On *Myxosoma catostomi* Kudo 1923, a myxosporidian parasite of the sucker, *Catostomus commersonii*[1].

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(With Plates 3—5.)

Contents.

Introduction.

Upon an examination of the literature on the development of the Myxosporidia, one would be impressed by finding a considerable

[1]) Contribution from the Zoological Laboratory of the University of Illinois. No. 282.

diversity of opinion among numerous workers concerning various phases of development, such as the behavior of the two nuclei in the sporoplasm prior to germination, the origin of the pansporoblast, the nature and significance of the deeply staining rounded bodies which appear in the developing sporoblasts, and so forth. This condition still exists although additional information has recently been supplied by Kudo (1922, 1923), Bremer (1922), Davis (1923), Debaisieux (1924, 1925) and Dunkerly (1925).

Developmental stages of *Myxobolus toyamai* which were the subject of two of my former papers (Kudo, 1915, 1917), were small both in number and in variety, and the observations were, consequently, far from being complete. In 1920 I studied the morphology and development of another myxosporidian, *Leptotheca ohlmacheri*, parasitic in the uriniferous tubules of the kidneys of frogs and observed certain interesting facts (Kudo, 1922), which led me to renew the study of tissue-infecting Myxosporidia. Under comparatively favorable conditions, I have had an opportunity to study a new tissue-infecting myxosporidian, *Myxosoma catostomi* Kudo, 1923, from a sucker, *Catostomus commersonii*. The work was completed early in 1922 and a brief summary of it published thereafter (Kudo, 1923). Since the host fish had been dead (although as I will state below apparently for a short time) before its fixation, it was feared that the developmental stages of the myxosporidian may have undergone degeneration and the findings were abnormal; hence the manuscript was put aside with the hope of completing it by studying the protozoan in life and in material fixed with different methods. The organism has, however, not been seen since. The manuscript has, therefore, been rewritten partly with additional reference to more recent publications which have appeared since 1922 and is published here in order to bring to light points of interest observed in the myxosporidian in question.

Material and methods.

The infected fish, *Catostomus commersonii*, was found dead on the shore of Douglas Lake, Michigan, in the vicinity of the University of Michigan Biological Station, in July, 1921 [1]). The sucker,

[1]) Thanks are due to Doctor H. J. VanCleave of the University who found the fish, and preserved and placed it at my disposal.

which had apparently not been dead long before its preservation as judged by the conditions of the tissues found in section preparations, attracted the attention of the collector by virtue of a conspicuous tumor which it exhibited near the pectoral fin on the left side of the body. The body was cut into two at a point just back of the tumor and the anterior portion containing the tumor was preserved in a mixture of 75 per cent alcohol, corrosive sublimate and acetic acid. The fixation was fairly good, and so allowed more or less satisfactory cytological studies.

Under the circumstances, studies of the myxosporidian in the fresh condition were impossible. A large number of smears and section preparations of the tumor, the part surrounding it, and the apparently uninfected part of the body were prepared. Besides the staining methods which I had used in my previous studies, DOBELL's (1914) alcoholic hematein method was used with good results. The time of staining was, however, made a little longer than that given by DOBELL for the amoebae, as the myxosporidian spores were somewhat harder to stain. I have kept the preparations for about thirty minutes to one hour in each of the two solutions. Recently I have examined some of the preparations mounted in ordinary Canada balsam, and found that the majority after five years still showed beautiful and distinct pictures. It may be worth stating here that 1 per cent solution of potassium hydrate, if added to smears containing preserved spores, brings out both the polar filament and spore membrane most distinctly. Ordinarily when the spores are treated with a fixative constaining sublimate, they do not extrude the filaments under the influence of mechanical pressure or of chemical reagents such as potassium hydrate. It is, however, possible for one to count the number of coils of the filament in each of the polar capsules, to measure the dimensions of each coil and further to calculate its approximate length, when the spores are treated with a solution of potassium hydrate (Fig. 112).

The tumor and the seat of infection.

The sucker measured about 18 cm in length[1]). On the left side of the body just posterior to and above the pectoral fin, a con-

[1]) This dimension is estimated by a comparison of the photograph of the entire body and the preserved part of the fish.

spicuous tumor was present (Fig. 1). The tumor, semispherical in shape, measured approximately 20 mm in diameter at its base and protruded for about 12 mm above the general surface of the body. The natural conditions of the tumor could not be studied, since the specimen was preserved in a rather small bottle and the tumor was very much flattened as compared with the state before preservation.

When a portion of the tumor was examined in alcohol, numerous spores and spore-filled parasitic masses were noted, which suggested that the growth was due to a myxosporidian infection. The parasitic masses were somewhat irregularly shaped, although mostly more or less rounded, and varied considerably in size, measuring from 50 to 350 μ in the largest diameter. Under a low magnification, each individual showed two regions, the peripheral and the spore-filled central portions, and was surrounded by a somewhat firm envelope composed apparently of the connective tissue of the host.

In section preparations, it was found that the tumor, instead of being composed of a single or a few individuals of the myxosporidian, consisted of an enormous number of the parasites which occupied the muscle fibers of the region (Figs. 2, 3). Each individual was found in the connective tissue of the muscle fiber and contained spores, developing pansporoblasts and nuclei of different form (Figs. 4, 5). The tumor contained exclusively sporulating trophozoites.

Unfortunately, the preserved specimen was very small and did not contain the visceral organs. Therefore the exact distribution of the parasite in the host body could not be determined. In the preserved portion of the body, the muscle was found to be the seat of infection. Just exactly what was the cause of the concentrated infection at this particular part of the body is not known as in numerous other species of tissue-inhabiting Myxosporidia which have been found to form cysts in the gills, fins and different parts of the subcutaneous tissues of varions fish, although the infection has always been believed to have occurred through the alimentary canal (Kudo, 1920).

The vegetative form.

The cytoplasm.

As was mentioned above, each individual of the myxosporidian was bound by the connective tissue of the host (Figs. 2—5). Its

outer surface is smooth and in direct contact with the host tissue. There is a peripheral layer of densely granulated cytoplasm, while the remainder of the body is coarsely vacuolated, a condition which has been noted in many species of tissue-infecting Myxosporidia I have studied previously. In some individuals there was seen a peripheral layer which showed radial striations (Fig. 5). I was first inclined to think that they were remains of peripheral plates of the muscle fiber of the host; but a comparison with control preparations showed that this was apparently not the case, and the striated protoplasm belonged to the myxosporidian itself. Somewhat similar conditions were observed in *Myxobolus toyamai* (KUDO, 1915, 1917), in *Myxobolus pfeifferi* (THÉLOHAN, 1895; KEYSSELITZ, 1908), in *Myxobolus gigas* (AUERBACH, 1906), in *Henneguya acerinae* (SCHRÖDER, 1906), in *Lentospora ovalis* (DAVIS, 1923) and in *Myxobolus notatus* (DEBAISIEUX, 1925).

The nucleus.

The vegetative nucleus.

Scattered throughout and particularly in the peripheral zone of the body, there are found the vegetative nuclei (Figs. 4—30). They are circular or oval in shape and measure from 3·5 to 6 μ in diameter. The delicate membrane is distinctly visible and small chromatin granules are attached to it. A few chromatin granules are distribudet over the nodes of the fine achromatin network which penetrates through the nucleus filled with nuclear sap. A large nucleolus is almost always present eccentrically. This is ordinarily rounded (Figs. 5, 6), but may be of different form (Figs. 6, 8). The nucleolus is mainly composed of plastin material and for this reason I call it a plasmosome or true nucleolus [1]). Typically the plasmosome is mixed with a karyosome which may be a rounded body in the central part of or may form a thin coat for the former (Figs. 8, 6, 28). The separation of these two nuclear components becomes more pronounced at the time of nuclear division, particularly when it divides into a vegetative and a generative nucleus. The greater part of the plasmosome seems to remain in the vegetative nucleus.

The vegetative nucleus multiplies by amitosis. The nucleolus becomes elongated (Fig. 6) and divides into two (Figs. 10—12) which may (Fig. 10) or may not (Figs. 11—13) be of equal size. These

[1]) I follow WILSON (1925) in using the terminology of plasmosome or true nucleolus and karyosome or chromatin nucleolus.

nucleoli become separated from each other, together with a portion of the scattered chromatin granules and achromatin network (Fig. 12). In the mean time the entire nucleus becomes elongated (Fig. 7). A constriction starts to form at the middle part (Fig. 18), which becomes deeper and deeper (Figs. 19, 20) and the nucleus divides finally into two (Figs. 21, 22). Another type of amitotic division is frequently met with. In this the nucleus divides by the formation of a transverse partition which cuts the mass into two (Figs. 6, 13—17, 21). Ordinarily a vegetative nucleus produces two vegetative nuclei of same or of dissimilar size.

The vegetative nucleus gives rise by division further to the generative nucleus. In this division the chromatin substance and plastin material become completely separated from each other into distinct masses (Figs. 10, 11). One half of the daughter nuclei receives more chromatin and less plastin (a generative nucleus), while the other half less chromatin and a large portion of plastin (a vegetative nucleus) (Figs. 10, 11, 19, 23). This division is also amitotic. The divisions of the vegetative nuclei as described here are abundantly seen in the present myxosporidian and are in striking contrast to the condition found in *Myxobolus toyamai* in which division stages of vegetative nuclei were absent in its cysts.

Earlier workers on tissue-infecting Myxosporidia, such as Thélohan (1895) and Doflein (1898), noted nuclei of variable size and appearance in the peripheral portion of large trophozoites or cysts. However, they did, not distinguish clearly the vegetative nuclei from the others. Keysselitz (1908) differentiated properly vegetative or somatic nuclei from generative or propagative nuclei in *Myxobulus pfeifferi*. He, however, did not see division of the somatic nuclei, stating „Kernteilung [of vegetative nuclei] habe ich in keinem Fall beobachten können". As to the formation of the generative nuclei from the vegetative, he writes as follows: „Die Entwicklung scheint sich demnach in der Weise zu vollziehen, daß der Kern der Copula eine Anzahl vegetative Kerne bildet. Dieselben besitzen als indifferente Nuclei gleiche prospektive Potenz. Bei der Propagationszellbildung werden nun nicht sämtliche Kerne direkt aufgebraucht, sondern nur eine Anzahl derselben differenziert sich durch Anreicherung des Chromatins und Sonderung des umgebenden Plasmas zu generativen Zellen." Keysselitz holds that the division of the vegetative nucleus and formation of generative nuclei are limited to a period during the early trophic life of the myxosporidian, stating that „er ist nicht mehr fähig neue vegetativen Zellen aus

sich hervorgehen zu lassen oder weitere Propagationszellen zu bilden". DAVIS (1923) seems to agree with KEYSSELITZ's view.

In Myxosporidia living in organ cavities of the host body, the vegetative nuclei are comporatively small in number due to the small size of the trophozoites. In *Leptotheca ohlmacheri* (KUDO, 1922), I have noticed the nucleus of a uninucleate amoebula divides into two, one of which becomes the vegetative nucleus and the other the generative. Although the generative nucleus divides repeatedly in the following stages of development, the vegetative nucleus remains ordinarily undivided. But after a certain period, the vegetative nucleus divides once and gives rise to another generative nucleus which by further divisions forms a trinucleate gemmule. AWERINZEW (1909) saw a mitotic division of the vegetative nucleus of a binucleate trophozoite of *Ceratomyxa drepanopsettae*. In other forms, such as *C. herouardi*, GEORGÉVITCH (1917) figures trophozoites with a number of vegetative nuclei, but the division ist not shown distinctly. In the polysporous form of *Sinuolinea* (*Sphaerospora*) *dimorpha*, DAVIS (1916) states that there are several vegetative nuclei, but their division is not well worked ont. In the disporous form of the same species, however, DAVIS, describes a type of amitosis which is somewhat similar to the one observed in the present myxosporidian.

ERDMANN (1917), in her second study of *Chloromyxum leydigi*, makes a statement to the effect that the vegetative nucleus divides by amitosis into two generative nuclei: „Bei *Chloromyxum leydigi* geht die bedeutsame Umwandlung der vegetativen Kerne in generative Kerne auf folgende Weise vor sich. Der Kern zeigt nicht mehr ein deutliches Caryosom und Außenchromatin, sondern ist, wie ich schon früher gesagt habe, umsponnen von zarten chromatischen Fäden. Sobald dieser Vorgang das Kerninnere verdeckt, grenzt sich dieser Kern mit einem kleinen Plasmasaum von seinem Mutterboden ab. Ein wenig später findet man Kerne, die zwei Caryosome haben, und nach kurzer Zeit entstehen zwei Zellen, die einen deutlich netzartigen Kern haben."

Recent investigators agree in that they have not seen division of vegetative nuclei, although they admit seeing a large number of them in the body of the species of Myxosporidia they studied. In his study on *Myxidium Lieberkühni*, BREMER (1922) writes „über die Vermehrung derartige Kerne kann ich nicht Sicheres aussagen" and „einwandfreie Kernteilungsbilder habe ich nicht gefunden". DAVIS (1923) stated simply for *Lentospora ovalis* "there is some evidence that they [vegetative nuclei] multiply by amitosis", without

giving any further description or figures of the process. Debaisieux (1924) does not make any definite statement on this point in his study of *Spharomyxa sabrazesi*. In his more recent paper on *Myxobolus notatus* he (1925) holds a view that some of the students of Myxosporidia had been using too ill-defined and heterogeneous terms in describing various stages of the sporozoans. With this I am in perfect accord; yet Debaisieux himself introduces in that paper "noyaux nus" as the synonym of "noyaux végétatifs". Of the vegetative nuclei of *Myxobolus notatus*, Debaisieux writes "les dessins 9 et 10 que nous croyons pouvoir interpréter comme représentant des amas en formation, permettent de supposer que les noyaux nus des amas naissant par des bipartitions successives, par des espèces d'amitosis", and further "les quelques aspects à allure mitotique que nous avons exceptionnellement observés sont trop rares et trop peu concordants pour que nous puissions y voir autre chose que des aspects plus ou moins artificiels". This author appears to have seen numerous cases of the division of vegetative nuclei which he interpreted as stages in karyogamy instead of those of nuclear division, of which a discussion is given elsewhere.

Dunkerly (1925) failed to observe the multiplication process of what he called "very large (vegetative?)" nuclei in *Agarella gracilis*, although he stated "the chief interest of *Agarella* is the apparent clearness of the stages in the development of pansporoblast and spore".

As was described above, in *Myxosoma catostomi*, the vegetative nuclei which doubtlessly become increased in number by divisions of a single nucleus of the amoebula stage, grow and multiply as the body becomes larger, giving rise to both vegetative and generative nuclei.

The generative nucleus.

The generative nucleus is easily distinguishable from the vegetative nucleus by its appearance and by an island of cytoplasm which surrounds it (Fig. 35). It is usually smaller than a vegetative nucleus, measuring 2·5 to 3·5 μ in diameter. It has a thick distinct membrane and the achromatin network penetrates through the nuclear sap which fills the nucleus. Chromatin granules are numerous and attached very densely to the membrane and to the nodes of the network. Ordinarily a somewhat large nucleolus, rich in chromatin substance and poor in plasmosome is located eccentrically in each nucleus and there is a clear zone around it. The island of cytoplasm

that surrounds the nucleus is reticulated and well defined peripherally, although it has no definite membrane.

The generative nucleus multiplies by division. The chromatin granules become connected with one another over the network and prominent (Fig. 36). This stage is followed by spireme stages (Figs. 37—40). In its earlier phase the spireme seems to wind around through the nuclear sap so that one may get an impression that the thread is more than one in number (Fig. 37). At the time the nuclear membrane disappears completely, it is, however, clearly seen that it is a single thread which thickens and shortens as the division advances. The spireme breaks up into four chromosomes (Figs. 41, 42) and each seems to divide into two in some manner unknown to me. In anaphase four chromosomes are grouped around the opposite ends of the cell (Figs. 43, 44). During the early part of these changes, the nucleolus seems to remain unconcerned (Figs. 36, 37, 41), but later becomes decreased in size (Fig. 42) or completely invisible (Fig. 43). No spindle fibers such as I observed in a microsporidian, *Thelohania legeri* (KUDO, 1923 a) or in a flagellate, *Lophomonas blattarum* (KUDO, 1926) are to be seen. In the later stages of nuclear division, there is, however, a slightly staining elongated structure running along the axis of division (Figs. 43, 44), which persists during the telophase (Figs. 45—48) and sometimes even after the complete reconstruction of the daughter nuclei (Fig. 49). When reconstructed, the daughter nuclei show the structure found in the mother nucleus before division. These stages are abundantly observed and the interpretation of different stages was more reasonably, and satisfactorily done than that of the stages found in a small number in cysts of *Myxobolus toyamai* (KUDO 1915, 1917), which were similar to the present form.

The two daughter generative nuclei become in most cases entirely independent from each other before further changes take place in them. In some cases, however, these nuclei divide once more before complete cytoplasmic separation, thus forming three (Figs. 51, 52) to numerous generative nuclei, each of which is enclosed in an island of cytoplasm. They may remain in groups of several individuals or scatter themselves throughout the outer portion of the body.

Mitotic division of generative nuclei has been observed in Myxosporidia living in organ cavities as well as in tissues of the hosts. Some observers described a typical mitosis in which centrosomes, spindle fibers and chromosomes occurred, which are considered as due to either erroneous observation or schematic presentation at

the present time. More recent observers such as Bremer (1922), Davis (1923), Debaisieux (1924, 1925) have not seen definitely a structure which may be compared with the centrosome.

With reference to the number of chromosomes which appear during the division, four were observed in *Myxobolus pfeifferi* (Keysselitz, 1908; Mercier, 1909), in *Henneguya gigantea* (Georgévitch, 1914), in *Myxobolus toyamai* (Kudo, 1915), in *Myxidium gadi* (Georgévitch, 1919), in *Myxobolus swellengrebeli* (Schuurmans Stekhoven, 1919), in *Myxobolus destruens* (Schuurmans Stekhoven, 1920) in *Myxidium lieberkühni* (Bremer, 1922) and in the present species, while six chromosomes were reported to occur in *Sinuolinea* (*Sphaerospora*) *dimorpha* (Davis, 1916), in *Sphaeromyxa sabrazesi* (Debaisieux, 1924) and in *Myxobolus notatus* (Debaisieux, 1925). The observations on the nuclear division in the present species do not give me sufficient data to consider in detail the findings of other workers.

The pansporoblast and development of the spore.

The generative nucleus becomes large and changes take place in it. The nucleolus becomes very large and the chromatin granules appear to increase both in number and in size. The latter are either attached to the membrane or to the nodes of the achromatin network. Such a nucleus measures 3·6 to 4 μ in diameter and the island of cytoplasm in which it lies measures 5 to 8 μ in diameter (Figs. 53, 54).

A similar body was observed by Thélohan (1895) in the endoplasm of *Myxobolus pfeifferi*, and this worker named it "sphère primitive". Gurley (1893) coined the term "pansporoblast" for this body, which has been properly used by several investigators. How the pansporoblast is formed? In order to solve this problem, students of Myxosporidia have spent much time on observations and on the interpretation of the observed facts. A brief historical review of opinions of several investigators will not be unnecessary if one is to understand clearly the present state of our knowledge on this point.

Thélohan (1895) considered the above-mentioned uninucleate body as the pansporoblast. Doflein (1898) seems to have agreed with this view. In *Myxobolus pfeifferi*, on which Thélohan made his obervations, Mercier (1906, 1909) distinguished two kinds of generative cells of unequal size, each containing a nucleus of a different character, which he called the macrogamete and the microgamete. Anisogamy takes place and a zygote is formed. Mercier

held this zygote to be a pansporoblast. SCHRÖDER, in his study of *Sphaeromyxa sabrazesi* (1907), found first „durch Zusammentreten eines großen und eines kleinen Kernes entsteht Pansporoblast, dessen Kerne sich bis auf vierzehn vermehren", but later (1910) expressed a view similar to that of KEYSSELITZ stated below. DEBAISIEUX (1924) worked on this myxosporidian and was inclined to think that the pansporoblast is formed by an association of two binucleate forms (which were originally uninucleate), such as shown in figure 60 which he called „sporocytes".

AWERINZEW (1907, 1909) thinks that in *Ceratomyxa drepanopsettae*, there are two vegetative and two generative nuclei in a young trophozoite; from the latter are differentiated two microgametes and two macrogametes; anisogamy takes place and two zygotes are produced, from each of which a spore develops. KEYSSELITZ (1908), by studying *Myxobolus pfeifferi*, came to the conclusion that „zwei Gametoblasten legen sich nun aneinander, ohne zu verschmelzen. Dagegen vereinigen sich die beiden kleinen Zellen und bilden eine dünne dichtanliegende Hülle um ihre Mutterzellen". For *Chloromyxum leydigi*, ERDMANN (1917 a) states "the nuclei with small cytoplasmic bodies approach each other and each cell divides up into a small and a big cell. The two small cells draw out in length and surround the two big ones, in this manner separating them from the other cells in the island. This quadruple group, two big cells and two small ones, is the starting point for the formation of the whole spore." LO GIUDICE (1912), in *Myxobolus ellipsoides*, holds similarly that the pansporoblast is formed by the union of two generative cells. Each cell buds off a small nucleus before the union, the nuclei remaining ununited. AUERBACH (1912) states that in *Myxidium bergense* the pansporoblast is formed by the union of a microgamete and a macrogamete, but without fusion of the two nuclei. The binucleate body thus formed was the sporoblast mother-cell. I held a similar view in *Myxobolus toyamai*. PARISI (1913) figures in his study on *Mitraspora (Sphaerospora) caudata*, stages in the union of a microgamete and a macrogamete, further coupling of the two binucleate cells and fusion of the two nuclei.

Of disporous forms living in the organ cavities of the host there have recently been three students who, contrary to the view advanced by AWERINZEW (1909) for *Ceratomyxa drepanopsettae*, agree, in general, regarding the formation of the sporoblast mother-cell. DAVIS (1916) in *Sinuolinea (Sphaerospora) dimorpha*, STEMPELL in *Ceratomyxa (Leptotheca) coris*, and myself (1922) in *Leptotheca ohlmacheri*

found that the nucleus of the uninucleate amoebula divides into three, one of which remains as a vegetative nucleus, while each of the remaining two develops into a spore. Schuurmans Stekhoven (1919, 1920) in *Myxobolus swellengrebeli* and *M. destruens* and Davis (1923) in *Lentospora ovalis* found that the pansporoblast is formed in a way similar to that as described for the present species.

On the other hand, in *Agarella gracilis*, a tissue-infecting species, Dunkerly (1925) supposed that a large cell with a large nucleus ("megalocyte") and a small cell with a small nucleus ("microcyte") become "associated in pairs" and two of these binucleated forms fuse into a group of four nuclei and become a pansporoblast. In regard to the formation of the propagative cell in *Myxobolus notatus*, Debaisieux (1925) writes as follows: „Les noyaux végétatifs sont très souvent groupés deux à deux et les couples qu'ils forment et qui ne sont pas attribuables à une rencontre fortuite, ne peuvent que représenter une fin de division dont les noyaux filles resteraient longtemps accolés, ou bien une association tendant à la fusion. Cette dernière interprétation nous paraît devoir être admise: en effet, le contact entre les deux noyaux, loin d'indiquer une tendance à se pédiculiser, comme cela se produirait en cas de division, est large et aplati comme s'il y avait pression des deux noyaux l'un contre l'autre. L'accolement paraît évoluer en fusion des deux noyaux et les éléments à deux caryosomes qui en résultent s'entourent d'une zone distincte de protoplasme et se transforment en cellules propagatives." This is indeed a very interesting view if supported by facts. To strengthen his view, Debaisieux quotes his and also my observations on some Microsporidia in which karyogamy of two schizonts results in the formation of a zygote or sporont. In *Thélohania légeri* (Kudo, 1923a) and in *Stempellia magna* (Kudo, 1925), there is positive evidence that this is the case, such as the extrusion of chromatic granules from the nuclei, the general appearance of the nuclei undergoing the change, etc., but none to show them as stages of nuclear division. It seems doubtful at least in the face of data published by Debaisieux, that karyogamy among the vegetative nuclei occurs in *Myxobolus notatus*. For instance, Debaisieux's figures 70, 71, 74 and 94 which he interpreted as stages in division, show two nuclei, the manner of association of which is exactly like that present in figures 23 to 26 and 31 to 34 which were supposed by this author to be stages in karyogamy. Further in his figure 27, two karyosomes are connected with each other by a conspicuous strand, which is apparently a division stage of a karyosome. These

figures and their comparison with stages found in *Myxosoma catostomi* lead me to think that those stages of karyogamy in *Myxobolus notatus* are in reality stages in amitotic division of the vegetative nuclei as are found in the present form.

As to the formation of the pansporoblast, DEBAISIEUX writes "nous admettons que deux sporocystes s'associent pour former le groupe à quatre noyaux qui représente le plus jeune pansporoblaste". Thus aside from DEBAISIEUX and DUNKERLY, recent workers of Myxosporidia agree with THÉLOHAN as to the origin of the pansporoblast.

The nucleus of the pansporoblast (Figs. 53, 54) undergoes a division. At the beginning the chromatin granules form irregularly coiled threads inside the nuclear membrane (Fig. 55). As the threads become more conspicuous, the nuclear membrane disappears completely. The chromatic mass divides into two unequal portions still connected by a chromatic strand (Fig. 56). The strand becomes less stainable and broader and finally disappears; the two chromatic masses of unequal size become completely separated from each other (Figs. 57—61). Frequently fine fibrous connections are found between these two masses (Figs. 57—59, 61), but no typical spindle fibers or centrosomes are to be seen. By this heteropolar division, the nucleus of the pansporoblast divides into one small and one large nucleus (Figs. 60—62) which not infrequently become separated by cytoplasmic constriction (Fig. 62). In *Myxobolus toyamai*, I (1915, 1917) saw numerous binucleate forms such as shown here in figure 62; but division stages described above were small in number, which led me to interprete that the binucleate form was produced by an association of two uninucleate generative cells of dissimilar dimensions.

To study the nuclear changes in the developing pansporoblasts smears must be used extensively to obtain the exact conditions and the number of the nuclei that are present in them. Section preparations, which seem to have been used exclusively by some investigators of tissue-infecting Myxosporidia, are of course of great importance in order to determine the arrangement of the pansporoblasts at different phases of development as found in the host body, and to study the minute structure of the individual nucleus in its natural location. But the multinucleate stages about to be described are cut in sections, and consequently it is not easy and certain to reconstruct the exact conditions of all the nuclei in a given pansporoblast from different sections. On the other hand,

in well-stained smears, one may with clearness and certainty be able to make out the number of nuclei and somewhat complicated nuclear changes even in late stages such as shown in figures 96—98.

The two nuclei of the above-mentioned binucleate form undergo further divisions. The small nucleus (Figs. 61, 62) divides once intwo two equal daughter nuclei (Figs. 63—69). This division may take place before (Figs. 63—69) or after (Figs. 75—78) that of the large nucleus. In some cases the small and the large nucleus may divide simultaneously, as is shown in figures 79 and 80. The small nucleus may not divide at all, as is probably the case in the pansporoblast in which a single spore has been formed (Fig. 98), or one of the daughter nuclei may divide again into two. Thus one, two or three nuclei are produced from the original small nucleus. They remain separated from the daughter nuclei of the large one throughout the whole development of the pansporoblast, and are found later between the two developing sporoblasts (Figs. 95—97). When the spore formation advances, these nuclei lose their typical appearance: the nucleolus, chromatin granules and achromatin network become diffused inside the nuclear membrane and the entire nucleus becomes less conspicuous (Figs. 94—98).

Thélohan (1895) saw two nuclei similar to those mentioned above in the developing pansporoblast of *Myxobolus pfeifferi* and called them „noyaux du sporoblaste, entourés d'une petite masse de protoplasme n'ayant pas pris part à la formation des masses sporogènes". Doflein (1898) called them „Restkerne", which term has been used by numerous workers. Judging from their behavior during the development of the two spores in the pansporoblast, and from their degeneration at the time of completion of spore-formation, these nuclei, which are located outside the developing sporoblasts, are, I believe, the vegetative nuclei which control the growth of the pansporoblast.

The large nucleus of the binucleate pansporoblast divides (Figs. 69—74). This division takes place ordinarily in a plane at right angles to the line connecting the two nuclei before the division. When completely divided, the nuclei are usually seen at opposite ends, as shown in figures 77 and 78. As their later development reveals, each one of the two is the nucleus which forms the nuclei of the sporoblast. The nuclei may divide simultaneously (Figs. 82, 85) or at different times (Fig. 84). Thus at the end of the second division, four equally large nuclei are produced (Fig. 86). Further divisions of these nuclei have not been seen to take place simultane-

ously. One therefore sees different stages containing four to twelve nuclei, excluding the vegetative nuclei, in a pansporoblast.

As the nuclei of a pansporoblast continue to divide, there appear small rounded bodies (Figs. 90, 93, 94, 95). These bodies stain deeply with HEIDENHAIN's iron hematoxylin, but less deeply with DOBELL's stain. They are more easily decolorized than chromatin granules. They seem to be fairly uniform in size. At the same time, the nucleolus which showed its typical appearance during the early part of the nuclear changes becomes very much smaller and compact. In DOBELL preparations nuclear changes such as shown in figures 90 and 93 are abundantly found. In figure 93, one distinguishes at least seven nuclei without a conspicuous nucleolus, five with a more or less large nucleolus and five extranuclear small rounded bodies which stain uniformly. In one of the nuclei shown in figure 81 and in four of those in figure 95, the nucleolus is located close to the membrane where the latter seems to be discontinuous. The best explanation of such conditions is that these nuclei are throwing off their nucleoli into the surrounding cytoplasm.

Similar bodies appearing in pansporoblasts of many other species of Myxosporidia have been reported in *Sphaeromyxa sabrazesi* (SCHRÖDER, 1907), in *Myxobolus pfeifferi* (KEYSSELITZ, 1908; MERCIER, 1909), in *Myxobolus ellipsoides* (LO GIUDICE, 1912), in *Myxobolus toyamai* (KUDO, 1915, 1917), etc. No one of the authors, however, has seen extrusion of nucleoli at this stage. ERDMANN (1917) saw numerous small bodies darkly stained (by hematoxylin) in the developing spores of *Chloromyxum leydigi* and regarded them as glycogen bodies which are used in the formation of the spore membrane. I have already shown that in the case of *Leptotheca ohlmacheri* there are no recognizable glycogen bodies used for this purpose (KUDO, 1922). The same is true in *Myxosoma catostomi*. Of the similar bodies appearing in the pansporoblast of *Lentospora ovalis*, DAVIS (1923) stated that he was "unable to find any evidence that they are of nuclear origin". They may, however, be found to have an origin similar to the present form.

Conditions observed in figures 81 and 95, together with the marked decrease in the amount of plasmosome in the nuclei and the appearance of apparently homologous bodies in the cytoplasm of the pansporoblast, lead me to consider that nucleoli are extruded from the nuclei during the course of spore-formation. What is, then, the significance of this phenomenon? Are the nucleoli thrown out to reduce the nuclear material? I have no evidence to show that

this may be the case. The fact that these bodies are located near the periphery of the sporoblasts (Figs. 90, 93—95), that stages such as shown in figures 96 to 98 in which they are lodged inside the developing spore membrane, are often present and that they disappear when the spore has completely been formed (Figs. 101—108), lead me to conclude that the extruded nucleoli which are composed chiefly of plastin material, are used in the formation oft he spore membrane.

These bodies are to be distinguished from the small deeply staining bodies which appear in the general cytoplasm of the myxosporidian (Figs. 5, 7, 26—34). As to the origin of these bodies, I am inclined to think that they represent different stages of degenerating nuclei. Throughout the general cytoplasm of a vegetative form, there are seen certain nuclei whose structure is quite variable as are shown in figures 28 to 34 where they are apparently undergoing degeneration. These bodies also seem to originate in the degenerating nuclei of pansporoblasts (Figs. 99, 100). Some authours report similar bodies in the cytoplasm of the cyst of other Myxosporidia. Davis (1923) noticed them in *Lentosora ovalis* and called them "chromatoid bodies". Their form and size suggest that similar changes of some nuclei occur in that species also.

Two of the four large nuclei divide once and become located in the peripheral portion of the sporoblasts, each sporoblast containing two nuclei. They produce the four valves of the two spores (Figs. 95, 96). Each of the remaining nuclei divides twice, producing eight nuclei. Four of these form the polar capsules and the polar filaments and the other four become the nuclei of the two sporoplasms. Ordinarily two spores are formed in each pansporoblast. But only one spore may develop in a pansporoblast as shown in figure 98.

The spore.

In front view the spore is oval with ordinarily equally rounded extremities (Fig. 109); in profile it is spindleshaped (Fig. 110); while in end view it is broadly ovoid (Fig. 111). The spore membrane is usually uniformly thick, although it is frequently thickened in places, particularly around the posterior margin. The spore membrane is composed of two valves as usual and the latter meet in a sutural plane. The valves are, however, quite frequently dissimilar in size, which condition is shown in figure 111. The sutural

line is usually straight; but when the valves are asymmetrical, it becomes somewhat curved to one side. There is a ridge on each valve running along the sutural line (Figs. 110, 111). This is not parallel to the latter, but takes an undulating course in which it approaches the sutural line as six to eight triangular markings (Fig. 109). These markings, which I called "markings on sutural ridge", are found in many species of the family Myxosomatidae and Myxoboidae (KUDO, 1920). In the species belonging to other families there are found ridges the plan of which is very different from that in the first two families.

Apparently the ridges on the spore membrane of Myxosporidia have been disregarded as folds on the spore membrane of deformed spores, especially when the methods of fixation were thought by investigators to be unsatisfactory. But the ridges, such as are found in the present species, are entirely different from the folds that may be produced mechanically for instance by dessication. In the first place, these ridges are distinctly observable both in fresh and in preserved conditions; for instance, I noted this condition in the spores of *Myxobolus mesentericus* (KUDO, 1920). Secondly, they are regularly present in every spore of the species and their structure and form are uniformly the same. In the third place, they become very often detached from the spores when the latter are treated with 1 per cent solution of potassium hydrate.

The dimensions of fully formed spores vary little. Measurements of a large number of spores in unstained condition: length 13 to 15 μ, breadth 10 to 11·5 μ, thickness 8 to 8·5 μ, polar capsules 5 to 6 μ long by 2·5 to 3·3 μ broad, calculated length of the filament about 40 μ.

The anterior part of the spore is occupied by two pyriform polar capsules which are usually of same size and form (Figs. 109, 111). They are divergent and are connected with the foramina in the spore membrane at the anterior tip (Figs. 108, 109). The polar filament which is coiled usually six times in the capsule is indistinctly visible in an ordinary preserved spore. But when the spore is treated with a solution of potassium hydrate, the filament becomes conspicuously visible against the finely granulated sporoplasm. The polar filament, however, is not extruded from the spore either by mechanical pressure or by potassium hydrate.

The rest of the cavity of the spore is occupied by a comparatively large sporoplasm which is coarsely reticulated and usually contains two refractive rings, which upon staining are shown to be

the two nuclei. Very rarely the sporoplasm appears to contain three or four nuclei, an apparently abnormal condition. When the spore is treated with a solution of potassium hydrate, the sporoplasm becomes finely granulated (Fig. 112). The sporoplasm does not show any structure which can be stained with LUGOL's solution.

The behavior of the nuclei of the sporoplasm and the question of auto-infection.

Concerning the changes which the two nuclei of the sporoplasm undergo later, I have given a full discussion in one of my papers dealing with *Leptotheca ohlmacheri* (KUDO, 1922). As may be seen there, the observations of different authors agree in one point, i. e., the two nuclei in the sporoplasm fuse into one either before or after the emergence of the sporoplasm as an amoebula, before the latter starts its trophic life. SCHUURMANS STEKHOVEN (1919, 1920) opposes this conception after studying chiefly section preparations of *Myxobolus swellengrebeli* and *M. destruens*, and states that the binucleate sporoplasm divides into two uninucleate bodies and each starts a new development. Yet the figures which he gives for the first-named species suggest strongly that the fusion of the two nuclei of the sporoplasm probably occurs in that species also. He saw uninucleate young amoeboid bodies and considered them as degenerating forms. If, as he thinks, the degeneration of the two nuclei really takes place, it is difficult to understand the peculiar condition that the degeneration of the two nuclei takes place not separately, but after their fusion into one mass. Furthermore, if "copulation" of the two nuclei occurs for the purpose of degeneration, why did he not find stages in which the process had advanced further? In figures 36 and 37, SCHUURMANNS STEKHOVEN (1919) gives stages which he called dividing individuals. These figures show that the two nuclei are not in the "resting stage". This is a rather strange condition if reviewed from our general knowledge of cytology, i. e., if a cytoplasmic body containing two independent nuclei in the resting stage divides into two uninucleate bodies, the nuclei do not show any dividing figures. The interpretation of the figures under consideration advanced by SCHUURMANS STEKHOVEN does not seem to be acceptable. According to my view, these figures appear as two stages in the division of uninucleate body

into two. If we arrange his figures on the plate attached to his paper in the following order, figures 32, 31, 37 and 36, we obtain four successive stages in the division of an uninucleate amoebula into two uninucleate bodies.

Therefore, the objection expressed by SCHUURMANS STEKHOVEN concerning the fusion of the two nuclei prior to germination of the sporoplasm as an amoebula is without solid foundation and cannot be accepted. That the fusion of the nuclei of the sporoplasm occurs at the start of the trophicl ife of an amoebula has been studied in *Myxosoma catostomi.*

In the sporoplasm of the of *Myxosoma catostomi* found in the tumor, there are usually seen two nuclei closely associated with each other (Figs. 101—103, 105, 109), although on some occasions they may be somewhat separated (Fig. 104). Spores with uninucleate sporoplasm are also noticed (Figs. 106, 107) particularly in the connective tissue around the tumor, where one or two spores are lodged in a clear space surrounded by the host tissue. A similar condition was noted by THÉLOHAN (1895) in *Myxobolus pfeifferi* and was described by him under the name of "diffuse infiltration". In *Myxosoma catostomi*, the condition mentioned above seems to arise in the following way: when the body of a vegetative form breaks up in the tumor, the developing pansporoblasts and other stages would be carried away from the mother body in which they were undergoing development through the blood or lymph stream. While they migrate, sporulation advances and the spores are completely formed, becoming lodged in the host's connective tissue.

Uninucleate sporoplasm is frequently found in spores situated in such locations. I am inclined to think that such spores seem to receive more influence from the host body than those that develop within the tumor, since they are directly exposed to the body fluids of the host. On two occasions, I have observed in section a condition which I interpreted as the uninucleate sporoplasm just leaving the spore membrane. In one of these spores, I noticed that one of the polar capsules extruded its polar filament which wound around the spore membrane, while the other showed only its basal portion. The sporoplasm was seen leaving the spore near the anterior tip. Spores without sporoplasm were frequently found near the periphery of the tumor. From these observations, it seems probable that auto-infection takes place in the present species. The development of the newly emerged amoebulae could not be studied satisfactorily, but the amoebula grows in size accompanied by nuclear divisions.

The phenomenon of auto-infection through spores has been kown for a long time. Lieberkühn (1854), Pfeiffer (1891), Thélohan (1895), Georgévitch (1914) and Kudo (1920) saw probable cases of auto-infection in the Myxosporidia they studied. In the case of *Nosema bombycis*, a microsporidian, I suggested that auto-infection is probably present (Kudo, 1916).

It may be worth recording here that on several occassions, leucocytes of the host fish were seen to contain a spore (Fig. 113). In every case, it appeared that the leucocyte engulfed a mature spore and the latter was being digested by it. The myxosporidian spore found in a leucocyte was much smaller than a normal free spore and in the case which is figured here, the sporoplasm was no longer visible, the polar capsules being the only structure which remained apparently unattacked by the phagocyte. Here one finds a typical case of phagocytosis.

Identification of the myxosporidian.

Since the work of Thélohan (1892), it has generally been recognized that the sporoplasm of the spore of species belonging to family Myxobolidae contains an iodinophilous vacuole, while that of the spore of species belonging to family Myxosomatidae does not contain this structure. Aside from a difference in this particular respect, these two families are practically indistinguishable from each other, since the general appearance of the vegetative forms, spores and habitat, are similar. Little is known about the significance of the iodinophilous vacuole, although I attempted te consider its chemical nature previously (Kudo, 1921). Davis (1923) considers that" the presence or absence of a distinct iodophile vacuole is evidently of only secondary importance and of little value in determining the natural relationships of different species". In the family Myxobolidae which I have studied up to date I have not seen a single species in which mature spores of one and the same species showed both characters, that is to say, some spores containing an iodinophilous vacuole, while others did not possess this structure. Therefore I hold that families Myxosomatidae and Myxobolidae should be distinctly separated from each other at the present moment.

On the basis of the absence of an iodinophilous vacuole in the sporoplasm, the present species should be placed in the first-named

family. In this family two genera, *Myxosoma* (THÉLOHAN, 1892) and *Lentospora* (PLEHN, 1904), have been created. These two genera are essentially the same in spore characters and should be united into one, retaining THÉLOHAN's generic name [see the definitions and figures of the two genera in one of my papers (KUDO, 1920)].

Ten species have been recorded in this family. The present species appear to differ from any one of them. Recently DAVIS (1923) described *Lentospora ovalis* from the gills of *Ictiobus bubalus* and *I. cyprinella* from Iowa, U. S. A. This myxosporidian forms cysts in the gills of the host fish and has according to DAVIS, spores which measure in fresh condition, 15 to 17 μ long, about 15 μ broad, and about 11 μ thick. The polar capsules are 8 to 9 μ by 6 μ broad. As is usually the case there are some variations in size, the smallest measured spore was only 12 μ long by 11 μ broad and contained capsules which were 6 μ by 4 μ. The spores of *Myxosoma catostomi* were preserved before they came to my hand and dimensions in fresh state are unknown. In a previous paper I (1921 a) showed that spores of *Leptotheca ohlmacheri* decrease in size when fixed. Assuming that the loss of the dimensions of the spores of *Myxosoma* (*Lentospora*) *ovalis* due to fixation was the same as that of *Leptotheca ohlmacheri*, the former species will have the following dimensions when fixed: length 12,6 to 14,3 μ, breadth about 10,7 μ, polar capsules 6,6 to 7,5 μ by 5 μ. Thus these dimensions resemble greatly those of the present species. As stated before, one finds regularly in the spores of the present form a ridge on each side of the sutural plane which takes an undulating course, while DAVIS apparently did not see any ridges on the spores of the species he studied. The spore membrane of the spores of these two species is different in thickness and the number of coils of the filament in the polar capsules is also different; in the present species it is regularly six (Fig. 112), while in *Myxosoma* (*Lentospora*) *ovalis* mostly four or some five according to figures drawn by DAVIS who counted „five to six turns" of the „coiled filament". With reference to the habitat and vegetative stages, one finds great differences between them. Hence I hold that *Myxosoma catostomi* and *Myxosoma* (*Lentospora*) *ovalis* should be held as distinct species.

In the literature one reads descriptions of a number of species placed in the genus *Myxobolus*, in which the authors stated that the „vacuole" could not be found. Of these, comparison must be made with *Myxobolus oblongus* observed and described by GURLEY (1894) from chub suckers (*Catostomus tuberculatus, Erimyzon sucetta*

oblongus) from North Carolina and Mississippi. Although a satisfactory comparison between GURLEY's form and the present species cannot be made, due to GURLEY's inadequate description, the spores of the two species differ in several respects. Therefore, in 1923 I considered that the species described in this paper was not identical with any recorded form, and proposed to name it *Myxosoma catostomi*.

Summary.

1. A myxosporidian, *Myxosoma catostomi* KUDO 1923, parasitic in the muscle fiber and intermuscular connective tissue of the body muscle of *Catostomus commersonii*, is described. The myxosporidian forms a conspicuous tumor in the host body.

2. Each spore contains a single binucleate sporoplasm. Fusion of the nuclei probably takes place in the spore prior to the emergence of the sporoplasm as an amoebula.

3. In the cytoplasm of a vegetative form, there are two kinds of nuclei: vegetative and generative. The division of the vegetative nucleus is direct, by which it produces either kind.

4. The generative nucleus becomes surrounded by an island of cytoplasm and divides by mitosis. By growth, a single nucleus transforms itself into a pansporoblast. The nucleus of the pansporoblast undergoes a heteropolar division. The small nucleus thus formed divides into two and the latter become the vegetative nuclei of the pansporoblast. These two nuclei are analogous to the vegetative nucleus of disporous species. The large nucleus divides three times and produce eight nuclei. Four of the eight nuclei produce the four valves of the spores. Two of the remaining four nuclei divide once more, and the latter four form the polar capsules and the filaments. The remaining two, after dividing again, become the nuclei of the sporoplasms.

5. During the later development of pansporoblasts, the nuclei throw off their nucleoli into the cytoplasm.

6. Normally a pansporoblast develops into two spores, but rarely into one only.

7. Auto-infection by spores and phagocytosis are of probable occurrence.

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Explanation of Plates.

All the figures refer to *Myxosoma catostomi* and except figures 1 to 4, were drawn, by means of ABBE's drawing apparatus attached to one of the compensation oculars of a binocular microscope. Figures 5 to 113 were originally magnified 2300 times and were reduced in reproduction. They should be measured by the scales placed under figures 30 and 108. Abbreviations used are as follows: D, DOBELL's alcoholic iron hematein; G, GIEMSA's staining; H, HEIDENHAIN's iron hematoxylin; Sc, section preparation; Sm, smear preparation.

Plate 3.

Fig. 1. The host fish, *Catostomus commersonii*, photographed from above, shortly after its collection, exhibiting a conspicuous tumor on the left side of the body. × about 1/3.

Figs. 2, 3. Microphotographs of part of the tumor in section preparations. H. × about 350.

Fig. 4. A microphotograph of parts of three individuals of the myxosporidian. H. × about 1500.

Plate 4.

Fig. 5. A part of a vegetative form, showing the radially striated periphery of the body, nuclei and developing pansporoblasts. Sc. H.

Fig. 6. Vegetative nuclei. Sm. D.

Fig. 7. Part of a vegetative form, showing division stages of vegetative nuclei, a generative nucleus and two degenerating nuclei. Sc. H.

Fig. 8. A vegetative nucleus. Sm. D.

Fig. 9. A vegetative nucleus. Sc. H.

Figs. 10—25. Stages in division of vegetative nuclei. Figs. 10—15, 19, 20, 22—25, Sc. H.; Fig. 18, Sm. H.; others, Sm. D.

Fig. 26—34. Parts of different vegetative forms in which degenerating nuclei are found. Figs. 26—28, Sc. H.; others, Sm. H.

Fig. 35. A generative cell. Sc. H.

Fig. 36—52. Stages in divisions of generative cells. Figs. 36—38, 46, 48, 50, 52, Sc. H.; Figs. 40, 42, Sm. D.; others, Sm. H.

Fig. 53, 54. Pansporoblasts. Fig. 53, Sm. D.; Fig. 54, Sc. H.

Plate 5.

Figs. 55—62. Stages in a heteropolar division of pansporoblasts. Figs. 58, 61, Sm. H.; others, Sc. H.

Figs. 63—69. Stages in division of the smaller nucleus. Figs. 63, 64, 68, Sm. H.; others, Sc. H.

Figs. 70—74. Stages in division of the larger nucleus. Figs. 70, 72, Sm. H.; others, Sc. H.

Figs. 75—78. Stages in division of the smaller nucleus. Figs. 77, Sc. H.; others, Sm. H.

Figs. 79, 80. Stages in simultaneous divisions of the two nuclei shown in figures 60—62. Sm. H.

Fig. 81. A tetranucleate pansporoblast. Sc. H.

Figs. 82—94. Further developmental stages of pansporoblasts. Figs. 82, 83, 85, 91, Sm. H. Figs. 89, 90, 93, Sm. D.; others, Sc. H.

Figs. 95—97. Further developmental stages. Fig. 95, Sc. H.; others, Sm. D.

Fig. 98. Monosporoblastic spore. Sm. H.

Figs. 99, 100. Degenerating nuclei of pansporoblasts. Sc. H.

Fig. 101. A spore. Sm. H.

Fig. 102. A spore. Sm. G.

Fig. 103. A spore. Sm. D.

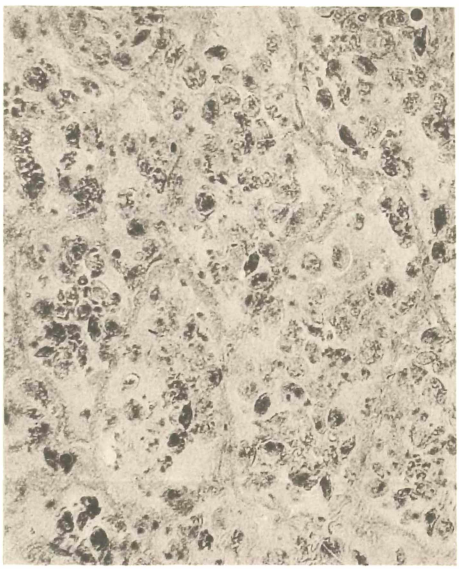
Figs. 104—107. Sections of spores. Sc. H.

Fig. 108. A spore. Sm. H.

Figs. 109—111. Different views of preserved, unstained spores as seen in water.

Fig. 112. A preserved spore treated with 1 per cent solution of potassium hydrate.

Fig. 113. A leucocyte of the host fish with an engulfed spore. Sc. H.



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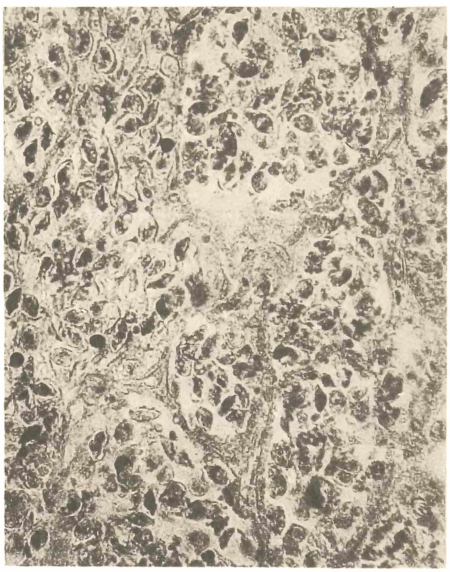

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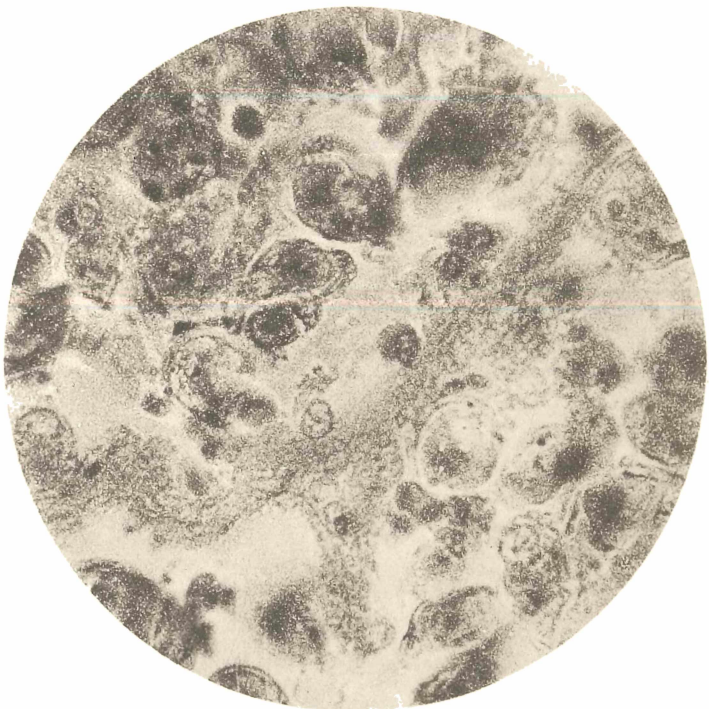

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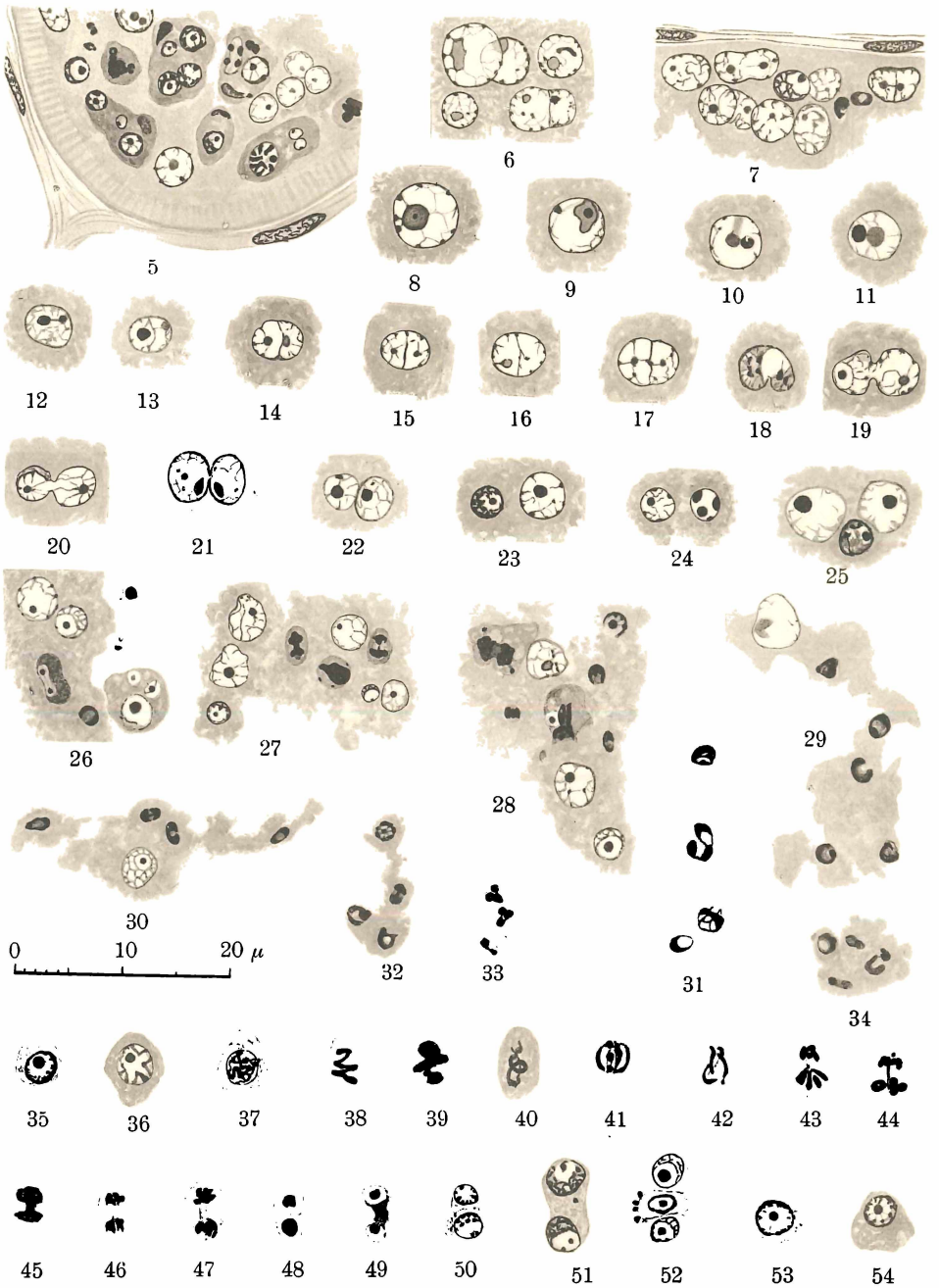

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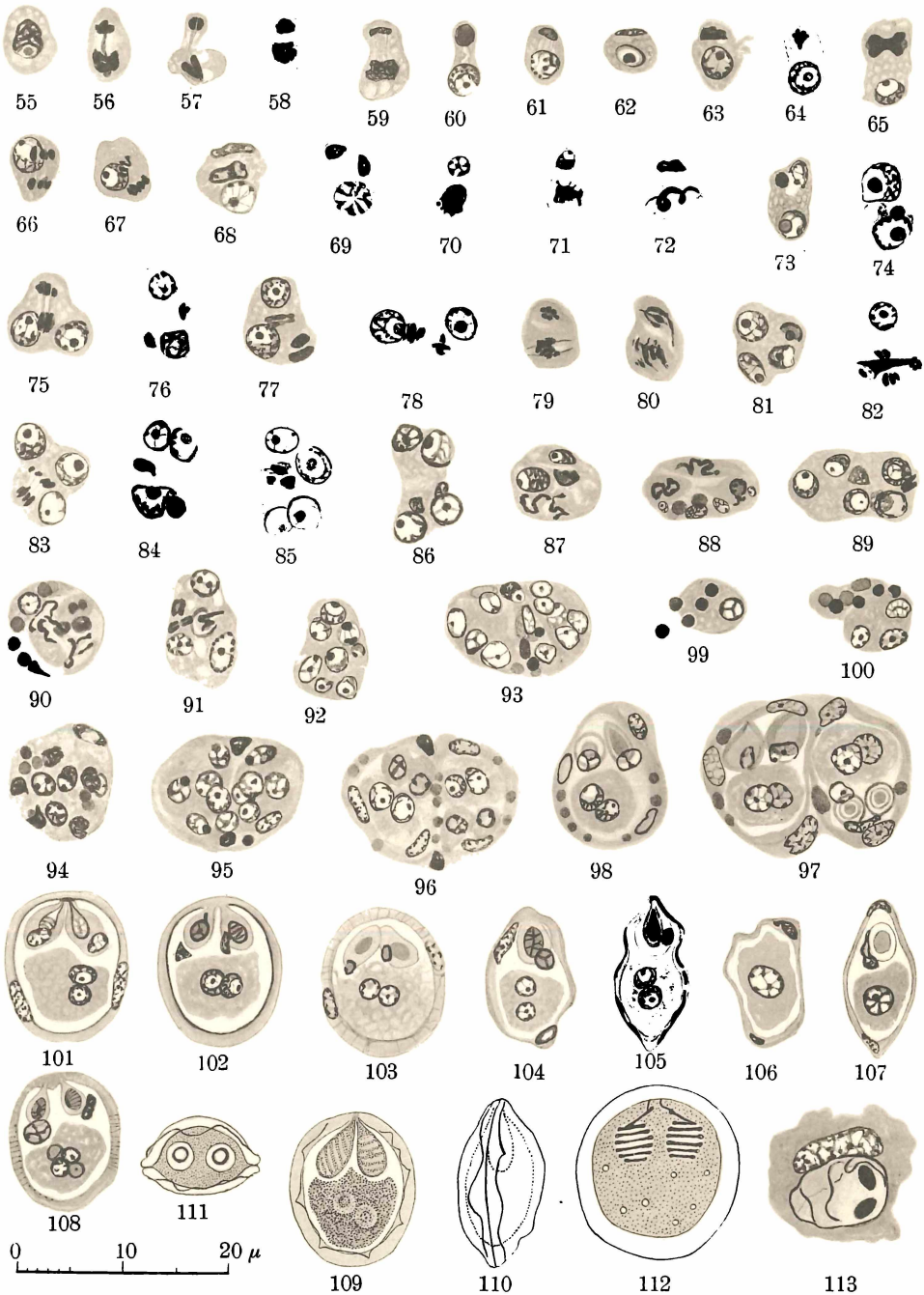

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