

ON A NEW MYXOSPORIDIAN *HENNEGUYA*
OTOLITHI N. SP. A TISSUE PARASITE
 FROM THE BULBUS ARTERIOSUS OF
 TWO SPECIES OF FISH OF THE
 GENUS *OTOLITHUS**

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Introduction

THE study of Myxosporidia, primarily a group of Sporozoan parasites in fish, has received considerable attention within recent years and much work in this direction has been done in different parts of the world, especially in North America and to some extent in Japan. Our knowledge about the

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Indian Myxosporidia is, however, very meagre, the only contributions on the subject being those of Bosanquet (1910), Southwell (1915), Southwell and Prashad (1918), Ray (1933), Ganapati (1936), and Chakravarty (1939).

The present investigation was taken up with a view to make a detailed study of the life-history, incidence of infection and host-parasite relations of a myxosporidian found as a tissue parasite in the heart of two common species of a local edible fish *Otolithus ruber* (Bl. Schn.) and *O. maculatus* (Kuhl and Hass.). The work was carried out during the years 1935–1938, in the University Zoological Research Laboratory under the direction of Professor R. Gopala Aiyar. I wish to express here my grateful thanks to him for his very helpful criticisms and constant encouragement. I am indebted to the University of Madras for the award of a research studentship which enabled me to carry out the work.

General Observations

There are no external indications to show infection, which is evident only on opening the animal and examining the heart. The parasite forms whitish opaque cysts in the wall of the bulbus arteriosus. The infected organ often presents a corroded appearance owing to the large number of cysts which are covered over by dark pigment granules (Photomicrograph 1). When the infection is localised the contour of the bulbus is considerably changed.

Myxosporidia though found in various tissues and organs have seldom been reported from the vascular system. The only previous record seems to be that by Keysselitz (1908) of *Myxobolus cordis* from the ventricle of *Barbus fluviatilis*. The present form belongs to an undescribed species of *Henneguya*, and is the first instance where the genus has been known to attack the vascular system. This fact together with other major characteristics of spore structure seem to me of sufficient importance to describe it as a new species of the genus for which the name *Henneguya otolithi* is proposed.

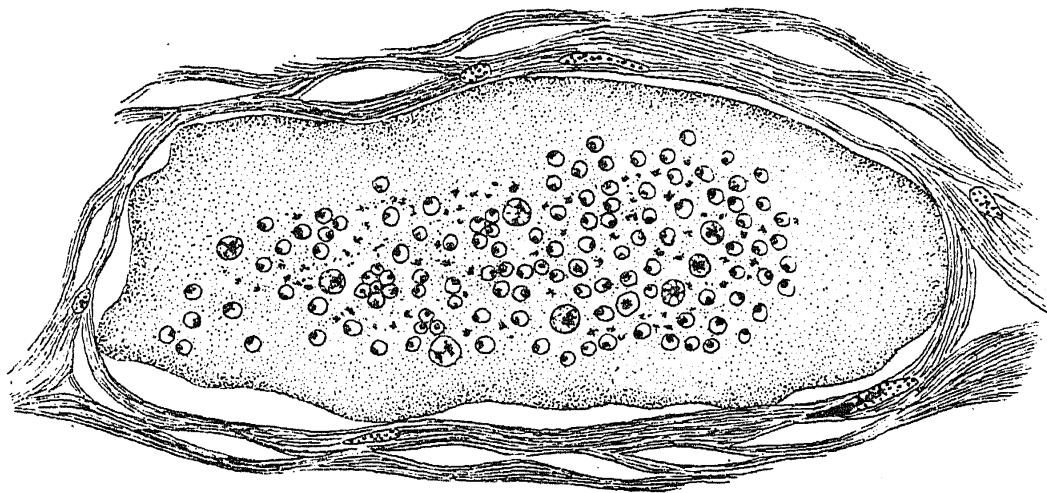
A large number of fish have been examined during the course of three years. *Otolithus ruber* is the commoner of the two species and *O. maculatus* though comparatively rare is equally susceptible to infection by this myxosporidian. Almost 75 to 80 percent. of the young and half-grown fish show infection in different degrees. Very young and full-grown fish are generally free from infection. It is therefore believed that the host, after passing through a certain stage in its life-time acquires immunity to the attack of the parasite. When infection is not heavy the fish appears to recover. It is not possible to say whether such fish establish immunity to further attacks.

Material and Methods

The fish used in this investigation were bought from local fishermen as soon as catches were brought to the shore. The Laboratory being situated very near the sea-shore, it was possible to examine the fish almost in the fresh condition. The infected organ was at once fixed for section cutting. Almost all the observations regarding the earlier stages and spore formation are based on sectioned material. Several fixatives like Bouin's, Zenker's, Schaudinn's, Flemming's and Worcester's fluid were tried. The last named fixative gave uniformly good results. Sections were cut 6 to 8 microns thick and ordinarily stained in Heidenhain's iron-alum hæmatoxylin followed by eosin or orange G. Ehrlich's hæmatoxylin counterstained by Van Gieson, Dobell's method of Mann's stain and Mallory's triple stain were also tried with success. A dilute solution of potassium hydroxide brings about filament extrusion in about 10 to 15 seconds. Examination of all the internal organs was carried out to study the distribution of the parasite within the host.

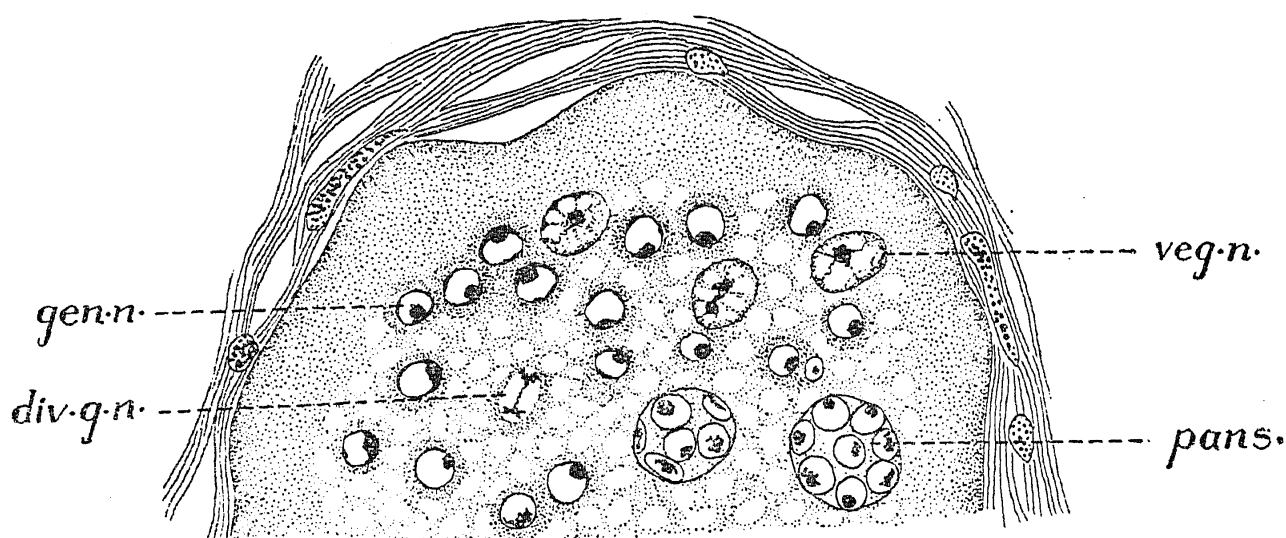
The Trophozoite

The trophozoites or vegetative forms are rounded, ovoidal or irregular. In the earlier stages a definite cyst wall formed of the host's connective tissue is not present and the parasite lies in between the muscle fibres. The body protoplasm is differentiated into an outer thickly granulated ectoplasm and



TEXT-FIG. I

a coarsely reticulated endoplasm (Text-Fig. I). In young trophozoites the endoplasm contains a few nuclei which are of two kinds—the generative and the vegetative. The vegetative nuclei are few in number and measure 3 to 4 microns in diameter. They are spherical to ovoidal in outline with a slightly ex-centric karyosome (Text-Fig. II). The vegetative nuclei are



TEXT-FIG. II

seen only in young trophozoites where spore formation has not commenced. The generative nuclei measuring 1.5 to 2 microns in diameter are much larger in number though smaller in size than the vegetative nuclei. The generative nucleus is rounded with a comparatively large karyosome often applied against a clear and well-defined nuclear membrane. The chromatin in both kinds of nuclei seems to be concentrated in the karyosome and very little of it is distributed in the nuclear sap. A thin layer of cytoplasm staining more vividly than the surrounding area, is present around each generative nucleus, and this feature together with its size and structure distinguishes it from the vegetative nucleus.

The differentiation of the nuclei in Myxosporidia into the two kinds, vegetative and generative, has been observed in all species that have been investigated in detail. Keysselitz (1908) differentiates the two kinds in *Myxobolus pfeifferi* and states that the division of the vegetative nucleus and the formation of generative nuclei are limited to a period in the early trophic life of the myxosporidian. Davis (1923) holds that the vegetative nucleus does not give rise to the generative nucleus after it has differentiated as such. The fact that the vegetative nuclei are present in the early stages of the trophozoite and that they are comparatively rare in older parasites seem to suggest that they give rise to the generative nuclei.

As the parasite grows, nuclear multiplication proceeds at a rapid pace, and the endoplasm in older trophozoites is filled with innumerable generative nuclei. The generative nucleus divides by a form of mitosis. The extremely small size of the nucleus renders it difficult to follow the details of the division. No asters or centrosomes have been made out at any stage of division. The older vegetative forms are more regular and a cyst wall composed of several

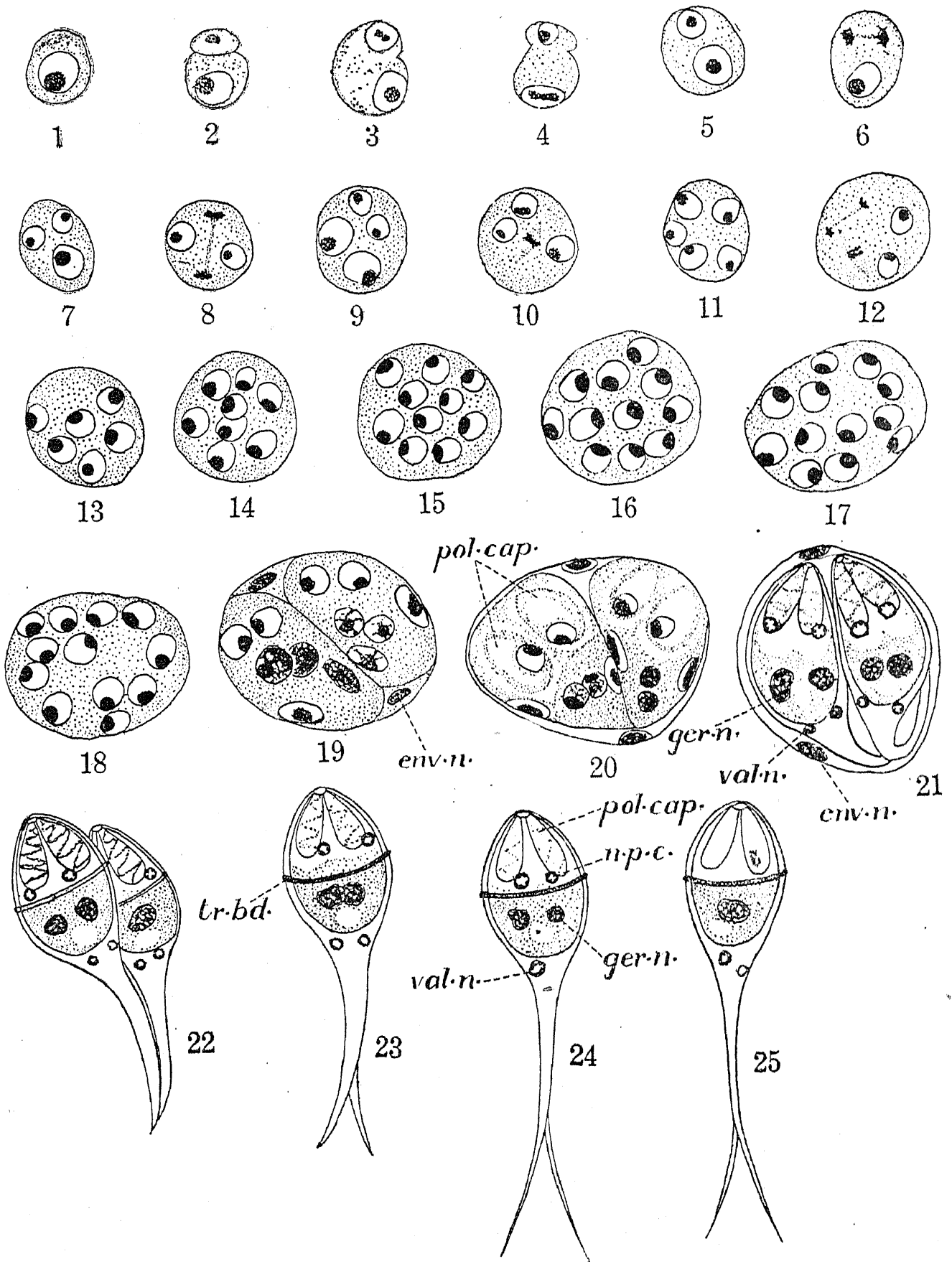
layers of the host's connective tissue is present round each parasite. Such a cyst examined in the fresh condition shows a number of developing pansporoblasts together with some refractile yellowish bodies and a few globules of fat.

Vegetative multiplication by plasmotomy appears to take place in the early stages of the trophozoite. Large numbers of young vegetative forms at the same stage of development have often been seen lying close together (Photomicrograph 2). It is difficult to imagine these forms could have originated by independent infection by different amoebulæ. Some of the pictures presented by these trophozoites show evidences of multiplication by plasmotomy. The occurrence of a multiplicative process of reproduction amongst tissue infecting Myxosporidia has been observed in *Myxobolus musculi* (Hahn, 1913), *M. pfeifferi*, Keysselitz, (1908), *Lentospora ovalis* and *Unicap-sula muscularis* (Davis, 1923 and 1924). This process of vegetative multiplication is common in Myxosporidia living in organ cavities.

Sporulation

There is considerable diversity of opinion with regard to the origin of the pansporoblast or sporoblast in those forms where it gives rise to a single spore. Much of the controversy centres round the question of the presence or absence of a sexual process in its formation. Naville (1930) gives an excellent review of the previous literature on the subject and by his independent researches on five species of Myxosporidia comes to the conclusion that in all these forms anisogamous gametes are formed preceded by a reductional phenomena of the chromosomes. The earlier stages of the parasite, according to this author, are diploid, while the gametes are haploid. The pansporoblast originates by the copulation of two anisogamous gametes not necessarily accompanied by nuclear fusion. When nuclear fusion also takes place, the zygote nucleus undergoes a reduction division in one of the subsequent divisions in the pansporoblast thereby again becoming haploid. The copulation of the two germ nuclei in the sporoplasm, either before or immediately after the germination of the spore, is said to restore the diploid number characteristic of the earlier stages of the parasite.

The gametogenesis and their chromosomic constitution have not been worked out in the present form. It has, however, been found that the pansporoblast originates by the copulation of a large and a small cell. These two presumably represent the macrogamete and the microgamete (Text-Fig. III, 1-2). In the two-celled stage a constriction, and in those cases where the protoplasmic fusion is incomplete, the faint outline of a cell wall is visible



TEXT-FIG. III

between the two nuclei (Text-Fig. III, 2-4). The two nuclei do not appear to fuse but proceed to divide independently. Either the smaller or the larger

nucleus may be the first to divide, so that three nucleated stages are common. In the succeeding divisions of the nuclei outlines of cell walls are not visible and pansporoblasts containing up to fourteen nuclei are met with. Two of these nuclei which are smaller than the others occupy a parietal position and they form the nuclei of the enveloping membrane when it is formed (Text-Fig. III, 19).

The different parts of the spore are constituted from the cells of the pansporoblast essentially as described by previous writers. The twelve nuclei separate into two groups, each of six cells, and a split appears in the cytoplasm separating the two groups (Text-Fig. III, 19-21). The envelope cells as already said occupy parietal positions. Each of the six cells is distributed relative to its position in the mature spore. The two anterior cells enlarge and gradually a vacuole is developed inside them. These two form the polar capsules (Text-Fig. III, 20). The spore valves are formed by the two parietal cells, while the protoplasm of the two remaining cells fuse and form the sporoplasm. In a few spores there was only a single germ nucleus formed by the fusion of the two originally separate germ nuclei. This is clearly a case of autogamy the significance of which is discussed in a later part of the paper. When the spores are fully formed, the enveloping membrane disintegrates and liberate the spores which lie free in the endoplasm of the parasite. The ectoplasm has meanwhile considerably shrunk and it is possible it is made use of by the developing pansporoblasts. When spore formation is complete the cyst is practically filled with spores and a few darkly staining bodies (Photomicrograph 4). Davis observed similar bodies in *Lentospora* and calls them "chromatoid bodies". Kudo (1926) noted these bodies in *Myxosoma catostomi* and is inclined to take them as different stages of degenerating nuclei. The staining reaction of these bodies evidently suggests their probable nuclear origin but beyond this it not possible to indicate their true nature.

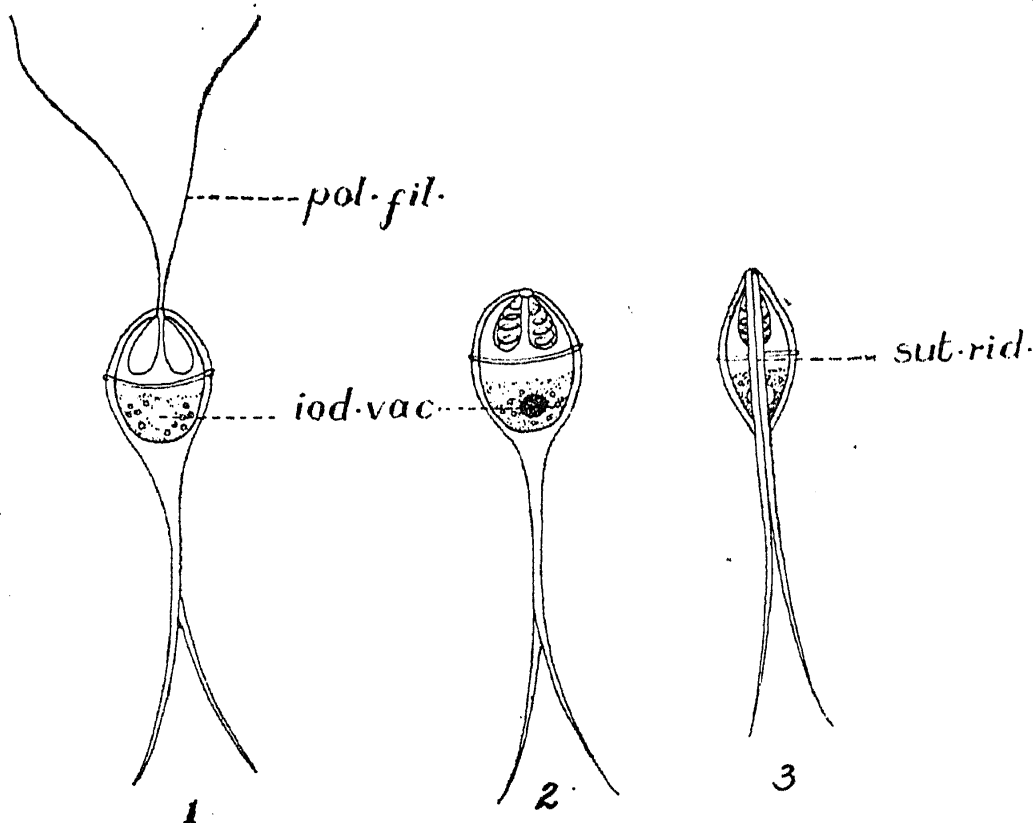
Description of Spore

The spores are oval in front view with rounded anterior ends. The two shell-valves are prolonged posteriorly into finely tapering tails. In profile, the spores are spindle-shaped. A thick, straight and simple sutural ridge is present. The shell is unstriated, but a characteristic thickening is present running transversely about the middle of the main part of the spore. The presence of this thickening or band enables the present species to be distinguished from other described species of *Henneguya*. Two pyriform polar capsules occupy the anterior third of the spore cavity and they are

convergent with a common opening. A spirally coiled polar filament showing five to six turns is visible in each of the polar capsules. The sporoplasm is finely granular and contains an iodophilous vacuole.

The dimensions based on measurements of fresh spores are as follows: length 10 to 12 microns; breadth 6 to 8.5 microns; thickness 4 to 5 microns; length of polar capsules 3 to 4 microns; breadth of polar capsule 2 to 2.5 microns; length of tail 35 to 40 microns.

The nuclei of the polar capsules and the spore valves persist for a considerable time after the spores are fully developed (Text-Fig. III, 22-25). The nuclei of the capsules are in the form of chromatin rings with a few beads of chromatin distributed along the nuclear membrane. The valve nuclei are much shrunk and lie at the root of the bifurcated tail. Spores in different stages of development show an interesting gradation of basophilic affinity as shown by their reaction to nuclear stains. With iron-alum hæmatoxylin, spores having the full quota of six nuclei, have their spore valves and appendages unstained, while their nuclei are stained deep. As the spores mature the basophil constituents seem to increase and ripe spores are stained intensely and remain so even after being strongly counterstained with acid dyes like eosin. The nuclei of the valves and appendage can scarcely be made out and presumably they degenerate when the spores ripen. The basophilic affinity indicates that the shell and appendage of the spores are constituted of a material very similar to chromatin in its composition.



TEXT-FIG. IV

By treating fresh spores with a dilute solution of caustic potash, the polar filaments may be made to extrude in about ten to fifteen seconds. The extrusion is accompanied by a sort of recoiling movement of the spore as a whole and it would appear as though a release of pressure has been effected inside the spore cavity. The sporoplasm in fresh spores shows a clear hyaline rounded area in the centre which goes dark brown when the spores are treated with Lugol's solution (Text-Fig. IV, 1-3). This is the iodophilous vacuole the content of which has been claimed by several observers as glycogen. In sections and smears the vacuole appears colourless and unstained.

Host-parasite Relations

1. *Infection*.—Attempts made to study how the infection of the fish takes place proved inconclusive. Great difficulty was experienced in getting the fish in the living condition and the few procured with difficulty did not survive for more than a few hours under Laboratory conditions. Treating the spores with the digestive juices extracted from various regions of the alimentary canal yielded only indecisive results. While in a few instances filament extrusion took place in about fifteen to twenty minutes, in no case was the sporoplasm seen to leave the spore cavity. Perhaps, this is due to the artificial conditions under which the experiments were carried out and it is probable that as in several other tissue infecting Myxosporidia, infection takes place by the ingestion of infected tissue containing spores by the host. The sporoplasm escapes from the spore cavity by the action of the digestive juices of the host and the liberated amœbula is carried through the blood stream to the primary site of infection.

2. *Autogamy and auto-infection*.—Almost all the writers have observed the copulation of the two germ nuclei in the sporoplasm, either before or immediately after the germination of the spore. This is clearly a case of autogamy. It has already been pointed out that a few spores were observed having only a single nucleus in the sporoplasm. Some of these spores were in an infiltrated condition, directly in contact with the host tissue. There are evidences to show that these spores germinate under favourable conditions and set up fresh infections in the same host. I have in my preparations young trophozoites having a single spore lodged in the cytoplasm, and with the two spore valves opened out (Photomicrograph 3). Since spore formation has not commenced in these forms, it is clear they are formed by the growth of the amœbulæ liberated from the spores under certain circumstances. This phenomenon of auto-infection partly accounts for the innumerable cysts seen in cases of heavy infections. Probable cases of

auto-infection in Myxosporidia have been described by Lieberkuhn (1854), Pfeiffer (1891), Thelohan (1895), Georgevitch (1914), Debaisieux (1922) and Kudo (1926). These writers hold that the spores could under favourable conditions, germinate setting up fresh infections.

3. *Pathogenesis.*—Histozoic Myxosporidia have long been known responsible for some epidemics in fish which bring about a high rate of mortality among the hosts infected. The "boil disease" in the barbel and other fresh-water fishes of Europe is known to be caused by the infection of the muscle and connective tissue of the body-wall by *Myxobolus pfeifferi* (Keysselitz, 1908). Davis (1924) showed the cause of the "wormy" halibut along the Pacific coast as due to the presence of an intracellular myxosporidian *Unicapsula muscularis* within the muscle fibres. The disease popularly known as the 'Drakkrankheit' bringing about a high rate of mortality especially in young fish of the Salmonidæ and the Gadidæ has been shown, through the efforts of Hofer and Plehn (1904, 1924) caused by a myxosporidian infecting the cartilage of the auditory organ.

There are no external pathological changes in the present instance to show infection. The vegetative forms grow between the muscle fibres in the wall of the bulbus and as their growth proceed the fibres turn through various angles from their original axial direction. The individual fibres get separated from one another and often present a frilled appearance. The muscle fibres immediately in contact with the parasite degenerate completely and their nuclei are hardly stained. When the vegetative forms are present in large numbers the destruction of tissue brought about is considerable. The fish reacts to the presence of the parasite by producing a number of active fibroblasts all round the parasite which give rise to several layers of connective tissue sheaths. This newly formed tissue completely shuts off the parasite from the muscle layer. The formation of a connective tissue cyst-wall seems to be induced by parasitic stimulation, a parallel to which is described by Mavor (1916) and Kudo (1929) in the case of the subdermal connective tissue of *Pimephalus notatus* infected by *Thelohanellus notatus* (Mavor), where an epithelial layer of cells is said to arise in the tissue immediately in contact with the parasite. Kudo describes this tissue as modified connective tissue cells of the host which modification took place as a result of parasitic stimulation. The muscle fibres of the barbel infected by *Myxobolus pfeifferi* are said to undergo hyaline degeneration leaving behind yellow granulations as degeneration products. The 'wormy' halibut (Davis, 1924) shows swollen muscle fibres that undergo a hyaline degeneration, but in spite of the abundance of the parasite, no fibre is said to be entirely destroyed.

A great increase of blood capillaries is noted in the infected organ. When infection is heavy the bulbus appears swollen and reddish in colour. The blood capillaries are fully distended and an exudation of leucocytes takes place suggesting inflammation. Dark pigment granules appear all over the cysts and these are absent in healthy fish.

The infected fish appears to recover when only a few cysts are present. An active multiplication of connective tissue cells takes place round the parasite and this newly formed tissue penetrates the cysts after the death of the parasite. For a time the newly formed tissue and the spores intermingle. Later on, the spores degenerate completely, leaving behind a few dark staining bodies (Photomicrograph 5). In heavy infections the wall of the bulbus contains nothing but numerous cysts and a few strands of muscle in between the parasites. It is difficult to imagine that in such cases the host recovers. Pathological change of a different kind was observed in a few cysts. In these the parasites had apparently degenerated leaving behind a number of yellowish refractile concretions, the exact nature of which could not be determined (Photomicrograph 6). These bodies are perhaps degeneration products of the parasite or calcification products due to myxosporidian infection. The proper functioning of the organ is impaired in yet another way. The cysts often project into the lumen of the bulbus practically obliterating the blood passage. Under these conditions the normal functioning of the organ cannot take place.

It may be interesting to note here that in the many instances recorded, the high rate of mortality through myxosporidian infection is said to be more through secondary invasion by bacteria and fungi through the lesions produced by the parasite, than by the myxosporidian itself. In the present case the site of infection happens to be an internal organ and as such the chances of secondary infection are remote. It is therefore probable that the mortality rate amongst the infected fish may not be high. This fact could not be ascertained since the host happens to be marine not confined to a limited area as in some fresh-water fishes.

4. *Diffuse and scattered infiltrations.*—The phenomenon of diffuse infiltration or the intermingling of the host tissue with the spores of the parasite is of wide-spread occurrence in many tissue infecting Myxosporidia. The cysts under certain conditions rupture liberating the spores and developing pansporoblasts that are carried by the blood and lymph stream into the surrounding tissue spaces. The pansporoblasts complete their development and a condition is reached when the spores and tissue appear to intermingle.

Scattered spores away from the primary site of infection have been located in the connective tissue of the kidney. They are generally found encapsulated in sheaths in groups of two. The scattering of the spores appears to take place by the rupturing of the cysts located near the lumen of the bulbus. The spores and developing pansporoblasts carried by the blood stream get lodged in the kidney. The pansporoblasts perhaps complete their development in the new surroundings which accounts for the presence of spores in groups of two.

5. *Seasonal occurrence.*—The host fish are available practically throughout the year though found in large numbers from October to December. From March to July they are comparatively rare. The fish generally attains a length of about three feet. The larger specimens are not caught from the coastal region. Examination of fish of different lengths shows that fish measuring 6 to 10 inches are most susceptible to infection. Very young and full-grown fish are generally free from infection. Table I gives the result of the examination of only a representative collection of 150 fish caught at different times of the year and kept preserved in the Laboratory. It may be seen from the Table that about 75 per cent. of the medium sized fish are infected in different degrees.

TABLE I
Showing relation between size of fish and infection

Length of fish	Number of fish examined	Number infected	Nature of infection
Inches 2-6	42	3	Fairly heavy infection in one.
6-8	52	44	All grades of infection.
8-10	44	29	Majority heavily infected.
10-15	12	Nil	

The vegetative forms are found in fish examined in the colder months of the year from November to February. The temperature of the coastal waters ranges from 23 to 27 degrees Centigrade in these months. Fish examined in the remaining months of the year contain only ripe cysts with mature spores. The temperature of the sea-water ranges from 27 to 30 degrees during these months. Table II gives a record of the examination of *Otolithus ruber* during the different months of the year 1937. It may be noted that from April on there is a fall in the percentage of fish infected

TABLE II

Showing seasonal occurrence of stages of parasite in 1937

Months	Size of the fish	Number examined	Number infected	Stages of parasite	Nature of infection
August	Small	8	1	Ripe cysts	Light
	Medium	7	4	Ripe cysts	Fairly heavy
	Big	3	Nil
September	Small	6	Nil
	Medium	15	9	Ripe cysts	All grades

October	Small	9	1	Ripe cysts	Fairly heavy
	Medium	22	16	Ripe cysts	All grades
	Big	6	Nil
November	Small	10	2	Vegetative forms	Heavy
	Medium	8	5	..	All grades

December	Small	5	Nil
	Medium	12	9	Sporulating forms	All grades

January	Small	16	2	Sporulating cysts and vegetative forms	Fairly heavy
	Medium	11	9	..	Mostly heavy

February	Small	5	Nil
	Medium	16	11	Ripe cysts and few vegetative forms	Fairly heavy

March
	Medium	8	6	Ripe cysts	Heavy

April-July	Small	11	2	Ripe cysts	Light
	Medium	33	17	Ripe cysts	All grades
	Big	5	1	Ripe cysts	Light

and it is believed this fall is due to a certain amount of mortality among the infected fish in these summer months when the maximum temperature is reached. Keysselitz found that the "boil disease" in the barbel is not manifest in winter and spring months, but appeared in April, the maximum mortality being reached in the warmer months of the year. This author showed that sporulation is accelerated in fish kept in aquaria maintained at a temperature of 25 degrees Centigrade or higher. Nemecek (1911) observed that after October, cysts of *Henneguya gigantea* do not contain spores but vegetative forms only. In *Henneguya similis* infecting the gills of *Perca fluviatilis* of Lake Constance, Zandt (1924) noted a periodic re-appearance of the parasite; the infection was first noted in December and January when mature spores are not found. The spores appear from February to March and infected fish were observed up to the end of May. From May to December no host fish carrying cysts were recognised. Keysselitz and Nemecek agree that spore formation is influenced by the temperature of water in which the host fish live and both authors are of opinion that a higher temperature accelerates spore formation. The observation on *Henneguya otolithi* confirm the findings of the above authors.

Summary

1. A new species of a polysporous tissue infecting myxosporidian, *Henneguya otolithi* is described from the bulbus arteriosus of two species of a marine fish, *Otolithus ruber* and *O. maculatus*.
2. Two kinds of nuclei, the vegetative and generative, are present in the earlier stages of the parasite. The vegetative nuclei are not present in later stages.
3. The pansporoblasts originate by the copulation of anisogamous gametes.
4. Evidences of multiplicative reproduction by plasmotomy in young vegetative forms are seen.
5. Autogamy is present and instances of auto-infection have been observed.
6. The histopathological processes in the infected organ are described.
7. Diffuse infiltration of spores is common and scattered spores have been located in the kidney.
8. A seasonal variation in the occurrence of the different stages of the parasite is noted. In general a higher temperature seems to accelerate spore formation.

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EXPLANATION OF TEXT-FIGURES AND PLATE

All figures in the text were drawn with the camera lucida at stage level, with Zeiss apochromatic 120 oil immersion objective and compensating oculars. All figures, unless otherwise stated, are from sections of the bulbus arteriosus fixed in formol-sublimate-acetic, and stained with iron hæmatoxylin and eosin.

- TEXT-FIG. I. A trophozoite where spore formation has not commenced. Note the clear differentiation of the ectoplasm and endoplasm. $\times 900$.
- TEXT-FIG. II. Part of a trophozoite highly magnified showing the vegetative and generative nuclei. $\times 1800$
- TEXT-FIG. III. Stages in spore formation, Figures 22 to 25 are from smears fixed in Schaudinn's fluid and stained with Giemsa. $\times 1800$.
- TEXT-FIG. IV. (1) Spore treated with dilute caustic potash solution. Note the extruded polar filament. (2) Spore treated with Lugol's solution showing the iodophilous vacuole. (3) Side-view of a fresh spore to show the thick sutural ridge.

PLATE VII

PHOTOMICROGRAPHS 1 TO 6

1. Section of bulbus showing a large number of rounded cysts. $\times 150$ approx.
2. Three trophozoites in section, with thin muscle strands of the host separating them. $\times 1350$ approx.
3. A trophozoite by auto-infection. Note the single spore in the cytoplasm with the spore valves opened out. $\times 1800$.
4. A ripe cyst showing mature spores. $\times 300$ approx.
5. An infected area showing a late stage of fibrosis. Note the newly formed fibrous tissue and the darkly stained degenerate remains of the parasite. $\times 1350$ approx.
6. Section passing through a number of cysts two of which contain dark concretions.

KEY TO LETTERING

<i>cal. con.</i>	.. Calcareous concretions	<i>n. p. c.</i>	.. Nucleus of polar capsule.
<i>cys.</i>	.. Cysts.	<i>pans.</i>	.. Pansporoblast.
<i>d. b.</i>	.. Dark bodies.	<i>pol. cap.</i>	.. Polar capsule.
<i>div. g. n.</i>	.. Dividing generative nucleus.	<i>pol. fil.</i>	.. Polar filament.
<i>env. n.</i>	.. Nucleus of enveloping membrane.	<i>sp. val.</i>	.. Spore valves.
<i>gen. n.</i>	.. Generative nucleus.	<i>sut. rid.</i>	.. Sutural ridge.
<i>ger. n.</i>	.. Germ nucleus.	<i>tr. rid.</i>	.. Transverse ridge.
<i>iod. vac.</i>	.. Iodinophilous vacuole.	<i>val. n.</i>	.. Valve nucleus.
		<i>veg. n.</i>	.. Vegetative nucleus.