On some Gregarine parasites of Indian Earthworms.

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(With Plates 7—9.)

I. Introductory.

One of us has already published a paper (Bhatia, 1924) on *Stomatophora*, a genus of Gregarine parasites of Earthworms remarkable for the possession of an epimerite. The genus *Rhynchocystis* is another such genus and it would be useful to add our observations to the already existing accounts, and to compare the nature of the epimerite with that of *Stomatophora*. A number of new species of *Nematocystis* and one new species of *Monocystis* have been studied by us, and an account of these also is included in this paper.

We have examined the contents of the seminal vesicles of *Pheretima barbadensis* (Beddard), *P. heterochaeta* (Mchlsn.), *P. posthuma* (L. Vaill.), *Allolobophora foetida* (Savig.) and *A. caliginosa* (Savig.), and a complete list of the parasites found so far in these hosts is given in the summary at the end of the paper.

Two of the species were discovered by the junior author from worms obtained at Kasauli and preliminary observations and preparations made by him at the Central Research Institute Kasauli, during the summer vacation of 1922. Our thanks are due to the Director of the institute for permission to work there, and to Dr. J. Stephenson of the Edinburgh University, for specific identification of the hosts.

II. The genus *Rhynchocystis.*

The genus *Rhynchocystis* was founded by Hesse in 1909 to include *R. pilosa* (Cuénot) and *R. porrecta* (Schmidt). Hesse defined the genus as follows: "Body ovoid or cylindroid. Anterior pole provided with a metabolic epimerite, most frequently elongated into a conical or cylindro-conical trunk. Sporocysts biconical with similar and non-appendiculate poles." He fully discussed the synonomy and previous work on these species and added a valuable account of his own observations. In 1911, Cognetti de Martiis described a new species which he named *R. hessei.* Since then no further work has been done on this genus, but a short description of a new species *R. piriformis* has recently been given by Berlin (1924). To the four known species, we are able to add another, an account of which is given below.

Rhynchocystis cognettii nov. spec.

Diagnosis: Monocystid with an epimerite. The shape of the body is variable, and may be pear-shaped, spherical or gregariniform, and the anterior end is provided with a mucron surrounded by a crown of sarcocyte. Trophozoite attains a maximum size of 129 μ by 46 μ. Hairs are found only in the region of the mucron and sometimes at the posterior end also. Position of the nucleus varies, but it is never situated in the epimeritic region. The nucleus is generally spherical, with a single spherical karyosome placed eccentrically and surrounded by a clear white area.

Host: *Allolobophora caliginosa* (SAVIG.) from Kasauli.

The parasite is by no means common in its occurrence, as only two out of thirty worms examined were found to harbour it in their seminal vesicles.

The parasite is found to be more or less spherical, pear-shaped or gregariniform (Figs. 3, 4, 8), and differs in its form from the species described so far. It is never active in its movements. During its sluggish movements, the cytoplasmic granules and the food grains are seen to move about from one pole to the other. The external form of the body is hardly affected during these movements. At times however the posterior region appears to be separated off from the rest of the body by a deep constriction (Figs. 8 and 10).

The animal always moves with the end bearing the mucron directed forwards. In the living condition, it presents a dark appearance owing to the reserve of food grains present in the cytoplasm. In the midst of these grains the nucleus is seen as whitish body moved along with the cytoplasm.

As in other species of the genus, the body is provided at its anterior end with a metabolic epimerite, which is most often elongated in the form of a conical or cylindro-conical trunk. The central portion which may be designated as the mucron, may be produced into a conical form (Fig. 5) or may be hemispherical (Fig. 4) or elongated into a short cylinder (Fig. 2). This is a zone of differentiated cytoplasm, and being free from endoplasmic inclusions, appears to be homogeneous. The mucron consists of endoplasm covered over by a thin epicyte, but there are no striations over this part. The mucron is marked off from the rest of the body by a curved surface, which presents its concavity towards the mucron. In individuals of a suitable size, the mucron is always surrounded by a crown. This is an ectoplasmic formation, containing sarcocyte and covered over by epicyte. The crown is cup-like and surrounds the base of the mucron (Fig. 2) and in certain cases extends beyond the mucron (Fig. 5), so that the latter is seen to be a prominence arising from the centre of the cup. The crown is marked by radial epicytal striations, which extend to the rim of the cup. The mucron and the crown of sarcocyte together constitute the epimerite. The crown of sarcocyte atrophies in the aged individuals, while in still older ones the mucron itself may disappear. The complete disappearance of the epimerite with age has not been noted by HESSE for other species of *Rhynchocystis* described by him.

In this species hairs are found only at the anterior end of the body and in certain cases at the posterior end of the parasite also (Figs. 1 and 7). In this respect it differs from *R. pilosa*, in which the young parasite at any rate is covered with hair all over the body. Hairs in the epimeritic region are directed forwards, and extend a considerable distance beyond the mucron. The endoplasm appears to run into the interior of the hair to about the middle of its length, beyond which the hair is reduced in thickness and tapers to a point.

The size of the parasites varies. The full grown individuals possessing an epimerite reach a maximum size of 82 μ by 36 μ. Forms still possessing a mucron but without the crown of sarcocyte, reach 74 μ by 46 μ, and older forms which have altogether lost the epimerite reach a maximum of 129 μ by 46 μ. The measurements of a number of individuals and their parts are given in the table below.

Trophozoite	Total length μ	Maximum width μ	Length of the mucron μ	Width of the mucron μ	Length of crown of sarcocyte μ	Width of crown of sarcocyte μ	Diameter of the nucleus μ	Diameter of the karyosome μ
A (Fig. 1)	36,8	18,4	7	7	—	—	9,2	4
B (Fig. 2)	46	20,2	13,5	13,5	—	—	9,2	5
C (Fig. 3)	46	37	6,9	16	—	—	9,2	4
D (Fig. 4)	55,2	29,5	6,9	13,8	—	—	13,8	5
E (Fig. 5)	82,8	36,8	9,2	13,8	13,8	18,4	13,8	9,2
F (Fig. 6)	82,8	36,8	9,2	18,4	13,8	13,8	13,5	9
G (Fig. 7)	87,4	36,8	9,2	13,8	—	—	13	6
H (Fig. 8)	74	46	9,2	13,8	—	—	10	6
J (Fig. 9)	83	46	—	—	—	—	12	7
K (Fig. 10)	128,8	46	—	—	—	—	10	7

The epicyte and sarcocyte are distinct over the whole body of the parasite; the sarcocyte is more distinct in the region of the epimerite, where it forms the portion described as the metabolic crown. The fibrils of myocyte are not seen distinctly and the slow movements of the parasite are due to the poor development of these fibrils. The body is not marked by any epicytal striations.

The endoplasm is excavated by alveoli, which contain numerous paraglycogen bodies of different sizes. These grains are elliptical or spherical.

The position of the nucleus in the body varies, but it is never found to be situated in the epimerite of the parasite, nor always

near the anterior end. It may occasionally be situated near the posterior end as well. So in this respect also, the organism differs markedly from the three varieties of *R. pilosa* as described by HESSE. There are also no channelled prolongations from the epimerite to the nuclear membrane. The nucleus is large, measuring from 9 μ to 14 μ in diameter. It is more often spherical, but sometimes ovoid. The nuclear membrane is not very sharply stained by iron-haematoxylin. There is a large spherical karyosome, which may be central or eccentric in position. When eccentric, it lies in the posterior half of the nucleus. Sometimes it is actually in contact with the nuclear membrane, while at other times, there is a clear space surrounding the karyosome. The karyosome is surrounded by chromatin grains dispersed over a network of linin.

Round about the karyosome are seen little masses of chromatin, some of which are in contact with the nuclear membrane, or having passed through the latter are lying just outside it (Fig. 9). In older parasites some of the chromatin masses lying in the cytoplasm are surrounded by a membrane and form secondary nuclei. In one of our preparations, we have come across a specimen (Fig. 10) which contains a secondary nucleus, which besides possessing a distinct membrane, shows some fine chromatin granules surrounding the central karyosome.

Of the reproductive phases, we are able to add little to the existing accounts of the genus. The association cysts are more or less ovoid, and measure up to 129 μ by 81 μ. The gametocytes are nearly equal in size.

III. The genus *Nematocystis*.

The genus *Nematocystis* was established by HESSE in 1909 to include monocystid parasites which attain a great length, reaching even up to to 5 mm, and in which the general form of the body is like that of a nematode worm. The nucleus is generally oval and contains one or more karyosomes. Trophozoites are solitary. Sporocysts are biconical with similar non-appendiculate poles. He referred *Monocystis magna* SCHMIDT to this genus, and described three new species, viz., *Nematocystis lumbricoides*, *N. vermicularis*, and *N. anguillula*. Since then COGNETTI DE MARTIIS has described two more species, viz., *N. cecconi* (1918) and *N. almae* (1921), and Berlin *N. anguillula* var. *gracilis* (1924). Two new species and occurrence of two others in new hosts are described below.

Nematocystis hessei nov. spec.

Diagnosis: Monocystid elongated like a worm and tapering at either end. Size up to 552 μ in length by 42 μ in thickness. No epycital striations. Nucleus long and fusiform, with two karyosomes, which are sometimes very unequal in size.

Host: *Pheretima heterochaeta* (Mchlsn.).

This parisite has been found in the seminal vesicles of *Pheretima heterochaeta* (Mchlsn.). The infection is very rare, so far we have come across only two specimens of the parasite in one out of a large number of smears from the seminal vesicles of these worms. Owing to the rarity of its occurrence, no observations could be made on the living parasite.

In the stained specimens the three layers of the ectoplasm are recognisable. The epicyte does not present any longitudinal or transverse striations. The sarcocyte is well developed and the fibrils of myocyte are visible only at the points the parasite has constricted itself. The endoplasm shows the usual alveolar structure, and paraglycogen grains are lodged in these alveoli. The paraglycogen grains are less voluminous than those found in other species of *Nematocystis*. The body is elongated like a worm, but is not quite cylindrical; it is deformable, and tapers to a point at either end, as in other species of the genus. The size of one trophozoite is 327 μ by 38,5 μ and of a second specimen 552 μ by 42 μ.

The characteristic feature of the parasite is the shape and structure of the nucleus, which is quite different from that in the other species. The nucleus is situated near the centre of the parasite. It is long and fusiform, and can attain a size of 79 μ along its long axis. The nuclear membrane is distinct and is deeply stained by basic stains. There are two karyosomes, which in one of the specimens (fig. 12) are very unequal in size. The larger of these measures 34 μ along its long axis and the smaller only 9 μ. Each karyosome is surrounded by a clear area. In the smaller one, we find a more deeply stained central area, but in the larger the central area is more lightly stained and contains within it a number of deeply stained bodies. Both the karyosomes in the other specimen (Fig. 11 a) show a vacuolated structure. Besides the karyosome, a number of chromatin grains are contained in the nucleus.

The parasite resembles in some respects *N. anguillula* but differs from it in the absence of epicytal striations and in the nuclear structure.

Nematocystis plurikaryosomata nov. spec.

While working at the Central Research Institute, Kasauli in 1922, Mr. CHATTERJEE came across an earthworm which has been identified by Dr. J. STEPHENSON, of Edinburgh University, as *Allolobophora (Eisenia) foetida* (SAVIG.). These worms are very abundantly found in cow dung and have been found to harbour specimens of a new species which is here described as *Nematocystis plurikaryosomata.*

Diagnosis: Monocystid with a long and extremely deformable body, reaching up to more than 1 mm in length and 100 μ in thickness. No epicytal striations. Nucleus elongated and oval, with several small spherical karyosomes. Cysts small attaining a size of 140 μ in diameter. Spores 6,5 to 8,5 μ by 3,4 μ in size.

Host: *Allolobophora (Eisenia) foetida* (SAVIG.)

In the living condition the organism presents a dark aspect owing to the great abundance of food-grains embedded in the endoplasm.

The nucleus is seen as a transparent oval body embedded in the central granular region of the endoplasm. The body of the parasite is very deformable and shows constrictions and bulgings during the progression of the parasite. The granular cytoplasm together with the nucleus appear to flow from one pole towards the other during the movements. The parasite reaches up to 1150 μ and 100 μ in thickness.

The parasite which lives in the seminal vesicles, is not found attached to any hypertrophied cell of the epithelium. There are no polar ornamentations and there are no hairs round the posterior pole of the parasite. In these respects as also in its nuclear structure it differs from *N. magna* and other species described by HESSE.

In stained preparations, the body exhibits the usual structure. Epicytal striations are wanting, and myocyte fibrils are visible at places where the body is constricted. Endoplasm is alveolar and the alveoli are filled with paraglycogen grains. A characteristic feature is that in these stained preparations, the paraglycogen grains are seen to aggregate along the central region, leaving a clear peripheral zone. The grains are more or less ellipsoidal and vary greatly in shape and size. The essential characteristic feature

however, is the structure of the nucleus. The position of the nucleus in the body of the parasite varies. The nucleus is large, elongated and oval, and measures on an average 60 μ along its long axis. The nuclear membrane is very distinct. The nucleus contains several large karyosomes, which vary in number in different specimens. They are generally spherical and may be from 6 to 12 in number. The linin network is not visible, nor are there any chromatin granules apart from the karyosomes. The ground substance of the nucleus is homogenously stained by acid stains.

The parasites contract considerably when associating. The cysts enclosing pairs of gametocystes are spherical and reach only up to 140 μ in diameter. The cyst-wall is thick and does not allow the associants to take up stain readily. Zygotes which are formed by the union of iso-gametes measure 5,8 μ in diameter. The spores are spindle-shaped and have similar non-appendiculate pointed poles. The size of the spores varies, the smallest measure 6,5 μ by 3,4 μ and the largest 8,5 by 3,4 μ, and there is a series with dimensions intermediate between the two.

Nematocystis lumbricoides Hesse.

Hesse described this species as a rather rare parasite of the seminal vesicles of *Helodrilus caliginosus* Savig. We have found a few parasites in the seminal vesicles of *Pheritima heterochaeta* (Mchlsn.) which resemble in all essential particulars with the description of this species as given by Hesse.

The general form of the parasite resembles that of an earthworm, being swollen in the middle and narrower at each end. The anterior end is somewhat rounded and the posterior more pointed. The body of the parasite is deformable, and presents several bulgings and constrictions. In addition the parasite can roll upon itself and look very much like an earthworm rolled upon itself as the result of being taken up in one's hand. Our specimens attain a maximum length of 1 mm and a width of 31 μ in the widest region.

Our specimens however differ from those described by Hesse, in the epicyte not being ornamented with parallel longitudinal striations. The nucleus is variable in position. It measures 46 μ along its long diameter. It is elongated and spindle-shaped, and contains a single large oval karyosome which is placed eccentrically. The karyosome is seen to be vacuolated. Fine chromatin granules can be seen scattered in the nucleus, but no linin network is visible.

Nematocystis vermicularis Hesse.

Hesse (1909) described this parasite from the seminal vesicles and coelom of *Helodrilus longus* Ude, but we have found it in the seminal vesicles of *Pheritima barbadensis* (Beddard). The infection is rather rare, as a single trophozoite has been found in one out of a large number of smears from the seminal vesicles of this worm.

The specimen is not covered over by phagocytes, and closely resembles Hesse's Fig. LXVIIIa. The size of the specimen is however much less, being only 446 μ in length by 69 μ in thickness. The nucleus is oval and situated more towards the blunt end of the parasite and measures 38,5 μ along its long axis. The nuclear membrane is not distinct. There is a central spherical karyosome. Linin network is not visible and chromatin particles are fine and closely dispersed round the karyosome.

IV. The genus *Echinocystis*.

Echinocystis globosa g. et nov. spec.

The seminal vesicles of *Pheretima heterochaeta* (Mchlsn.) contain a spherical gregarine with two spine-like prolongations from the surface of the body. We have assigned it to a new genus *Echinocystis* and the specific name *globosa* is given to the parasite.

Diagnosis: Monocystid with a more or less spherical body with two spine-like structures radiating from the surface of the animal. The nucleus is large and generally ovoid and contains a single large karyosome. Trophozoites are solitary. Sporocysts biconical, with two similar truncated poles.

The infection is very rare. The young parasite developes within the blastophore and in this stage it has no spine-like structures. The spines are observed in adult parasites only, which are living free in the seminal vesicles.

The parasite presents a dark aspect when examined under the microscope in the living condition in 0,75 % salt solution. It does not at all present active movements; the external form is hardly affected during the slow movements the animal presents. Paraglycogen reserve food grains are found in the body of the parasite and this accounts for the dark aspect of the parasite in the living condition.

The shape of the body of *Echinocystis globosa* is more or less like a globe, insensibly drawn along one axis. The average measurement of the adult forms is 74 μ by 65 μ. From the surface of the body spring out two spine-like structures which gradually narrow towards the distal end. The two spines are not always of the same length. These are covered by epicyte, lining which is a layer of sarcocyte; fibrils of myocyte are not visible. Lying in the interior of the spines is the non-granular endoplasm. In their constitution the spines of *Echinocystis globosa* somewhat resemble the epimerite met with in *Rhynchocystis*, in which however only one prolongation is found at the anterior end of the parasite. In *Rhynchocystis* also the mucron is free from paraglycogen grains and the granules of the endoplasm are so close, that it appears homogeneous. The separation between the homogenous endoplasm of the spine and the granular endoplasm of the body is marked by a curved surface which presents its concavity towards the body of the parasite.

Epicyte and sarcocyte are visible whereas fibrils of myocyte are not observed and this absence of fibrils of myocyte accounts for the complete loss of active movements of the parasite. The layers of epicyte and sarcocyte are visible throughout the body of the parasite and also in the spine-like prolongations. The endoplasm is marked by alveoli and in these alveoli are lodged paraglycogen grains which take on a deep stain with Iron haematoxylin. The reserve food grains assume various shapes and vary also in size.

The nucleus is generally large, slightly elongated and oval; it is often situated near the middle of the body; at times it may approach one or the other side of the sphere. It attains a size up to 24 μ along its long axis. The nuclear membrane is very distinct. In the nucleus there is a single, fairly large karyosome placed near its centre. Generally the shape of the karyosome is ovoid but at times it is spherical. The karyosome in the adult is not vacuolated. The karyosome may reach a size of 15 μ.

Development. In the blastophore the trophozoite assumes a spherical form; later on it becomes ovoid. In these early stages, the nucleus is spherical in form and contains a single spherical karysome. Chromatin granules are not visible. The young parasite grows at the expense of the surrounding attached spermatozoa. Gradually the parasite assumes the form characteristic of adult individuals, the nucleus becoming oval at the same time. The exact

stage at which the spine-like processes make their appearance is not known.

Two adult trophozoites when they are ripe for reproduction come into close association with each other. Then they secrete a common cyst wall around them. During this stage the spine-like processes, like the sucker of *Stomatophora* and the epimerite of *Rhynchocystis* are lost and no trace of them can be seen in the associated individuals. We have not come across any of the stages in gamete formation. One cyst containing the zygotes was met with and the zygotes found to measure 7,5 μ across. Just a few spores are also found in one of our preparations. These spores are unusually large, measuring 28 μ by 14 μ. In one of these spores there are seen two distinct sporozoites. We are not certain if these spores belong to this species, but if they do, the spores are quite remarkable, considering the fact that in Monocystids the spores are invariably octozoic.

V. The genus *Monocystis*.

Monocystis pheritimi nov. spec.

Diagnosis: Monocystid of a variable form, attaining a size up to 220 μ by 50 μ, and showing active movements. Paraglycogen grains are few. Nucleus generally spherical, sometimes ovoidal, with a single spherical karyosome, placed eccentrically. Cysts spherical reaching 80 μ in diameter.

Host: *Pheretima posthuma* (L. Vaill.) from Lahore and Bombay.

Ghosh (1923) has recently described two new species of *Monocystis* viz., *M. bengalensis* and *M. lloyodi* from the seminal vesicles of *Pheretima posthuma* from Calcutta and its neighbourhood. We have studied many smear preparations made from the seminal vesicles of this same worm from Lahore, and find the monocystid parasite differing markedly in form and size of the trophozoite and its nuclear structure from the previously known species of *Monocystis*, and also from the two species from this host as described by Ghosh. Our preparations were fixed in Schaudinn's fluid and stained by iron-haematoxylin. We have also had the opportunity of examining a series of preparations of the contents of the seminal vesicles of the same host from Bombay, made by Mr. Sam Setna, M. Sc. These preparations were stained by Ehrlich's haematoxylin. These preparations also contain the same parasite as the Lahore worms do.

Our thanks are due to Mr. Sam Setna for placing his preparations at our disposal.

When examined in the living condition the parasite presents a bright aspect. The body of the parasite is extremely deformable and it shows movements characteristic of the genus *Monocystis*. It constantly changes its shape and assumes quite different aspects at different times; at times it is spherical, then it becomes ovoid and then ellipsoidal and at other times it may be dumb-bell shaped. Paraglycogen grains are comparatively less in amount; these are quite often spherical but the shape and size may vary. The nucleus is seen as a white area moving along with the granular cytoplasm, first towards one pole, then back to the other. The adult trophozoite attains a size up to 220 μ by 50 μ.

The layer of epicyte is thin and there are no epicytal striations. Sarcocyte is rather well developed; the fibrils of myocyte are extremely delicate structures and visible only at places. The endoplasm is excavated by alveoli; these are quite large and within these are lodged grains of paraglycogen which take up both acid and basic stains.

The position of the nucleus in the body varies. In spherical and oval forms it is situated more or less near the centre; in ellipsoidal forms the nucleus may be in the middle or may approach one or the other pole of the body. It is generally spherical, but may also be ovoid. There is a distinct nuclear membrane which takes up chromatin stains. The karyosome is fairly large and is placed eccentrically in the nucleus. There is linin net work in the nucleus and chromatin granules are seen scattered over the net work. The diameter of the nucleus may reach 15 μ.

Cysts are spherical, being on an average 80 μ in diameter. Zygotes measure about 3 μ in diameter. Spores and sporozoites are as usual.

General.

Brasil (1908 and 1909), Hesse (1909), Bastin (1919) and Bhatia (1924) have already discussed the structure and significance of an epimeritic structure found in certain so-called acephaline gregarine parasites. As pointed out by Hesse the epimerite in such Monocystid genera as bear them, e. g. *Stomatophora* and *Rhynchocystis*, differs from the epimerite of the typical cephaline gregarines, in (1) being changeable in form and (2) in not being abandoned

within the host cell. He also pointed out that in the epimerite of Polycystids there is never any sarcocyte nor myocyte, whereas these two layers not only exist in the epimerite of *Rh. pilosa*, but are even more devoloped than over the rest of the body. These points need not be emphasized any further.

In our studies of the three species of *Stomatophora* and of *Rhynchocystis cognettii* (described in this paper) we are struck by the essential resemblance between the epimerites in these two genera. The structure in its well developed form always consists of (1) a mucron projecting from the anterior end of the body, which consists of epicyte covering a core of homogeneous entocyte (but no sarcocyte or myocyte) and (2) a peri-mucronal zone of differentiated cytoplasm in which sarcocyte and myocyte are also present. This latter zone assumes a variety of forms in different species, and may even be wanting. In *St. simplex* there is no crown of petals surrounding the sucker, while in *St. coronata* there is a well-developed petaloid crown and in *St. diadema* the whole body is marked by furrows and divided into a number of irregular lobes. Similarly in *Rhynchocystis porrecta* (vide HESSE'S figs. 23 and 29) the mucron in well developed, but the crown of sarcocyste is wanting. In *Rh. hessei* (vide COGNETTI'S fig. 3) both the mucron and the differentiated zone in which sarcocyte is present are well developed. In *Rh. pilosa* (HESSE'S figs. 28, 22 and 24) and *Rh. cognettii* (figs. 2, 5, and 22 in this paper) the structure reaches its full development and recalls comparison with *St. coronata.*

Strictly speaking as remarked by HESSE "the mucron only would be comparable with the epimerite of the Polycystids, the rest of the structure would then be comparable with a very peculiar protomerite, with differentiated cytoplasm, the granules of which are so fine and close that it appears somewhat hyalin and much more dense in consequence than the cytoplasm of the deutomerite, but not separated from the latter by an ectoplasmic partition". So the epimerite in *Stomatophora* and *Rhynchocystis* is no doubt a reduced structure, indicating clearly at the same time that the monocystids are degenerate descendants of Polycystid forms.

Monocystis striata (HESSE'S fig. 32) shows a distinct anterior sucker with a central mucron and bears a close resemblance with the young stages of *Rh. pilosa.* Besides this *M. lumbrici* (HESSE'S fig. 1) and *M. agilis* (BASTIN'S fig. 1) show a distinct mucron. So while certain species of *Monocystis* show a close approach to *Stomatophora* and *Rhynchocystis*, these latter in their aged stages lose their

epimeritic structures and then become hardly recognisable from *Monocystis* or *Nematocystis*.

Discussing the probable role of the mucron in *M. agilis*, Hesse (p. 73) remarked that most probably it is a rudimentary organ, the vestigial remains of a voluminous structure, which indicates that *M. agilis* is derived from a fixed form (Monocystid or Polycystid) provided with a well developed epimerite. There is ample evidence to show that genera like *Doliocystis*, *Lankesteria* and *Gonospora* on the one hand and *Stomatophora* and *Rhynchocystis* on the other, are intermediate forms between *Cephalina* and *Acephalina*. Such primarily dicystid genera should be included with the *Acephalina* and placed in the sub-order *Haplocyta*, as advocated by Bhatia in his previous paper.

Summary.

1. A number of gregarine parasites from the seminal vesicles of several species of Indian earthworms are recorded in this paper.
2. The genus *Rhynchocystis* is closely related to *Stomatophora* as it possesses a well developed epimerite. Three species are previously known, and a fourth one named *Rhynchocystis cognettii* is described in this paper. This species possesses a distinct mucron surrounded by a crown of sarcocyte, and differs from the other species in the trophozoite bearing no hairs except on the mucronal region and occasionally at the posterior end, and the nucleus never occurring in the epimeritic region, nor being connected with the latter.
3. Two new species of *Nematocystis* viz., *N. hessei* and *N. plurikaryosomata* are described. The former has no epicytal striations, and possesses a long and fusiform nucleus containing two karyosomes. The latter also is not marked by epicytal striations, and possesses an oval nucleus containing several karyosomes.
4. A remarkable genus of monocystids, with two spine-like structures arising from the body is described under the name of *Echinocystis*. The spines resemble the epimerite of other monocystids, but no explanation can be offered for the existence of two spines.
5. A new species of *Monocystis* is also described.

A complete list of the hosts examined by us so far, and the parasites found in them by other workers or ourselves is given below.

Pheretima rodericensis (Grube)	*Stomatophora coronata* (Hesse)	Vesiculae seminales
	S. simplex Bhatia	
	Nematocystis anguillula Hesse	
	Monocystis macrospora Hesse	Coelome
**Pheretima barbadensis* (Bedcard)	*S. coronata* (Hesse)	Vesiculae seminales
	S. diadema Hesse	
	†*N. vermicularis* Hesse	
**Pheritima heterochaeta* (Mchlsn.)	*Echinocystis globosa* Bhatia & Chatterjee	„
	N. hessei Bhatia & Chatterjee	
	†*N. lumbricoides* Hesse	
Pheritima posthuma (L. Vaill.)	*M. bengalensis* Ghosh	„
	M. lloyodi Ghosh	
	M. pheretimi Bhatia & Chatterjee	
Allolobophora (Eisenia) foetida (Savig.)	*M. lumbrici olidi* Schmidt	„
	M. agilis s. str. Stein	„
	M. ventrosa (cysts) Berlin	„
	M. arcuata Boldt	„
	M. hurculea Bosanquet	„
	M. suecica Berlin	„
	M. densa Berlin	„
	Rhynchocystis piriformis Berlin	„
	R. porrecta Schmidt	„
	N. plurikaryosomata Bhatia & Chatterjee	
Allolobophora caliginosa (Savig.)	*N. lumbricoides* Hesse	Vesiculae seminales
	M. Le Memei Hesse	Vesiculae seminales & Coelome
	Zygocystis cometa Stein	Vesiculae seminales
	Pleurocystis cuenoti Hesse	Ciliated Pavillion
	Rhynchocystis cognettii Bhatia & Chatterjee	Vesiculae seminales

* These hosts have been examined for the first time for their Gregarine parasites by the authors of this paper.

† Known species which are recorded from this host for the first time.

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Explanation of Plates.

Illustrating paper entitled "On some Gregarine parasites of Indian earthworms". By M. B. L. Bhatia and Mr. G. B. Chatterjee.

Plate 7.

Figs. 1—10. *Rhynchocystis cognettii* nov. spec.

All figures are free hand sketches drawn under Zeiss microscope provided with 2 mm. Leitz apochromatic objective. All are from stained preparations. Fig. 7 from preparation stained with iron alum haematoxylin, all others from preparations stained with iron alum haematoxylin and eosin. Figures are drawn to a magnification of about 450.

Fig. 1. A young trophozoite showing hairs round the mucron; the hairs seem thickened at their bases owing to continuation in them of the endoplasm from the body of the parasite. Hairs are also visible round the posterior extremity. Just below the horn there is a narrow differentiated area. Nucleus is more towards the posterior region.

Fig. 2. Young trophozoite possessing hairs round the mucron only. The mucron and the crown of sarcocyste are seen at the anterior end, the latter is marked by epicytal striations.

Fig. 3. The shape of the mucron and the from of the body are markedly different from parasites shown in Figs. 1 and 2. There is a secondary nucleus at the posterior extremity.

Fig. 4. A young trophozoite showing a different from of the mucron.

Fig. 5. A trophozoite showing the structure of the metabolic crown of sarcocyte extending beyond the mucron. The striations end in definite points. Paraglycogen grains are seen completely filling the body of the parasite. There are no hairs round the epimeritic region.

Fig. 6. A trophozoite showing different form of the epimerite. The crown of sarcocyte is extending beyond the mucron and shows epicytal striations. The hairs are seen surrounding the mucron. The eccentric karyosome is towards the posterior region of the nucleus.

Fig. 6a. Showing the structure of the nucleus. The karyosome is placed eccentrically, and contains a number of vacuoles and deeply stained grains. The karyosome is surrounded by a clear space. Chromatin grains are scattered within the nucleus in definite peripheral rings.

Fig. 7. A trophozoite showing hairs round the mucron, as well as at the posterior end of the body.

Fig. 8. An adult constricted trophozoite in which the anterior end is clearly differentiated into two regions.

Fig. 9. An old trophozoite having no mucron. Many deeply stained chromatoid bodies are seen scattered in the body of the parasite, and are surrounded by clear areas. In the nucleus some chromatin masses are seen in contact with the nuclear membrane.

Fig. 10. A still older trophozoite showing a deep constriction near the middle of the body. A secondary nucleus with a distinct nuclear membrane, chromatin grains, and a central eccentric karyosome is also seen.

Plate 8.

All figures are free hand sketches from permanent preparations. Figs. 12 to 16 are from preparations stained with iron alum haematoxylin and eosin; figs. 18, 19 and 21 from preparations stained with iron alum haematoxylin; figs. 11, 17 and 20 are from preparations stained with DELAFIELD's haematoxylin and eosin. Figs. 14 and 16 are drawn under 16 mm objective; figs. 11, 13, 15, 17—21, under 4 mm objective; fig. 12 under 2 mm apochromatic objective and No. 10 eye-piece.

Nematocystis hessei nov. spec.

Fig. 11. An adult trophozoite with its nucleus showing two karyosomes. × 660.

Fig. 11a. Nucleus of the above parasite showing the vacuolated structure of the two karyosomes. Chromatin grains are seen scattered uniformly within the nuclear membrane. × 660.

Fig. 12. Nucleus of another individual, more highly magnified. Each karyosome is surrounded by a clear white area. Inside the smaller of the two karyosomes there is a deeply stained ovoid body. In the other there is a similar but lightly stained body which contains several deeply stained grains and other grains

which are united to form a rod like body. Chromatin grains are scattered uniformly in the nucleus. × 1470.

Nematocystis plurikaryosomata nov. spec.

Fig. 13. An adult trophozoite with an oval nucleus containing twelve spherical karyosomes. Chromatin grains are not clearly visible. × 660.

Figs. 14, 15 and 16. Different shapes which the parasite can assume, each having an oval nucleus with small karyosomes.

Nematocystis lumbricoides Hesse.

Fig. 17. A part of the adult trophozoite showing the great deformability of its body and containing an oval nucleus with a large, oval, vacuolated karyosome. Chromatin grains are seen scattered uniformly in the nucleus of the parasite. × 660.

Echinocystis globosa g. et nov. spec.

Fig. 18. An adult trophozoite showing the two spine-like structures and a large oval nucleus with an oval karyosome. Chromatin grains are seen scattered in the nucleus. × 660.

Fig. 19. An adult individual having an oval nucleus with a spherical karyosome placed eccentrically. The karyosome is surrounded by a clear space. Chromatin grains concentrate at several places and fine particles of chromatin are distributed rather unevenly. × 660.

Monocystis pheretimi nov. spec.

Fig. 20. An adult individual with a spherial nucleus having a large spherical karyosome placed eccentrically. The karyosome is surrounded by a clear white area. Chromatin grains are scattered uniformly. × 660.

Fig. 21. Another adult individual.

Plate 9.

The microphotographs in this plate were prepared for us by Mr. A. R. Binns, B. Sc. to whom our thanks are due. The photographs were taken under Leitz's microscope provided with apochromatic objectives, and with Leitz's micro-photographic outfit.

Fig. 22. *Rhynchocystis cognettii.* An adult trophozoite. 2 mm objective. × 6 oc.

Fig. 23. *Rhynchocystis cognettii.* An old individual showing a deep constriction in the middle of the body, and a secondary nucleus. 2 mm objective. × 6 oc.

Fig. 24. *Nematocystis hessei.* The anterior part has not come in the photograph. The nucleus is seen with the vacuolated karyosomes. 4 mm objective. × 12 oc.

Fig. 25. *Nematocystis plurikaryosomata.* With a nucleus containing several karyosomes. 4 mm objective. × 6 oc.

Fig. 26. *Echinocystis globosa.* Showing the two spines and the oval nucleus containing an oval, eccentric karyosome. 4 mm objective. × 12 oc.

Fig. 27. The same as in Fig. 26. Less highly magnified. 4 mm objective. × 6 oc.

Fig. 28. *Monocystis pheretimi.* Showing the nucleus with an eccentrically placed karyosome. 4 mm objective. × 6 oc.

Fig. 29. *Monocystis pheretimi.* Showing an oval nucleus placed more posteriorly. 4 mm objective. × 6 oc.

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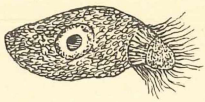

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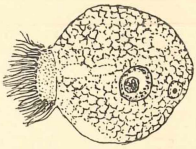

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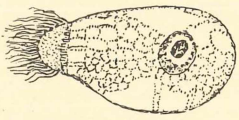

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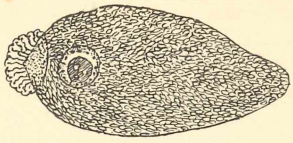

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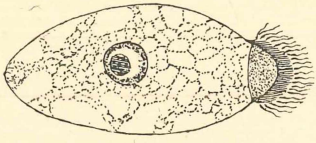

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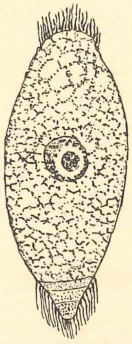

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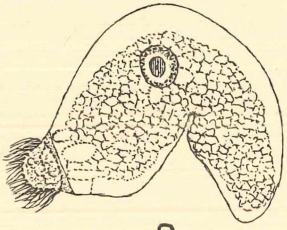

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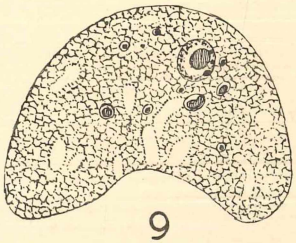

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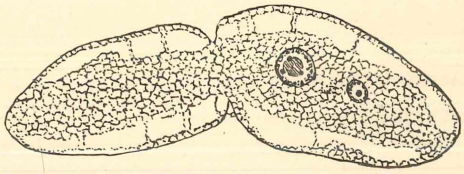

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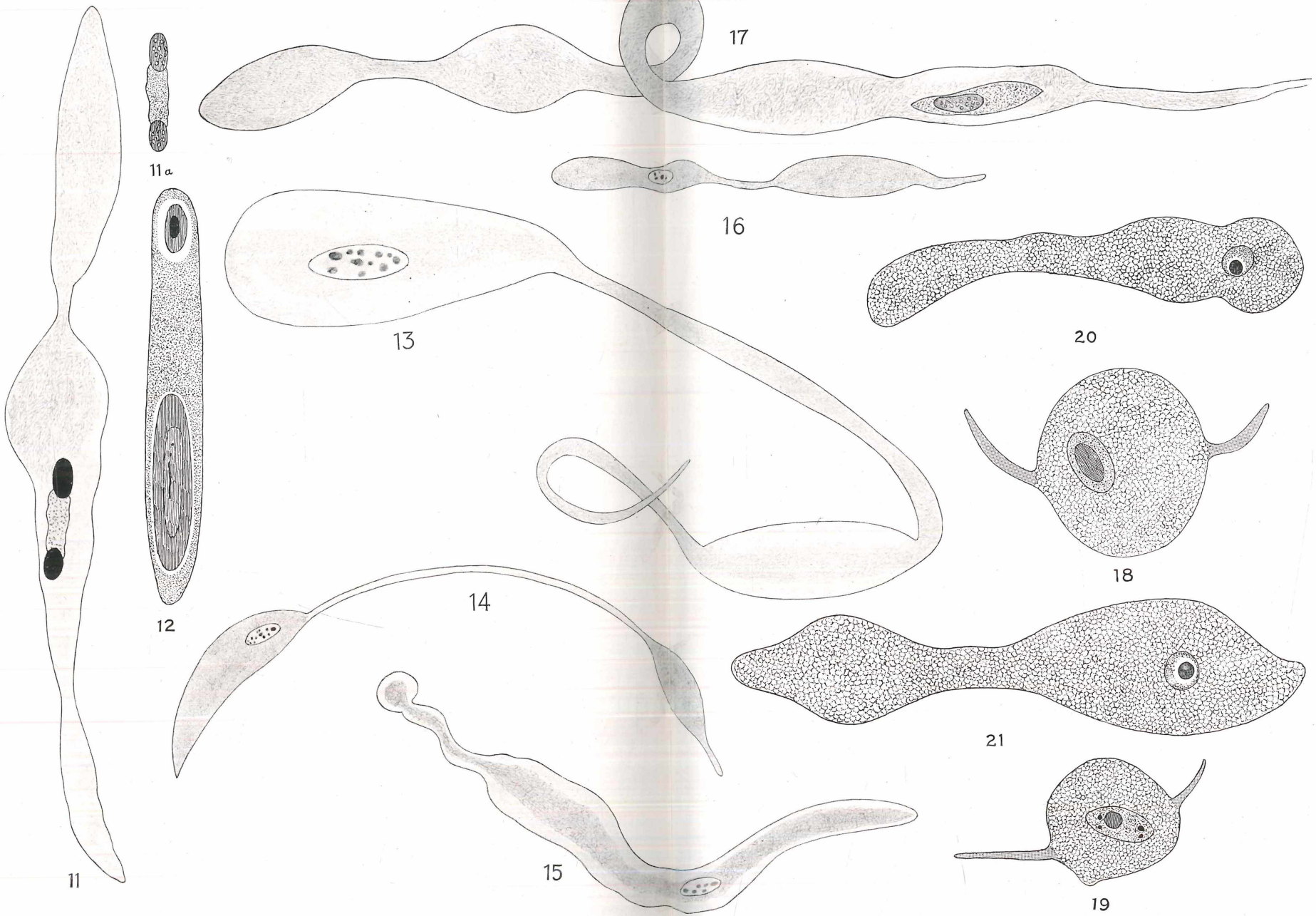
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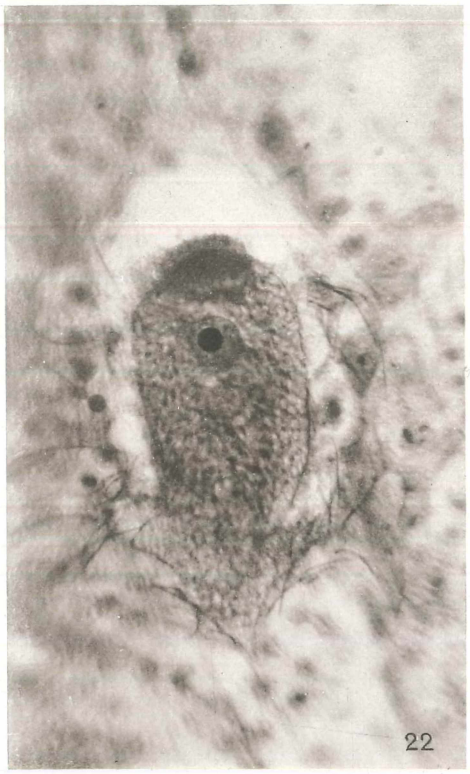

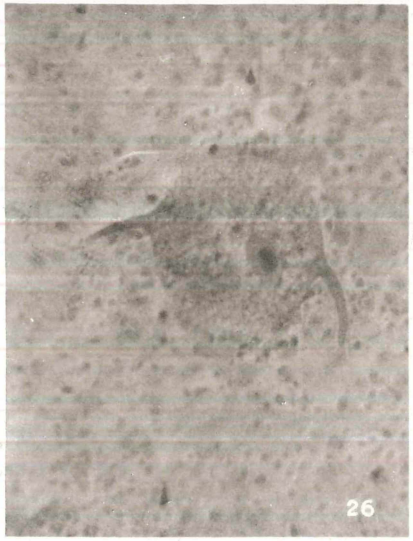

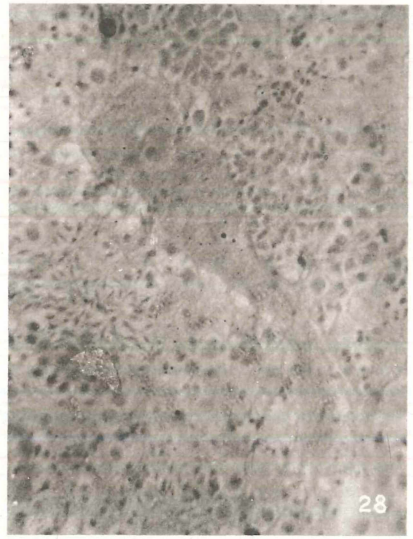

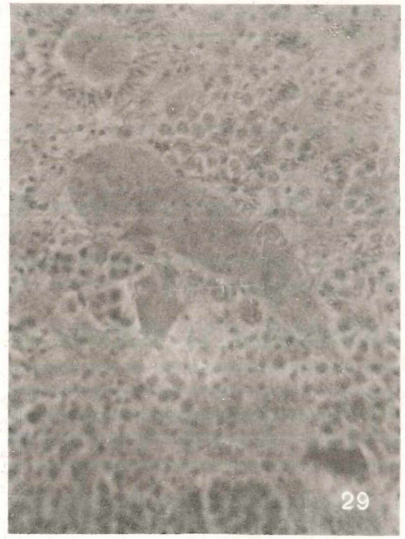

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