PART III

HYPERPARASITES IN GREGARINES

OF EARTHWORMS

INTRODUCTION

Like metazoan organisms the protozoa are also susceptible to parasitic infection. The problem of organisms living on and in protozoa was discussed at length by Kirby (1941) From his review it would appear that various protozoa might be infected by Schizomycetes, by organisms of uncertain systematic position viz. Phycomycetes, by certain fungi viz. Sphaerita and Nucleophaga as well as by hyperparasitic protozoa.

Review of literature shows that Monocystid gregarines are no exceptions. They too have often been found infected by micro-organisms which often led to their destruction. For example, Hesse(1909) found that Monocystis lumbricilli, Monocystis striata, Monocystis agilis, Rhynchocystis pilosa, and Stomatophora coronata had their own peculiar bacterial parasites. Such bacteria, apparently belonging to Schizomycetes, varied in different species of gregarines ovoid to filamentous forms. The presence of such sitic bacteria in the majority of cases appeared ental to the gregarines. For example, Hesse(1909)

mentioned that bacterial parasites noted by him were uncommon and when present attacked host individuals of the species often leading to their death. To date, the association between a bacteria and its host (especially gregarines) have usually been cases of endoparasitism. Recently a case has been reported where bacteria have been seen lying attached to the surface of Porospora, a gregarine (Desportes & Theodorides/1977). Bacteria in this case have been found inserted between the epicytic folds of the gregarines, apparently inducing morphological changes similar to those noticed at the level of the junction between two biassociative gregarines. The author believed that in this case the gregarines constituted a substrata for the bacteria, all of them using for their food the intestinal contents of the host.

Mackenzie and Walker (1979) reported the presence of bacteria- like structures in the endoplasm of Gregarina garnhami. These structures were gram- negative.

According to Leger and Duboscq (1909), Frenzelinae conformis, a gregarine, parasitised by Nosema frenzelinae, developed normally up to a certain point only, the

formation of the gametes not taking place at all.

Caullery and Mesnil (1919) stated that Metchnikovellidae, haplosporidians, parasitic on gregarines (host-specific), had little pathogenic action on the host, particularly on the vegetative stages, though the author did not rule out mechanical injury in cases of heavy infection. According to Mackinnon and Ray (1931) the spores of Metchnikovellida escaped from their cyst into the endoplasm of the gregarines.

Kirby (1941) pointed out that according to a manuscript prepared by Stubblefield (1937), Amphiacantha, a haplosporidian, released gametocytes within the host gregarine Ophiodina elongata, by rupture of the cyst. Reduction division took place, resulting in the formation of the gametes, which underwent fertilization leading to further development; all these taking place within the remnant of the host gregarine.

According to Ganapati and Aiyar (1937), cytoplasm of Lecudina brazili, a dicystid gregarine, could be packed with cysts of a haplosporidian resulting in a mis-shapen

body of the host and a degenerating nucleus. Such parasitised gregarines were not found to associate, thus giving rise to the belief that heavily infected gregarines could not complete sexual development. (see Caullery and Mesnil 1919).

The presence of crystalline inclusions resembling virus particles have also been reported in certain parasitic protozoans including malarial parasites (Dasgupta 1968, Terzakis 1969, Davies & Howells 1971) . Malarial parasites harbouring such inclusions have been reported to undergo death and degeneration, and the significance of the presence of such inclusions remains to be clarified. Similar virus like particles have also been reported in certain gregarines and coccidia by Porchet & Richards (1969) and Porchet and Vivier (1971).

Pathogenic effects were also described in protozoa other than gregarines; for example, Bourne (1891) and Penard (1893) mentioned that Pelomyxa was generally always parasitised by endobiotic bacteria. Heavy infection led to hypertrophy, alteration in the body, and finally to the disintegration of the host (Leiner 1924).

Similar observations were made by Nagler (1910) and Epstein (1935) who worked on two different varieties of amoeba parasitised by micrococci.

Fatal effects were noticed by Wenrich (1937) on Iodamoeba butschlii, Endamoeba citelli (Becker 1926) and Nyctotherus which had macronuclear infection by microorganisms (Bassuchin 1928). Protozoa parasitised by Nucleophaga continues its activity with no change in protoplasmic structure. Lavier (1935) noticed hypertrophy of the nucleus of the parasitised Endamoeba ranarum and relegated it to a sort of defense mechanism on the part of the amoeba.

Marked physiological change was observed in paramecia infected with Dreparospira, Spirillaceae. The development in the host was rapid followed by the drying up of the nuclear contents. Bozler (1924) and Fiveiskaja (1929) noted vacuities in Paramecia infected with macronuclear infection. Fat droplets increased in amount, trichocysts were disarranged, food vacuoles stopped forming, leading to the disappearance of the mouth, gullet and the cytopyge. The host died eventually. It seemed that the above bacteria had a certain indirect

action on the host cell, the secretions and waste products of the hyperparasites seeping into the cytoplasm and resulting in the intoxication of the nucleus.

Stentor coeruleus infected with bacilli, lost their bright green colour and their capacity for motor responses (Hetherington 1932). According to Kirby (1941) many observers had reported the death of the host infected with Sphaerita. Dangeard (1889, 1895) Puymaly(1927), Mitchell (1928) and Jahn (1933) stated that Euglena when parasitised by Sphaerita lost chromatophores, suffered inhibition of flagellar movements and changes in nuclear structure. It finally burst to liberate the zoospore.

In view of all the facts stated above, it would appear that there is enough scope of work on the presence of parasitic infection in various gregarines occurring in nature. In the course of the present work an effort has been made to detect any such cases of hyperparasitisation and abnormalities in staining reactions in the gregarines studied and an effort has been made to elucidate the causes of such differences in staining characteristics.

Observations

The abnormal forms of Zygocystis n.sp.(a), were characterised by lightly stained areas in the cytoplasm, as seen in iron alum haematoxylin stain. In quite a number of cases it was observed that the entire trophozoites were lightly stained. Such lightly stained individuals were the abnormal forms of the species.

The abnormality arose as a small lightly stained patch in the cytoplasm of an otherwise normal trophozoite. The patch extended and such extended abnormal areas tended to lose compactness. As a result the trophozoites tended to lose characteristic shape. More patches developed and expanded. Such patches later on became confluent until the whole body of the trophozoite assumed a lightly-stained condition. In this final stage the trophozoite was again seen in its old characteristic shape.

These lightly-stained trophozoites appeared to harbour bacteroids, possibly of the kind reported by Hesse (1909). These imparted a faintly rosy hue to the cytoplasm. Some of these bacteroids were seen to be in a stage of binary fission. Lightly-stained

single trophozoites were occasionally found. In such cases the nucleus was either in a fragmented or in a diffuse condition. Furthermore, in some of the lightly stained individuals, black refractile pigments were found to be present. Those possessing these black refractile pigment granules had the nucleus in a disintegrating condition. The phenomenon of autotomy is also noticed in the abnormal forms of Zygocystis n.sp.(a).

When stained with Feulgen's method, the abnormal patches took up faint green stain with light green, in contrast to the surrounding areas which took up a deeper stain. No trace of Feulgen-positive body was noted in the cytoplasm of these forms.

When stained with pyronin-methyl green, the abnormal patches took up lighter colour with pyronin than the surrounding areas. With toluidine blue stain, the abnormal patches did not take up any stain as compared to the normal areas. The nucleus in the abnormal gametocyte was negative to toluidine blue but in some cases metachromasy has been observed in the nucleolus.

With acridine orange (Fluorescence microscopy),

P L A T E XXVIII

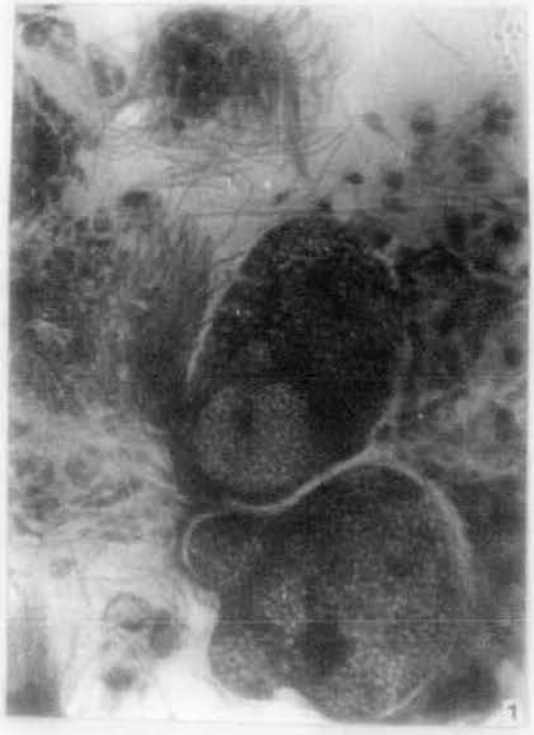
Fig.1. Patch forming in a trophozoite, which tends to lose its shape.
15 X 40X.

Fig.2. Association between a normal and an abnormal trophozoite. Note the nuclear change in the abnormal one.
15 X 40X.

Fig.3. Degenerating trophozoite. Nucleus has disintegrated. 15 X 40X.

Fig.4. Inclusion bodies in the cytoplasm of Apolocystis n.sp(d) 15 X 40X

PLATE XXVIII



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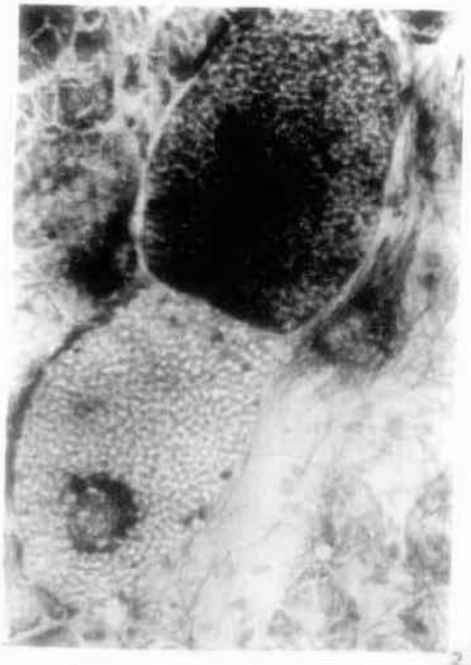

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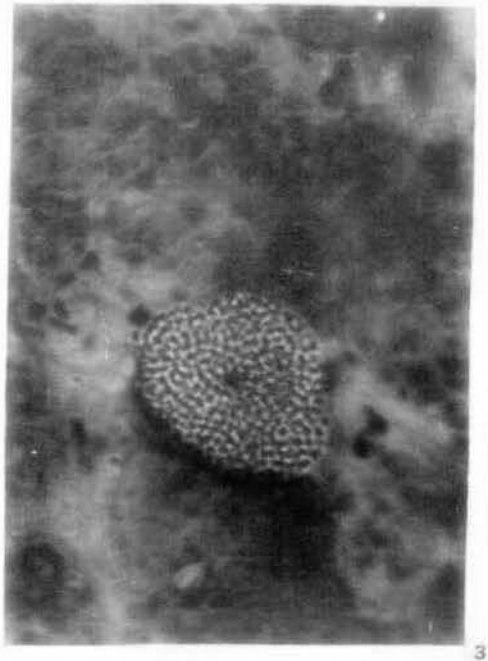

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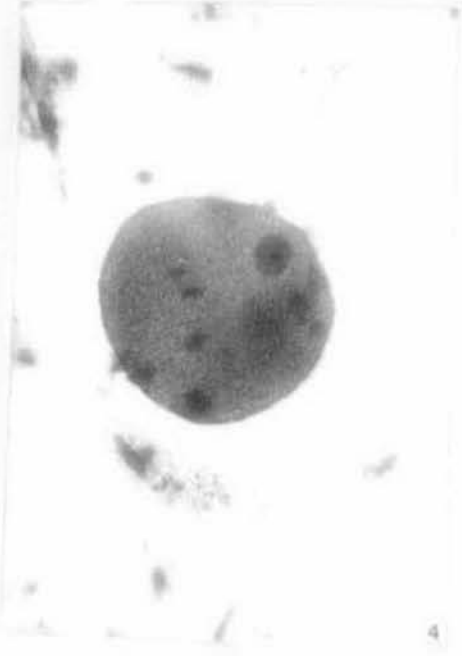

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an interesting phenomenon has been observed. The cytoplasm of these forms did not exhibit orange fluorescence as seen in the normal trophozoites. But scattered in the cytoplasm were a number of minute bodies, which fluoresced bright yellow apparently representing DNA present in the micro-organisms. The nucleus of such trophozoites showed no indication of having any trace of DNA or RNA.

Apolocystis n.sp.(d)

98% of the above gregarines possessed one or more darkly-stained patches scattered at random in the cytoplasm. These patches were circular or irregular in outline. It was noticed that a very young trophozoite (50.4 μ in diameter) might have such a patch, while an older one (104.4 μ in diameter) was without such a patch.

With Feulgen's method, the nucleus did not take up the stain. Some trophozoites were encountered in which Feulgen-positive bodies appeared in the cytoplasm. In one instance these Feulgen-positive bodies were so numerous that the gregarine took up a pinkish hue.

The patches in the cytoplasm, which represented various shapes (generally spherical) and which stained deeply with iron alum haematoxylin, corresponded to Feulgen-positive areas. These areas occurring in patches did not appear homogeneous, as seen in the iron alum stain. The patches harboured granular inclusions which were strongly Feulgen - positive, and which appeared in a faintly Feulgen-positive matrix. Prominent red metachromasy was observed in areas corresponding to Feulgen- positive patches (when stained with toluidine blue), and these areas are believed to represent inclusion bodies of bacterial or viral origin. Such patches did not stain with alcian blue. One or two orthochromatic dots were noticed in the general cytoplasm.

When pyronin-methyl green was used, the entire cytoplasm took up the pyronin stain, while areas corresponding to the Feulgen-positive patches took up methyl green. The nucleolus took up the pyronin stain very deeply.

With acridine orange (Fluorescence microscopy), strong yellow fluorescence was noticed in the Feulgen-positive patches occurring in the cytoplasm.

Besides the above two, other abnormalities were encountered in the course of the present investigation.

In A (a), innumerable Feulgen-positive bodies occurring in dot-like forms were found to lie scattered in the cytoplasm. In one instance it was noted that these bodies were elongated in shape. Both the larger as well as the smaller forms of the parasite appeared to have these Feulgen-positive bodies in the cytoplasm. In about two instances, however, a very faint patch of Feulgen positive area was seen. The nucleus was Feulgen-negative. These bodies also took up methyl green stain.

With acridine orange, the entire cytoplasm fluoresced orange. However, the bright orange cytoplasm did not appear homogeneous but was bunched up in lumps, presenting a blotchy appearance. The nucleoli looked pale orange with a slightly darker area in the centre. In some cases, however, the nucleus fluoresced faintly yellow.

Minute Feulgen-positive bodies were also found scattered in the cytoplasm of Stomatophora n.sp.(a). Likewise, a large number of similar Feulgen-positive bodies which stained with methyl green were found in the

cytoplasm of Nematocystis n.sp.(d).

DISCUSSION

The difference in staining reaction either in one or both the members of a pair of Zygocystis n.sp.(a), could possibly be due to the difference in the quantity and distribution of cytoplasmic inclusions. Similar observations were also made by Mühl(1921) and Joyet- Lavergne (1926) who used neutral red. These authors attributed the differences, to the difference in the oxidation-reduction potentials of the two gametocytes and also in the quantity and distribution of the cytoplasmic inclusions.

The finding of the fragmented or diffuse nucleus in the abnormal forms may lead to another assumption that the breaking down of the nucleus is in some way responsible for the difference noticed in the staining reaction of the cytoplasm.

We may however, rule out the above possibilities on account of the fact that all these lightly stained parasites showed extensive cytoplasmic invasion by the bacteroids,

which are definitely not normal inclusions in the species. Micro-organisms resembling these bacteroids and referred to as bacteria occurring in Monocystis species, and also in Rhynchocystis pilosa and Stomatophora coronata were reported by Hesse (1909). The bacteroids under consideration are variable in form i.e. are spherical, while others are rod-shaped. These divide by binary fission. Gradually the increase of the hyperparasites within the host, leads to the alterations in the nucleus. The changes are degenerative. The nucleus soon fragments and eventually disintegrates. Thus finally the invasion of the bacterial parasites leads to the destruction of the gregarine host.

Similar kinds of nuclear disintegration, which led to the death of the host, have been reported in Amoeba verrucosa (see Mattes 1924) and Endamoeba disparta (see Kirby 1941), which were infected by Nucleophaga. Leiner (1911) described Pelomyxa hyperparasitised by Cladothrix pelomyxa, a bacterium.

Caullery and Mesnil (1897) stated that Metchnikovella

caulleryi, a hyperparasite in a gregarine, "seemed to have little pathogenic effect on the host; particularly on the vegetative stages. What injury there is, is mechanical, when infection is heavy."

Heavy parasitisation might result in the waste products and secretions of the hyperparasite accumulating in the cytoplasm of the host gregarine, which might upset the metabolism of the gregarine leading to the lightly-stained condition. This directly or indirectly, has an effect on the nucleus which soon starts to fragment and disintegrate.

The death of the host leads to the death of the hyperparasites. Chitinization occurs around the dead micro-organisms resulting in the formation of black pigment which are refractile by nature, and the body of the host slowly disintegrates.

The association of the normal and the abnormal trophozoites, does not lead to the formation of the gametocyst. A similar observation was made by Caullery and Mesnil (1919) and Ganapati and Aiyar (1937). The latter however mentioned that the parasitised gregarines

did not associate in the case of Metchnikovellidae. In the course of the present study it has been found that association of this sort took place in Zygocystis n.sp.(a), but development ceased altogether after this. A few cases, where both the gametocytes were abnormal were also encountered. An assumption can be made that when association just occurred, only one of the members of a pair was parasitised by the bacteroids; infection spread rapidly to the other member, until both of them were heavily infected and did not take up the stain. The nuclei in such cases are not normal and are in a state of disintegration.

The above instance of hyperparasitisation proved to be fatal to the host. However in the course of the study, we have come across another case of hyper-parasitisation, which does not appear to cause any deleterious effect. For example about 98% of the trophozoites of Apolocystis n.sp.(d) harboured bacteroids, which were not spread throughout the cytoplasm, but remained in aggregates, forming some sort of inclusion bodies. No definite membrane was observed around these aggregates. The aggregates

consisted of granules. These granules were grouped in masses of irregular form.

The association between the parasites and the host is more or less a constant one, and can often be mistaken for normal structures of the host itself. Cytochemical studies have revealed no drastic changes in the polysaccharide contents of the host; this in all probability could mean that the inclusion bodies are not detrimental to the host. In Kirby's words (1941), ' They have come to occupy a normal place in the metabolism of the combination'.

Such aggregates of micro-organisms have also been found to be present in Devescovina glabea by Grassi and Foa (1911).

The micro-organisms in the other gregarines reported here also do not cause any harmful effect on the host. Their variability in number and the fact that they do not occur in all specimens is evidence enough that they are not normal inclusions of the host body. However, gregarines harbouring such inclusions appear to lead a normal life.