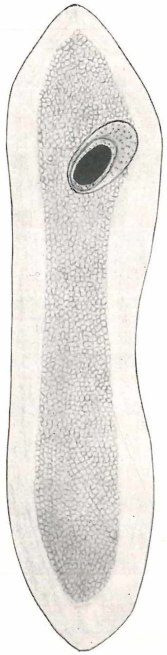

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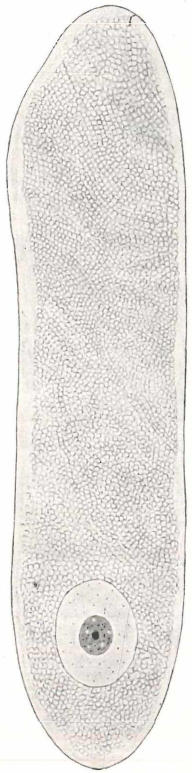

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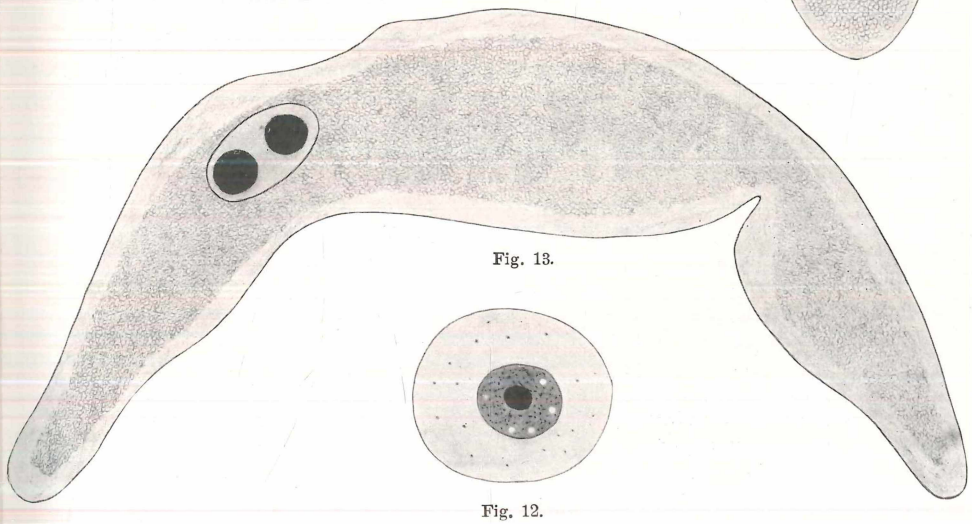

Fig. 13.

Fig. 12.

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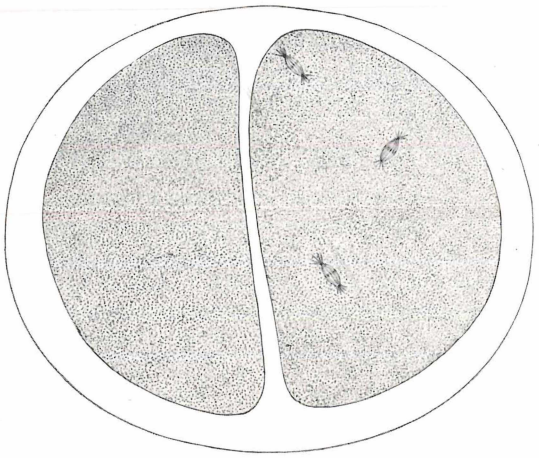

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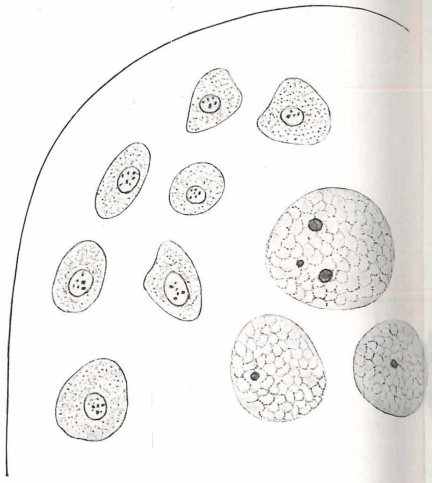

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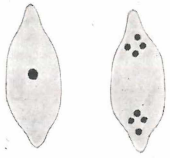

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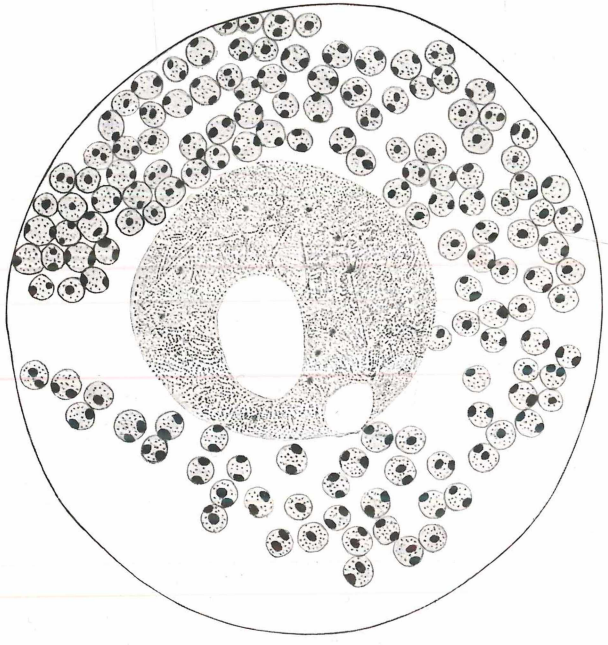

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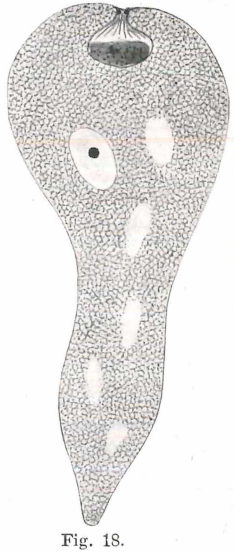

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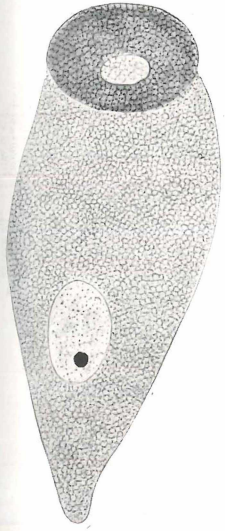

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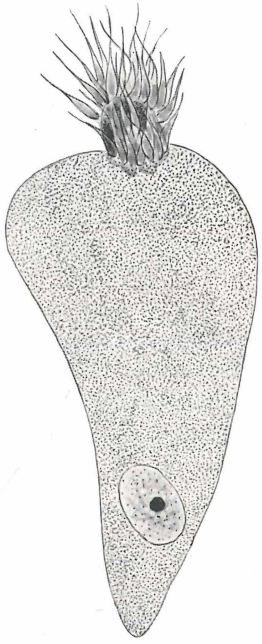

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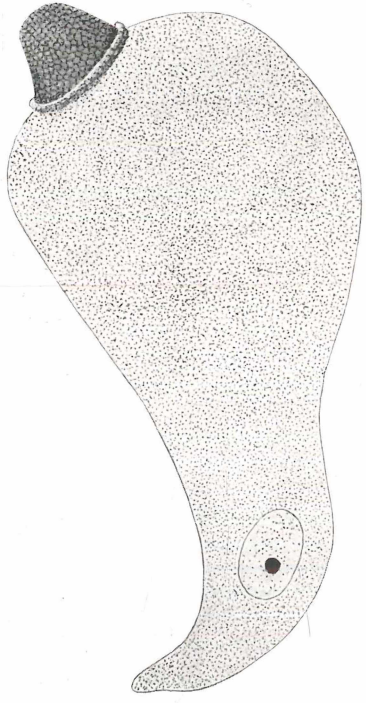
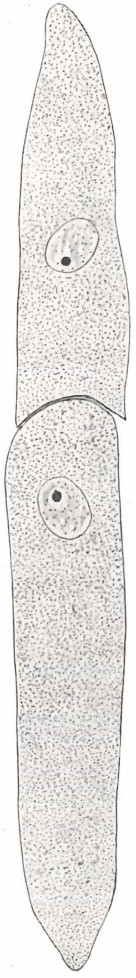


Fig. 22.

Fig. 19.

Fig. 23.



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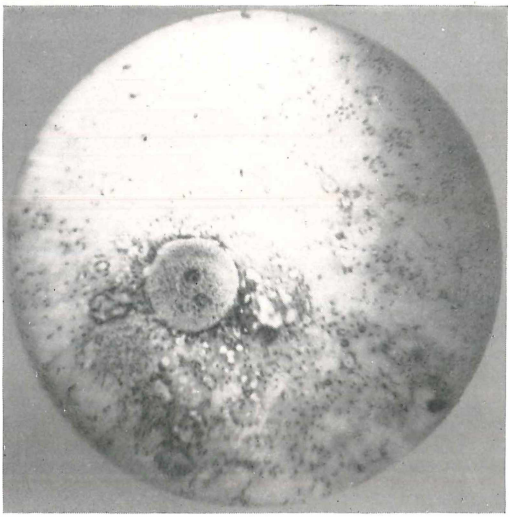

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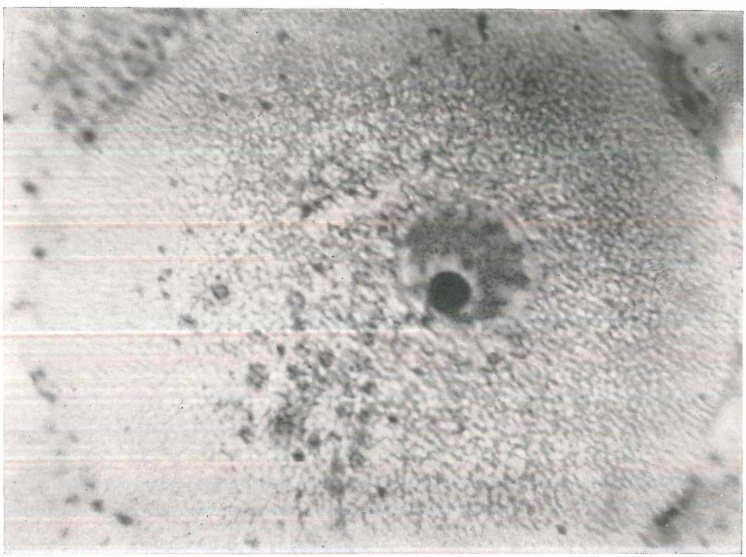

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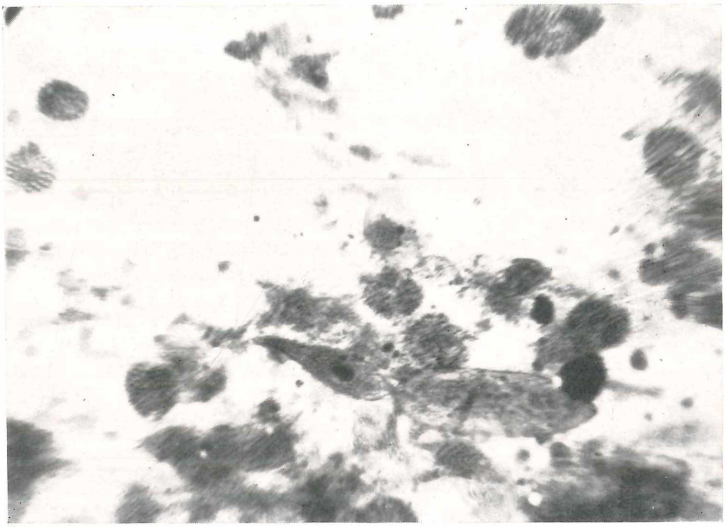
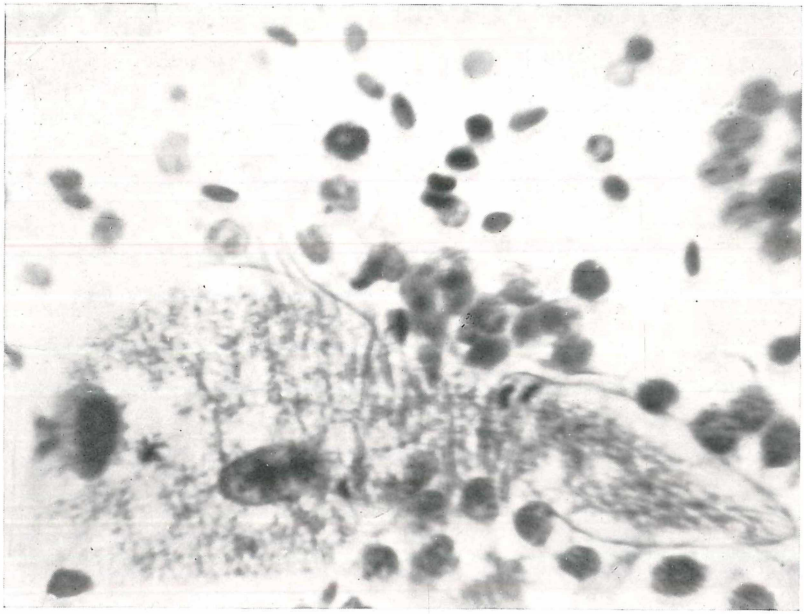


Fig. 28.

Fig. 27.

Fig. 29.



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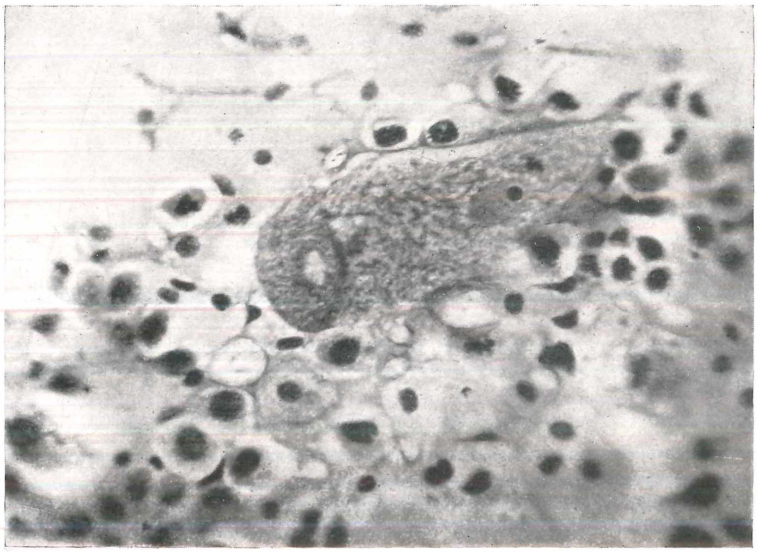

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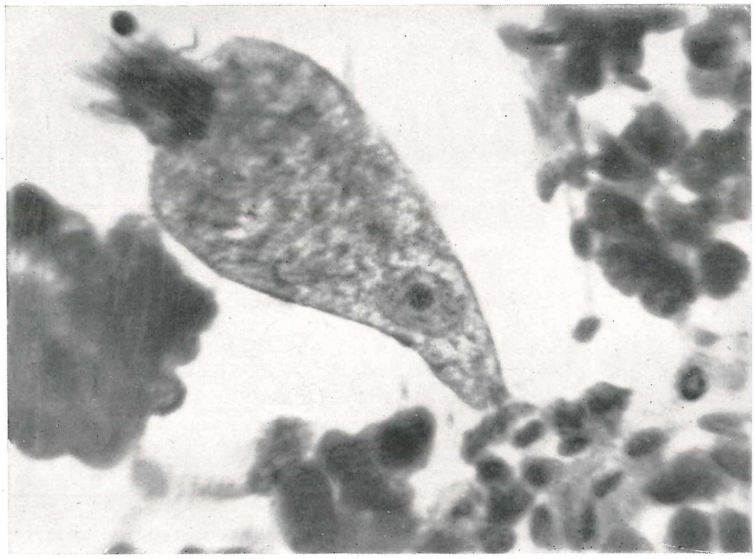

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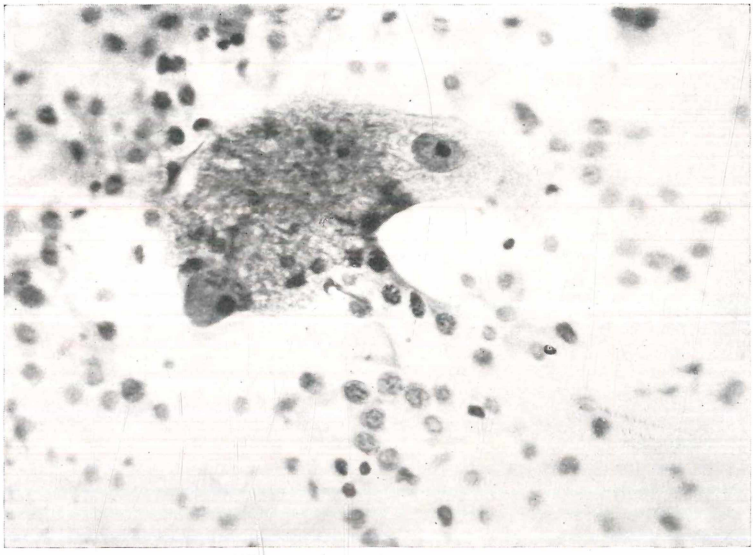

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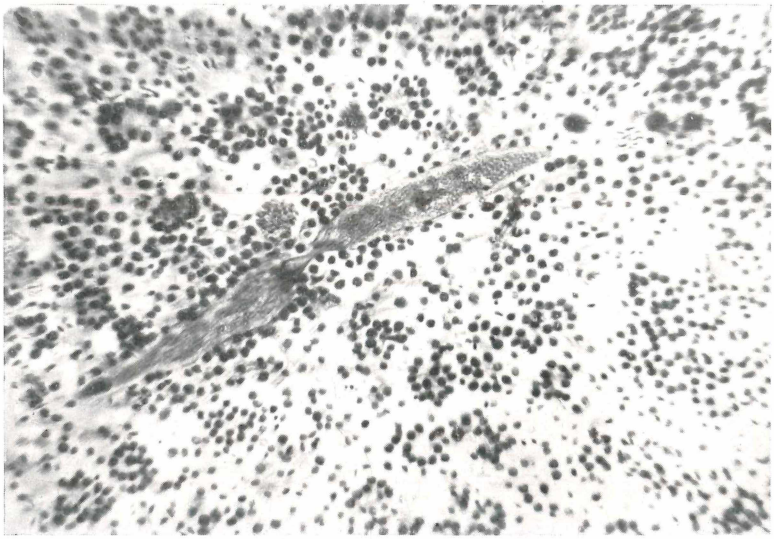

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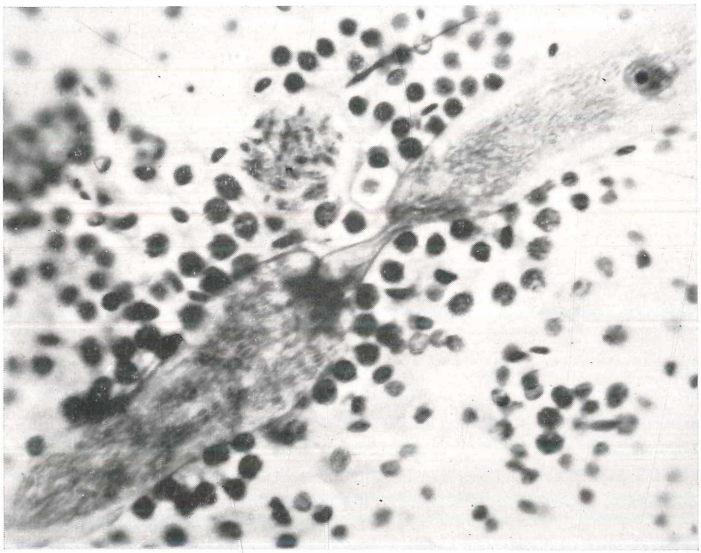

Fig. 35.