THE DEVELOPMENT AND SPOROGONY OF A COCCIDIAN *MYRIOSPORA GOPALAI* N.SP., PARASITIC IN THE GUT OF THE POLYCHÆTE *CIRRATULUS FILIFORMIS* KEFERSTEIN

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INTRODUCTION

IN a previous paper (Ganapati, 1939) I described a Coccidian *Myriospora polydoræ* from the body-cavity of a polychæte worm *Polydora ciliata* Johnst. An important phase in the development of Coccidiidæ in general, namely, asexual multiplication by schizogony was not seen in this form and, as the parasites were observed only from worms collected during a particular season of the year, the possibility of this aspect of development taking place at some other time of the year could not then be verified. I had also reasons to believe that the infection by this parasite may have been a sporadic one since the hosts examined subsequently were completely free from the parasites.

During the Summer of 1939, while examining some polychætes collected from the Madras Harbour, my attention was drawn to some large Mono-

cystis-like sporozoans in the gut of *Cirratulus filiformis*. A preliminary examination of this parasite in the living condition and in sections soon convinced me that I was dealing with a Coccidian very similar in many respects to the one from *Polydora ciliata*. The parasites confine themselves to the posterior third of the gut and they are seen attacking the sub-epithelial layer of the intestine. All stages leading to the formation of the oocyst were similar to those of *M. polydoræ*, the differences being mainly of details. For a long time my attempts to get stages of the ripe oocysts containing sporocysts and sporozoites from the worm were unsuccessful, and this, as I realised later on, was owing to the fact that the oocysts escape from the host along with the fæcal matter and complete their development outside, in the sea-water. The number of sporocysts inside each oocyst varies from 8 to 16, and the sporocysts are polyzoic. Compared with *Myriospora trophoniæ* and *Myriospora polydoræ* this number is much smaller, but for reasons discussed in another part of the paper I feel justified in including the present form in the genus *Myriospora* though it is admittedly a new species. I propose the name *M. gopalai* for this Coccidian from *Cirratulus filiformis*.

When I undertook a detailed investigation of the present form, I had in my mind the hope of solving the question of schizogony which has been missed both in *M. trophoniæ* and in *M. ploydoræ*, owing to lack of material in the former, and to the sporadic nature of the infection in the latter. Though both these difficulties were not encountered in the parasite from *Cirratulus*, the problem of schizogony remains unsolved. No trace representing this phase has been encountered in the hundreds of parasites examined from hosts collected during the several months from 1939-42. I have therefore come to the conclusion that schizogony does not take place in the worm and if such a phase in the life-history is really present, it should take place in another host. The difficulties in investigating this aspect of the problem is discussed elsewhere in this paper. The present study has in the main served to confirm many of my observations on *M. polydoræ* and incidentally to get details regarding the earlier stages leading to sporogony, which were missed in that form. At the end of the paper I have discussed the systematic position of the genus *Myriospora* from a comparison of the three species so far known.

In addition to the Coccidian, *Myriospora gopalai*, *Cirratulus filiformis* harbours three other Sporozoans and an astomatous Ciliate inside its gut. Of the Sporozoans two are Schizogregarines belonging to the genus *Selenidium* while the third is a dicystid Gregarine of the genus *Sycia*. The astomatous ciliate is a species of *Anaplophrya*, probably *A. brasili*. These parasites are described in another paper (Ganapati, 1942 unpublished).

Material and Methods

The host worms are fairly abundant in the Madras Harbour boring on shells of oysters or living amidst the debris collected on sponges and tunicates. The presence of the worms could easily be detected by their bright orange colour and by their characteristically long cirri usually found waving in the water outside their burrows. They are gregarious in habitat and have the habit of collecting together in clusters. The most successful method to induce the worms to come out of the burrows is to keep the shells after proper washing in a large basin of sea-water. The worms presumably get asphyxiated in about two hours and begin to crawl out. These are picked out and kept in clean sea-water contained in finger bowls. With daily change of water the worms remained healthy in this condition for about three weeks.

The infected worms could easily be picked out from healthy ones by examining them under a microscope when under the slight pressure of the cover glass the parasites become readily visible. For the study of the parasites all the methods described in a previous paper were employed (Ganapati, 1939). In addition Feulgen's technique was tried with success for the study of the nucleus. Fixation with Flemming's, Carnoy's or Hermann's fluid followed by suitable staining was used for special purposes. For general morphological study sections of material fixed in Bouin-Duboscq's fluid stained with iron-alum hæmatoxylin were the most satisfactory.

The oocysts were collected from the fæcal matter of the worm, using a pipette with a fine nozzle, under the binocular microscope, and kept in clean sea-water or in a 5 per cent. solution of potassium dichromate. In this condition the oocysts normally completed their development in about four days forming ripe sporocysts and sporozoites.

The Merozoites

The merozoites form the starting phase in the life-history of *Myriospora gopalai*, as it develops in the gut of *Cirratulus filiformis*. How these merozoites came into being, whether directly as a result of the growth of the sporozoites, through ingestion of the ripe sporocysts by the worm, or whether they are the ultimate products of a previous asexual multiplication in another host, are facts which shall be examined in a later part of the paper, where the question of schizogony is discussed.

The merozoites are the most conspicuous stages indicating the presence of the parasite within the worm. They are large gregariniform bodies reaching a size of nearly 150 × 25 microns. The body is long, cylindrical

and broadly rounded at either extremities (Figs. 5–7). The cytoplasm is coarsely granulated throughout excepting for a small zone at one end which in fresh preparations appears clear and hyaline. In sections stained with hæmatoxylin this end stains deep showing a large number of siderophilous granules. The nucleus is a clear rounded vesicle placed in the middle of the body and containing a large rounded karyosome. The karyosome stains dark with hæmatoxylin and the nucleoplasm is clear without any chromatin. There is a distinct nuclear membrane. Scattered in the general body protoplasm are a few deeply staining siderophil grains. The parasite is covered by a thin pellicle.

The smallest and presumably the earliest merozoites were found free in the gut or attached to the intestinal epithelium (Fig. 1). They are more or less of the same size as the sporozoites (Fig. 2) measuring about 15 microns in length. In structure they show the same features as those of the full-grown merozoite except that there is no distinct nuclear membrane surrounding the nucleus. The karyosome is large and conspicuous and there is a clear halo surrounding it. The difficulty of interpreting these free merozoites in the gut as those belonging to the Coccidian is obvious when it is known that there are three other sporozoans which occur side by side with the parasite under consideration. I have based my conclusions using as criteria two unmistakable morphological features constantly present in the merozoites. These are the structure of the nucleus and the presence of siderophil grains at the anterior end of all the merozoites. The other sporozoans have their own peculiar morphological features which preclude the possibility of confusing stages of these as belonging to the Coccidian.

The young merozoites penetrate the gut epithelium before reaching their normal habitat, namely the sub-epithelial layer. I have seen a few of these merozoites actually in the act of entering the epithelium. Inter-epithelial stages were, however, very rare and I have observed such stages only on two occasions. The rarity of this stage leads me to conclude that the penetration of the epithelium is rapid and that this is the normal means by which the parasite gains entry to the primary site of infection.

Once safely lodged the merozoites begin to grow. Each merozoite is surrounded by a sheath formed of the host's connective tissue and, in sections, a clear space is visible all round the parasite (Photomicrograph 1). This space, I believe, is produced at the time of fixation and that in the living condition the parasite is in direct contact with the host's tissue and nutriment is absorbed by the general surface of the body, which is made use of for growth.

The living merozoites do not show any movement when freed from the gut and examined in a medium of sea-water or in the body fluid of the worm. They also appear quite inert in their natural surroundings.

A comparison of the merozoite of *M. gopalai* with those of the other two species, *M. trophoniæ* (Lermantoff, 1913) and *M. polydoræ* (Ganapati, 1939) brings out the following similarities. The shape of the body, the gregariniform appearance, the presence of siderophil grains at one end, and the structure of the nucleus are identical in all the three forms. The merozoites of *M. trophoniæ* are however, much larger than the other two species. Lermantoff states that the merozoites in *M. trophoniæ* reach a length of nearly 700 microns which is four to five times the average size of the present form and of *M. polydoræ*.

Another question which we have to consider at this stage is the occurrence of a sexual differentiation of the merozoites. Lermantoff (1913) states that such a difference may be present according to the shape and the relative size of the karyosome. The males are said to be more curved and to possess a smaller karyosome than the female. I have said that in *M. polydoræ* there are no constant differences between the karyosomes of the supposed male and female and that the differences consist in the structure of the cytoplasm and the relative abundance of its inclusions. This view has been further substantiated by the study of the present form. Once the merozoites get transformed into the gametocytes it is readily possible to distinguish the male and the female.

Microgametocyte

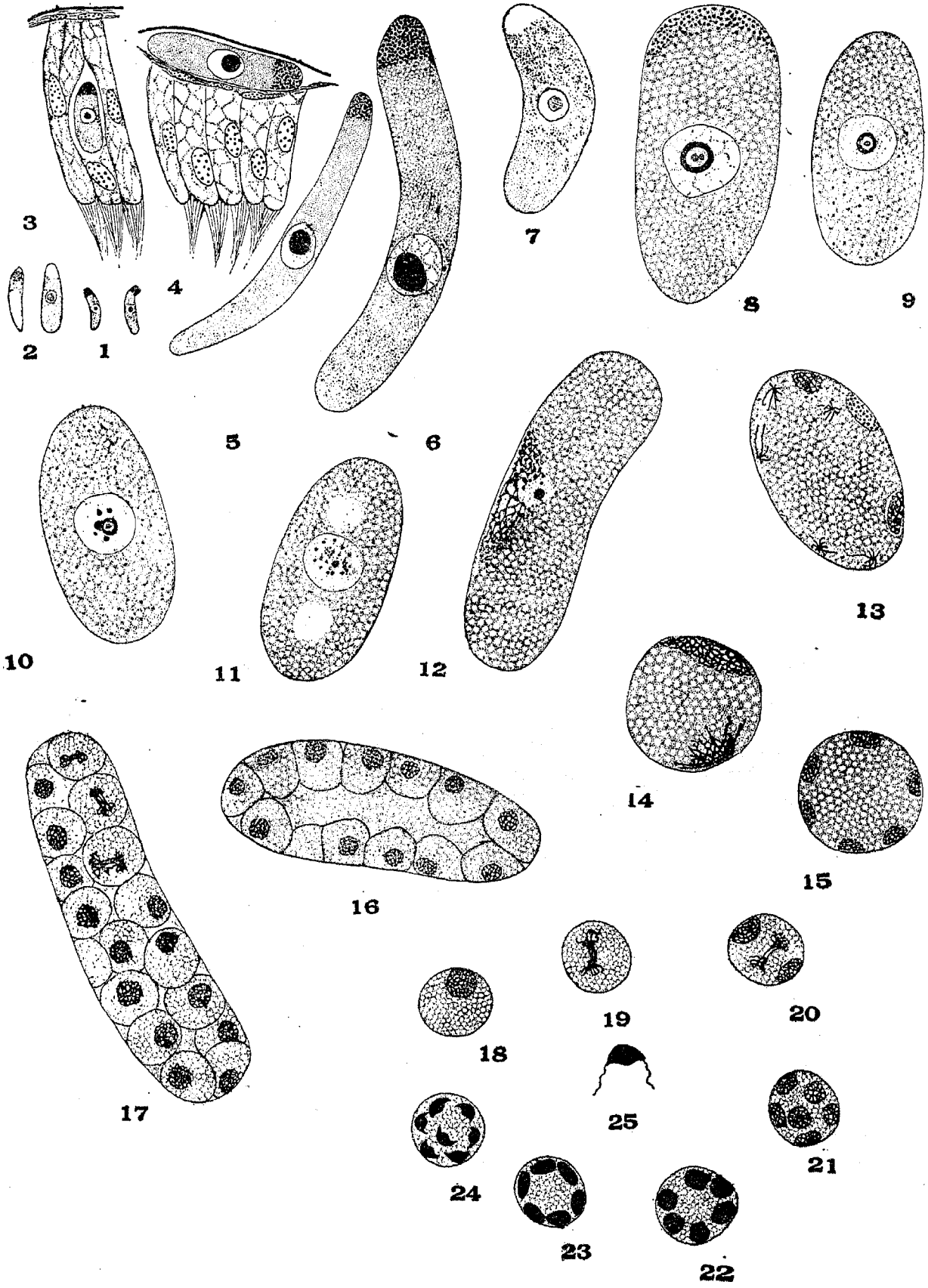
After the merozoites have attained their full growth they get transformed into the gametocytes. The transformation takes place in a manner essentially as in *M. polydoræ*. The main changes involved are a slight shortening in length accompanied by a growth in width. This is soon followed by a scattering of the siderophil grains concentrated at the anterior end into the general body protoplasm (Fig. 8). The nucleus is essentially the same as in the merozoite except for a slight increase in size. In well differentiated sections the karyosome shows an outer deeply staining rind or cortex and an inner lighter medulla. The medulla contains one or at times two small rounded bodies which reminds one of the micronucleus of *Aggregata* described by Dobell (1925). In *Aggregata* there is only a single micronucleus.

The maturation phenomena or the ripening of the male before it gets ready to form the gametes consist of some changes taking place in the nucleus. The single karyosome extrudes a number of daughter karyosomes through temporary openings formed on the cortex of the main karyosome (Fig. 10).

It would appear that these daughter karyosomes are formed from the substance of the medulla, the cortex remaining intact and entire after the process is completed. Thirty or more daughter karyosomes are formed in this manner. There is still no indication of any chromatin grains in the nuclear sap.

At this stage in the development of the parasite two clear areas of cytoplasm simultaneously make their appearance a little distance from the nucleus, at either pole along the longer axis of the animal (Fig. 11). In fresh parasites these are transparent and hyaline while in sections they are seen as made up of finely granulated cytoplasm which stands in sharp contrast with the alveolated cytoplasm of the body. The appearance of these two areas is the prelude to the next stage in development, namely, the division of the nucleus. I am unable to assign any exact significance to the appearance of these clear zones but am convinced that they have to do something with the division of the nucleus. It is possible they may represent the achromatic figures which have partly a cytoplasmic origin in many of the Protozoa. Though the division of the nucleus takes place by a form of mitosis, in which chromosomes are formed, there are no typical achromatic figures in these divisions, like the centrioles, centrospheres and asters. The chromosomes are distinct and well defined only in the anaphase stage and in the early telophase. It is significant that these characteristic areas are also present in the mature female. I have not seen these clear areas in the gametocytes of *M. polydoræ.*

The nucleus when ready for division migrates to the surface and occupies a position immediately below the pellicle. The chromatin grains at first appear as fine grains which stream into the nucleoplasm from the main karyosome. I am not able to say what part of the karyosome contributes the chromatin and whether the micronuclei have any part in its formation. It is significant that the 'micronuclei' disappear when the chromatin makes its appearance. The chromatin grains soon collect into small clumps which later get distributed on a highly tangled network. The karyosomes have meanwhile dwindled in size and only two or three are visible. The nuclear membrane disappears and the whole network gets drawn out (Fig. 12). I have not seen stages showing the completion of the first division. Parasites showing four up to sixty nuclei have been met with and the details of their division observed. The division process is exactly as in *M. polydoræ.* Chromosomes are distinctly seen in late anaphase and telophase stages. As in *M. polydoræ* the chromosome number is four. The ends of the chromosomes intercross forming spider-shaped masses and not more than eight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free ends are to be seen (Fig. 13). The nuclear divisions take place on the surface of the parasite and when these divisions are completed the nuclei occupy positions immediately below the pellicle. Each such nucleus consists of a few large globules of chromatin connected together by fine strands. The cytoplasm of the parasite forms a number of furrows, which, starting from the surface extend inwards between the nuclei. Finally each nucleus is separated with an area of cytoplasm around it (Photomicrograph 2). These secondary bodies are the real microgametocytes each of which gives rise to a few male gametes (Fig. 17). When these bodies are formed the pellicle of the male disintegrates and each one hereafter completes the development independently. Lermantoff (1913) calls these secondary bodies of the male microgametocysts; Siedlecki (1907) calls them cytomeres or microgametocyte proper, when describing similar structures in *Caryotropha* I have used the term microgametoblasts for these bodies in *M. polydoræ.* I am inclined to consider that the microgametoblasts, though in the strict sense may be compared to the microgametocytes of other Coccidians, are however, to be distinguished from the latter by adopting another nomenclature and the term microgametoblast appears to me to be the most explanatory. After the disintegration of the pellicle of the male parent the microgametoblasts get scattered to some extent and are often seen far removed and isolated from their place of origin.

Microgametogenesis

Each microgametoblast is the starting phase in the formation of 6 to 8 male gametes. The body is spherical, surrounded by a thick pellicle. The cytoplasm is closely alveolated and the nucleus lies ex-centrically. A few siderophil grains are found scattered in the cytoplasm. The single nucleus repeatedly divides into 6 to 8 nuclei (Figs. 18–24). In the later stages of division the chromatin gets more and more condensed and ultimately the whole nucleus stains homogeneously with hæmatoxylin. Each nucleus gives rise to a single gamete. The fully formed male gamete is crescent-shaped with a swollen belly and drawn out ends, each end carrying a flagellum (Fig. 25). The microgamete is composed mostly of chromatin and stains homogeneously deep with hæmatoxylin (Photomicrograph 3). When the microgametes are fully formed a spherical mass of residual protoplasm is left unused in the middle. In fresh preparations the microgametes are seen to be very active rotating inside the enveloping membrane of the microgametoblast, with the aid of the flagella. Lermantoff (1913), on the strength of Professor Dogiel's observation, states that the male gametes in *M. trophoniæ* show so much movement that the residual body inside the covering also rotates along with them. The microgamete of *M. gopalai* resembles closely

that of *Angeiocystis audouiniæ* described by Brasil (1907) from the heart-body of *Audouinia tentaculata*. The fully developed microgametes are liberated by the disintegration of the enveloping membrane of the microgametoblast and the free male gametes are often seen adhering to the surface of the female. The microgametoblasts themselves often undergo their development close to the female and this, combined with the active motility of the male gametes, facilitates the phenomenon of fertilisation.

Macrogametocyte

The female parasite gets transformed into the macrogametocyte in a manner similar to that of the male gametocyte. The merozoite destined to become the female stops growth when it reaches a length of about 120 microns, and thereafter increases in width. The cytoplasm is more coarsely granulated, the alveoli larger, and the cytoplasmic inclusions are more than in the male (Fig. 26). The nucleus has the same structure as in the merozoite except for an increase in size. The karyosome shows two regions, an outer cortex and an inner medulla. There are one or two round bodies inside the medulla corresponding to the 'micronuclei' of *Aggregata*. The karyosome extrudes a number of daughter karyosomes in a manner similar to that in the male (Fig. 27). These changes in the nuclei constitute the maturation phenomena. The nucleoplasm is free from chromatin up to a late stage in the extrusion of the karyosomes. The two clear areas of cytoplasm mentioned in the male, at either end of the nucleus, along the longer axis of the parasite are seen in the ripening female also. In the next stage the nucleus migrates to the surface. The nuclear membrane nearest the surface of the parasite gets raised up into a blunt cone-like elevation (Fig. 28). Meanwhile the chromatin makes its appearance in the form of a number of globules connected together by thin strands of the same material. The female at this stage is ripe for fertilisation or, in other words, it is now a macrogamete.

Sporogony

Very careful search of large numbers of the female parasites has not shown the actual process of fertilisation or the entrance of the male gamete into the female. I have seen the male gametes adhering to the surface of the macrogamete and the indications are that one of them succeeds in piercing through the pellicle of the female and fuses with the nucleus of the latter. The immediate result of fertilisation is the formation of a relatively thick fertilisation membrane which later becomes the oocyst wall. There appears to be a stage of rest of the zygote nucleus before division commences, and I have seen a few such stages in which the oocyst wall is present but the

nucleus still showing no signs of division. Immediately after fertilisation the cytoplasm recedes or shrinks away from the wall of the oocyst leaving a clear space between the cyst-wall and the parasite. In sections this space extends all round and the wall itself appears wavy as a result of fixation.

Though I have not seen a typical fertilisation spindle, described in many Coccidians, I was successful in getting a stage in which the zygote nucleus showed a late anaphase stage of division (Fig. 29). The chromosomes were very distinct and well defined and the karyosomes were found discarded in the cytoplasm after the distintegration of the nuclear membrane. Fig. 29 is a reconstruction of this stage from four serial sections. The chromosomes intercross at either pole of the division (Fig. 29). The number of chromosomes is four, as there are eight free ends, and this, as we have seen from the male during microgametogenesis, is the haploid number. I was unable to see an earlier stage in the division of the zygote nucleus, which is necessary to decide whether there is a reduction division at this stage as has been proved to be the case in *Aggregata* (Dobell, 1925). Since the chromosome number in microgametogenesis is haploid there is every reason to believe that the diploid number is restored at the time of fertilisation and that the first zygotic division is the reduction division. Parasites showing subsequent stages of division of the nuclei have been examined in large numbers. The details of division are the same as in the male. In all cases where it was possible to make a count the chromosome number was four. There is no indication of polymitosis. By repeated divisions 8 to 16 nuclei are formed and these come to lie on the surface immediately below the pellicle of the parasite. The nuclei are dense and rich in chromatin. The segmentation of the cytoplasm to form the sporoblasts starts from the surface as furrows which extend inwards between the nuclei. Each nucleus is ultimately separated with a bit of protoplasm around it resulting in the sporoblasts. No residual cytoplasm is left behind in the oocyst after the formation of the sporoblasts. The sporoblasts acquire a thick double-contoured wall or envelope and after its formation it was found impossible to get satisfactory fixations though all the known fixatives which have a rapid penetrating power were tried. The fixatives produced considerable shrinkage of the oocyst wall but failed to penetrate further into the sporoblasts. I had therefore to restrict my further observations on the development of the sporoblasts as revealed by an examination of fresh oocysts kept either in sea-water or in a 5 per cent. solution of potassium dichromate.

The oocyst, as stated already, is passed to the outside along with the castings of the worm, at a stage either soon after fertilisation or immediately after the sporoblasts are formed. Oocysts showing the latter condition

are more commonly met with. The gut epithelium of the host is ruptured while the oocysts are liberated and when the infection is heavy the damage produced is great. The fæcal matter of the worm was examined at regular intervals of the day and the oocysts picked out under a binocular microscope. When kept either in sea-water or in a 5 per cent. solution of potassium dichromate they completed their development in about 4 days. The walls of the oocyst and of the sporocyst are perfectly transparent and therefore it is easy to observe the changes inside by examining them at intervals under the microscope.

The wall of the oocyst is double and at one pole there is a cap-like thickening of the outer wall (Fig. 35). This pole corresponds to the micropylar end of typical Eimerian oocysts. The cytoplasm either completely fills up the space inside or in some cases a clear space is seen between the oocyst wall and the cytoplasm, at either pole, which is filled with a clear fluid. After the segmentation of the cytoplasm has taken place there is no cystal residuum and the sporoblasts completely fill up the space inside. Within the oocyst 16 sporoblasts are usually formed though, frequently, the oocyst segments even at the 8-nucleate stage producing only 8 sporoblasts (Photomicrograph 4).

Those containing intermediate numbers like 10 or 12 are extremely rare. I have examined more than 200 oocysts but the number of sporoblasts has never exceeded 16. The inconstancy in the number of sporoblasts and sporocysts led me at first to doubt whether I am dealing with more than one species of Coccidian. All evidence concerning the endogenous stages in development proved, however, that the oocyst belonged to one and the same parasite. The discrepancy in the number within the small range may be either due to the incomplete segmentation of the cytoplasm before forming the sporoblasts or it may be explained by the failure of some of the sporoblasts to complete the development.

Table I shows the measurements of 24 oocysts picked at random, 12 of which contained 8 sporocysts, and the other 12, 16 sporocysts.

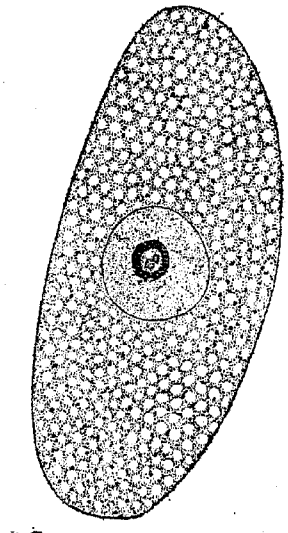
It will be seen from the table that the largest oocyst containing 16 sporocysts measures $115 \cdot 2 \times 54$ microns while the largest containing 8 sporocysts measures $90 \times 39 \cdot 6$ microns. It can also be seen that the smallest oocyst containing 16 sporocysts is only slightly smaller than the largest containing 8 sporocysts. On an average the former measure $91 \cdot 8$ microns by $53 \cdot 7$ microns, and the latter $72 \cdot 6$ microns by $42 \cdot 9$ microns. In general oocysts containing 8 sporocysts are smaller than those containing the full complement of 16.

TABLE I

Serial number	Oocysts with 16 sporocysts		Oocysts with 8 sporocysts	
	Length in microns	Width in microns	Length in microns	Width in microns
1	86·4	54·0	68·4	43·2
2	93·6	54·0	82·8	39·6
3	86·4	54·0	68·4	46·8
4	86·4	50·4	68·4	43·2
5	86·4	50·4	68·4	46·8
6	93·6	54·0	68·4	39·6
7	86·4	54·0	72·0	43·2
8	86·4	50·4	64·8	36·0
9	86·4	50·4	79·2	46·8
10	86·4	50·4	72·0	43·2
11	115·2	54·0	90·0	39·6
12	108·0	68·4	68·4	46·8
Average	91·8	53·7	72·6	42·9

The sporoblasts complete their development in about 4 days producing the ripe sporocysts and sporozoites. The sporocyst is ovoidal measuring 25 × 20 microns (Fig. 38). There is a thick double-contoured wall around each sporocyst and the sporozoites are seen arranged regularly inside. Each sporocyst forms 24 to 32 sporozoites which are disposed all round within the sporocyst. There is a spherical sporocyst residuum at one pole. The sporozoites may be liberated by applying gentle pressure on the cover-glass, when the sporocyst membrane ruptures (Photomicrograph 5). The sporozoites show active twisting movements for a short time after liberation, when examined in sea-water. The body of the sporozoite is stave-shaped, one end being sharply pointed while the other end is blunt and rounded. Near the blunt end the body is transparent for about half the length of the parasite, while at the pointed end a few refractile spherules are seen (Fig. 38). Smear preparations of the sporozoites fixed in Bouin-Duboscq's fluid stained with hæmatoxylin bring out their structure very clearly. In these it will be seen that the nucleus occupies about half the length of the body and that the position of the nucleus corresponds to the transparent zone in fresh preparations (Fig. 40). The nucleus is near the rounded end. The refractile spherules at the opposite end stain deeply with hæmatoxylin and form a cap (Photomicrograph 6).

A careful examination of the sporozoite and the early merozoite would convince that there is a striking similarity in structure between the two. The size of the sporozoite and the earliest merozoite are about the same and there is a similarity in the shape also (Figs. 40 and 1). The presence of



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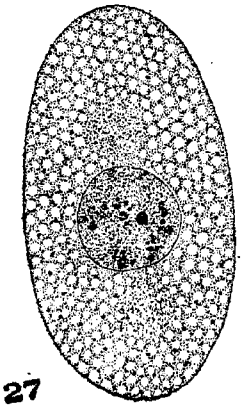

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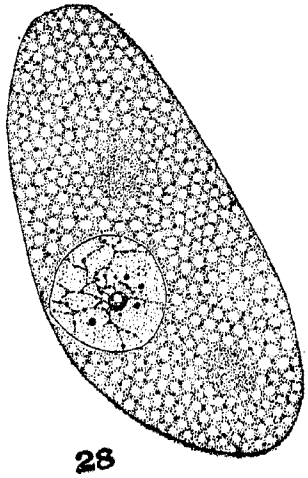

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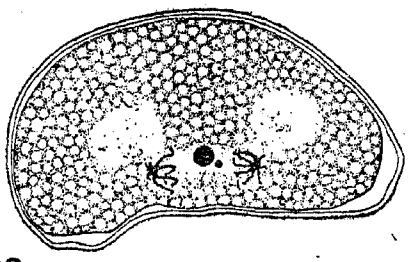

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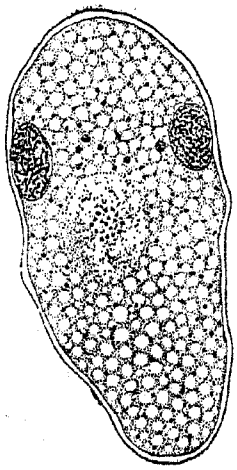

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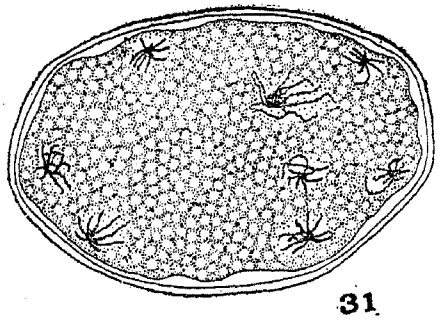

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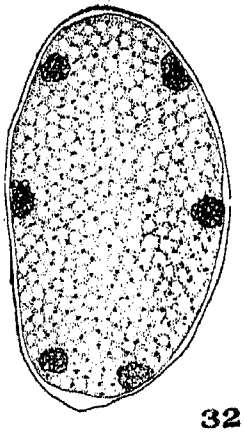

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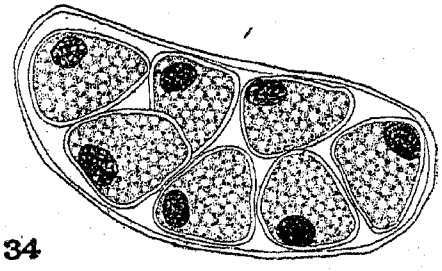

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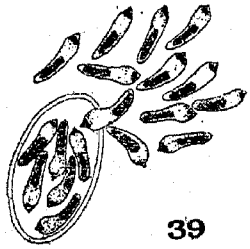

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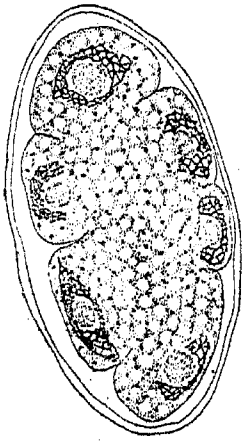

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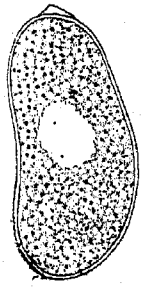

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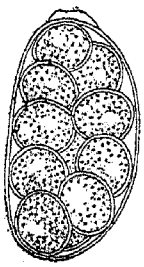
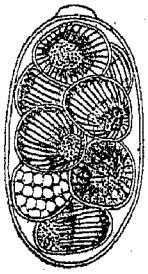
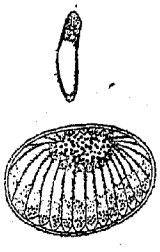


36



37



38

40

siderophil grains at one end is common to both, and I have no doubt these grains in the sporozoites are the forerunners of the same bodies in the merozoites. If it is imagined that the elongated nucleus of the sporozoite undergoes condensation into a rounded body it will be typically like the large spherical karyosome of the merozoite. I am inclined to consider that as soon as the sporozoites are liberated into the gut lumen this change in the structure of the nucleus takes place.

Infection of the worm presumably takes place by the ingestion of the ripe sporocysts which liberate the sporozoites into the gut lumen. The sporozoites develop into the merozoites and these into gametocytes and the life-cycle is repeated.

SCHIZOGONY

I have analysed (Ganapati, 1939) briefly the question of schizogony in *Myriospora polydoræ*, and tentatively concluded that such a phase may be absent in that form. My main reason in arriving at such a conclusion was the close similarity that existed between the sporozoite and the early merozoite. I had, however, to leave the question open on account of the limited material available, collected during a particular season of the year. In considering the question of schizogony the following possibilities have to be borne in mind, if we are to judge from known forms of Eimeriid Coccidia. In the large majority, schizogony takes place in the same host where sporogony is observed. The only known exception to this is *Aggregata* where there is an alternation of hosts, the schizogony taking place in a crab while the sporogony is in a cuttle-fish. A third possibility is that schizogony may take place only during particular seasons of the year as in *Orcheobius herpobdellæ* described by Kunz (1907) from the leech *Herpobdella atomaria*, where schizogony was observed only in material collected in the spring months of April and May. A fourth possibility is the occurrence of schizogony in some organ other than the one where sporogony takes place.

I have examined the host worms during the years 1939-42 at all the seasons of the year without finding any trace of schizogony. Serial sections of the different organs of the worm have also been examined with the same result. The only other possibility is an alternation of the host as in *Aggregata*. In the case of *Aggregata* it is well known that crabs form a major constituent in the diet of the cuttle-fish and it is quite natural to look for the alternative host in the crustacean. With this in mind I examined the gut of several worms to find out their feeding habits. The worm is essentially a vegetable feeder and the intestine contained only large quantities of minute diatoms and algæ with an occasional foraminiferan shell, which

might have got in by accident. This made the problem all the more difficult. It is beyond all reason to imagine that schizogony may take place in an animal larger than the worm itself and that the merozoites which are the end products of schizogony may have a free existence before entering the worm. The possibility of infection by inoculation by the merozoites by an alternate host may also be dismissed as far-fetched. My observations on *M. gopalai* have served to confirm my conclusion in regard to *M. polydoræ* that there is every indication to show that schizogony is absent. The peculiar method of microgamete formation by which an unusually large number of male gametes are produced by a single male and also the large number of sporozoites inside the oocyst are factors which would compensate for the absence of schizogony.

The Genus *Myriospora*

Lermantoff in the year 1913 created the genus *Myriospora* to accommodate a polysporocystid Eimeriid Coccidian which he discovered in the heart-body of a polychæte *Trophonia plumosa*. Among the characters which he gives as diagnostic of the genus are the large and gregariniform merozoites, the peculiar method of microgamete formation from secondary bodies produced as a result of the breaking up of the male parasite, and the development of a large number of sporocysts within the same oocyst, each sporocyst containing several sporozoites. An asexual method of multiplication by schizogony was not observed in the form. The extremely large size of the merozoites, the formation of a large number of sporocysts and sporozoites within the same oocyst and the absence of schizogony, so characteristic of the majority of known Coccidians, made Lermantoff doubt whether *M. trophoniæ* is a Coccidian at all or whether its proper place should not be among the Gregarines. There are, however, a few typical unmistakable Coccidian features in *Myriospora*. These are, its intra-cellular habitat during the early part of development, the widely separated nature of the gametocyte when producing the gametes, the production of numerous small male gametes composed mostly of chromatin and the development of the female parasite into a single macrogamete which in its turn gives rise to a number of sporocysts. The failure to see the schizogony is not unique in *M. trophoniæ* when it is known that of the six genera in the subfamily Aggregatinæ to which *Myriospora* belongs, schizogony has been observed only in two. In *Angeiocystis* from the Polychæte *Audouinia tentaculata* schizogony has not been observed.

Nearly twenty-five years after Lermantoff's original description of *M. trophoniæ*, I rediscovered a Coccidian in the body-cavity of *Polydora ciliata*

which showed some striking resemblance to *M. trophoniæ* in all its developmental features and described it as *M. polydoræ* (Ganapati, 1939). The sporocysts of *M. polydoræ*, while being polyzoic as in *M. trophoniæ*, are not so numerous in the oocyst, as in the type species. The general course of development and morphology, however, left no doubt as to its position in the genus *Myriospora.*

The Coccidian which I have here described essentially differs from the other two species in the fewer number of sporocysts developed within the same oocyst. This number is more definite, varying within a small range of 8 up to 16. The sporocysts are polyzoic as in the other two species. Schizogony was not observed to take place in this form also though the investigation has been more thorough and comprehensive extending over a period of three years.

Reviewing the genus as a whole we find that it occupies a unique position among the Coccidiidæ, its nearest ally being *Caryotropha mesnili*, described by Siedlecki (1907) from the spermatogonial cells of another polychæte *Polymnia nebulosa.* The resemblance between the two forms is strikingly seen in the microgamete formation. *Caryotropha,* however, does not possess large Monocystis-like merozoites, and the oocyst contains only about 20 sporocysts, each sporocyst having 12 sporozoites. Asexual reproduction by schizogony has been observed in *Caryotropha,* to take place in the same host where sporogony occurs.

Two other forms which may invite comparison with *Myriospora* as regards microgametogenesis are *Dorisiella scolelepidis* (Ray, 1930) from the polychæte *Scolelepis fuliginosa* and *Merocystis kathæ* (Patten, 1935) from the whelk *Buccinum undatum.* In both these the number of male gametes formed by a single parasite is not so large as in *Myriospora.* It is also not possible to extend the comparison beyond this similarity.

Hoare (1933) suggesting a new classification of the family Eimeriidæ bases the division of the sub-families according to the number of sporocysts formed in the oocyst, and these into genera differing from each other in the number of sporozoites in each sporocyst. The number of sporocysts in the oocyst is a constant factor in all the genera of the Eimeriidæ except the forms belonging to the group Polysporocystidea comprising of the two sub-families Barroussinæ and Aggregatinæ, where this is a variable factor. The polysporocystid *polyzoic** group includes forms ranging from monozoic sporocyst genera like *Barroussia* and *Echinospora* to polyzoic sporocyst

* The term polyzoic is used to denote the total number of sporozoites in the oocyst and not in each sporocyst.

forms like *Myriospora*. The genera *Merocystis, Pseudoklossia, Aggregata* and *Caryotropha* represent the intermediate forms in the series. Gousseff (1937) has created a new sub-family Yakimovellinæ to include a form *Yakimovella erinacei* from a hedgehog (*Erinaceus europeusp*). The oocysts of this form are octosporocystid and polyzoic. The sub-family is placed between the tetrasporocystid Eimeriinæ and the polysporocystid Barroussinæ. In creating the new sub-family Gousseff has made provision for hypothetical forms which are heccaidecasporocystid and heccaidecazoic or polyzoic. If this system is adopted it is apparent that *M. gopalai* may fit in either into the sub-family Yakimovellinæ Gousseff or it may admirably serve to take the place of Gousseff's hypothetical form which is heccaidecasporocystid and polyzoic, leaving out of consideration those rare cases where the oocyst may contain a number between 8 and 16. To say the least adopting this criterion alone, namely, the number of sporocysts in the oocyst, in determining the systematic position of any particular form of Coccidian sounds most unnatural taking into consideration other morphological and developmental similarities in the endogenous phase of the life-history. I therefore feel that while in those forms where the sporocyst number in the oocyst is a constant factor, Hoare's system of classification may be the most convenient, it is not so in the case of the polysporocystid sub-families where the sporocyst number is variable. In all such cases it is necessary to know the endogenous life-cycle before a particular form is assigned to the generic rank. A study of the three species of *Myriospora* (two by me and one by Lermantoff) has clearly shown that while the endogenous development is identical in all the three, there is considerable variation in the structure of the oocyst especially in the number of sporocysts formed, though, in all of them the sporocysts are polyzoic.

Myriospora gopalai Spec. Nov.

Diagnosis.—Merozoites large, elongated, with siderophil bodies at one end. Microgametocysts long and cylindrical breaking up into 60-80 microgametoblasts, each of which forms 6-8 male gametes. Microgametes crescent-shaped and biflagellate. Macrogametocyte shorter and broader than the male. Oocysts ovoidal, measuring 90-100 by 50–60 microns. The wall of the oocyst has a cap-like thickening at one pole. 8–16 Sporocysts are formed in each oocyst. Sporocysts ovoidal measuring 25 × 20 microns. Sporocysts polyzoic producing 24–32 sickle-shaped sporozoites. A sporocyst residuum present.

Schizogony not known.

Habitat.—Gut of *Cirratulus filiformis,* Madras Harbour, Madras.

Summary

1. The development and sporogony of a new Coccidian, *Myriospora gopalai* n.sp., are described, from the gut of a polychæte *Cirratulus filiformis*.

2. Merozoites are large and elongated with characteristic siderophil bodies at one end.

3. Microgametocyte is long and cylindrical producing 60–80 microgametoblasts. Each microgametoblast develops independently, forming 6–8 crescent-shaped biflagellate male gametes.

4. The females are shorter and broader than the males.

5. The chromosome number during microgametogenesis and in the nuclear division of the oocyst is haploid and it is believed that the diploid number is restored at the time of fertilisation. The first zygotic division is probably a reduction division.

6. Oocysts are ovoidal with a characteristic thickening of the wall at one end. 8–16 Sporocysts are formed by each oocyst. Sporocysts are polyzoic containing 24–32 sporozoites.

7. Schizogony was not observed and this question is discussed.

8. The systematic position of the genus *Myriospora* Lermantoff is discussed and it is pointed out that the criterion of the oocyst structure alone cannot be emphasised in separating the polysporocystid Eimeriid genera.

Acknowledgments

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Explanation of Text-Figures

All figures were drawn from sections with the aid of a camera lucida at stage level, with Zeiss apochromatic oil immersion objective and compensating oculars. Unless otherwise stated, the material was fixed in alcoholic Bouin-Duboscq and the sections stained in Heidenhain's iron-hæmatoxylin. The magnifications given are those at which the drawings were made but figures (1–40) have been reduced to a little less than one half.

Fig. 1. Section of two merozoites free in the gut lumen. ×1800.

Fig. 2. A sporozoite and an early merozoite (fresh preparation). ×1800.

Fig. 3. Section of gut to show an intra-epithelial merozoite. ×1800.

Fig. 4. A merozoite in the sub-epithelial tissue. ×1200.

Figs. 5 & 6. Two stages in the growth of the merozoite. ×1200.

Fig. 7. A fresh merozoite. Note the clear zone of protoplasm at the anterior end. ×800.

Fig. 8. A merozoite transforming into the gametocyte. Note the siderophilous grains at the anterior end spreading into the cytoplasm and also the structure of the nucleus. ×1800.

Fig. 9. A male gametocyte. ×1800.

Figs. 10 & 11. Maturation of the male. Note changes in the nucleus and the clear areas of cytoplasm at either pole of the nucleus. ×1800.

Fig. 12. A male showing the nucleus dividing on the surface. ×1800.

Fig. 13. A later stage of the male showing a few nuclei in different stages of division. ×1800.

Fig. 14. A cross-section of a male at the above stage. ×2400.

Fig. 15. A cross-section of a male at a later stage to show the nuclei arranged on the surface. ×1800.

Fig. 16. Formation of microgametoblasts. ×1800.

Fig. 17. Male showing the fully formed microgametoblasts. ×1800.

Figs. 18–24. Different stages in microgametogenesis. ×2400.

Fig. 25. A male gamete. Note the two flagella. ×2400.

Fig. 26. A macrogametocyte. ×1800.

Fig. 27. A ripening female. Note changes in the nucleus. ×1800.

Fig. 28. A macrogamete. ×1800.

Fig. 29. Oocyst showing division of the zygote nucleus. ×1800.

Fig. 30. A later stage of oocyst showing two nuclei. ×1800.

Fig. 31. A multinucleate oocyst showing the nuclei in various stages of division. ×2400.

Fig. 32. Oocyst showing the resting nuclei on the surface. ×1800.

Fig. 33. Oocyst showing formation of sporoblasts. ×1800.

Fig. 34. Oocyst with the sporoblasts inside. ×1800.

Fig. 35. A fresh oocyst. Note the cap-like thickening of the wall at one pole. ×600.

Fig. 36. Oocyst with sporoblasts inside (fresh). ×600.

Fig. 37. A ripe oocyst with sporocysts and sporozoites (fresh). ×600.

Fig. 38. A ripe sporocyst. Note the sporocyst residuum (fresh). ×1200.

Fig. 39. Sporocyst showing escape of sporozoite after rupture (smear). ×1200.

Fig. 40. A sporozoite. ×2400.

Explanation of Plate XI

Photo-micrograph 1. A merozoite. Note the outpushing of the epithelium by the growth of the parasite. ×about 600.

„ 2. A male showing the microgametoblasts. ×about 1200.

„ 3. A few microgametoblasts showing the microgametes. × about 1200.

„ 4. An oocyst which is ruptured showing 16 sporocysts (fresh). about × 1200.

„ 5. A ruptured sporocyst showing the escape of the sporozoites. ×about 1200.

„ 6. A sporozoite (smear).