Notes on a new Coccidian (Merocystis kathae n. gen. et sp.) occurring in the Renal Organ of the Whelk.

By
Dr. **W. J. Dakin**
Assistant Lecturer in Zoology in the University of Liverpool.

(With 14 figures in the text.)

During the course of an investigation into the anatomy and histology of the common whelk *Buccinum undatum*, peculiar small white spherules were observed in the renal organ of specimens collected from a point in the Irish Sea some little distance west of Port Erin, Isle of Man. In every specimen examined these bodies were more or less frequent and they became very easily detached, so that at first sight it appeared as if the granules were some concretions which were being set free into the lumen of the renal organ. They appeared, however, too large and too regular in shape to be such, and sections showed them to be parasitic protozoa. They infect the renal organ to an enormous extent in some cases, but do not appear to injure it at all except in so far as the actual cells invaded by the parasite are concerned. Specimens of *Buccinum undatum* have been obtained now at close intervals throughout a period of one year, and the same conditions have always prevailed.

Methods.

The following methods have been used with success. The whelks, which are difficult animals to narcotise without injury so far as histological structure is concerned, were taken living and

the shell was gradually broken away with bone forceps until the renal organ was exposed. Small pieces of the renal organ were fixed in Zenker's and Bouin's Fluids respectively. Both these fixatives gave excellent results and Bouin's Fluid was particularly used when it was desired to stain with Methyl-blue Eosin (Mann).

For stains, Heidenhain's Iron Haematoxylin with eosin as cytoplasmic stain, and Ehrlich's Haematoxylin were very useful, particularly for nuclear structure. Mann's Methyl-blue Eosin was also frequently used, and as mentioned above always after Bouin's Fixative. Sections have been depended upon almost entirely for information as to the structure and life history and many thousands of these have been cut. Some of the living parasites have however been examined. It is impossible to see through them, but, by isolating them and compressing under a coverglass it has been possible in some cases to set free the spores and to observe the solitary sporozoite in each.

The renal organ of Buccinum.

The renal organ of the Whelk is essentially a tube which opens into the pericardium and connects this cavity with the pallial cavity. It lies on the right side of the animal in close contact with the digestive gland and rectum, and the pericardium with the heart lies in a notch midway along its length. The epithelial layer which forms the outer wall of this tube is not thrown into folds, and is perfectly continuous with that covering the rest of the body. The internal epithelial layer is the true renal epithelium and is thrown into a most complicated series of foldings and processes, projecting into the lumen and reducing its size. This folding however only takes place on the outer side, so that in a transverse section the renal organ would appear to have a very thick outer wall and delicate inner wall. Between the folds of renal epithelium, and underlying the latter is a mass of connective tissue richly traversed by blood lacunae.

The renal cells are thin walled and intensely vacuolated, and the free margin (facing the lumen of the organ) is drawn out so that it appears very ragged. This is due to an actual shedding of cells or parts of cells with waste matter into the cavity. The parasites may number as many as 15—20 in the field of view at once (Leitz $^2/_3$" Objective and No. 4 Eyepiece) and various stages may be seen therefore at one glance.

The parasite.

In describing the parasite a difficulty arose at the outset which will be discussed later. It is however necessary to refer to it here since it concerns the nomenclature to be used. In the typical coccidian life history there are two methods of reproduction — a sexual life cycle resulting in the formation of resistant spores with sporozoites (Sporogony) and an asexual endogenous multiplication which takes place within the host and results in the formation of merozoites (Schizogony). The sporozoite becomes a motionless trophozoite gradually increasing in size until full grown. This then proceeds by schizogony to divide and as a result large numbers of small bodies are formed termed merozoites. The merozoites infect cells in a similar manner to the sporozoites, and also give rise to trophozoites, but the trophozoite may become differentiated into sexual mother cells of gametes instead of dividing to form merozoites.

Now in the case of *Merocystis kathae* the first stages of division resemble so closely the Schizogony of that interesting Coccidian *Caryotropha* described by SIEDLECKI [1]) that it was only after some detailed examination that one could be convinced it was really sporogony. In the description which follows it will be understood that it is the sporogonic life cycle which is being de scribed.

The earliest stages of the parasite (Fig. 1) which have been made out are of considerable size compared with the sporozoites. They are pear shaped or spherical bodies already as large as a renal cell and it is impossible to say whether one or more renal cells have been infected because even at this early stage the nucleus or nuclei of the host cell or cells has hypertrophied to such an extent that the parasite is completely enveloped by an intensely staining chromatin mass. This covering of hypertrophied nucleus is particularly characteristic of these early stages. The young trophozoite at this stage possesses a nucleus, which is relatively much larger than in the full grown parasite. The cell is limited by a delicate membrane staining blue with MANN's Methyl-Blue Eosin, and the Cytoplasm is finely granular and stains a dark greyish blue with the same stain. Plastinoid granules have not yet been formed and thus the cytoplasm presents a very different appearance from that of the adult trophozoite. No other signs of differentiation

[1]) SIEDLECKI, M.: Über die Struktur und die Lebensgeschichte von Carytropha mesnili. Bull. Ac. Sc. Cracovie Sc. math. et. nat. Mai 1907.

are to be observed in the cytoplasm. The nucleus is spherical in shape, is bounded by a delicate nuclear membrane and is 10 μ in size in young stages of 18 μ diameter.

It already possesses a large nucleolus which stains bright red with methylblue eosin. The nucleoplasm is granular and the sharply marked chromatin of later stages is not obvious as yet.

Round this young trophozoite can be seen the remains of the hypertrophied renal cells — extremely vacuolated and possessing a very characteristic appearance. They disappear completely when the trophozoite has grown larger.

Growth of the trophozoite.

Trophozoites of all sizes may be observed in any one section scattered in an irregular manner throughout the tissue, and as growth proceeds in the majority of cases the parasites are preparing slowly to become the macrogametocytes. In the cytoplasm, reserve stores begin to make their appearance quite early, in the form of refringent granules-extremely short rod like bodies. In the full grown trophozoite (Fig. 3) these are so numerous that they mask the stained cytoplasm unless the latter be stained very intensely. The increase in size is very great, the average adult attaining a diameter of 162 μ and thus being readily visible to the naked eye. In addition to the refringent granules mentioned above, considerable

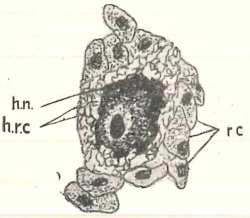

Fig. 1.

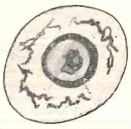

Fig. 2.

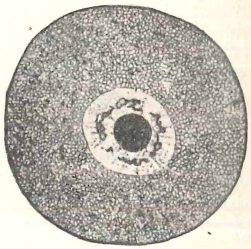

Fig. 3.

numbers of equally small granules, staining an intense black with Heidenhain's iron haematoxylin are to be found in the cytoplasm. The nucleus becomes more differentiated as growth takes place, and a very conspicuous mass of chromatin appears in the form of granules and irregular wisps which are arranged in a more or less regular layer surrounding the nucleolus.

On no occasion has more than one nucleolus been observed.

The Nucleolus (Fig. 2) is formed of a central part which often

appears more faintly stained and a cortical dense portion, but in the central area may sometimes be observed deeper staining granules. It is probable that these bodies are finally emitted into the nucleoplasm, but this has never been actually seen in the sections examined. They would correspond to the „nucleolites“ of *Aggregata eberthi* (Labbé).[1])

The microgametocytes (Fig. 14) are comparatively very few in number and the early stages of nuclear division leading to the formation of microgametes have not even been observed. The microgametes are formed at the periphery of the spherical gametocyte.

Division of the zygote.

The division of the zygote taking place after fertilisation is remarkably like the schizogony of *Caryotropha*. Actual fertilisation has never been observed, and at first it was doubtful whether or not the stages observed represented sporogony or schizogony. The structure of the macrogametocyte is however extremely suggestive and the various stages following the first division of the nucleus of the zygote can be followed without any difficulty. No trace of merozoite formation is to be seen and division ends with the formation of true spores.

1st Stage. The macrogametocyte becomes the macrogamete. The chromatin in the nucleus becomes much more distinct and forms

Fig. 4.

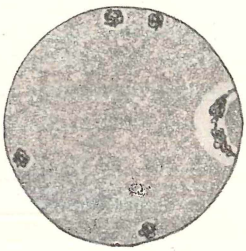

Fig. 5.

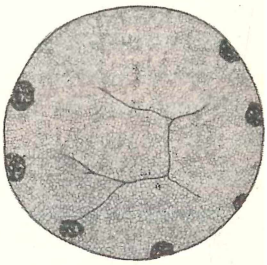

Fig. 6.

a tangled skein, and the nuclear membrane disappears. The nucleolus now somewhat smaller in size seems to be cast out into the cytoplasm and is finally lost altogether. Division after fertilisation commences by a drawing out of the chromatin threads into a kind of spindle (Fig. 4) at either end of which they tend to become aggregated.

[1]) Léger et Duboscq: L'évolution schizogonique de *Aggregata eberthi* (Labbé) Arch. f. Protistenkunde. Band 12. Heft 1.

There may be observed a delicate arrangement of radiating fibres proceeding as if from a centriole or granule of some kind.

Division of the spindle takes place transversely and the halves move away towards the periphery. Continued division takes place in the same way, by a process which is therefore not mitotic, but direct, and which results in the formation of a number of peripheral nuclei (Fig. 5).

2nd Stage. The second stage in the division of what must now be termed the zygote is marked by the appearance of highly characteristic septa which divide the whole into a series of compartments in each of which a peripheral nucleus may at first be found (Fig. 6). This feature reminds one of the formation of

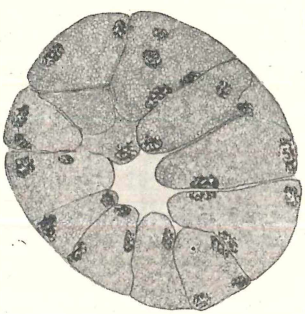

Fig. 7.

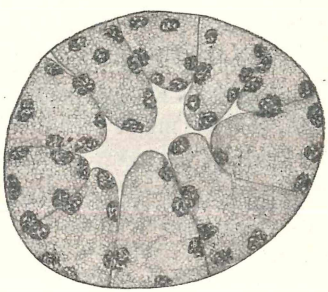

Fig. 8.

schizontocytes in *Caryotropha*. Inside these segments bounded by the delicate membranous septa the nuclei continue to divide, remaining however more or less close to the periphery. All stages of this division may be found leading up to the one depicted in Figs. 7 and 8

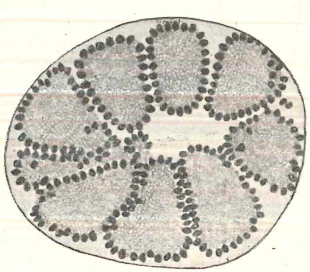

Fig. 9.

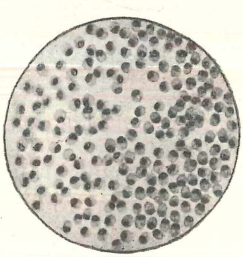

Fig. 10.

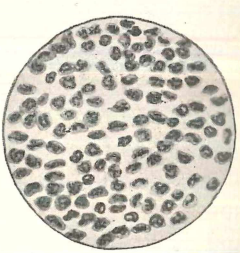

Fig. 11.

where all trace of the membrane of the segments seems to have disappeared, but where each of the latter remains enclosed by a layer of small close lying nuclei (Fig. 9).

3rd Stage. The next stage which has been observed consists of a spherical cyst enclosing a number of small bodies each with a nucleus (Fig. 10). It is natural to assume that the nuclei of Stage 2 have each separated with a certain amount of cytoplasm. This is however the only point where some considerable change takes place without many stages illustrating the development having been found. In a few cases clumps of two or three nuclei can be seen still unseparated from the protoplasm.

It would appear that when the nuclei do begin to separate with a portion of the cytoplasm this separation is very quickly completed. These bodies or sporoblasts are 9 μ in diameter. In some few cases bodies have been seen resembling the sporoblasts, but showing a nucleus in a peculiar condition (Fig. 13). It is impossible to say whether this is a preparation for another division that would come before the actual definite sporoblast or whether it is to be placed after fig. and represents the elongating of the nucleus and formation of the sporozoite. The examination of additional sections seems to throw no further light on the matter for though the parasites are so common, the same stages are continually to be seen.

Fig. 12.

Fig. 13.

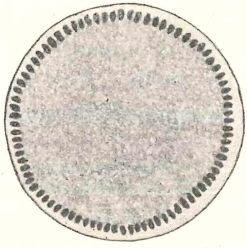

Fig. 14.

4th Stage. The last stage in the sporogonic life cycle results in the formation of true spores (Fig. 11). The sporoblast secretes a sporocyst, which appears as a delicate refringent nonstaining case. It is somewhat angular and disc shaped so that in sections one sometimes sees the face view, sometimes the edge, or a transverse section, if the sections have been thin. Inside this spore (Fig. 12) a single long sporozoite is formed. In sections the nucleus occurs as a long deeply staining mass, which is arranged in the form of a horse shoe or coiled to a greater degree, near the wall of the spore, and in the centre is to be found a certain amount of residual protoplasm. The sporocyst is perfectly smooth and as far as sections and teased preparations go, does not appear to be bivalve.

Conclusion.

It has already been pointed out that *Merocystis* occurs in large numbers in all whelks obtained from Port Erin. It is possible that the parasite is confined to *Buccinum* from this area. One certainly would have expected a protozoan of such size and interest to have been observed long before now, if it occurred in *Buccinum undatum* as a general rule. This however is not the only parasite whose host appears to be the Port Erin *Buccinum* for a parasitic turbellarian discovered there about fourteen years ago, has not yet been recorded elsewhere, though still just as common in this Irish Sea district.

The position of *Merocystis* as far as at present determinable is as follows: Coccidiidea

Fam. Polysporocystidae

Genus *Merocystis*; species *M. kathae.*

The genus is characterised by the division of the zygote by septa into secondary cysts, in each of which numerous spores are found. These all lie in the later stages, loosely in the larger cyst. It belongs therefore to the family Polysporocystidae, Léger. The spores are monozoic, somewhat flattened and slightly angular, and the sporocyst is smooth and not bivalve. It occurs in the renal organ of the whelk (*Buccinum undatum*).

The Sporogonic life cycle which has been described is the only one to be observed in the whelk. Schizogony may take place in another host or it may be absent altogether. In any case the whelk is such an omnivorous feeder that it would be difficult to trace the life history any further without some additional clues. It is rather striking that in the large number of sections made and examined, each crowded with parasites, one finds the same stages always turning up and the same little gaps are left.

Explanation of Figures.

Figs. 3 to 10 and Fig. 14 are drawn to the same scale. All figures have been drawn with the camera.

Fig. 1. Young trophozoite, r. c. renal cells, h. r. c. hypertrophied renal cells, h. n. the black mass of hypertrophied nuclei round parasite. × 500.

Fig. 2. Nucleus of adult trophozoite (stained with EHRLICH's Haematoxylin). × 350.

Fig. 3. Adult macrogametocyte. × 250.

Fig. 4. Division of nucleus of zygote after fertilisation. × 250.

Fig. 5. Formation of first peripheral nuclei (zygote). × 250.

Fig. 6. First appearance of septa in the cytoplasm of the zygote. × 250.

Fig. 7. Septa completely formed, the whole is now divided up into segments in each of which several nuclei have been formed. × 250.

Fig. 8. A slightly later stage, otherwise the same as Fig. 7. × 250.

Fig. 9. Completion of nuclear division in the secondary cysts. × 250.

Fig. 10. The nuclei have separated with a small amount of protoplasm to form sporoblasts. × 250.

Fig. 11. Cyst with spores. × 250.

Fig. 12. Spore (stained with iron haematoxylin). × 1000.

Fig. 13. Sporoblast-like bodies from cyst (see text). × 850.

Fig. 14. Microgametocyte with microgametes. × 250.