6. Brief notes on two Myxosporidian organisms (Pleistophora hippoglossoideos, n. sp. and Myxidium mackiei n. sp.).

By W. Cecil Bosanquet.
(From Professor E. A. Minchins Laboratory of Protozoology, Lister Institute of Preventive Medicine, London, S. W.)

(With 13 figures.)

eingeg. 3. Januar 1910.

The material on which the following brief notes are based was given me for examination by Dr. H. M. Woodcock, to whom my best thanks are due for this and for his assistance and advice throughout the investigation.

1. *Pleistophora hippoglossoideos* n. sp.

The material in this instance consisted in three small portions of tissue taken from the fin-muscles of the flat-fish, *Hippoglossoides limandoides*. It had been hardened in alcohol and was already embedded in paraffin. On cutting sections of these blocks, there were seen small whitish nodules lying in the substance of the muscular tissue. The nodules were round or oval in shape, and from 1 to 2 mm. in diameter.

Fig. 1.

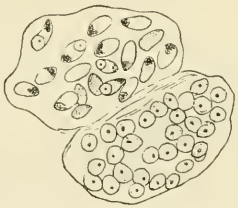

Fig. 2.

Fig. 1. Spores (above) and Sporoblasts (below) of *Pleistophora hippoglossoideos*.
Fig. 2. Spores of *Pl. hippoglossoideos*, × 2000.

The sections were stained with iron-haematoxylin, with Delafield's haematoxylin, and with thionine, some being counter-stained with eosine and others with orange.

Microscopical examination showed the nodules to be made up of a honeycombed mass of small cysts, most of which contained ripe spores (Fig. 1, upper part). The cysts lay in a small amount of structureless or fibrillated reticulum, apparently derived from the host, among which remains of muscle-fibres were here and there visible. A slight degree of cellular infiltration was seen at the edge of the nodules, but the muscle-fibres in the neighbourhood seemed normal and unaffected. The individual cysts measured some 20 to 25 μ in diameter.

The spores were very minute, measuring about $3^1/_2$ μ in length by

2 μ in breadth (Fig. 2). They were oval or pear-shaped, having a single polar capsule situated at the smaller end. No details of the latter nor even its exact shape could be made out, owing to the minute size of the spore. Towards the broader end of the spore (in some cases there was no observable difference in the size of the two ends) there was often visible a clear, rounded space, looking like a vacuole, and in most

Fig. 3. Fig. 4.

Figs. 3 and 4. Division-stages (?) in formation of spore.

instances there was in this a central dot. Between this and the polar capsule was a small mass of protoplasm, in which in very favourable specimens it was possible to detect two minute nuclei.

Lying in the reticular substance between the cysts containing ripe spores were a certain number of others filled with small, rounded bodies (Fig. 1, lower part.) which appeared to be the final sporoblasts the stage just before the formation of the spore-coat. These little bodies were

Fig. 5. Fig. 6.

Figs. 5 and 6. Fragmentation of individual and (?) commencement of endogenous spore-formation.

usually provided with a single nucleus and were somewhat smaller than the mature spore. In one or two cases they were provided with two minute nuclei, lying close together. Whether the single nucleus usually divides before the spore is formed, I cannot say. The material available being small in amount and already fixed and embedded, observations of developmental stages were necessarily fragmentary. A few of those which preceded the sporoblasts just mentioned were visible, but the

whole process could not be followed out. In some instances 4 fragments, each with a single vesicular nucleus, were seen within a single compartment or cyst: in another there were 7 smaller fragments, one of which contained two nuclei and was apparently dividing (Figs. 3 and 4). In these it would seem as if the whole body of the sporont had broken up as a preliminary to spore-formation. In others again there were irregular masses of protoplasm containing many nuclei (Fig. 5), or a single mass of this nature, representing the original sporont (Fig. 6). In these multinucleate forms the majority of the nuclei were generally solid in appearance, but among them were often one or two vesicular nuclei, possibly representing the rudiments of developing spores.

The closely packed mass of cysts in this infection points undoubtedly to a stage of schizogony preceding that of sporulation. The possibility that some of the forms just described (e. g. Figs. 3 and 4) were stages in the former cycle cannot be excluded. A priori, however, it would seem more probable that they belong to the process of spore-formation, as the great majority of the parasites were at the end of this phase.

2. *Myxidium mackiei*, n. sp.

The material in this instance was sent home from India by Capt. J. Percival Mackie, I. M. S., and was obtained in the Bombay

Fig. 7.

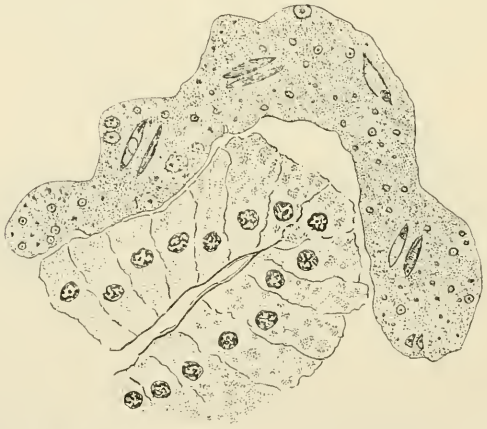

Fig. 7. *Myxidium mackiei* (above) in contact with cells of a renal tubule.

Bacteriological Laboratory. It consisted in three slides bearing mounted sections of the kidney of the tortoise, *Trionyx gangeticus*. Mackie noted the presence of paratites in the urinary tubules, and these were identified by Woodcock as a species of myxidium. Mackie recorded in a note

accompanying the specimens that the spores were almost constantly 14 μ in length by 4 μ in breadth, the extreme range being from 12 to 16 μ in length and from 3,5 to 5 μ in breadth; that the ends tapered to a blunt point, and that at each end was situated a deeply stained polar capsule; that the spore-coat was markedliy striated; and that the parasites did not appear to excite any reaction in the tissue of the host, the animal's health being unaffected.

The tissue was not very well preserved, but the main features could be made out fairly satisfactorily. The largest specimen of an individual *myxidium* measured 160 μ in length by 27 μ at its broadest part (Fig. 7). In the majority of instances no distinction between ectoplasm and endoplasm could be drawn, but in a few in which almost the whole animal was converded into spores there was some appearance of a cuticle or cyst-vall (Fig. 8). The protoplasm contained a large number of nuclei, apparently of two varities some vesicular, others smaller and solid-looking. It was difficult, however, to make sure that the latter were not merely granules of deeply-stained material. Two forms of nuclei

Fig. 8.

Fig. 9.

Fig. 10.

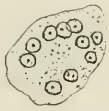

Fig. 8. Portion of *myxidium*, almost completely converted into spores, showing thickening of cuticle at upper part.
Fig. 9. Portion of *myxidium* in section, showing 2 sporoblasts in a cavity.
Fig. 10. Disporoblast showing 10 nuclei, one of which is dividing (?).

are described by Schröder in *Sphaeromyxa labrasesi*, in which conjunction, but not conjugation, takes place between a large and a small nucleus as a preliminary to sporulation.

Spores are formed in pairs, at many points within the *myxidium* simultaneously. Ultimately almost the whole substance of the animal is converted into spores (Fig. 8). With but limited material in this case also, only a few stages in the development of the spores could be observed. A portion of protoplasm becomes rounded off and lies in a definite space within the parasite (Fig. 9). This portion (disporoblast?) contained in one instance 10 nuclei, of which one was possibly dividing (Fig. 10). The final sporoblasts into which this divides appear to contain usually 6 nuclei (Fig. 9), so that it seems likely that 12 is the full number of nuclei in the disporoblast; but the sporoblast depicted on the right hand in the figure (which had been broken in making the section) appears

to contain 7 nuclei, 3 vesicular and 4 solid, suggesting that the number may not be constant. This would correspond with what occurs in other genera (e. g., chloromyxum, according to Joseph), in which the number of nuclei in the sporoblast seems to vary.

Of the 6 nuclei of the sporoblast 2 go to form the valves of the sheath, 2 attach themselves to the polar capsules, and 2 remain in the protoplasm of the spore (amöboidkeim) (Fig. 11). This is the same arrangement as is described by Schröder in *Sphaeromyxa*. The two last-mentioned nuclei may fuse into one (Fig. 12*c*). In the protoplasm of the spore two large vacuoles are often seen one near to each polar capsule (Fig. 12*a*). The spore-coat, as noted by Mackie, is ribbed or striated: the striations are best seen in some spores which have been divided transversely in cutting the section (Fig. 13).

According to my own measurements the spores average 16 μ in length by 5 μ in breadth. A few measure as much as 17 μ. Examples

Fig. 12.

Fig. 11.

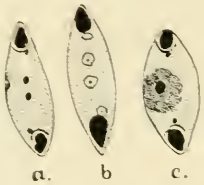

Fig. 13.

Fig. 11. Sporoblasts, showing nuclei and formation of polar capsules.
Fig. 12. Spores of *Myxidium mackiei*. *a*, showing vacuoles and remains of one capsular nucleus; *b*, vesicular nuclei in protoplasm and attached to polar capsules; *c*, single central nucleus in rounded mass of protoplasm (Amöboidkeim).

broader than 5 μ are not uncommon, and seem to represent immature spores, as the sporoblast elongates and becomes thinner in the process of development (Figs. 11 and 12*c*).

The only species of *myxidium* previously recorded as existing in a chelonian host is *M. danilewskyi*, described by Laveran in the kidney of *Emys orbicularis* (L.) (*Cistudo europoea*), in which the spores measure 12 μ in length. If, as it would seem, the present species has not been previously described. I would venture to suggest for it the name of *Myxidium mackiei*, after the name of its discoverer.

References.

Schröder, 1907. Arch. f. Protistenk., IX. 359.
Joseph. 1907. Arch. f. Protistenk. VIII. 398.
Laveran, 1897. C. R. Soc. de Biologie, July 17; and 1898. Jan. 8.