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THE STRUCTURE AND DEVELOPMENT OF A MYXOSPORIDIAN PARASITE OF THE SQUETEAGUE, CYNOSCION REGALIS[1]

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SEVEN PLATES AND SEVEN TEXT-FIGURES

CONTENTS

I. INTRODUCTION

During the course of a somewhat extended study of the Myxosporidian parasites of the marine fishes of Beaufort, N. C., an undescribed species was found in the urinary bladder and ureters of the common squeteague, Cynoscion regalis, which showed so many points of exceptional interest that an extended investigation was made of its structure and development.

[1] Published with the permission of the Commissioner of Fisheries. Contribution from United States Fisheries Biological Station, Beaufort, N. C.

II. MATERIAL AND METHODS

The Myxosporidian, which I have named Sphaerospora dimorpha, is abundant in the urinary bladder of Cynoscion regalis, extending into the Wolffian ducts in large numbers, but I have never found it in the kidney tubules proper. The parasite occurs as two very different forms, one disporous, the other polysporous, although both forms are usually present together in the same host. A large number of fish were examined and in no case was the bladder found to be entirely free from infection although there was great variation in the abundance of the parasites. The results incorporated in this paper are based on the study of a large amount of material, both fresh and preserved.

For studying the living trophozoites a small amount of material was taken from the bladder in a pipette and transferred to a slide where it was studied at once.[2] To prevent evaporation the cover was at first ringed with vaseline, but later paraffine of a low melting point was found to be much superior for this purpose and was thereafter used exclusively. Under these conditions it was possible to keep the trophozoites for several hours without any appreciably bad results. However, after six or eight hours the trophozoites usually showed distinct signs of degeneration, probably due to the rapid increase of bacteria in the preparation. Attempts were made to grow the trophozoites on agar culture media but without success, although they lived somewhat longer under these conditions than on the slide.

In studying preserved material both dried smears and sections were used. In preparing the smears a small amount of material was removed from the bladder with a pipette and smeared thinly over a coverglass which was then exposed to osmic vapor for 30 to 60 seconds, after which the preparation

[2] Since the parasites are commonly attached to the lining of the urinary bladder it was found in practice that to insure getting a large number of trophozoites it was first necessary to allow most of the urine to escape. This was done by slitting the wall of the bladder with the scissors, allowing the urine to flow out, and then by applying the mouth of the pipette to the inner wall and forcibly sucking up the remainder of the contents the parasites could invariably be obtained in large numbers.

was dried, then placed in absolute alcohol 15 to 30 minutes, then washed in distilled water to remove any soluble substances present in the urine which might interfere with the stain. After washing the preparations were stained with Giemsa's stain over night, decolorized with acetone and then mounted in neutral balsam or damar. The latter was found to be preferable since the stain was much more permanent than in balsam.

For sectioning, the infected tissues were removed from fish which had not been dead more than 5 to 10 minutes, placed in Worcester's formol corrosive acetic fluid for several hours. After washing and dehydrating in the usual manner the tissues were imbedded in paraffine and sectioned. The sections were stained with iron-hematoxylin and Congo red. This method gave excellent results, both the nuclear and cytoplasmic structures being well preserved. Material killed in Schaudinn's fluid and also in Hermann's fluid was inferior to that obtained with Worcester's fluid.

Most of the work was done at the Bureau of Fisheries Laboratory, Beaufort, N. C. during June, July and August, 1911 and 1912.[3]

III. DESCRIPTION OF TROPHOZOITES AND SPORULATION

1. Disporous form

a. Living trophozoites. When the contents of the urinary bladder are placed on the slide the trophozoites can usually be found in large numbers. In most cases during June, July and August both the younger stages previous to spore formation and older trophozoites containing spores in all stages of development, are abundant.

The non-sporulating trophozoites are very distinctly amoeboid but sluggish in their movements (fig. 1). When attached to the lining of the bladder they are usually somewhat rounded and closely applied to the free, rounded ends of the epithelial cells, with one to several pseudopodia extending down between

[3] Grateful acknowledgment is hereby made to the Bureau of Fisheries for the opportunity to do this work.

the cells (fig. 21). On being transferred to a slide they become actively motile, often sending out pseudopodia in several directions at once, and soon became very irregular in shape.

The ectoplasm is easily distinguished as a homogeneous, hyaline layer surrounding the endoplasm which is filled with rather indistinct vacuoles (fig. 1), separated from each other by a denser, homogeneous substance which has much the same appearance as the surrounding ectoplasm. In fact the endoplasm gradually merges into the ectoplasm by the vacuoles gradually becoming less abundant until in the latter they entirely disappear. Usually the endoplasm also contains a few yellowish, refractive globules, evidently composed of fat since they stain intensely with Sudan III. These globules vary considerably in size and numbers, being, in general, more abundant in older individuals; although there is considerable variation in this respect in trophozoites of approximately the same age. In some cases, even in full grown individuals, there are only a few fat globules present while in others they may be very abundant.

Occasionally the endoplasm contains one or more erythrocytes which are evidently obtained from the blood of the host and are ingested by the parasites (fig. 2). I am convinced that the erythrocytes are actually taken in and digested by the trophozoites since I have often found individuals, both in fresh and preserved material, in which they were unquestionably inclosed in the endoplasm. Erythrocytes in various stages of disintegration occasionally occur within the endoplasm so there can be little doubt that this species actually utilizes solid food. Cohn ('96) found that erythrocytes were ingested by Myxidium lieberkuhni Bütschli, a parasite of the urinary bladder of the pike, but his statements have been questioned by later writers. The evidence in the case of S. dimorpha is, however, so clear that there can be little doubt that this species forms a notable exception, as regards the character of its food, to most of the Myxosporidia. On the other hand, individuals containing erythrocytes are too few to justify the conclusion that this is the only means of obtaining food. In all probability they absorb

liquid food as well. There is also evidence that the trophozoites may ingest any small, solid particles present in the urine.

I have been unable to find any case where the bladder epithelium showed appreciable injury by the parasites, although the presence of blood corpuscles in the urine would indicate that this must occasionally occur.

The size of the full-grown, vegetative trophozoites varies somewhat, the average diameter being from 25 to 30μ. As sporulating trophozoites were found to have approximately the same diameter this is believed to be the maximum size reached by this form.

Large numbers of trophozoites were observed in which the spores were in various stages of development. These showed practically the same characteristics as the vegetative forms, except that most of the endoplasm is used up in forming the spores, the fat globules which remain being crowded to one side between the two spores (fig. 44). Trophozoites in which the spores were matured usually disintegrated very quickly after being placed on the slide, thus setting the spores free.

The spores (figs. 45 and 46) are approximately spherical with an average diameter of about 15μ. In a few cases the diameter was found to be slightly greater in one direction than in others, but this is not believed to be a constant character. The spores contain two polar capsules, each having a diameter of 4.5μ. The length of the polar filaments after extrusion varies from 27 to 35μ. The sporoplasm is plainly seen within the spore as a rounded, finely granular mass below the polar capsules. Numbers of fat globules are often present in the spore, part of them being inclosed within the sporoplasm. The sutural line, formed by the junction of the halves of the spore membrane, is not straight, as is the case in many species of Myxosporidia, but takes a characteristic sinuous course around the spore which can best be understood by consulting figure 46. Usually one valve of the spore membrane is slightly smaller than the other into which it appears to telescope.

b. Preserved material. In giving an account of the results obtained from the study of stained material it will simplify mat-

ters to begin with the small, mono-nuclear trophozoites. This stage occurs only rarely in my material, and is characterized by the relatively large amount of dense, finely granular cytoplasm containing a large, well defined nucleus (figs. 8 and 9). Within the nucleus the chromatin forms a well defined network composed of irregular granules varying greatly in size and especially abundant around the periphery. A conspicuous nucleolus is always present and often (especially in strongly decolorized specimens) can be seen to be composed of a deeply staining outer portion enclosing a central mass which stains less intensely. Usually—possibly in all cases—the nucleolus is attached to the nuclear membrane, and sometimes, as in figure 9, the side in contact with the membrane is somewhat separated from the rest with which it is connected by a faintly staining substance.

The next stage in the development of the trophozoites is shown in figures 10 to 15. The trophozoite now contains two nuclei of equal size and similar appearance, each surrounded by a layer of dense cytoplasm which, in most cases, can be more or less clearly differentiated from the less deeply staining material composing the remainder of the individual. The denser, more deeply staining area of cytoplasm around each nucleus is, however, apparently not surrounded by a definite membrane at this time. While it can usually be more or less easily distinguished from the surrounding cytoplasm, in some cases the two merge together so gradually that no distinction can be made (figs. 12 and 16). That even in such cases there is a specially differentiated area around each nucleus is clearly shown in figure 26, where, owing to a defect in fixation, the cytoplasm immediately surrounding the nucleus has shrunken away from the adjacent endoplasm. These two nuclei are, I believe, derived by mitotic division from the single nucleus of the preceding stage. In a dried smear stained by Giemsa's method, I found a mono-nuclear trophozoite in which the nucleus was dividing mitotically. Moreover, the bi-nucleate stage is often scarcely, if any larger, than trophozoites which contain but a single nucleus and thus can scarcely be formed by the fusion of two such trophozoites. Although the two nuclei are similar in size and appearance, yet

each has a very different later history. One becomes the vegetative nucleus and, in most cases, undergoes no further division, while the other divides a number of times to form the spores.

At a little later stage each trophozoite contains three nuclei, one being considerably larger than the other two (figs. 16 to 21). The two smaller nuclei are derived from one of the nuclei of the preceding stage by mitotic division while the other nucleus remains undivided. The latter gradually increases in size, the specially differentiated area of surrounding cytoplasm becoming indistinguishable from the rest of the trophozoite. This forms the vegetative nucleus and ordinarily undergoes no further changes, but rarely may, in later stages, divide amitotically (fig. 30). This division of the vegetative nucleus is very exceptional and only in rare cases have two vegetative nuclei been found in the same trophozoite. The two smaller nuclei are the generative nuclei and soon divide by mitosis to form four nuclei of equal size (figs. 23 and 24). One of the generative nuclei usually divides before the other (figs. 20 to 22) so that trophozoites with but four nuclei are common. As a result of these divisions trophozoites are formed with five nuclei, one larger vegetative nucleus, and four generative nuclei of equal size, each surrounded by a denser more deeply staining area of cytoplasm. The generative nuclei then become grouped in pairs (fig. 24), each pair forming a sporoblast. At the same time one of the nuclei of each pair divides mitotically, resulting in sporoblasts with three nuclei, one nucleus in each being considerably larger than the other two (figs. 25, 27, 28, 29 and 32). Trophozoites with two sporoblasts, each with three nuclei, are more abundant in my preparations than any other stage. At this time the cytoplasm immediately surrounding the sporoblast nuclei is much denser and more clearly defined than at any preceding stage, and is sharply marked off from the surrounding cytoplasm which now stains less deeply than before. Each sporoblast is, I believe, composed of three distinct cells corresponding to the three nuclei, but in many cases the cells are so closely associated that they can only be distinguished with difficulty, and sometimes not at all. In all cases during this and later stages the

two sporoblasts are entirely distinct, and are often quite widely separated.

The later history of each sporoblast is the same, although one usually lags a little behind the other in development. The two smaller cells divide by mitosis, one usually a little after the other (figs. 29, 33 and 34) forming sporoblasts with five cells, usually lying in approximately the same plane and arranged in a definite manner (figs. 30, 31, 35 and 36). The division in this case is unequal, one of the daughter cells from each mother cell being slightly larger than the other. These two cells later form the capsulogenous cells which give rise to the polar capsules; the two adjoining smaller cells fuse to form the sporoplasm, while the large cell at the end opposite the capsulogenous cells, divides by amitosis into cells which later form the valves of the sporocyst. In this division the nucleolus divides first, after which the nucleus divides by a simple constriction (figs. 37 and 38). Thus in each three-celled sporoblast the larger of the three cells is destined to produce the parietal cells which form the valves of the sporocyst, while each of the smaller cells will give rise to one of the capsulogenous cells and one of the sporoplasm cells.

Up to this time the resting nuclei show a similar structure throughout the different stages, but shortly after the division of the sporoblast into six cells the nuclei present a quite different appearance (figs. 39 and 40). Definite nucleoli are no longer to be distinguished, but are probably represented by several deeply staining masses closely apposed to the nuclear membrane. These masses are probably composed chiefly of chromatin, the achromatic part of the nucleolus having disintegrated. Throughout this and later stages there is a marked tendency in all the nuclei for the chromatin to collect in larger and larger masses.

At this time there is also a general rearrangement of the cells in each sporoblast, each cell assuming the position relative to the others which it occupies in the mature spore. During the formation of the spore all the nuclei decrease in size and stain more deeply as a result of the concentration of the chromatin in larger masses. The two parietal cells become flattened and

enclose the others, while the sporoplasm cells fuse into a common mass of protoplasm; the nuclei, however, remain distinct even in the mature spore (figs. 41 to 43). The capsulogenous cells can easily be distinguished by the fact that the cytoplasm becomes condensed at one side of the nucleus and contains numerous minute granules which stain deeply with the plasma stain (fig. 41). A little later a small, rounded, deeply staining body appears in the concentrated cytoplasmic mass (fig. 42). At a somewhat later stage this body becomes differentiated into a clear space surrounding a central, deeply staining part which is evidently the developing thread of the capsule (fig. 43).

Sporoblast cells undergoing mitotic division are common in sectioned material so that the details of the process can be easily worked out. During the prophase (figs. 48 and 49) the chromatin forms a coiled spireme. Apparently there is more than one of these chromatin threads, although there are certainly not as many as there are distinct chromosomes later. Usually the spireme can be traced into one or more irregular masses of chromatin imbedded in achromatic material. These masses are probably disintegrating nucleoli, the chromatin of which is taking part in the formation of the chromosomes. As indicated on page 338—the nuclei are composed of two distinct materials—an outer portion which shows a great affinity for chromatin stains and is, in all probability, chromatin; and an inner portion which stains less readily and is evidently composed of plastin.

During the metaphase the chromosomes become arranged around the equator of the spindle in the usual manner (figs. 50 to 52). The chromosomes are usually so closely crowded together as to make it difficult to count them with certainty but polar views of a few exceptionally favorable cells show that there are, without doubt, six chromosomes. As regards size these chromosomes can be readily grouped into three pairs, one of which is considerably larger than the other two, one smaller, while the third pair is intermediate in size. The spindle is poorly developed and at no time have I been able to find any trace of centrosomes. Figure 53 shows an early, figure 54 a later anaphase. In the latter the well-defined remains of the nucleolus

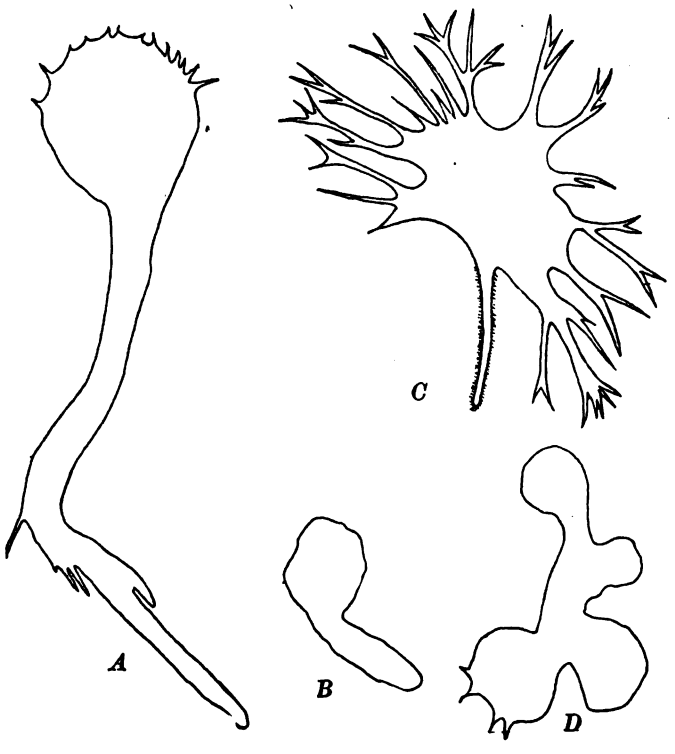
can be distinguished at one side of the spindle. In many cases as in figure 53 the nucleolus has entirely disappeared at this time and I have never been able to distinguish it at a later stage. In all cases, when present, the nucleolus lies at one side, and at some distance from the spindle, never on it. Figure 55 shows a telophase in which the interzonal filaments can be plainly distinguished and there is a slightly developed 'zwischenkörper.' In some cases the interzonal filaments may persist for a short time after the daughter cells have otherwise entirely separated.

2. *Polysporous form*

a. Living trophozoites. In addition to the forms described above there are almost invariably present a number of trophozoites which have a very different appearance. These trophozoites, which are usually much larger than the disporous form, are sometimes relatively rare, in other cases they were found to be equally, if not more abundant than the smaller form. I am uncertain whether or not they are ever entirely absent. According to my notes a few fish examined during the early part of the investigation contained none of this type, but I do not feel at all certain that they were not present in small numbers, since in that case they could easily be overlooked. At first I considered that the larger trophozoites were specifically distinct, but was later forced to the conclusion that both the large and small types of trophozoites are but different forms of the same species. The reasons for this conclusion will be given later.

The polysporous trophozoites have a very characteristic appearance, and in all but the youngest stages can readily be distinguished from the disporous form already described. When attached to the inner lining of the bladder they are usually elongated and cylindrical, but considerably larger at the base where they are attached to the epithelium (figs. 61 and 62). The free end of the body is commonly drawn out a long, tail-like process, rounded at the end (fig. 56). The size varies greatly. Some are no larger than the disporous trophozoites while one of the largest measured was 575μ long with a maximum diameter of

90μ. Another individual measured 270μ long with a maximum diameter of 60μ. However, since they vary so greatly in shape and, especially, in the length of the slender free portion of the body, measurements can only give a very inaccurate idea of the size (text figs. A and B).



Text-figures A to D Various forms assumed by polysporous trophyozoites after being removed from the urinary bladder. × 350.

The body is covered with short hair- or bristle-like processes of the ectoplasm which are larger and more abundant near the free end (figs. 56 and 60 to 62). These processes are similar to those which cover the body of Myxidium lieberkühni and the writer has seen similar processes in several other species. They are often well developed in Chloromyxum leydigi, common in the gall bladder of many species of sharks and rays. While not

movable these processes are readily absorbed, and may later be reformed. When the trophozoites are attached to the bladder epithelium the ectoplasmic processes usually cover the entire free surface of the body, but after being placed on the slide the processes are usually rapidly absorbed, until after a short time they may disappear over the entire body.

The trophozoites are attached to the epithelium of the urinary vessels by a specially modified part of the larger end of the body. The ectoplasm, which is here more abundant and probably denser than on other parts of the body, forms irregular, thin, plate-like processes (pseudopodia) which extend in between the outer ends of the epithelial cells (figs. 61 to 64). Between the plate-like psuedopodia are depressions which receive the free, rounded ends of the epithelial cells. Although the trophozoites thus become very closely applied to the epithelium, I have been unable to find any evidence that the cells are injured thereby. This is the more remarkable when we consider that in cases where the parasites are very abundant almost the entire epithelium may be covered with them.

Often the trophozoites are more or less completely covered with small granules and débris of various kinds. In some cases almost the entire surface of the body is thickly covered with extraneous particles, and even erythrocytes can occasionally be seen clinging to it. This viscid character of the ectoplasm is undoubtedly due to the short, bristle-like processes, since particles only adhere to the body where these are present.

The ectoplasm is abundant, clear, homogeneous or faintly granular and usually covers the entire body but is more abundant around the enlarged basal portion (figs. 56 and 60). The endoplasm is distinctly granular and, usually, more or less vacuolated. Small fat globules are commonly present but may be entirely absent in the smaller individuals, although abundant in the larger trophozoites. In addition to the fat globules, small, irregular, refractive granules are usually rather abundant in the endoplasm. Occasionally a few yellowish crystals can be seen, but these are never abundant and in the majority of individuals are entirely wanting. They are apparently similar

to those described as hematoidin in Myxidium lieberkühni, but they always lie free in the endoplasm and are never enclosed in fat globules as in that species. In many cases erythrocytes were observed in the endoplasm (fig. 57). In fact they are more frequently found in the polysporous than in the disporous trophozoites. As in the disporous forms a careful study of both living trophozoites and sections has convinced me that erythrocytes are actually enclosed within the endoplasm and are not always simply adhering to the exterior of the body as often happens. This is clearly shown in the case of sections where there can be no doubt as to their position. Many of the refractive and other granules in the endoplasm are indistinguishable from those found floating freely in the urine and attached to the exterior of the body, and have probably been ingested in the same way as the erythrocytes.

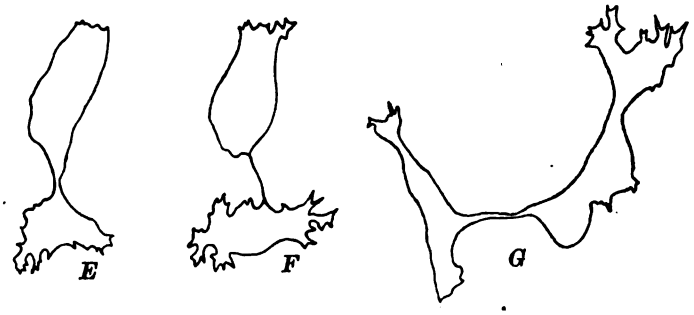
Very often the endoplasm contains several rounded bodies (fig. 59) composed of an outer hyaline layer surrounding a granular central portion which contains numerous refractive granules. As will be described later, these bodies are gemmules which make their way out of the parent and develop into daughter tropozoites.

Occasionally a large trophozoite was found containing a number of spores which were in all respects identical with those formed by the small disporous individuals. In the large trophozoites, however, there are always more than two spores and sometimes they are so abundant as almost to fill the entire body. Large trophozoites containing spores were never abundant and many fish were examined in which no sporulating individuals of this type were found.

Soon after being removed to the slide the trophozoites begin to send out numerous long, slender, branched pseudopodia from the large anterior end of the body. These pseudopodia when first formed are composed entirely of ectoplasm but later endoplasm may flow into the proximal portion. Still later, especially in smaller individuals, nearly the entire body may take part in the formation of pseudopodia so that the trophozoites may assume very irregular and grotesque forms (text figs. C and

D). In several cases after being on the slide for some time they became so irregular in shape that the body was divided into two or more parts connected only by a small strand of protoplasm, and in two or three instances the parts were observed to separate by moving in opposite directions until all connection was destroyed (text figs. E to G). This is evidently a case of plasmotomy but whether it occurs under normal conditions I am unable to say.

After being on the slide for several hours the branched, attenuated pseudopodia disappear and are replaced by a few rounded,



Text-figures E to G Division of polysporous trophozoites after being on the slide for some time. × 350.

lobe-like pseudopodia (text fig. D), the trophozoites by this time having become practically motionless.

In a few cases a phenomenon was observed which is apparently similar to that described by Cohn ('96) in Myxidium lieberkühni, which he believed to be a method of reproduction. Several of the largest trophozoites, after being placed on the slide for some time, formed numbers of rounded, bud-like protuberances, varying greatly in size, along the posterior part of the body where the ectoplasmic hair-like covering was well developed (fig. 58). These rounded processes were, in most cases at least, composed entirely of ectoplasm. In a very few of the larger processes a faintly granular central portion could be distinguished which may possibly have been endoplasm. Many of the processes were observed to change their position on the trophozoite,

indicating that they were simply adhering to the body by means of the viscid ectoplasmic covering. The formation of these bud-like structures was only rarely observed and was, I believe, an abnormal process due to the removal of the trophozoites to the slide. They were never observed until after the trophozoites had been on the slide for some time, usually several hours. No trace of bud formation has ever been observed in the preserved material, where great care was always taken to prevent degenerative changes. Moreover the fact that these bodies were so variable in size and, with few exceptions, were almost certainly composed entirely of ectoplasm, would indicate that they can scarcely be reproductive bodies. I interpret them as being simply abnormal extrusions of the ectoplasm (possibly produced by the pressure of the coverglass) analogous to the lobe-like pseudopodia which often appear after the trophozoites have been on the slide for several hours, and as utterly without any significance in the life-history of the species.

b. Preserved material. In sections of the urinary bladder trophozoites of the large form are often seen attached to the epithelial lining. Figure 61 represents a section through one of the younger, while figure 62 is from a section through a later stage. In the later stages there are always a much larger number of nuclei than in the smaller, disporous forms and these nuclei are of two very different types: viz., vegetative and generative.

The vegetative nuclei are much larger than the others from which they can be readily distinguished at a glance (figs. 61, 62, 65 to 67). They are filled with a well defined and evenly distributed reticulum of chromatin, and also contain at least one (rarely two) large rounded nucleolus which stains deeply with iron hematoxylin, but in strongly decolorized sections the interior is considerably lighter than the peripheral portion. In dried smears stained by Giemsa's method the vegetative nuclei stain very differently from the generative (fig. 65). In such preparations the chromatin of the generative nuclei take the purple stain as usual while the chromatin of the vegetative nuclei stain a light red and the nucleoli a light, uniform blue. The vegetative nuclei decolorize much more rapidly than the others in

acetone so that in strongly decolorized preparations they can scarcely be distinguished. Moreover, the vegetative nuclei are never surrounded by a specially differentiated area of cytoplasm as are the generative. The number of vegetative nuclei varies greatly, the older trophozoites containing more than the younger, although there are probably never less than four. Usually they are confined to the larger, attached end of the trophozoite where the cytoplasm stains more deeply and is evidently much denser than in other parts of the body.

The generative nuclei, although varying considerably in size, are always much smaller than the vegetative and have a very different appearance. They may occur singly or in groups and, like the generative nuclei of the disporous form, are surrounded by a specially differentiated area of cytoplasm forming a distinct cell (figs. 66 and 67). The chromatin is scattered in irregular clumps through the nucleus and there is usually no nucleolus. The generative cells divide mitotically and in a large trophozoite there may be a number of these cells present in the endoplasm. They all have a similar structure but may develop into two different types of reproductive bodies.

Occasionally a degenerative cell is seen, in which division of the nucleus is not followed by a corresponding division of the cytoplasm. Successive nuclear divisions follow in rapid succession until eight nuclei are formed, all enclosed in a common cytoplasmic mass (figs. 67 to 71). Meanwhile, the entire structure increases considerably in size, forming a very characteristic rounded body, sharply marked off from the surrounding endoplasm (figs. 59, 60, 69 and 70). These bodies are probably homologous with the pansporoblasts but have a very different history from the ordinary structures of that name. They are, in reality, similar to the gemmules formed by many species of Protozoa.

The gemmules at this stage have eight similar nuclei which are closely crowded together at the center. Surrounding them is a layer of cytoplasm which is more finely granular and stains more deeply than the undifferentiated endoplasm in which they are imbedded. Often, as in figures 69 and 70, a clear

space separates the gemmule from the surrounding endoplasm. This is no doubt due to shrinkage and indicates that the gemmule lies in a distinct cavity formed in the endoplasm of the mother trophozoite. In dried smears stained by the Giemsa method the cytoplasm of the gemmules stains a light blue while the cytoplasm of the mother trophozoite stains a light red. In the younger stages the nuclei of the gemmules contain no nucleoli, but by the time the 8-nucleate stage is reached there is usually a well defined nucleolus in each nucleus. There may be several gemmules present at the same time in a trophozoite as shown in figure 71 where there are nine, and trophozoites with even larger numbers are not rare.

The 8-nucleate state is usually as far as the gemmules develop within the mother trophozoite. They now make their way to the periphery (fig. 69), thence to the exterior by rupture of the ectoplasmic layer. I have several times observed the escape of gemmules from living trophozoites (fig. 60). Moreover, gemmules which have evidently just emerged from the mother trophozoites are common.

After the gemmules become free, four of the nuclei enlarge to form vegetative nuclei, while the other four become surrounded by a differentiated area of cytoplasm and develop into generative cells (figs. 72 and 73).

Occasionally the gemmules may be retained in the body of the mother trophozoite for a longer time. In such cases they increase in size and begin to develop in the same way as those which have become free (figs. 62, 74 and 75). The cytoplasm loses its dense, finely granular structure and no longer stains more deeply with plasma stains except near the center, where the denser material may persist for some time. Around the periphery the cytoplasm becomes vacuolated and exhibits a characteristic radiate structure (fig. 74). The nuclei increase in number by mitotic division and become differentiated into vegetative and generative as in the free forms.

This method of reproduction obviously furnishes a means for the rapid increase of the parasites within the host. Apparently the gemmules always develop into the large form of tropho-

zoites for I have never seen anything to indicate that they may develop into the smaller disporous type. During the months of June, July and August, when these observations were made, the formation of gemmules is certainly the principal method of reproduction in the case of the polysporous trophozoites, for sporulating individuals were always rare, and in many fish none at all were found, while trophozoites containing gemmules were always abundant. Whether this be true at other times of the year I am unable to say.

The spores are developed from generative cells similar to those which form the gemmules. Unlike the disporous form, the spores always develop in pairs from a common pansporoblast, the sporoblasts of which are never separated. Each pansporoblast originates from a single cell, but in this case the entire cell divides, so that the developing pansporoblast can be easily distinguished from the gemmules, in which only the nuclei divide. During the multiplication stage the generative cells separate soon after division, but in the pansporoblasts the daughter cells remain closely associated (figs. 76 and 77) although the outlines of the individual cells can usually be easily distinguished. Unlike the sporoblasts of the disporous form, the cytoplasm of the pansporoblasts does not ordinarily stain more deeply than the surrounding endoplasm. However, in a few cases I have noticed pansporoblasts in which the cytoplasm was denser and stained more intensely, while the outlines of the individual cells were indistinct and difficult to make out (fig. 79). Such pansporoblasts appear strikingly like the sporoblasts of the disporous trophozoites except that the nuclei never contain a nucleolus.

The cells in the pansporoblasts do not have any such definite arrangement as in the sporoblasts of the disporous form, and it is, therefore, impossible to trace the derivation of the different cells of the spores, but there is no reason to think that it is essentially different. The fully developed pansporoblast contains twelve cells as in figure 78, in which eleven cells occurred in the section; the other cell which was in the next section is not shown. A careful study of a number of pansporoblasts which were sufficiently separated from adjoining cells to admit of an accurate count

has convinced me that there are never more than twelve nuclei present and that there is no trace of 'residual nuclei' at any stage. This is, of course, in accordance with the conditions found in the disporous forms.

The cells of the fully developed pansporoblasts are much alike except that the capsulogenous cells are larger and there is a deeply staining region in the cytoplasm at one side of the nucleus (fig. 78). The nuclei in these cells are also somewhat larger and the chromatin is less compact. Two of the nuclei shown at a lower level in the figure are larger and more flattened than the others and are probably in 'parietal cells' which form the valves of the spore membrane.

Figures 81 and 82 show later stages in the development of the spores which lie in a common cavity formerly occupied by the pansporoblast. Each spore is made up of six cells and here is no trace of 'residual nuclei' (figs. 81 to 83). In all essential respects they are like the spores of the disporous form (figs. 41 and 42, 80 to 82). Practically the only difference is in the capsulogenous cells which, in the polysporous forms contain, previous to the formation of the polar capsules, numbers of rounded granules staining deeply with the plasma stain. As previously stated I have been unable to distinguish any difference between the mature spores of the disporous and polysporous forms.

The process of mitotic division in the cells of the polysporous trophozoites is essentially the same as in the disporous, so that a detailed description is unnecessary. Figures 84 to 89 show the different stages of the process. The most striking difference is in the larger size of the chromosomes and the better development of the mitotic figure. As in the disporous form no sign of a centrosome was seen at any stage. I wish to emphasize the fact that while the chromosomes are much larger, the number is the same as in the disporous form. Moreover, as in the latter, the chromosomes are evidently in three pairs differing in size (figs. 86 to 87). One pair is considerably larger than the others; one pair is somewhat smaller; while the other pair is intermediate in size.

IV. GENERAL DISCUSSION AND REVIEW OF LITERATURE

Auerbach ('10) has recently given a very complete review of the literature on the Myxosporidia and it is, therefore, superfluous to go into a full discussion of it at this time.

1. Polymorphism

That the polysporous and disporous forms of trophozoites described in the preceding pages are one and the same species is, I believe, certain. At first I naturally took them for entirely distinct species, but as the work progressed it became more and more evident that only the assumption that both forms belong to the same species would account for the facts. In the first place the two forms are practically always found together. Moreover, the spores in both cases are practically identical, and this is, of course, the strongest evidence for considering the two types of trophozoites only different forms of the same species.[4] The development of the spore in the two types is very similar, differing only in unessential details. Also I would lay especial stress on the fact that although the chromosomes in the polysporous forms are much larger than in the disporous, the number and shape is the same in both cases. In both forms the chromosomes can be grouped in three pairs differing somewhat in size, the relative sizes of the chromosome pairs being the same. Furthermore, both types of trophozoites ingest the erythrocytes of the host, a phenomenon known to occur in only one other species of Myxosporidia. These two species form, so far as I know, the only examples of the ingestion of solid food in the entire group of the Sporozoa.

On the other hand I do not wish to minimize the fact that in appearance and structure the two types of trophozoites are unlike. In the examination of living material numerous examples of small trophozoites were seen which could not with certainty be assigned to either form. In cases where the ectoplasmic

[4] It is well known that the structure of the spore is very constant in the Myxosporidia, each species having its own characteristic spore which shows little variation. So constant is the form of the spore that it is the principal character used in distinguishing between the different species.

processes are absent, the small polysporous trophozoites are often strikingly like the larger vegetative trophozoites of the disporous form. However, when stained there is no difficulty in distinguishing between the two types since as regards the nuclei they are very distinct. In a very few instances I have found individuals in which the nuclei were not typical for either type, and it may be that they were transition forms, but such trophozoites occurred so rarely in my preparations that I have been unable to satisfy myself as to their significance. Possibly transition forms occur only at certain seasons, but this must, for the present, remain pure conjecture.

Auerbach ('09 and '10) finds that Myxidium bergense forms both monosporous and polysporous trophozoites, but there is no such difference in the appearance of the two forms as in S. dimorpha. According to the same author (Auerbach '09a) Zschokella may be either monosporous or disporous, while Amerizew ('11) finds that the trophozoites of a species of Myxidium in the gall bladder of Cottus scorpius may form from one to three spores.

2. Multiplicative reproduction

Doflein ('98) has distinguished between multiplicative and propagative reproduction in the Myxosporidia. The former results in the increase of the organism within the host, or in other words in autoinfection, while the latter furnishes a means by which the parasite may pass from one host to another.

Plasmotomy is held by most writers to be the common method of multiplicative reproduction. However, in S. dimorpha, while plasmotomy has been observed in individuals after being removed from the bladder, there is no evidence that this is a common occurrence under normal conditions. In sections and Giemsa smears I have never seen multinucleate individuals which showed any evidence of having recently divided. In the disporous forms the character of the nuclei is such that plasmotomy, if it occur, should be easily detected; while the large numbers of this form which are practically always present indicate that some form of multiplicative reproduction is common,

I believe it must occur not later than the binucleate stage. In all later stages the nuclei are distinctly differentiated into vegetative and generative nuclei which are very characteristic, and no trophozoites have been seen which could have been formed by the division of such an individual. On the other hand there is evidence that the young binucleate forms may divide, and it is interesting to note that such a method of reproduction has been found by Auerbach in Myxidium bergense. However, the evidence of division is not conclusive, and for various reasons this species furnishes very unfavorable material for the determination of this question. I believe it is questionable whether plasmotomy as defined by Doflein, occurs normally in the disporous Myxosporidia. The whole subject is in need of careful investigation.

Turning to the polysporous form we find that here, also, there is practically no evidence that plasmotomy is a normal occurrence. It has been observed on the slide under abnormal conditions, but there is little warrant for believing the process to occur normally. A few individuals were observed on the slide which showed rounded processes strikingly like those described by Cohn ('96) in Myxidium lieberkühni and which he believed to be a process of budding.[5] As previously stated, I believe these processes are abnormal; formed as a result of the pressure of the overlying coverglass, and that they have no significance in connection with reproduction.

The normal method of multiplicative reproduction in the polysporous form is, I believe, by the formation of gemmules. Trophozoites containing gemmules were so abundant as to leave no room for doubt that multiplication by this method is rapid and sufficient to account for the large number of polysporous trophozoites found in many instances.

[5] Laveran and Mesnil ('02) deny that such budding occurs. They find that the trophozoites formed by plasmotomy may attach themselves to the larger individuals, thus giving rise to the appearance erroneously interpreted by Cohn as budding. In S. dimorpha the exterior of the large trophozoites are distinctly viscid and small trophozoites may become temporarily attached to them. However, there is no doubt that the processes shown in figure 58 are due to extrusion of the ectoplasm.

It would be strange indeed, if this method of multiplication is confined to the species under discussion. Erdmann ('11) has described peculiar protoplasmic bodies which sometimes emerge from disintegrating trophozoites of Chloromyxum leydigi, and which she considers 'vegetative reproductive bodies.' These bodies are apparently homologous with the gemmules of S. dimorpha, although Erdmann believes that their function is to provide for reproduction under unfavorable conditions only. This belief is apparently based on the fact that she observed their formation only in trophozoites growing on a gall agar culture medium, or introduced into the digestive tract of the host.

I have evidence (which will be reserved for a later paper) indicating that a similar method of endogenous budding, resulting in the formation of gemmules, occurs in a species of Ceratomyxa found in the gall bladder of a shark, Carcharhinus sp., and also in a Myxosporidian (probably a new genus) occurring in the urinary bladder of the flounder, Paralichthys albiguttus.

3. Propagative reproduction

Probably the sole method of propagative reproduction among the Myxosporidia is by the formation of resistant spores which can live for some time outside the body of the host. The process of sporulation, which is in all cases very complicated, has been studied by a number of investigators. Auerbach ('10) has given a very full review of the work on sporulation, and as the earlier accounts are so evidently erroneous in many respects, it is unnecessary to consider them here in detail. The most complete of the earlier accounts is that of Thélohan ('95), according to whom sporulation begins in Myxobolus by a single nucleus becoming surrounded by a spherical, condensed mass of protoplasm, which is in turn surrounded by a membrane. The nucleus then divides by mitosis until ten nuclei are present in the pansporoblast. The pansporoblast then divides into two parts (sporoblasts), each containing four nuclei. The remaining two nuclei remain outside the sporoblasts and gradually disappear. Each sporoblast becomes converted into a spore,

one nucleus passing into each of the two polar capsule cells while the other two nuclei remain in the sporoplasm.

In recent years a number of detailed studies of spore formation have appeared, notably those by Amerinzew ('09 and '11), Keysselitz ('08) and Schröder ('07 and '10). All these writers agree that six cells (eight in Chloromyxum) take part in the formation of the spore instead of four as held by earlier writers. Of these six, two form the valves of the spore membrane, two form the capsulogenous cells in which the polar capsules develop, and the remaining two the sporoplasm. With this conclusion my results are in perfect accord. There are, however, important differences in the history of the nuclei during sporulation. According to Amerinzew ('09) in Ceratomyxa drepanopsettae the trophozoite has at first two nuclei which are derived from the division of a single nucleus. Each nucleus then divides by mitosis into two nuclei of unequal size. The two smaller nuclei are vegetative, the two larger generative. The protoplasm becomes concentrated around each of the two generative nuclei to form cells inclosed in the endoplasm of the trophozoite. These two cells differ somewhat in size, the larger being a macrogametocyte, the smaller a microgametocyte. Each gametocyte then divides to form two gametes. Each gamete extrudes a quantity of chromatin into the cytoplasm after which each microgamete unites with a macrogamete, the single celled zygotes thus formed being the sporoblast from which the spores are developed later. In the formation of the spores each sporoblast divides into two cells differing in size. The smaller then divides again, giving a sporoblast with three cells, a larger which ultimately gives rise to two 'parietal cells' which form the valves of the spore membrane, and two smaller cells, one of which divides to form the two capsulogenous cells. The nucleus of the other small cell divides to form the bi-nucleate sporoplasm.

According to Schröder ('07) in Sphaeromyxa sabrazesi and Keysselitz ('08) in Myxobolus pfeifferi the conjugation of the gametes during sporulation takes place at a quite different stage. According to these writers the spores arise from 'propagative cells' which originate by generative nuclei of the plasmo-

dium becoming surrounded by a condensed mass of protoplasm. The propagative cells may multiply for a time by mitosis, but eventually proceed to spore formation. The propagative cell then divides into two cells of unequal size. Two such cell couples become associated and the two smaller cells form an envelope around the two larger which form the pansporoblast. The pansporoblast is thus from the first composed of two cells. Although the cytoplasm of these cells may fuse later, the nuclei remain distinct and divide repeatedly until the pansporoblast consists of twelve nuclei which may or may not be located in distinct cells inclosed within the envelope cells. The nuclei of the envelope cells, the so-called 'residual nuclei,' degenerate and take no active part in spore formation. The pansporoblast divides into two cell groups of six cells each, two cells in each group are parietal cells, two are capsulogenous cells, while the remaining two unite to form a binucleate cell which becomes the sporoplasm. Later, after the spore is fully formed, the two nuclei unite. Presumably in each sporozoite one nucleus is derived from each of the two original propagative cells but there is no direct evidence that this is the case. On the other hand, Mercier ('10) holds that in Myxobolus pfeifferi the pansporoblast is a zygote formed by the conjugation of a micro- and macrogamete. The single nucleus of the zygote then divides several times to form fourteen nuclei. Twelve of these separate into two groups, the sporoblasts, each containing six nuclei. Each sporoblast eventually forms a spore while the remaining two nuclei are thrown out and degenerate.

The principal discrepancy between the accounts of Amerinzew on the one hand, and Schröder and Keysselitz on the other is in regard to the time at which the gametes unite. According to Amerinzew copulation occurs previous to the formation of the sporoblasts while Keysselitz and Schröder hold that it is delayed until after the spores are formed. It is significant that in Myxobolus pfeifferi on which Keysselitz worked, Mercier found that copulation takes place previous to the formation of the pansporoblasts. This would bring the sporulation of the Polysporea in essential agreement with that of the Disporea.

On the other hand, in S. dimorpha, I have been unable to find any traces of sexual phenomena during sporulation in either the disporous or polysporous forms, although careful search has been made in both forms for evidence of a sexual process at this time. In the disporous form there is certainly nothing like the process described by Amerinzew in Ceratomyxa drepanopsetta. As previously described there can be little doubt that in S. dimorpha the sporoblasts are formed by the division of the nucleus of a uninucleate trophozoite, one of the daughter nuclei forming the vegetative nucleus, the other forming a 'propagative cell' which divides later to form the sporoblasts. Such trophozoites as are shown in figures 17 to 22 can, I believe, be explained in no other way. There is some evidence (which will be considered in detail later) that the original uninucleate cell is a zygote. If this be true, copulation must take place at a much earlier stage than in Ceratomyxa. It might be held that the binucleate trophozoite is formed by the fusion of two uninucleate trophozoites, although I have seen no evidence that this is the case. But in that event we would be forced to the remarkable conclusion that the nucleus of one gamete becomes the vegetative nucleus, while the nucleus of the other alone gives rise to the spores. At present I see no way of harmonizing my results with those of Amerinzew in C. drepanopsettae. However, in Myxidium sp. from the gall bladder of Cottus scorpius, according to Amerinzew ('11), a binucleate trophozoite may form sporoblasts directly without any previous sexual phenomena. In fact, judging from the short account which he gives of sporulation in this species, it differs from S. dimorpha only in relatively unimportant details.

In the polysporous forms sporulation in S. sabrazesi according to Schröder, and in M. pfeifferi according to Keysselitz, is in several respects quite different from the same process in S. dimorpha. In all three species the 'propagative cells' may multiply by mitosis. The daughter cells in S. dimorpha are, however, always of equal size and the pansporoblast originate from a single 'propagative cell'. There is no evidence of a preliminary fusion of cell couples as in S. sabrazesi and M. pfeifferi. In

S. dimorpha each pansporoblast eventually forms twelve—not fourteen—cells and a careful study of a large number of pansporoblasts has failed to show any trace of 'residual nuclei.' It seems strange that there should be such fundamental differences in the process of sporulation in forms so closely related to each other as the species under discussion, but in the present state of our knowledge it is impossible to harmonize the discordant accounts.

4. Mode of infection

I have no direct evidence of the method by which S. dimorpha gains entrance to a new host. The investigations of Auerbach ('09, '10) on Myxidium bergense and Erdmann ('11) on Chloromyxum leydigi have shown that, in all probability, the infection of new hosts takes place through the digestive tract. The sporozoite becomes free in the intestine and, as an amoebula, wanders actively up the bile duct to the gall bladder. Probably the infection of new hosts by S. dimorpha is accomplished in the same way, although the universal occurrence of the parasite in the urinary bladder of Cynoscion regalis is difficult to explain as the result of purely accidental infection.

There can be little doubt that the spores pass to the exterior soon after becoming free from the mother trophozoite, for in no case were large numbers of spores found in the bladder, although trophozoites in all stages of sporulation were abundant. Spores of S. dimorpha, when placed on the slide without previous exposure to sea water, and mixed with a drop of fluid from the pyloric caeca of the host, usually germinated within five to fifteen minutes. In most cases of germination the valves of the spore membrane separated along the sutural line followed by the emergence of the sporozoite which crept out, by an active amoeboid movement (fig. 47). In some cases the sporozoites were observed to disintegrate shortly after emergence (probably due to the action of the digestive fluid); others remained intact for the length of time they were under observation. In most cases, but not all, the filaments were extruded from the polar capsules on the addition of the caecal fluid. A few spores

failed to germinate or extrude their polar filaments after being exposed to the caecal fluid for three hours, when the experiment was discontinued.

It appears probable, therefore, that the free spores, when taken into the intestine of the host, germinate, and the sporozoites, as free amoebulae, actively make their way into the urinary bladder. Whether the amoebulae then go through an intercellular stage as has been found by a number of investigators to be the case in other species, it is impossible to say. While I have noted a number of instances in which the epithelial cells of the bladder contained an intracellular parasite I am not at all certain that the parasite was a stage of S. dimorpha. The urinary bladder and Wolffian ducts of C. regalis are often infected with a species of Leptotheca in addition to S. dimorpha, and while there is no difficulty in distinguishing the free forms of the two species, owing to their very different structure (especially as regards the nuclei), I am by no means certain that it is equally easy to distinguish the intracellular forms. Certainly the nuclei of the intracellular parasites are quite different from those of the free-living trophozoites of S. dimorpha.

5. Fertilization

Finally comes the question: Is there any evidence of a sexual process in the life-history of S. dimorpha? We have already seen that no sexual phenomena are associated with sporulation in this species, so that if there be a sexual process, it must occur at some other stage. Although the two nuclei of the sporoplasm are still distinct in the fully matured spore, it is probable that they fuse later (possibly at the time of germination), as has been found to be the case in so many species of Myxosporidia. Such a fusion would be a case of endogamy, but there is some evidence that exogomy also occurs.

In Giemsa smears there are occasionally seen small mononuclear cells which are quite different in appearance from the mononuclear trophozoites shown in figures 8 and 9. Not only are they much smaller, but the amount of cytoplasm is relatively

much less and appears perfectly homogeneous, never granular as in the ordinary mononuclear trophozoite (fig. 3). Occasionally such cells lie side by side while similar cells containing two nuclei are not uncommon (figs. 4 and 5). The appearance of these binucleate cells strongly suggests that they are formed by the fusion of two cells like that shown in figure 3, while figure 6 may plausibly be interpreted as a somewhat later stage in which the nuclei are partially fused, and figure 7 may represent a later stage after complete fusion of the nuclei. If the above interpretation be correct figure 7 represents the earliest stage of the definitive trophozoite, formed by the fusion of two similar gametes, in which the cytoplasm has not yet developed the granular structure so characteristic of the later stages.

On the other hand, were it not for figure 6, the cells shown in figures 3 to 7 might just as plausibly be interpreted as different stages in the division of a cell like figure 7, in which case the sequence of the different stages would be just the reverse of that suggested above. Although I have devoted much time to the study of these stages, I am still unable to decide which is the correct interpretation. The cell shown in figure 6 is the only case I have found where the nuclei appeared to be fusing. On the other hand I have found no case in which the nuclei appeared to be undergoing division in cells characterized by a small amount of homogeneous cytoplasm. Several cases of nuclear division in the larger mononuclear trophozoites have been observed, but they can always be distinguished by the relatively larger amount of granular cytoplasm.

For the present, then, it must be considered doubtful whether the stages shown in figures 3 to 7 represent a case of copulation or simply a method of multiplication by division of small mononuclear trophozoites.

V. SUMMARY

1) The urinary bladder and Wolffian ducts of the squeteague, Cynoscion regalis, contain large numbers of a myxosporidian parasite, Sphaerospora dimorpha, n. sp.

2) This parasite occurs in two very distinct forms, one of which is disporous, the other polysporous.

3) Both disporous and polysporous forms ingest solid food.

4) The spores produced by each form are identical and the number and form of the chromosomes are the same in both cases.

5) In both disporous and polysporous forms the trophozoites contain both vegetative and generative nuclei.

6) In the disporous form the generative or 'propagative' cell forms two sporoblasts, each containing six cells, two of which form the sporoplasm, two the capsulogenous cells, while the other two form the spore membrane.

7) In the polysporous form the generative or 'propagative' cells may form either gemmules or pansporoblasts.

8) Sporulation in the polysporous form is essentially the same as in the disporous, differing only in unimportant details.

9) There is no evidence of a sexual process connected with sporulation in either form.

10) In all probability the only method of multiplicative reproduction in the polysporous forms is by the formation of gemmules.

VI LITERATURE CITED

AMERINZEW, S. 1909 Studien über parasitische Protozoen. I. Die Sporenbildung bei Ceratomyxa drepanopsettae, mihi. Arch. f. Protistenk., Bd. 14, pp. 74–112.

1911 Studien über parasitische Protozoen. VII. Über Sporenbildung bei Myxidium sp. aus der Gallenblase von Cottus scorpius. Arch. f. Protistenk., Bd. 23., pp. 199–204.

AUERBACH, M. 1909 Biologische und morphologische Bemerkungen über Myxosporidien. Zool. Anz., Bd. 35, pp. 57–63.

1909 a Die Sporenbildung von Zschokkella und das System der Myxosporidien. Zool. Anz., Bd. 35, pp. 240–256.

1910 Die Cnidosporidien. Eine monographische Studie. Leipzig.

COHN, L. 1896 Über die Myxosporidien von Esox lucius und Perca fluviatilis. Zool. Jahrb., Abt. f. Anat., Bd. 9, pp. 227–272.

DOFLEIN, F. 1898 Studien zur Naturgeschichte der Protozoen. III. Über Myxosporidien. Zool. Jahrb., Abt. f. Anat., Bd. 11, pp. 281–350.

ERDMANN, RH. 1911 Zur Lebengeschichte des Chloromyxum leydigi, einer mictosporeen Mixosporidie. (Teil I.) Arch. f. Protistenk., Bd. 24, pp. 149–162.

KEYSSELITZ, G. 1908 Die Entwicklung von Myxobolus pfeifferi, I u. II. Arch. f. Protiztenk., Bd. 11, pp. 252 u. 276.

MERCIER, L. 1910 La sexualité chez les Myxosporidies et les Microsporidies. Mem. Acad. Roy. Belgique., Ser. II, T. 2, No. 6.

SCHRÖDER, OLAW 1907 Entwicklungsgeschichte der Myxosporidien, Sphaeromyxa labrazesi (sabrazesi). Arch. f. Protistenk., Bd. 9, pp. 359–381.

1910 Über die Anlage der Sporocyste (Pansporoblast) bei Sphaeromyxa sabrazesi, Laveran et Mesnil. Arch. f. Protistenk., Bd. 19, p. 1–5.

THÉLOHAN, P. 1894 Recherches sur les Myxosporidies. Bull. Scien. de la France et de la Belgique, Tom. 26, pp. 100–394.

PLATE 1

EXPLANATION OF FIGURES

All figures, with the exception of figure 47, were drawn with the aid of the camera lucida at the level of the base of the microscope. The magnification, where not otherwise specified, is 1550 diameters. All figures, where not otherwise noted, were drawn from sections of the urinary bladder fixed in formol-corrosive-acetic and stained with iron hematoxylin and congo red.

Figures 1 to 55 are of the disporous, figures 56 to 89 of the polysporous form.

1 Living vegetative trophozoite shortly after being placed on the slide. × 1400.

2 Living vegetative trophozoite with two erythrocytes in the endoplasm. × 1400.

3 Small mononuclear cell the nucleus of which is surrounded by a thin layer of homogeneous cytoplasm. Giemsa smear.

4 and 5 Cells with two nuclei but otherwise similar to figure 3. Giemsa smear.

6 Cell with homogeneous cytoplasm and two nuclei which appear to be fusing. Giemsa smear.

7 Mononuclear trophozoite with homogeneous cytoplasm which is not as abundant as is usually the case at this stage. Giemsa smear.

8 Mononuclear trophozoite in which the cytoplasm is more abundant than in figure 7 and is also distinctly granular. Giemsa smear.

9 Mononuclear trophozoite similar to figure 8. At one side can be seen the pseudopodia by means of which it was attached to the urinary epithelium.

10 to 12 Binuclear trophozoites larger than figures 4 and 5 and with granular cytoplasm.

13 to 15 Binuclear trophozoites in which each nucleus is plainly surrounded by a specially differentiated area of the endoplasm. Figure 13 from a Giemsa smear.

16 Trophozoite with three nuclei of approximately same size. No specially differentiated area of endoplasm could be distinguished around the nuclei.

17 Trophozoite with three nuclei one of which is considerably larger than the others. Giemsa smear.

18 Similar to figure 17 except that a specially differentiated area of endoplasm could be distinguished around each of the smaller nucler. Giemsa smear.

19 Similar to figure 18 except that the structure of the larger (vegetative) nucleus is plainly unlike that of the other two. Giemsa smear.

20 Two trophozoites, one with only three, the other with four nuclei. Giemsa smear.

21 Two trophozoites, one with three, the other with four nuclei. In each trophozoite the vegetative nucleus can be easily distinguished. At the right can be seen the pseudopodia by means of which they were attached to the urinary epithelium.

22 Trophozoite with four nuclei. The three generative nuclei are inclosed in distinct cells.

23 Trophozoite with five nuclei. Three of the four generative nuclei are inclosed in distinct cells, which can be made out only with difficulty. No definite sporoblasts can be distinguished.

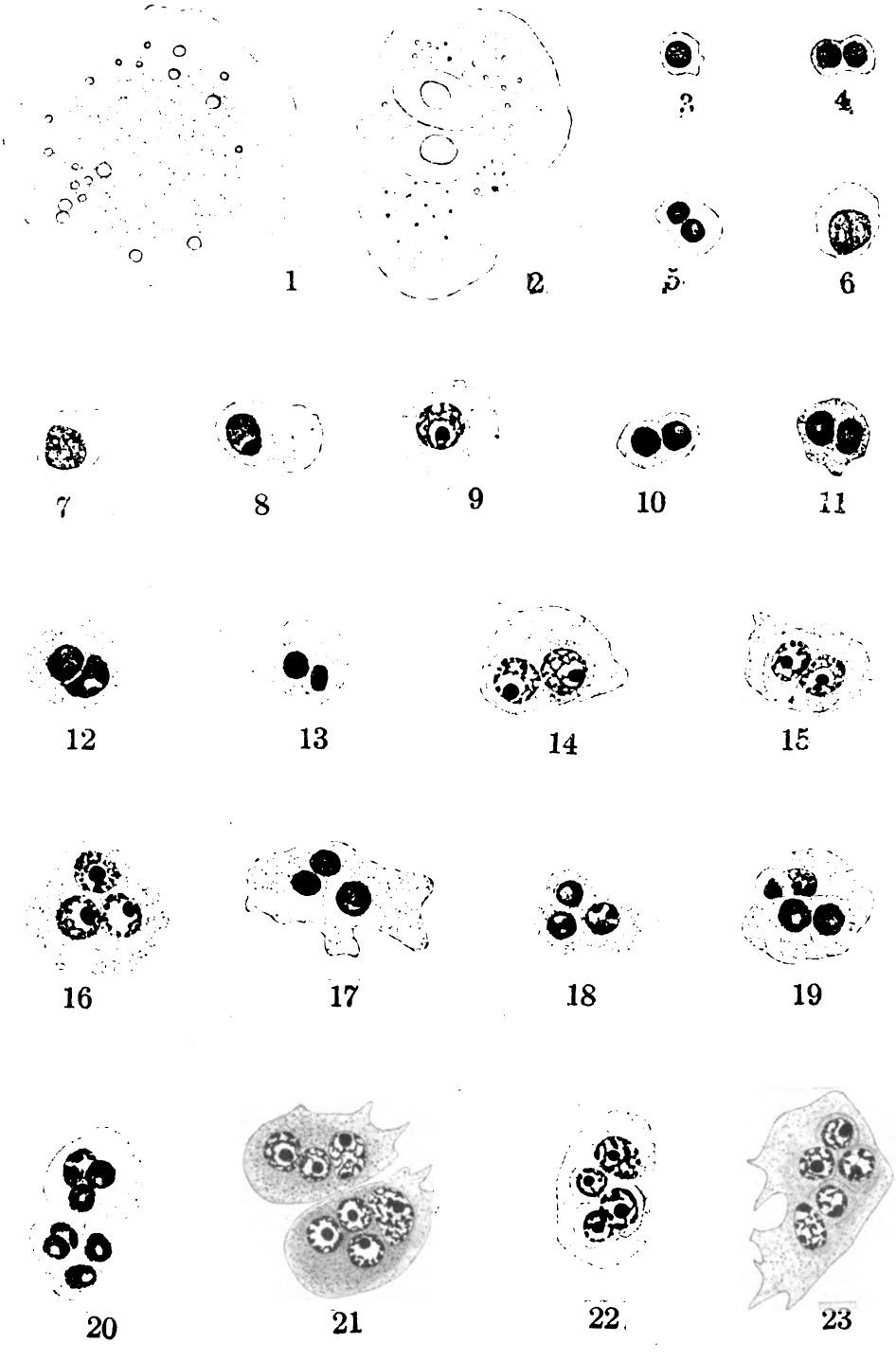


PLATE 2

EXPLANATION OF FIGURES

24 Trophozoite with one vegetative and four generative nuclei, the latter associated in pairs to form the sporoblasts. Giemsa smear.

25 Trophozoite containing one vegetative nucleus and two sporoblasts, one with two, the other with three nuclei. Giemsa smear.

26 Trophozoite in which the sporoblast has shrunken away from the surrounding endoplasm. Only two of the three nuclei of the sporoblast are in the section.

27 Trophozoite showing one entire sporoblast composed of three cells. Only part of the other sporoblast occurs in the section.

28 Trophozoite containing two sporoblasts, each with three nuclei but no distinct cells could be made out. One vegetative nucleus present. All the nuclei in the trophozoite are shown.

29 Trophozoite with one four-celled and one three-celled sporoblast. Only one vegetative nucleus present.

30 Trophozoite in which the vegetative nucleus is dividing amitotically. Only one sporoblast with five nuclei occurs in the section.

31 Trophozoite with one three-celled and one five-celled sporoblast. Only one vegetative nucleus present.

32 Three-celled sporoblast drawn under a higher magnification. Note that one cell is distinctly larger than the other two. × 2000.

33 Three-celled sporoblast, in which the two smaller cells are dividing mitotically. × 2000.

34 Sporoblast in which one of the smaller cells has just divided while the other is in a late anaphase. × 2000.

35 and 36 Five-celled sporoblasts formed by the division of the two smaller cells of the three-celled sporoblasts. × 2000.

37 and 38 Five-celled sporoblasts in which the large end cells are preparing to divide amitotically. Note that in figure 38 the nucleolus shows a median constriction and that in figure 37 there are two nucleoli. × 2000.

ABBREVIATIONS

cap.c., capsulogenous cell.
gem., gemmule.
g.c., generative cell.
par.c., parietal cell.
sp.c., sporoplasm cell.
v.n., vegetative nucleus.

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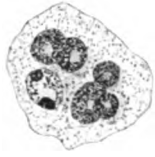

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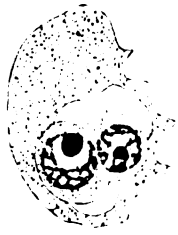

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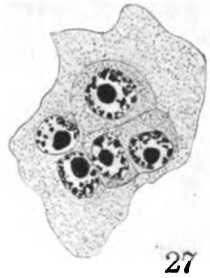

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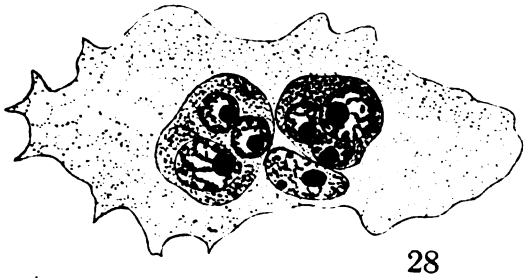

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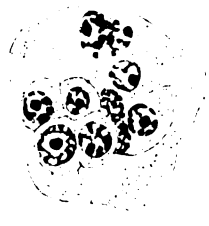

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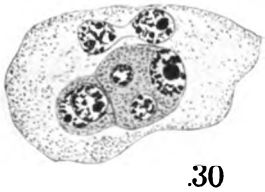

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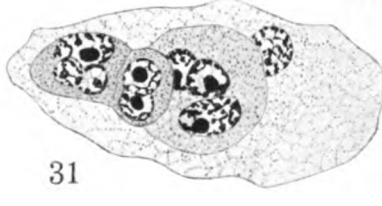

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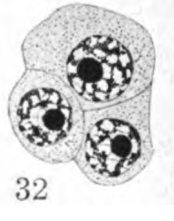

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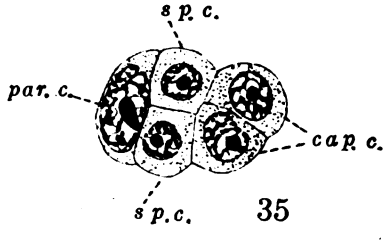

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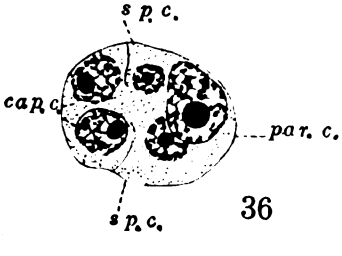

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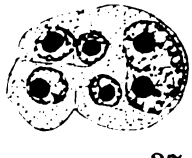

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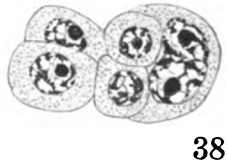

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PLATE 3

EXPLANATION OF FIGURES

39 and 40 Optical sections through the same trophozoite showing all the nuclei. There are twelve cells in the sporoblasts becoming arranged to form the spores. Only one vegetative nucleus is present. × 2000.

41 Trophozoite in which the cells have become definitely arranged to form the spores. All the nuclei are shown. The parietal cells surround the capsulogenous and sporoplasm cells. The capsulogenous cells can be easily distinguished by their larger nuclei.

42 Trophozoite in which the spores are slightly more advanced than in figure 41. Only one spore is shown. A deeply staining body is present in the cytoplasm of the capsulogenous cells.

43 Nearly mature spore. Only one capsulogenous cell is shown.

44 Living trophozoite containing two mature spores. × 640.

45 Mature spore as it appears when set free in the urine. × 1400.

46 Mature spore slightly compressed under the cover-glass. × 1400.

47 Free-hand drawing of germinating spore.

48 to 55 Different stages in the mitotic division of the sporoblast cells.

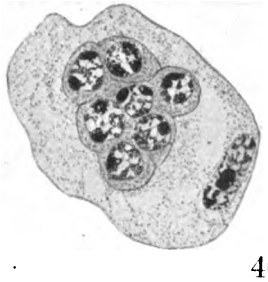
48 and 49 Prophase. × 2650.

50 to 52 Polar views of the equatorial plate showing six chromosomes. × 2650.

53 and 54 Anaphase. × 2650.

55 Telophase. × 2650.


39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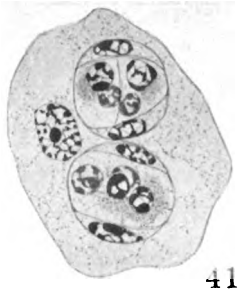
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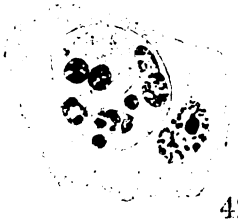
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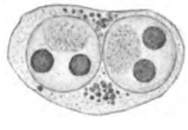
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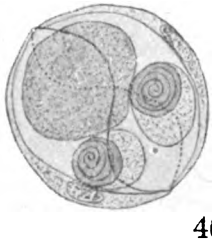
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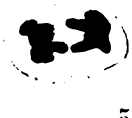
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PLATE 4

EXPLANATION OF FIGURES

56 Living trophozoite of the polysporous form shortly after being placed on the slide. × 640.

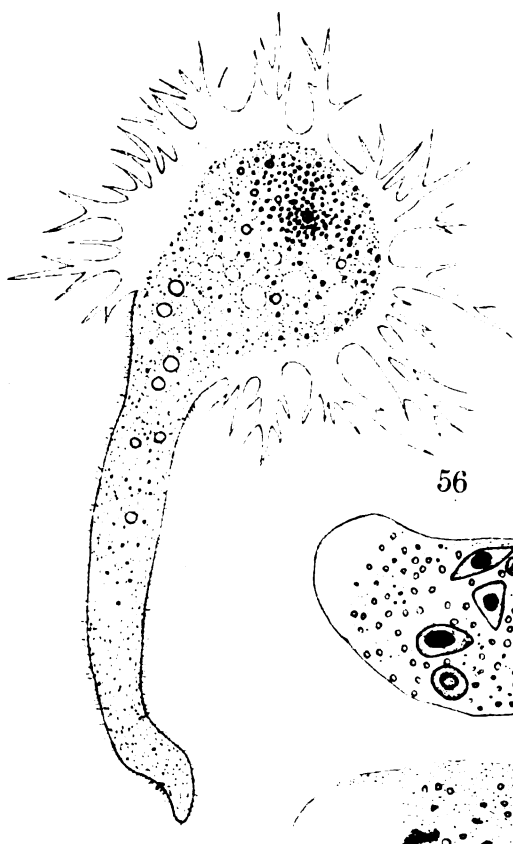
57 Small living trophozoite of the polysporous form containing several erythrocytes in different stages of disintegration. × 640.

58 Living trophozoite with a number of bud-like ectoplasmic processes. × 640.

59 Living trophozoite with a number of gemmules in the endoplasm. × 640.

60 Living trophozoite from which a gemmule is just escaping. × 640.

63 Section through base of young trophozoite showing protoplasmic processes by which it is attached to the urinary epithelium.



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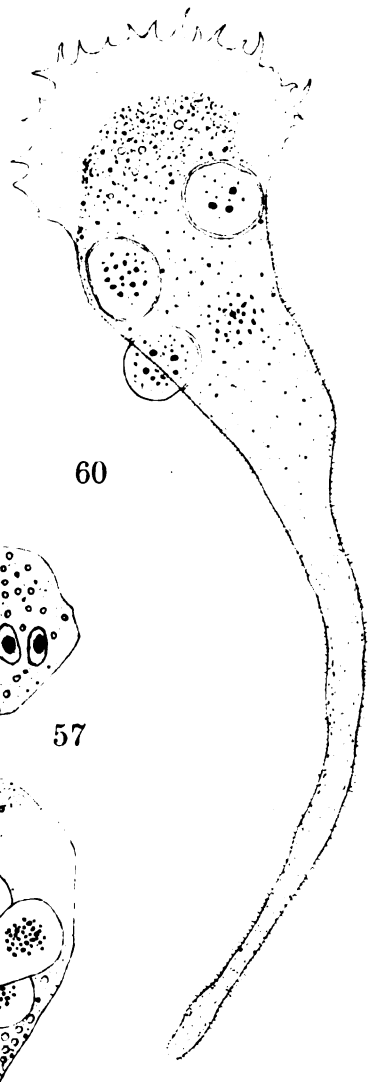

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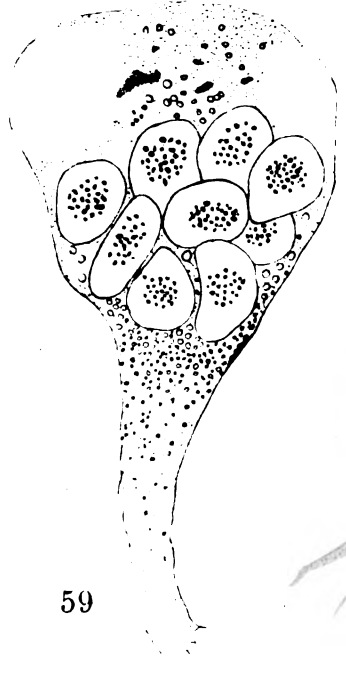

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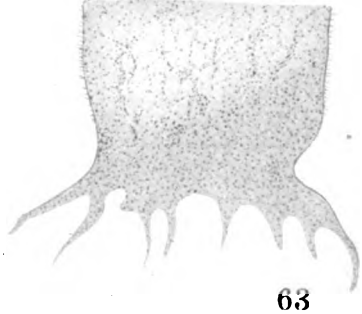

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PLATE 5

EXPLANATION OF FIGURES

61 Section through trophozoite attached to lining of the urinary bladder. × 525.

62 Section through older trophozoite than in figure 61. × 525.

64 Section through the base of an older trophozoite showing organs of attachment more highly developed.

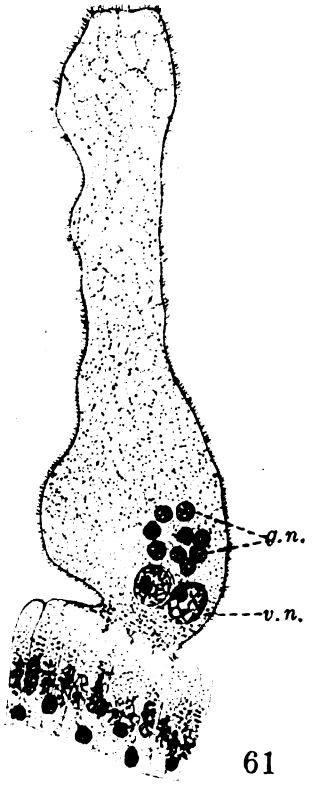
65 Portion of a trophozoite from a dried smear showing vegetative and generative nuclei. Two of the generative nuclei are dividing mitotically. × 1400.

66 Portion of section through trophozoite showing generative cells and vegetative nuclei.

68 Section through small part of trophozoite containing a gemmule with only four nuclei.

69 Section through gemmule containing eight nuclei only five of which are shown. The cavity in which the gemmule lies can be distinctly seen.

70 Section through gemmule at periphery of trophozoite and apparently just ready to emerge. One nucleus is dividing mitotically. Only part of the nuclei are shown. At one side the gemmule is shrunken away from the surrounding endoplasm.



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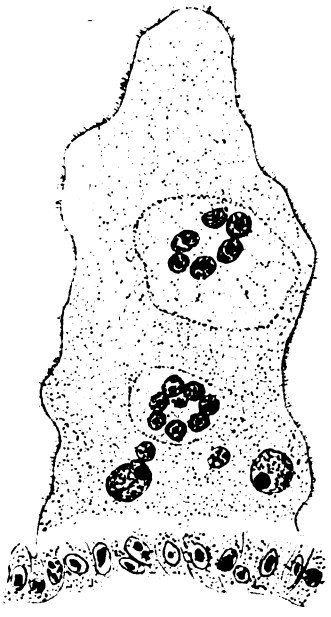

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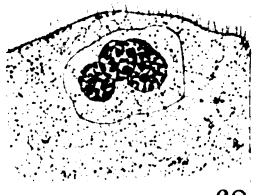

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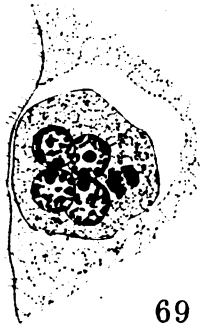

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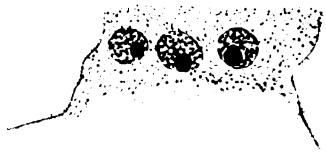

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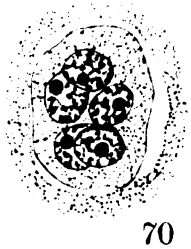

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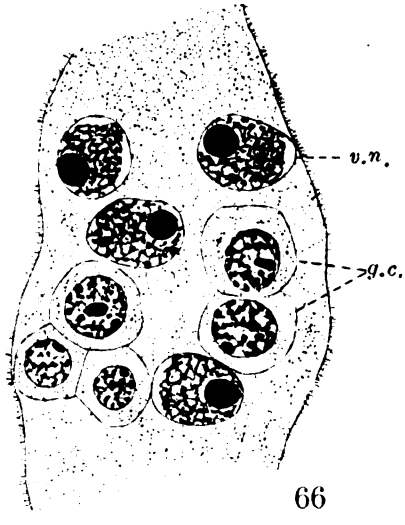

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PLATE 6

EXPLANATION OF FIGURES

67 Section through small portion of trophozoite showing a number of generative cells, two gemmules and one vegetative nucleus.

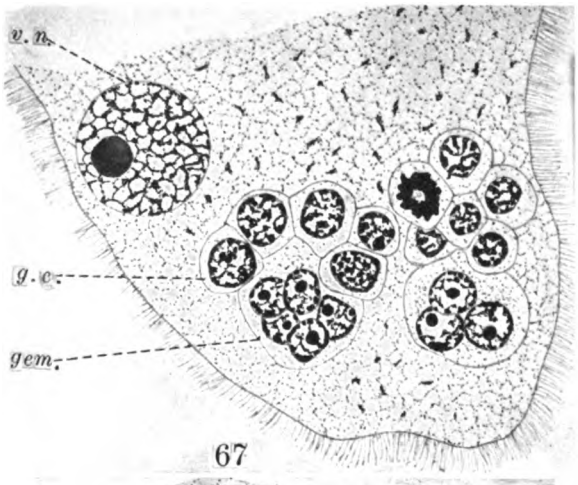
71 Portion of trophozoite showing a number of gemmules. Drawn from a dried smear stained by Giemsa's method. × 1400.

72 and 73 Gemmules which have recently emerged from the mother trophozoite. Note that four of the nuclei are becoming enlarged to form vegetative nuclei.

74 Section through gemmule which has been retained in the mother trophozoite longer than usual. The nuclei are already differentiated into vegetative and generative nuclei, the latter being inclosed in distinct cells.

75 Similar to figure 74 except that the gemmule is in a somewhat later stage of development.

76 Portion of trophozoite showing several pansporoblasts. All the nuclei are inclosed in distinct cells. Only part of the cells are shown.



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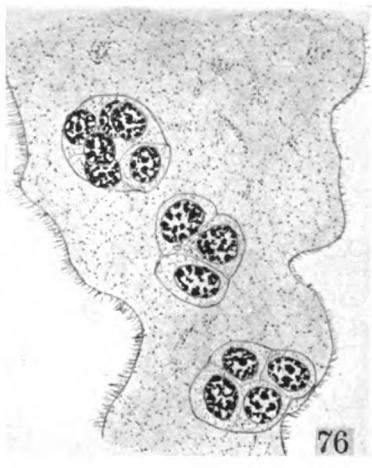

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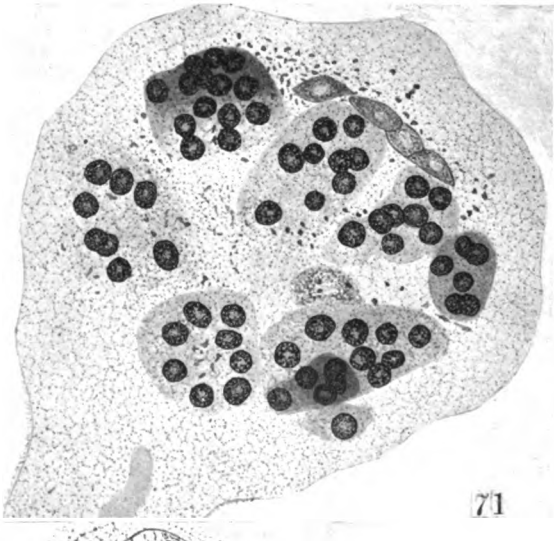

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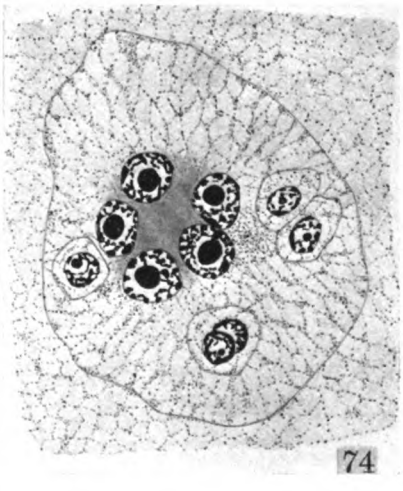

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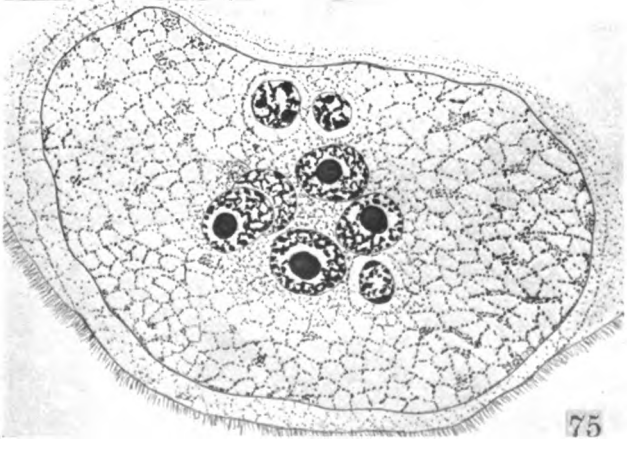

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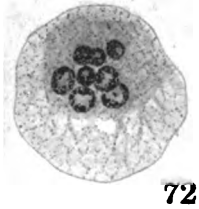

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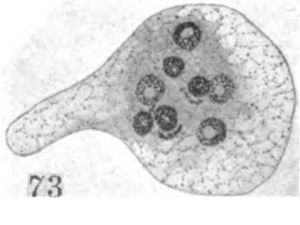

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PLATE 7

EXPLANATION OF FIGURES

77 Portion of a trophozoite showing parts of several pansporoblasts and one gemmule.

78 Portion of trophozoite containing a pansporoblast. There are only eleven nuclei in the section the twelfth being in the adjoining section.

79 Portion of trophozoite containing parts of two pansporoblasts. Note that the cytoplasm of the pansporoblasts stain deeply while the cell outlines are very difficult to distinguish.

80 Nearly mature spore. All the nuclei in the spore are shown.

81 and 82 Each figure shows two spores formed from the same pansporoblast. Slightly later stage than in figure 80. All the nuclei are shown.

83 Portion of sporulating trophozoite in which the cells of the pansporoblast have become definitely arranged to form spores. Drawn from a dried smear stained by Giemsa's method. × 1400.

84 to 89 Different stages in sporoblast cells of the polysporous form.

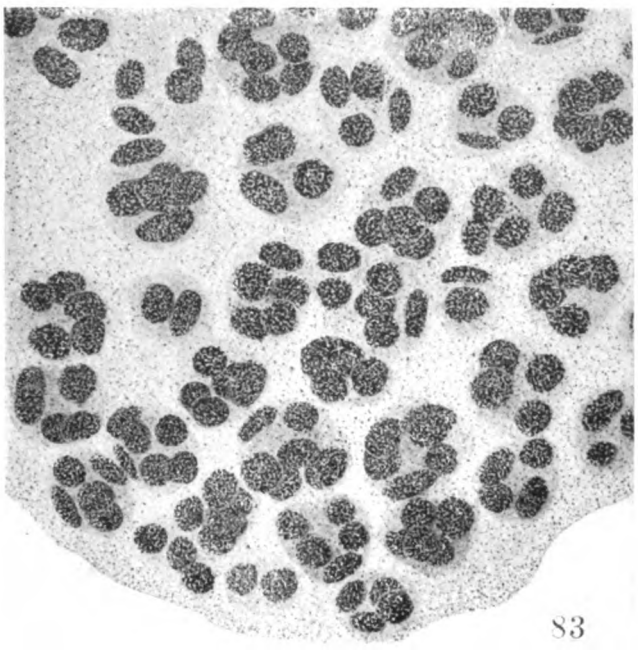
84 Prophase. × 2650.

85 Side view of metaphase. × 2650.

86 and 87 Polar views of equatorial plates showing six chromosomes in each case. × 2650.

88 Anaphase. × 2650.

89 Telophase. × 2650.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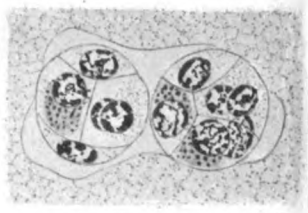
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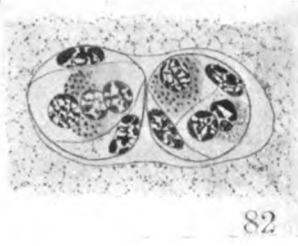
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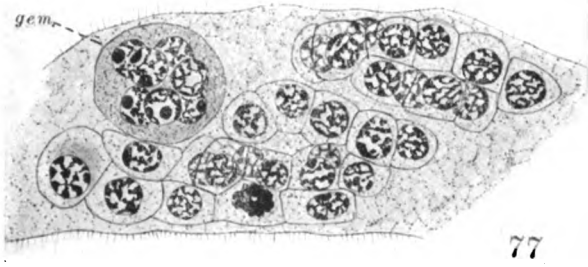

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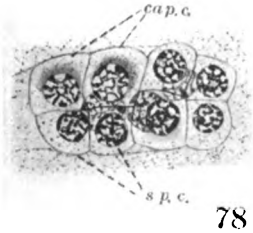

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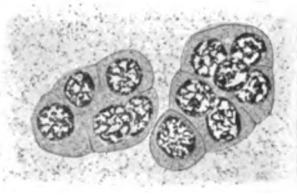
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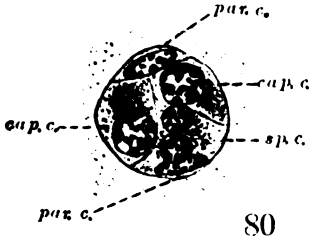

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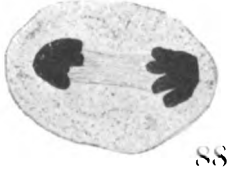
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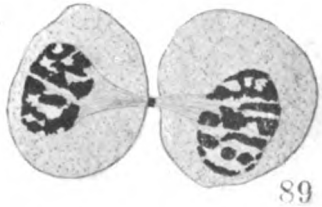
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