ON THE SPOROZOON PARASITES OF THE FISHES OF WOODS HOLE AND VICINITY

II. ADDITIONAL OBSERVATIONS UPON MYXOBOLUS MUSCULI OF FUNDULUS AND A NEARLY RELATED SPECIES, M. PLEURONECTIDAE OF PSEUDOPLEURONECTES AMERICANUS

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Reference to the multiplicative stages of this parasite was made in a former paper (Hahn, 1913). At that time the true parasitic nature of the trophoblasts of both the multiplicative and propagative stages was insufficiently established. The relative virulence of the protozoon and the bacteria also needed further confirmation. Subsequent studies leave no doubt as to either of these points.

In almost every diseased integument, gill, or flesh wound which one examines, there will be found among the decadent tissues a few or many clear, white, even-contoured bodies which rarely take up any stain, no matter what treatment the tissues may be subjected to. The bodies are therefore in strong contrast with the surrounding tissues. If conditions are such that the parasites can be seen at all, the tissues must have taken up more or less of the stain. It was hoped that by using a variety of stains in different combinations with a wide range of fixatives, one might succeed in finding a treatment that would reveal the nucleus and perhaps other cytoplasmic contents of the parasites. No very encouraging results were obtained with the reagents that follow.

After fixation with alcohol (Abs. 62 per cent), ether (32 per cent) and 40 per cent formaldehyd (6 per cent), I used Giemsa, toluidin blue, methylene blue, thionin, Bismarck brown, fuchsin, anilin blue, Bordeaux red, neutral red, dahlia violet, sudan III, indigo carmin, methyl violet, alizarin, rose anilin violet, carbol fuchsin, picro-nigrosin, safranin, and hematein combinations. With corrosive sublimate solutions in different solvents and after chromic, chromosmic, and many other common and some unusual fixatives, such as tannic, malic and formic acids, the following stains were employed: Ehrlich's hematoxylin, Mayer's hematein, safranin, fuchsin, Heidenhain's hematoxylin, and brazilin. Both Mayer's hematein and Heidenhain's hematoxylin give to the cytoplasm of the parasite a slight clouded effect which renders it visible throughout. Rarely a medium or large-sized trophoblast has a faint blue nucleus, and less frequently a small dense spherical nucleus. Brazilin has given promising results when used in connection with a 5 per cent aqueous chromic acid fixation.

The trophic stages of the multiplicative cycle are much more frequently encountered in all the tissues I have examined. They also occur in much larger numbers, especially the minute stages. Thousands of them are frequently distributed more or less equally throughout the myoplasm of certain areas of muscle fibers (Fig. 9). A few are interfibrillar. Such muscle fibers may or may not give evidence of hypertrophy. The size of the parasites in one and the same tissue may vary from 1.5μ to 80 or 90μ in diameter. A very good picture of them has already been published in Figure 12, Plate XX, of the paper mentioned above. The trophic stages of *Chloromyxum clupeidae* (Fig. 8) appear to be very nearly the same in appearance as those of *M. musculi.*

In shape the multiplicative trophoplasts are circular or oval when small. Older ones have slight blunt extensions here and there over the surface. Occasionally a long pseudopod is encountered. Since these observations are made from fixed smears, it is probable that in life the display of activity on the part of the pseudopods would be very striking, could it be seen. As yet I have observed no striking activity in numerous fresh tissues. In very large parasites the cytoplasm is finely granular. The smaller ones appear to be structureless. Trophoplasts of moderate size frequently have a thin border of stainable material covering a part or all of the surface. This suggests an excretion or surface deposit, but is in reality what remains of the muscle nucleus which has been atrophied under the action of the parasite (Fig. 4). This can be demonstrated by the study of a large number of cases, when it will be found that there is a complete series of stages between the condition here described and normal nuclei.

Multiplicative trophoblasts have been found in muscle epidermis, gill epithelium, and connective tissue. All of these tissues are attacked and undergo cellular degeneration. The nuclei and mucous cells usually remain in various stages of hypertrophy and constitute a very misleading series of artifacts.

The staining reaction and the general appearance of the multiplicative trophoplasts are such as to suggest strongly that these bodies are some fatty or lipoid degeneration product. After many months of doubt, preceded by many more during which they were overlooked because it was assumed that the bodies in question were oil globules, it finally proved impossible to exclude them from the myxosporidian life-history. Authority may be found in the literature in support of both interpretations. It is an accepted fact (Adami, 1910) that with the hypertrophy of muscle, uniformly distributed fat bodies are to be expected. It has also been shown that the hypertrophy of the nucleus sets up changes in its immediate vicinity that result in lipoid substances.

The association of hypertrophied nuclei and Myxosporidia described above fits these specifications very well. On the other hand, small globular bodies within and between the muscle bundles were taken by Pfeiffer (1891: 106) to be germs of a myxosporidian.

The evidence upon which I have based my decision is (1) the failure of either osmic acid or sudan III to give a fat reaction, whereas oil globules on the same slides give a typical reaction. For the sudan III tests the tissues were fixed in aqueous formaldehyd solution, treated with a low-grade alcoholic solution of sudan III, and preserved in glycerin. (2) The large trophoplasts show granular cytoplasm and a faint nucleus at times, when stained with Mayer's hematein and Heidenhain's hematoxylin. (3) The trophoplasts occur in graded sizes as if belonging to the same stage of growth. (4) Many trophoplasts have pseudopodial extensions that have a strong motile suggestion. (5) Many muscle fibers in an advanced stage of hypertrophy are free from the bodies in question; they have migrated or operated in some other part. The products of degeneration would be expected to be uniformly distributed in all atrophied muscle fibers. (6) The sporoblasts of both *M. musculi* and *Chloromyxum clupeidae* have exactly the same oil-like appearance as the multiplicative trophoplasts and reactions, but contain some characteristic body that belongs to the sporogenesis, such as the myxospore itself (Chloromyxum) or the sporoblast nuclei. (7) When one compares the trophic stages of the multiplicative cycle with the propagative cycle of the Myxobolus or both with similar stages of the Chloromyxum, four kinds of bodies may be recognized. If the structures in question are artifacts, this distinction into two classes would not conform exactly to the conditions required by the protozoon life cycle as to equality of development of all individuals present. This is exactly what is found in regard to both of the genera here described. Either all the parasites are young trophoblasts of the multiplicative cycle, or all are in some phase of the propagative cycle. (8) Many of my preparations have been treated with ether and absolute alcohol. Oils are extracted by this treatment. Yet most of the structures in question show some evidence of a solid content, whereas casts of fat bodies, when encountered, are clear and structureless.

Many observers have found in fresh tissues small motile, structureless bodies, and also cells with nuclei, which they have assumed were parasites. I have examined fresh infected tissues of both the herring and Fundulus. While able to recognize the trophoplasts and sporoblasts, it has never been possible to be certain that the suspected objects were parasites until they were either connected by stages to sporocysts containing myxospores or until they had been verified in fixed and stained preparations. Pathological tissues frequently con-

tain artifacts resulting from the products of degeneration (Hahn, 1913: 197) which are very misleading. There are also numerous tissue cells and ameboid cells with well-developed pseudopods in atrophied tissue, especially in the epidermis of such fish as the flounder. Under these circumstances, one is inclined to place little confidence in observations based upon fresh tissue alone. It is probable that the observations of Pfeiffer (1893 and 1891), Thélohan (1893), and Megnin on the trophic stages of *M. pfeifferi* were correct, but one must always feel doubtful about the reliability of one's interpretations when good and sufficient reasons for considering any fresh cell as a parasite are not given.

The multiplicative trophoplast continues to grow in size until it is over 50μ in diameter. Although not observed alive, the shapes and general appearance lead to the conclusion that they are motile. They usually occur singly in comparatively uninfected portions of the tissue. In shape they vary from a long gregarine-like structure with very finely granular endoplasm and a shallow clear cytoplasm, to a smooth oval or circular mass when seen in profile. These large individuals may reach a diameter of 50μ (Fig. 14). Associated with them in the same tissues one rarely finds schizonts containing minute spores. The schizonts range in size from 40 to 55μ. These bodies are embedded in the muscle fiber in a cavity which they completely fill, giving precisely the same appearance as the large immature schizonts (Fig. 12). The multiplicative spores within are about 1.5μ in diameter. In the few cases which I have examined, they have not taken up the stain, but are visible owing to the presence of a residual material which retains a moderately intense stain. Free multiplicative spores are common, and like all multiplicative stages, they are also characterized by the non-staining quality. Occasional schizonts containing spores are encountered in fresh tissues. They have also been seen and distinguished from propagative stages in sections. As yet none of the latter were so large as those here figured. The scarcity of sporulating schizonts is no doubt to be attributed to the rapidity of the dissemination of the spores under the muscular activity. As previously noted, the schizonts have already migrated into fresh tissues by the time they have reached any considerable size.

One might suspect that the bodies produced by the so-called schizogony and figured here are bacteria. Very similar colonies of bacilli have been described elsewhere (Hahn, 1913). These bacilli do not occur in muscle which is essentially normal, and they are not accompanied by interstitial material when isolated and embedded in the myoplasm. The individual here figured was fixed with Flemming's fluid and stained with safranin. This combination cannot be expected to stain bacteria. Failure to stain with Giemsa and methylene

blue does occur in certain bacilli which are common in the necrotic region of these sores. The stain is therefore not so reliable a criterion as location, uniformity of size, association with other free individuals, etc.

The time required for one complete multiplicative cycle is approximated in the discussion of inoculation experiments.

Schizogony in *M. musculi* would be expected if the schizonts containing spores had not been seen, since the peculiar distribution of the trophoplasts cannot be readily explained by any other kind of multiplication. The smallest individuals are usually very numerous in localized regions and differ but little in size. Older stages occur in fewer and fewer numbers unaccompanied by the small forms, proving that they have migrated from the focus of the multiplicative process.

Multiplicative reproduction in Myxosporidia was demonstrated by Cohn (1896) in *M. lieberkuhni* and by Doflein (1898) in *Glugea lophii.* Minchin (1903) describes fission and budding and refers to the multiplicative schizogony of *Glugea lophii* as a kind of schizogony, adding that "this kind of reproduction is probably very common, if not universal, in the tissue and cell-infecting Myxobolidae and Glugeidae." Doflein (1911) makes provision in an outline of the life-cycle of a typical myxosporidian for schizogony and suggests it is typical as a preliminary to sporogenesis, but gives no specific illustrations and does not elaborate this as a process of multiplicative reproduction independent of the formation of sporoblasts.

Laveran and Mesnil (1902) review the various methods of reproduction in Myxosporidia, referring to budding as described by Cohn in *Myxidium lieberkuhni,* also to binary fission as described by Doflein in *Chloromyxum leydigi,* and to the simultaneous division of the nuclei in the process of plasmotomie, but cite no typical cases of schizogony.

The occurrence of multiplicative schizogony in a species of Myxobolus in the bile of the flounder has been observed by the writer. Plehn (1905) figures and describes a schizont with a large number of multiplicative spores in *Lentospora cerebralis* from the salmon. It causes the so-called "twist disease" (drehkrankheit). He supposes that the spores develop into a cell which has a conspicuous nucleus that is lacking in the spores. In view of what has been learned about *M. musculi,* it seems more probable that Plehn's nucleated cells are in the line of the propagative cycle. They are probably sporoblasts or gametoblasts. The non-nucleated spores are perhaps multiplicative spores.

A schizont with ten multiplicative spores has been described by Nemeczek (1911) in *Henneguya gigantea.*

It is now certain that the propagative cycle starts with a spore which is unlike the meront of the multiplicative stage. Beginning with

the spore, the staining properties of the propagative trophoplast are distinctly different. In the paper already cited, Figure 14, Plate XX, represents a schizont with differentiated spores. They are probably not multiplicative spores as there stated. The latter are smaller and their nuclei do not stain. The propagative spores occur free in the myoplasm in fewer numbers, but with about the same pathological effects and habits as the multiplicative spores. Because of the intimate and constant association of the small propagative spores having nuclei with large ameboid trophoplasts (Fig. 14), I have concluded that the former develop into the latter. This view might be less tenable if there was not a sharp limitation to the range of development which the parasites have attained in any given tissue.

The fate of large propagative trophoplasts such as are shown in Figure 14 is probably some form of multiplication which results in moderate-sized sporoblasts. It is possible that they develop directly into primary sporoblasts, such as are undoubtedly represented in a trophic condition in Figure 5, and in a quiescent condition in Figures 6 and 7. Such an interpretation conforms to the accepted life-history for other species of Myxobolus. But if one is right in supposing that certain elongated spores of moderate size which have been occasionally encountered isolated (Hahn, 1913: 113, Figs. 17 and 19, Plate XXI), and in small sporogenic cells (Ibid.: Fig. 16, Plate XX), are to be included in the life cycle of *M. musculi,* then it is difficult to reconcile the stages represented with the sporogenesis as hitherto described by Mercier (1908), Keysselitz (1908), Schröder (1907, 1910), and others. There are apparently three different kinds of spores in *M. musculi.* One belongs to the multiplicative cycle and may without doubt be called an asexual type. The other two are very probably to be associated with the propagative cycle, and one may expect that they have some sexual significance. Of these, one is a spherical spore 2.5 to 3μ in diameter, which has a small well-defined nucleus and faintly staining protoplasm. They occur in large schizont cysts (Hahn, 1913, Fig. 14, Plate XX), and are produced in rather large numbers. They no doubt become the sporoblasts that are so numerous in tissue adjacent to them in the one tissue where they have been encountered. The latter are identical to sporoblasts such as are figured in this paper (Figs. 5, 6, and 7). The other type of propagative spore was encountered in the same slide as the above and in the immediate vicinity of them. They are contained in cells having a diameter of about 12μ. These sporocyte cells appear to be of independent origin. They occupy the space left by an atrophied muscle fiber. The contained spores are 2.5 by 4μ in size and have rather large nuclei. Each sporocyte contains from four to twelve spores.

The elongated type of spore not yet has been satisfactorily explained. If the spherical spores which contain a stainable nucleus are identical with what was assumed to be multiplicative spores, the elongated spores may prove to be sporocytes. I am not altogether certain that the one tissue represented was not harboring a double infection. A third hypothesis is that the small spherical spore is a microgamete and the larger elongated spore is a macrogamete. In this connection it is interesting to note that in the muscle fibers where typical medium-sized sporoblasts are abundant, occur also several small elongated cells with pointed, densely staining nuclei, having a terminal position (Hahn, 1913, Fig. 18, Plate XXI). One may suppose that these are motile microgametes, but at present no evidence is available to substantiate the hypothesis.

One may conclude with reasonable assurance that the sporoblasts do arise from a very common type of spore which arises by a process of schizogony, and that the propagative sporoblasts are sufficiently differentiated from the multiplicative spores to be easily distinguished while yet in the schizont cyst. I believe that after a succession of multiplicative cycles ending in multiplication by schizogony, there follows a schizogony which generates spores that become differentiated very early into either gametes or primary sporoblasts. (See also page 102 in the first section of this paper for time relations.)

The primary generative cells of *M. musculi* certainly do not arise by free cell formation in large myxoplasms, such as is the case in *M. pfeifferi* of the barbel, and *Sphaeromyxa labrazesi* (Lav. and Mesnil), according to Schröder, 1907. The primary propagative cells of *M. musculi,* on the other hand, are set free simultaneously by one or the other of the schizonts described above. This conclusion is based not only upon the existence of two or more types of schizonts, but upon the fact that in four tissues where sporoblast stages occur, they are very numerous and at approximately the same state of development.

The propagative stages have not been encountered so frequently as the multiplicative stages. This is probably due to the fact that they are not nearly so abundant. In some tissues one may find both kinds present, but according to my observations, one or the other is always greatly predominant. With the exception of the elongated spores which occur in certain small cysts that have been figured and described elsewhere (Hahn, 1913: 204, Fig. 16, Plate XX), there is no evidence that the multiplicative and propagative trophoplasts do not have practically the same structure and appearance when small.

There is absolutely no evidence that they are generated consecutively by budding or fission or plasmotomie, but quite the contrary. The propagative stages gradually differentiate from the multiplicative type, and by the time one can positively identify them as such, they are dif-

ferent both in appearance and staining reaction. When unmodified by the contraction of the muscle fibers, they are more or less spherical bodies with almost transparent glassy cytoplasm and a small vaguely staining nucleus (Hahn, 1913). Older conditions are shown in Figure 18 of the paper just referred to. They have a large well-stained nucleus and fit loosely in the space which they have eaten in the myoplasm. The shape varies from round to oval, and evidence of active mobility or of pseudopods is often lacking. Somewhat earlier stages, when compressed by the shortening of the muscle fiber, have long extensions of the cytoplasm (Fig. 5). The nucleus is also extended into a long slender mass and sometimes extends into the thicker portions of the protoplasmic branches. This condition does not seem to be quite normal. Many cases of less compressed myxoplasm occur as regularly distributed spindles.

Besides very small sporoblasts, there are numerous good examples of larger sporoblasts and sporocysts in all stages of sporogenesis and sporocysts with immature and more or less mature myxospores. Stages not figured in the plate of this paper will be found in my paper of 1913.

When unmodified by the contraction of the muscle fibers, the sporoblasts are probably more or less spherical with a small nucleus (Fig. 7), or a large one (Fig. 6), and almost transparent vitreous cytoplasm. The nucleus does not stain intensely, but is more or less free from characteristic stainable bodies (Hahn, 1913, Figs. 18, 21 and 35, Plate XXI). Presumably these are the same stage of the organism as those which are encountered frequently in an ameboid condition fitting loosely into irregular transverse clefts of hypertrophied muscle fibers (Fig. 5). The conditions in some cases, such as Figures 6 and 7 here and Figure 18 (Hahn, 1913), suggest that there is an advanced condition in which ameboid activity is lost. If so it is probably just preceding the process of sporogenesis. There is a transition between the ameboid condition and the inactive condition wherein the myoplasm is divided into narrow transverse partitions by very numerous spindle-shaped cells which lie with their long axis at right angles to the length of the fiber. It is unsafe to say to just what extent the mechanical action of the muscle and the number of parasites are responsible for these alterations in shape. Thélohan (1891) figures and describes exactly the same condition in fish muscle fibers. He also interprets them as sporogenic cells.

Sporoblasts are sometimes so closely packed in the space once occupied by a muscle fiber that, though the form of the fiber remains, the myoplasm can be seen only rarely (Fig. 18). When thus packed together, these cells form a pseudo-epithelium which can be distinguished from a slightly degenerated epidermal or mucous epithelium with the greatest difficulty. Practically one must depend in many

cases upon a general resemblance to other epithelial masses in the same tissue, the cells of which have entered upon some easily recognizable stage of sporogenesis. Such pseudo-tissues are either more or less obscured by the hypertrophied myoplasm, muscle, and vascular nuclei, or are so closely packed that unless spread out mechanically in smear preparations, suitable specimens for drawings cannot be found. It is such a scattered group that was selected for the camera drawings represented in Figures 7 and 18. For purposes of reproduction it was necessary to exaggerate the detail of both nuclei and the cytoplasm of the parasites. The disinclination to stain is still retained to a limited degree in the propagative stages.

The epithelioid tissue just referred to must not be confused with another condition which has already been described (Hahn, 1913), in which the hypertrophied nuclei of vascular and connective tissue occupy the mold of a muscle fiber and, mingled with the remnants of the myoplasm, resemble a bit of degenerating epithelium.

The identity of the cells of which these pseudo-tissues are composed rests upon very positive evidence. Not only can one easily find obvious differences between them and true epithelium, but there are many such masses lying among the atrophied muscle fibers, many of which are in stages of sporogenesis like that represented here (Fig. 3). On a single slide one cannot fail to connect stages identical to those of Figures 3 and 6 (below) with the less obvious stages in Figures 7 and 18. There are also interesting isolated groups of sporoblasts identical in appearance to those forming the epithelioid masses that occupy small spaces in the myoplasm (Fig. 6). Differences in the size of the nuclei are to be expected when it is recalled that we are comparing primary and secondary sporoblasts with pansporoblasts and possibly other stages of the propagative cycle. Figure 7 is magnified 560 diameters and Figure 6, 750 diameters. It is noteworthy that the group in Figure 6 is accompanied in the same fiber by a pansporoblast with ten or eleven nuclei. Between the former and the latter the hypertrophied myoplasm has lost the fibrillae. That the bodies represented in this fragment of muscle fiber were invading parasites is clearly obvious. The muscle hypertrophy alone is significant. Adjacent fibers have numerous isolated parasites, while the epithelioid masses and numerous stages of sporogenesis like Figure 3 are on the same slide from which the group in Figure 6 are taken.

It is rather by analogy with other Myxobolus than by direct observation that one must interpret the various propagative stages which have been encountered in the tissues of Fundulus. The majority of the older stages such as those in the pseudo-epithelium are probably sporoblasts. As already stated, those with large and small nuclei may prossibly be gametoblasts. There are some very large spherical stages

with two large and two small nuclei from which the sporogenesis starts. With numerous succeeding stages leading up to Figure 3 one has, at least, ample proof that trophoblasts whose nuclei stain are destined to give rise to propagative spores, i. e., myxospores.

It is of considerable interest that the early propagative stages like trophoplasts have a destructive career. Their scattered distribution in the younger stages is due to a rather extensive motility either upon the part of the parental schizont or upon their own activity. But when nearly mature they evidently become less active. The masses which occupy the mold of the muscle fibers suggest in a general way pseudocyst formation such as has been found in the gill (Textfigs. 1, 2, and 3), and is common in many of the other species (*M. pfeifferi* of the barbel disease).

The pseudo-epithelium (Fig. 18) formed by the propagative stages of *M. musculi* is a most remarkable condition and deserving of more attention. The simulation of normal or slightly hypertrophied host tissues is a most deceiving circumstance. When a parasite having such qualities occurs in small numbers and more or less isolated, the most careful observer will fail to recognize it. Moreover, if a sufficient number of tissues is not available, suitable stages for a positive identification will be wanting. The facts just noted are important because of their possible bearing upon the epithelioid tissues of mammalian cancer. Adami (1910) states that cancer tissue resembles nothing so much as a parasite upon the mammalian tissues. The propagative stages of *M. musculi* frequently give the appearance of a typical epithelioma.

SUMMARY

For the results of inoculation experiments bearing upon the life-history see the summary at the end of the first section of this paper.

1. *M. musculi* has a series of multiplicative cycles starting with the myxospore, followed by a propagative cycle, ending in the myxospore.

2. There are two or more types of schizonts and schizogony.

3. Multiplicative reproduction is carried out by means of a large schizont which gives rise to a very numerous progeny of very minute spores.

4. The multiplicative spores and trophic stages do not take up any stain thus far utilized, with one not very satisfactory exception.

5. Multiplicative trophoplasts and schizonts migrate into uninfected tissue, particularly just before the quiescent period preceding schizogony.

6. All propagative stages possess a nucleus which reacts to basic stains.

7. The schizonts which give rise to primary propagative spores also migrate into new tissues before undergoing schizogony.

8. Another process of schizogony exists in which the schizont is very large and the spores, though larger than multiplicative spores, are small and have a small nucleus which reacts to a basic stain.

9. A third type of propagative schizogony may possibly exist in which the schizont is small and the spore very large, with a large nucleus which reacts to a basic stain.

10. If the conditions in 8 and 9 are trustworthy, there is a differentiation of gametes into macro- and microspores.

11. Sporoblasts, whether arising from conjugation or destined to conjugate, are ameboid, trophic, having the ability to migrate to a limited extent only when immature, and losing this property later.

12. Multiplicative stages perforate muscle fibers extensively and bring about profound hypertrophy. Propagative stages while yet trophic are also predacious, but to a less degree. The latter give rise to characteristic irregular transverse clefts in the fibers. Such clefts vary in number, shape and size, and occur in more or less atrophied fibers only.

13. The passive propagative sporocytes pass through all the characteristic stages of sporogenesis such as have been described for *M. pfeifferi* (Keysselitz, 1908).

14. Closely packed primary and secondary sporoblasts form an epithelioid tissue which at times has the appearance of integumentary epithelium and closely resembles mammalian epithelioma.

15. Pseudocysts occur, having many myxospores in a common sporocyst plasm. They probably arise by the fusion of closely packed sporocysts.

MYXOBOLUS PLEURONECTIDAE OF WINTER FLOUNDER

A winter flounder (*Pseudopleuronectes americanus*) having open sores was collected by Dr. W. E. Sullivan in the vicinity of Woods Hole. When examined, the flesh proved to have undergone pathological changes almost identical to what has already been described in Fundulus. The flounder was 8 inches long and had three lesions. One on the dorsal side was ¾ inch wide and 1 inch long; the other two were smaller. The integument was either white and partially decomposed or completely gone. The underlying flesh was red and vascular at the surface and less transparent than normal. These external conditions resemble the appearance of the myxosporidian disease of Fundulus as much as one could expect, considering the difference in the integument, skin, and color of the flesh of these fish.

Suitably stained smear preparations of the flesh present almost the same pathological conditions as are found in the fundulus disease.

There are present hypertrophied muscle fibers and epithelium cells, degenerated nuclei, mucus cells, and numerous bacteria limited to the most disintegrated parts. Numerous fibers contain considerable numbers of unmistakable trophic stages of the multiplicative cycle of a Myxobolus. One could not distinguish these from the same stages of *M. musculi* of the Fundulus. Large multiplicative schizonts, almost mature sporoblasts, and myxospores are also to be found in the same tissues. With the exception of the myxospores, there is no noticeable difference in the propagative stages and those of *M. musculi.*

The myxospores are not very abundant, but they are suitably stained for comparison with other species. One has no difficulty in distinguishing them from the myxospores of *M. musculi* by their shape (Fig. 2). The latter are tapered more at the polar end and the polar capsules are drawn out into a narrow apex.

The flounder parasite has myxospores which are 14.8μ long and 11.9μ wide. Those of *M. musculi* are 14.3μ long and 6.7μ wide. Immature myxospores of *M. musculi* are 12 by 7.5μ by actual measurement. In both cases the figures here given are the average of several different spores. The flounder myxospore has polar capsules which are 6μ long by 3.7μ thick, and the fundulus parasite has polar capsules 6.5 by 2.0μ. The flounder myxospore is therefore 2μ shorter than *M. pfeifferi* and 1.9μ narrower. In shape and appearance it resembles the latter closely. Allowing for slight variations in size and shape due to difference in maturity, the discrepancy between the myxospores of the two fish is too great to consider them as belonging to the same species. The inoculation of one host species by myxospores from the other will easily settle this question. In the meantime, the name *M. pleuronectidae* is proposed for the flounder parasite. It is probable that many species of the flatfish are subject to attacks by this parasite.

It is interesting to note that one Chloromyxum myxospore was encountered in the tissues of this flounder.

The articles cited in this portion of the paper will be listed at the conclusion of the paper in the September number of the JOURNAL.

EXPLANATION OF PLATE

Fig. 1.—Five sporoblasts of *C. clupeidae* from the same slide as Figure 16. Note the unstained cytoplasm of the sporoblasts with thin filaments of host tissue residues separating one sporoblast from another. Compare this non-staining material with that in Figures 8, 11 and 16. Note the square form of both myxospores and sporoblasts. The capsule nuclei are applied to the polar capsules in the right hand lower sporoblast. The sporoplasm is unstained. The sporoblasts each contain a developing spore the nuclei of which are probably imperfectly stained. × 1575.

Fig. 2.—A myxospore of *M. pleuronectidae* from a lesion of the back of a winter flounder (*Pseudopleuronectes americanus*). ×1575.

Fig. 3.—A sporoblast of *M. musculi* undergoing sporogenesis. The specimen here represented is one of many in a mass of cells similar to that in Figure 7. The dark border is stained serum. A space exists between the latter and the sporoblast, due to shrinkage. There are about 30 well defined nuclei. A few appear to be elongated as if about to divide. ×560.

Fig. 4.—Three trophoplasts of *M. musculi* from the eye muscles of an inoculated Fundulus that had died from a general infection of the head region. The parasite has not taken up any stain while the host tissue has. These three cases show as many stages in the hypertrophy of muscle nuclei which the parasites have apparently attacked. Note the small trophoplast is associated with a nucleus showing normal alveoli while the larger trophoplasts are associated with nuclei from which alveoli have partially or completely disappeared. ×1575.

Fig. 5.—A fragment of an atrophied muscle fiber from a large open lesion of Fundulus containing propagative trophoplasts, possibly sporoblasts of *M. musculi*. I regard these as earlier than in Figure 6. The position of the long axis of the sporoblasts and their cavities, which is at right angles to the length of the muscle fiber, is due most likely to the contraction of the fiber. Compare the granular nuclei in this with Figures 6 and 7. ×300.

Fig. 6.—A muscle fiber from Fundulus with several sporoblasts of *M. musculi* in the same cavity and one isolated. The many small nuclei of the latter indicate that it is in an advanced stage of sporogenesis. The large size of the nuclei in the others indicates that they are in a much later condition than those shown in Figures 5 and 7. Atrophy of the myoplasm is just beginning. ×750.

Fig. 7.—A group of sporoblasts of *M. musculi* at about the same stage of development as in Figure 5. The group lies adjacent to an epithelioid tissue which has replaced a completely atrophied muscle fiber. These cells are drawn out of the mass sufficiently to permit drawing details which are not possible in the compact masses, one of which is shown in Figure 18. ×560.

Fig. 8.—Portions of four muscle fibers from the dorso-branchial region (not body muscle) of the young of *Clupea harengus* which had numerous pseudocysts of myxospores of *C. clupeidae*. No myxospores occur in the head region. All the muscle is thus riddled with the trophoplasts of the Chloromyxum. They are both inter- and intra-fibrillar. When intercellular, note they have crowded the muscle fibers. Fibrillation and striation of the muscle fibers is entirely lacking. ×300.

Fig. 9.—A portion of a muscle fiber from the body muscle of *F. heteroclitus* having a typical infection of very young multiplicative trophoplasts of *M. musculi*. ×300.

Fig. 10.—Three mature myxospores of *C. clupeidae* showing the four polar capsules stained. ×1650.

Fig. 11.—Sporocyst of *C. clupeidae* in an atrophied myoplasm from anterodorsal body of muscle of young *C. harengus*. Compare with Figures 1 and 16. Note the increase in size. Sporocyst plasm is unstained. Sporoplasm has assumed a more or less rectangular form. ×1575.

Fig. 12.—A multiplicative schizont of *M. musculi* in the myoplasm of an atrophied fiber from Fundulus. ×750.

Fig. 13.—A medium sized trophoplast of *C. clupeidae* migrating from an old tissue to new. ×300.

Fig. 14.—A large schizont of *M. musculi* from the same slide as Figure 12 which has not yet undergone schizogony. ×560.

Fig. 15.—A large schizont of *C. clupeidae* from the body muscle of young *Clupea harengus* in which no pseudocysts are present and no myxospores were found. These schizonts are abundant in comparatively normal muscle fibers. ×300.

Fig. 16.—Four sporoblasts of *C. clupeidae* from inflamed body muscle in the ventrolateral region. The two left-hand sporoblasts are enclosed in the sporocyst and the right-handed sporoblasts are free. The latter are comparable to the shaded portions in Figures 1 and 10. ×1650.

Fig. 17.—Photograph of a typical lesion in *F. heteroclitus* which afterwards proved to be caused by a typical infection of *M. musculi*. Note the swelling, the loosened and projecting scales, and the open central area from which the integument has disappeared.

Fig. 18.—A mass of sporoblasts of *M. musculi* giving the appearance of an epithelium. The truncated form of the mass is due to the fact that these sporoblasts have occupied the space left by the muscle fiber whose hypertrophy they have brought about. ×300.

PLATE

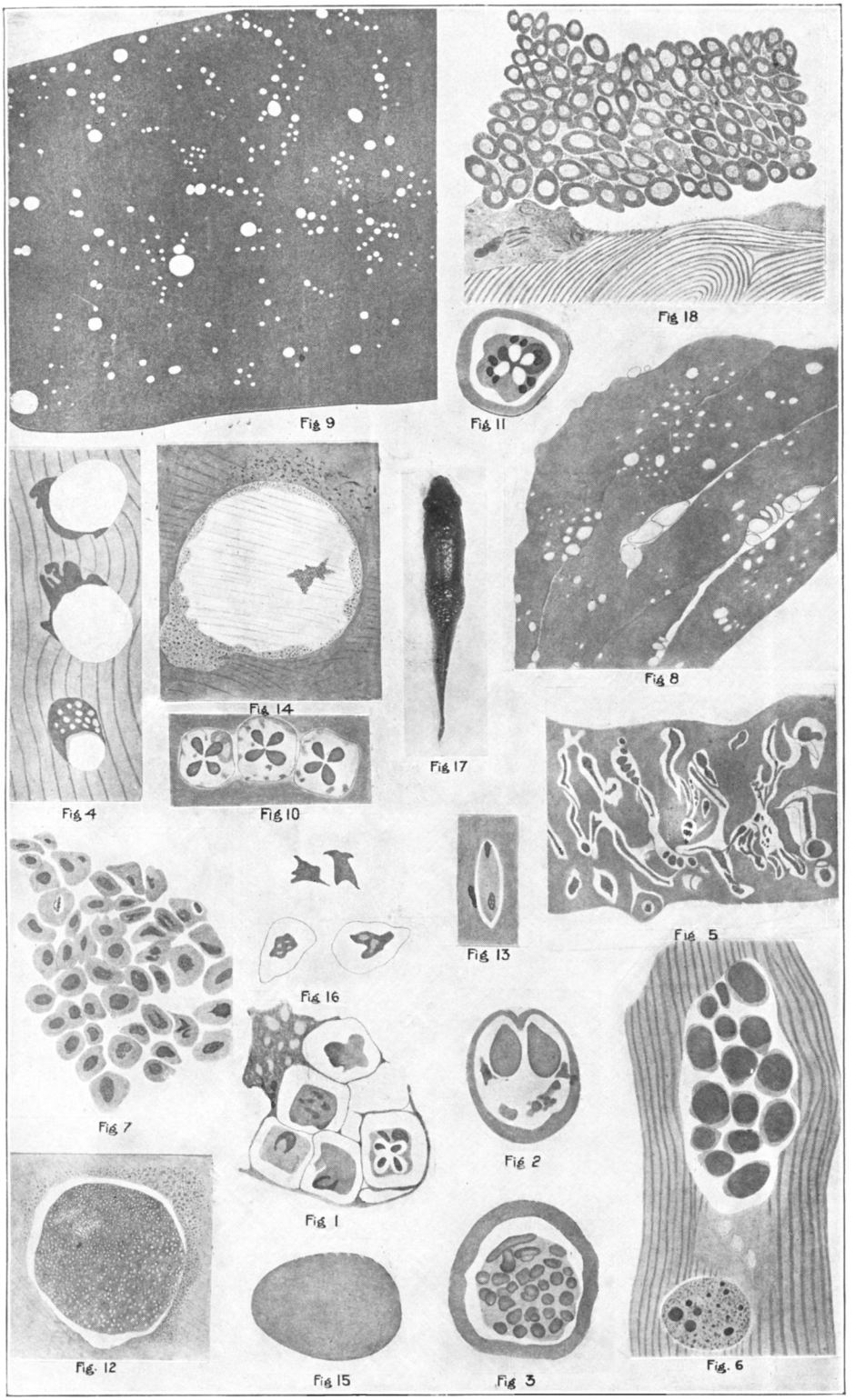
Fig 9
Fig 18
Fig 11
Fig 8
Fig 14
Fig 17
Fig 4
Fig 10
Fig 5
Fig 13
Fig 16
Fig 7
Fig 2
Fig 1
Fig. 12
Fig 15
Fig 3
Fig. 6