OCTOMITUS SALMONIS, A PARASITIC FLAGELLATE OF TROUT

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INTRODUCTION

It is well known that when any animal is confined under unnatural conditions various difficulties are encountered, which are of little importance in nature. This principle is well illustrated in the artificial propagation of fish, which is becoming more and more important because of the rapid rate at which the fish life of lakes and streams is becoming depleted. As a result of herding together large numbers of fish at the hatcheries, various parasites, which cause little or no injury in nature, have taken advantage of the exceptionally favorable conditions for their rapid increase and now present one of the most important problems with which the fish-culturist has to deal. Unless methods for controlling the various parasites and diseases so prevalent at hatcheries can be developed, the artificial propagation and rearing of fishes is threatened with failure. This is especially true of trout, which, owing to the ease with which the eggs can be handled, are crowded together in large numbers in a very limited space.

Among the parasites of trout probably none is more important to the fish-culturist than the flagellate *Octomitus salmonis* Moore, since this species infests the young fish at an early age before they are large enough to leave the hatchery. The conditions in the hatchery troughs, where thousands of fingerlings are crowded together in a narrow space, afford an ideal environment for the development of this parasite at a time when the fish are least able to resist its ravages. These facts, coupled with its wide distribution, have made Octomitus a serious menace to the success of trout culture.

NOMENCLATURE

Octomitus salmonis was first described by Dr. Emmeline Moore (1922), who found it in several species of trout at the New York State hatcheries. Investigations of the parasite were begun almost simultaneously in the spring of 1922 by Doctor Moore and the writer, who had independently discovered it in rainbow and brook trout from the White Sulphur Springs (W. Va.) hatchery.

Octomitus salmonis belongs to the family Hexamitadæ, which, according to Alexeieff (1914), includes the four genera, Hexamitus, Octomitus, Giardia, and Trepomonas. It is not the writer's intention at this time to enter into the controversy relative to the distinctive characters of the genera Hexamitus and Octomitus. This matter has been discussed at length by Dobell (1909), Alexeieff (1914), Swezy (1915), and Moore (1922). It is believed that there are valid grounds for distinction between the two genera as pointed out by Alexeieff. This distinction should be based not on a difference in habitat, as proposed by Dobell, but on strictly morphological characters, since the three genera, Hexamitus, Octomitus, and Giardia, can be clearly distinguished by the character of the flagella. As pointed out by Alexeieff (1914), the three pairs of anterior flagella in Hexamitus arise directly from the basal granules. In Octomitus there is a short rhizoplast surrounded by a differentiated layer of protoplasm between the basal granules and the flagella, while in Giardia the rhizoplasts are much longer and better developed than in Octomitus. The second characteristic—that of the fusion of the axostyles for part of their length—is believed to be based on an error in observation, since it is very doubtful if the axostyles are ever fused in either of the three genera, although they may sometimes be so close together as to appear fused on casual observation. The writer has several undescribed species of Octomitus and Hexamitus from various fishes, but in all cases the axostyles are distinct throughout their length. Probably another characteristic of Octomitus is a specially modified region at the anterior end, which forms a primitive cytostome but without the dorso-ventral flattening so characteristic of Giardia.

It will thus be seen that Octomitus occupies an intermediate position in the phylogenetic series between Hexamitus and Giardia. Hexamitus undoubtedly represents the primitive type from which the other two genera have been developed, while in Giardia we have the forms that are most highly specialized for a parasitic mode of life.

DISTRIBUTION

Octomitus salmonis is widely distributed throughout the country, having been reported from trout hatcheries in New England, New York, New Jersey, Virginia, West Virginia, Iowa, and Missouri, and it is probable that very few hatcheries are free from the parasite. It has also been found in young salmon from hatcheries in Washington, Oregon, and California. The parasite has not yet been found in wild trout except under circumstances that indicate that the infection was probably derived from hatchery fish. However, there is no reason to doubt that it does occur naturally in wild trout, but it is probably rarely abundant under such circumstances. No doubt the hatcheries first became infected from wild fish, the

greater abundance of the parasites in hatchery fish being due to the exceptionally favorable conditions for its spread and development. This conclusion is borne out by the fact that allied species of flagellates occur in other fishes, both fresh-water and marine.

Not only is *Octomitus salmonis* widely distributed geographically, but it has also been found in a considerable variety of hosts. It evidently occurs in all species of trout, having been reported from the brook trout (*Salvelinus fontinalis*), rainbow trout (*Salmo shasta*), brown trout (*Salmo fario*), Loch Leven trout (*Salmo levenensis*), and lake trout (*Cristivomer namaycush*). The writer has also found the flagellate in considerable numbers in fingerlings of the chinook salmon (*Oncorhynchus tschawytscha*) and the silver salmon (*Oncorhynchus kisutch*).

SYMPTOMS OF THE DISEASE

The disease caused by infection with *Octomitus salmonis* and known as octomitiasis is not characterized by well-defined symptoms by means of which it can be readily distinguished from other ailments of trout. There are no external lesions, and the most common indication of the presence of the disease is the appearance of thin, emaciated fish, usually somewhat darker in color than normal, although in some instances they may be lighter. Owing to the relatively large head in comparison with the emaciated body, such fish are commonly referred to as "pinheads" by fish-culturists. Many of the "pinheads" may improve after a time and eventually resume their normal rate of growth, but others gradually grow weaker and weaker until finally death supervenes.

More rarely the disease may occur in an acute form accompanied by a heavy mortality. Dr. Emmeline Moore (1923) refers to the disease as the "whirling sickness" and states that "Balance seems easily lost and the fish turn over repeatedly with a 'whirling' or 'corkscrew' motion in the water; too weak to make headway against the current, numbers of them are found in the corners at the foot of the trough or nosing along the sides near the surface; they lie on their backs with gills distended and in feverish action." This whirling motion has been observed only rarely in our investigations, and in no case was it a prominent feature of the disease. It should be remembered in this connection that whenever fish become so weakened from any cause as to be unable longer to maintain their balance they turn on their sides, and any effort to swim necessarily results in their taking a spiral course. In one instance where the disease was exceptionally severe the dying fish were observed to lie on the bottom of the trough and bend the body from side to side in quick spasmodic movements. Similar movements were also noticed when the fish were swimming at or near the surface, but in no case did they exhibit a whirling or spiral motion.

Doctor Moore also refers to diarrhœal symptoms as being specific for the disease, but this has not been noticeable in any of the diseased fish observed by us. It is true that the intestinal contents of infected fish are usually more fluid than normal, but this is believed to be due simply to the fact that such fish take comparatively little food.

The simplest and most reliable method of diagnosing the disease consists of a microscopical examination of the contents of the anterior end of the intestine.

This should be mounted on a slide in a drop of water, in which the parasites will remain alive and active for 10 to 15 minutes. Since no other parasites are likely to be encountered, which could be confused with Octomitus, examination with the low power of a compound microscope usually is sufficient. At this magnification the parasites can be distinguished readily as colorless, minute, pear-shaped organisms darting rapidly about in all directions.

When the parasites are very abundant, the intestinal wall just behind the stomach usually is thin and transparent and distinctly yellowish rather than brownish, which is the normal color. In acute cases it may exhibit a distinctly reddish color due to congestion. However, since the appearance of the intestinal wall is to a considerable extent dependent on the character of the food, too much reliance should not be placed on the yellow transparent appearance as a diagnostic character.

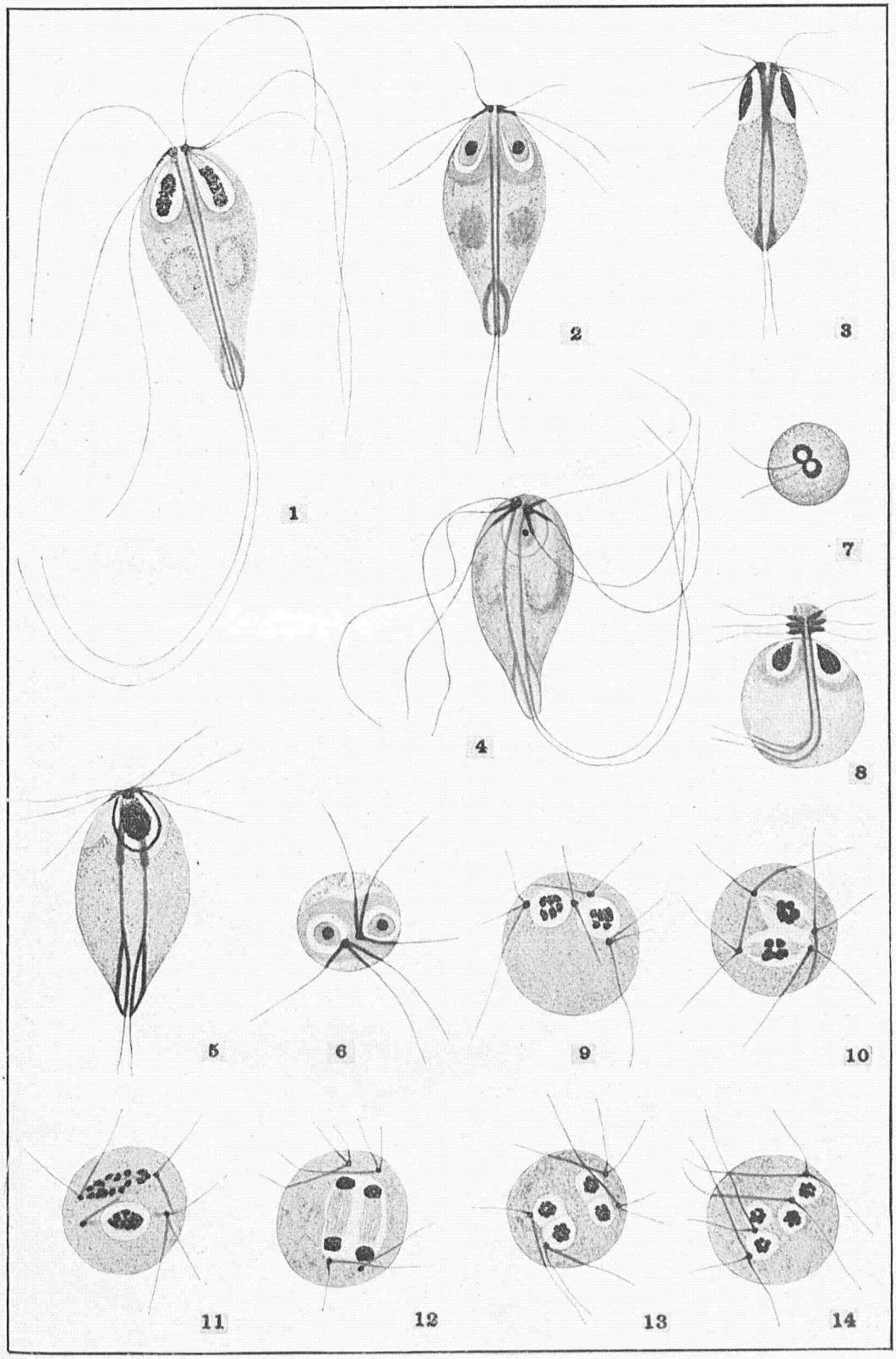
MORPHOLOGY OF THE FLAGELLATE

The flagellate usually is pyriform or ovoidal in shape, tapering gradually toward the posterior end, which is more or less distinctly truncate (figs. 1 to 4). Rounded individuals are quite common, however, and other variations in form are by no means infrequent. They vary considerably in size, also, the majority being about 6 to 8 microns in width by 10 to 12 microns long.

But little of the internal structure can be distinguished in the living flagellates, which are usually very active and swim swiftly about by means of rapidly moving flagella. Furthermore, the animals are so minute that even when fixed and properly stained it is very difficult to make out the finer details of their structure.

A thorough study of more carefully fixed and stained material has convinced the writer that his previous description (Davis, 1923) of the structural details was inaccurate in several respects. This is especially true of the cytostome, which was described as a slightly concave structure on one side of the anterior end. It is now believed that there is no constant flattened or concave region, nor is there a definite ventral side. On the contrary, the anterior end is usually uniformly rounded on all sides. Although there is no flattened cytostome, the protoplasm in this region has a quite different structure from the rest of the body and can be plainly distinguished in both living and stained individuals. In the former it appears as a clear hyaline region occupying the anterior third of the animal, which is sharply marked off from the more opaque and granular protoplasm composing the remainder of the body. When stained with hematoxylin, this region usually appears darker than the rest of the body, but sometimes may be lighter in color (figs. 1 to 4). In the living animal this region is very mobile and changes shape rapidly, sometimes being thrust out for some distance in front and again retracted so that the anterior end becomes enlarged and rounded. It seems probable that this mobile region has been specially modified to enable the animal to cling to the intestinal wall and possibly even to bore into the tissues. In other words, it is apparently a rudimentary cytostome and homologous with the more complex structure characteristic of Giardia. A similar mobile region has been described by Schmidt (1920) in *Octomitus intestinalis truttæ*.

When in active movement, the organism rotates rapidly on its long axis and, as noted above, there is no definite ventral side as described in a previous paper.



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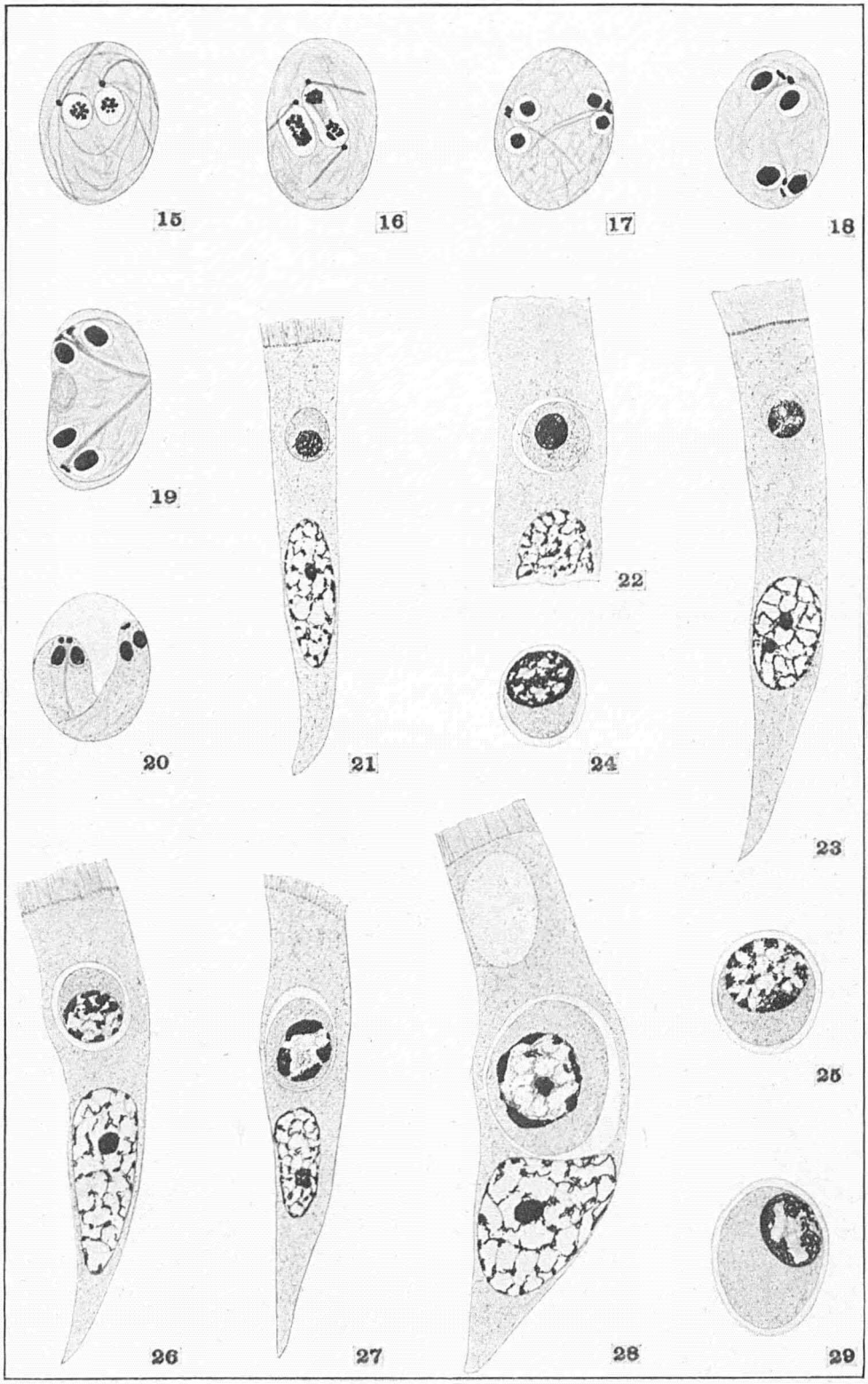
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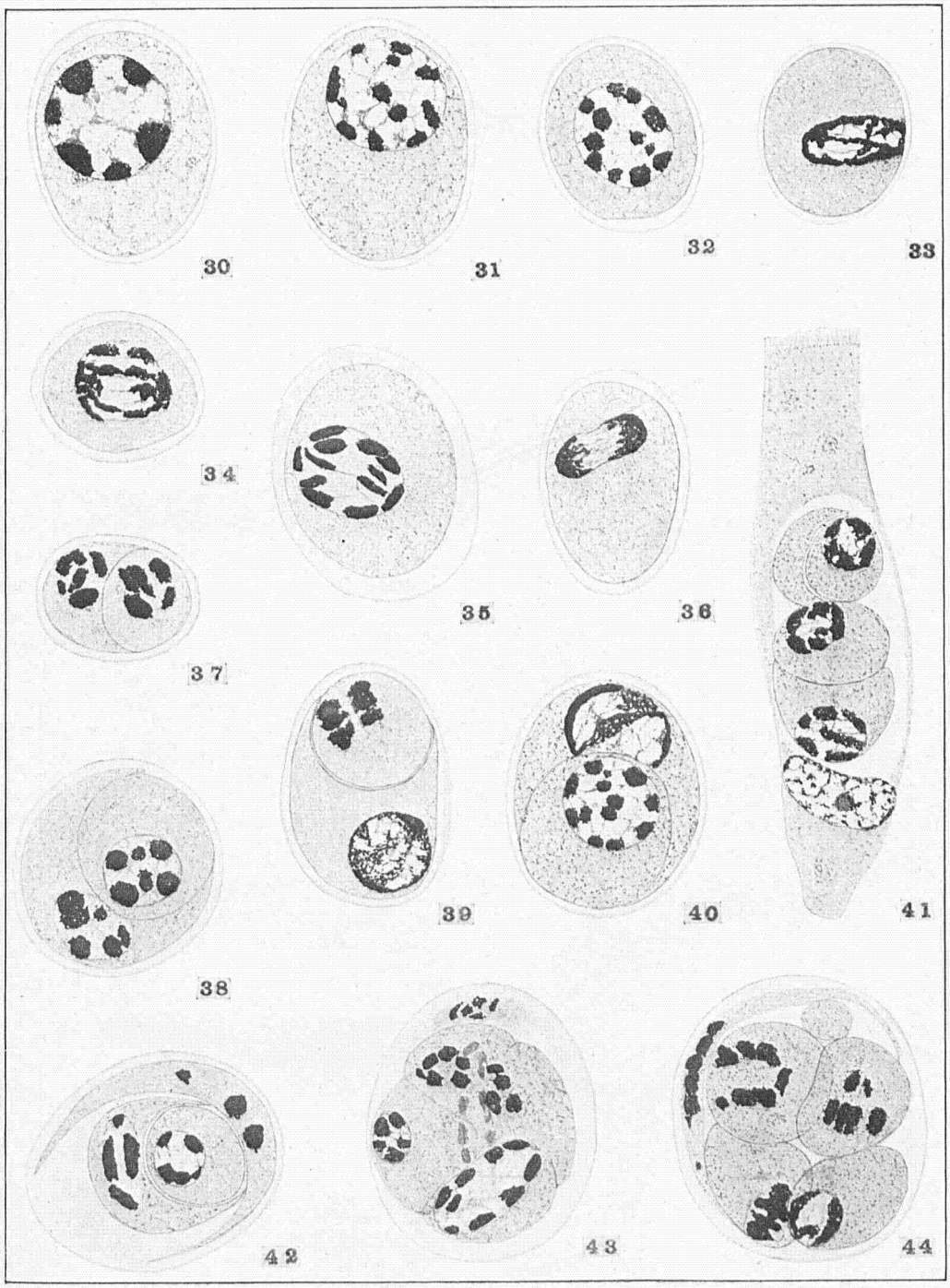
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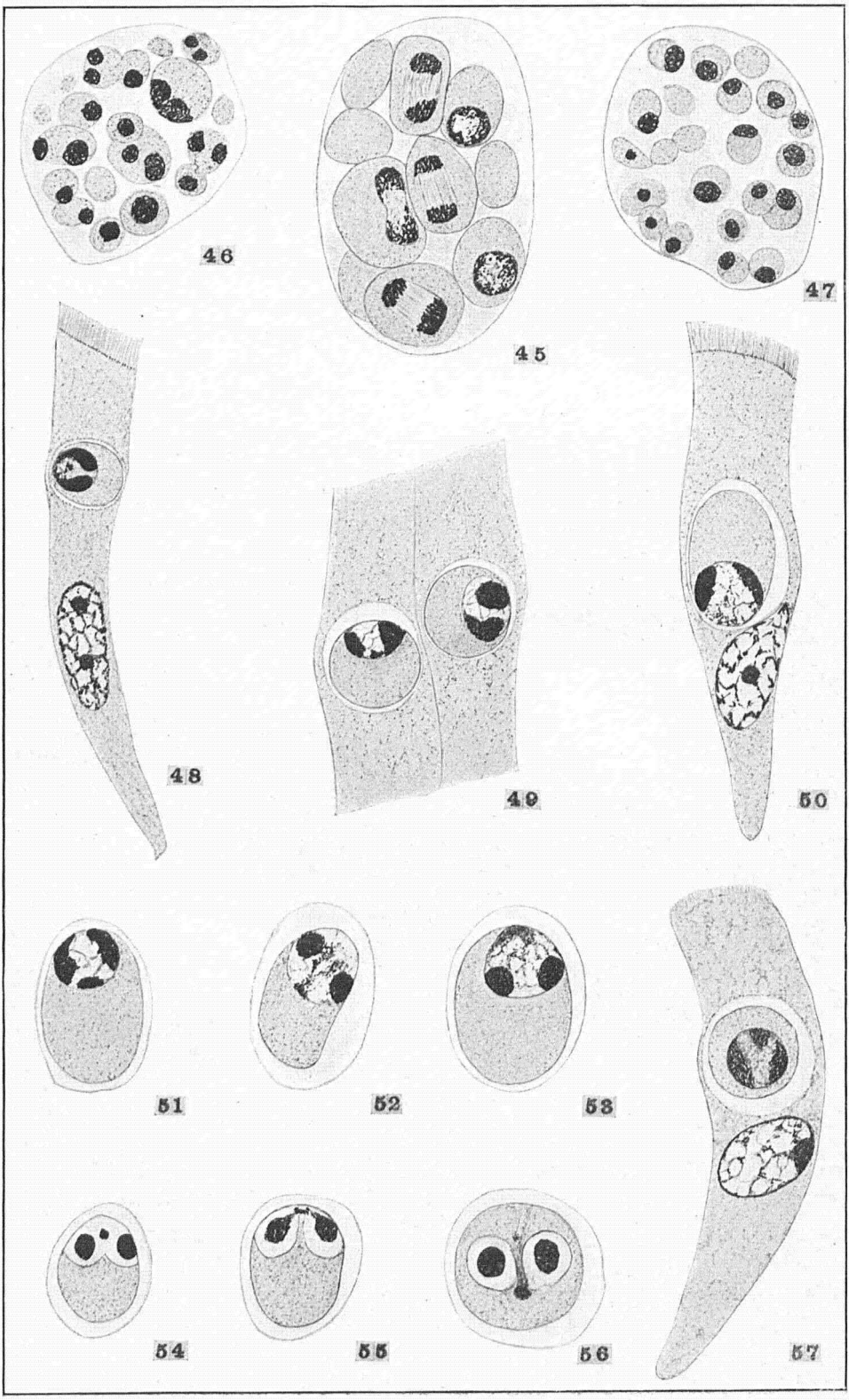
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While a ridge can sometimes be seen on one side of the body, this is evidently due to a temporary displacement of the axostyles.

Attached to the anterior end are three pairs of flagella, which vary considerably in length but are usually about one and one-half times the length of the body. These flagella arise from the blepharoplast complex attached to the anterior ends of the axostyles. Surrounding the base of each flagellum is a deeply staining layer, which extends from the axostyles to the point at which the flagellum leaves the body (figs. 4, 6, and 8). Owing to this layer, it is often very difficult, if not impossible, to distinguish a definite granule at the base of the flagella, and one gets the impression that the blepharoplast complex is formed by the greater development of the deeply staining layer at the junction of flagella and axostyles. A fourth pair of flagella arise from the axostyles at the extreme posterior end of the body. These flagella are considerably longer than those at the anterior end and trail behind the animal when it is in motion.

The axostyles are a pair of slender flexible rods which extend the entire length of the body and can usually be distinguished in the living animal. Their relative position is very variable, and while they may sometimes lie nearly parallel (figs. 1 and 2) they often extend across each other as shown in Figure 3. At the anterior end the axostyles are surrounded by a chromatic layer which in deeply stained specimens may be conspicuous (fig. 5), but is usually indistinguishable in strongly decolorized individuals. Near the posterior end is a characteristic structure, also composed of chromatic material, which is easily recognized even in strongly decolorized individuals. This structure appears as three or four granular chromatic bands extending along the axostyles for about one-third their length (figs. 1 to 5). These bands often appear to form the walls of a pair of longitudinal grooves and were so described by Moore (1922). However, when viewed from the posterior end (fig. 7) it is evident that the chromatic material in reality forms a pair of funnel-shaped structures attached to the axostyles with the smaller end pointed forward. Owing to the movements of the axostyles, the appearance of these structures varies greatly in different individuals.

At the anterior end of the body is a pair of ovoidal or egg-shaped nuclei, with the smaller end extending anteriorly to connect with the blepharoplast complex and axostyles. Surrounding the nucleus is a delicate membrane, within which a layer of chromatic granules can be seen in deeply stained individuals (fig. 5), but these readily lose the stain when the preparations are properly decolorized. Within the nucleus is a central karyosome surrounded by a clear space or halo. The karyosome is elongated and stains deeply with hematoxylin. When properly decolorized it can be seen to be composed of deeply stained granules embedded in a lighter matrix—the plastin—which extends anteriorly to connect with the blepharoplast. In strongly decolorized individuals the chromatic material is limited to a single spherical granule near the middle of the karyosome (figs. 4 and 6). When further decolorized, this chromatic body gradually grows smaller and eventually disappears.

There can often be seen, near the middle of the body, a pair of irregular, finely granular structures, which stain somewhat darker than the surrounding cytoplasm (figs. 1 and 2). These structures vary greatly in shape and appearance, and, while

sometimes quite prominent, flagellates are often seen which show no trace of them even when fixed and stained in the same way (figs. 3 and 5).

MULTIPLICATION OF FLAGELLATES

Multiplication takes place by a complicated process of binary fission, which in its essentials is very similar to that described by Swezy (1915) in Hexamitus. In preparation for division, the organism becomes rounded and the chromatin in the karyosomes collects in a number of rounded granules, which are evidently chromosomes (fig. 9). These granules are so small and close together that it is very difficult to determine the exact number, but there are probably five or six in each nucleus. The differentiated region forming the rudimentary cytostome becomes indistinguishable from the rest of the protoplasm, but the clear areas surrounding the karyosomes are a prominent feature throughout the process of division. Early in the prophase each blepharoplast and axostyle divides into two parts, which quickly separate so as to lie on opposite sides of the nucleus (fig. 9). No paradesmose connecting the blepharoplasts, as described by Swezy in Hexamitus, could be distinguished. The flagella fail to take part in this division, with the result that one of the blepharoplasts has two flagella, the other only one, and one axostyle has none. A primitive type of spindle, which extends across the nucleus connecting with the blepharoplast centrosomes outside the nuclear membrane, is then formed from the plastin of the karyosome (fig. 10). The chromosomes are at first grouped at the equator of the spindle, but later become distributed along its entire length (fig. 11). At a somewhat later stage the daughter chromosomes become grouped at each end of the spindle a short distance from the blepharoplast centrosomes (fig. 12). At first interzonal filaments can be plainly seen connecting the two groups of chromosomes, but these gradually disappear with the formation of the daughter nuclei. The telophase appears strikingly like the prophase, except that there are two pairs of nuclei instead of one, and, just as in the prophase, each karyosome is composed of five to six rounded granules embedded in a homogeneous matrix (figs. 14 and 15). All the axostyles now bear flagella, but no new ones have yet been developed from the blepharoplasts. Just when these appear has not been determined.

It will be noted that dividing individuals retain their flagella throughout the process and for that reason are actively motile. Flagellates in different stages of division have often been observed in fresh preparations, swimming about as actively as the others, but with considerably more irregularity in their movements. On one occasion a rounded individual, which was evidently in the last stages of division, was observed to separate in less than 60 seconds into two typical flagellates, which swam away in opposite directions.

It is evident from the foregoing account that fission in this species is essentially a longitudinal division very similar to the process described by various authors in Hexamitus and Giardia. It should be pointed out, however, that no evidence has been found of a multiple division such as has been described in Giardia and Hexamitus, and it is not believed that it occurs in this species.

The writer has found no reason to believe that a transverse division, as described by Moore (1924), ever occurs. Individuals showing a transverse constriction in some part of the body are quite common in some of the writer's preparations, but there is every reason to believe that such a constriction has nothing to do with division. It has been noticed frequently that when an individual is passing through a small opening the body may become strongly constricted as the animal squeezes through the narrow space. When mounted in the thick mucus from the posterior end of the intestine, numerous individuals that are worming their way through small openings of this kind can often be seen in the same field.

It is a notable fact that fission is evidently more or less periodic, since in the majority of the writer's preparations dividing forms are rare, while in a few instances they are very abundant. Just what factors are concerned in stimulating division is still somewhat doubtful, but there is evidence that there may be a sudden increase in the rate of fission when the host fishes are subjected to unfavorable conditions, such as a rise in temperature or a deficiency of oxygen or food.

DEVELOPMENT OF CYSTS

The cysts of *Octomitus salmonis* are ordinarily rare in the intestines of infected fish, even though the flagellates may be abundant, but a few can usually be found if a diligent search is made. In several instances they were present in large numbers, and it is worthy of note that these cases were all found in early spring. However, the evidence at hand is too meager to justify the conclusion that the cysts are more abundant at this season, although this may very well be the case.

When first formed, the cysts are ovoidal, rarely spherical, and are surrounded by a very thin transparent membrane. No definite structure can be made out in the living cysts, but in some cases rapid vibratile movements can be plainly seen. These are evidently due to the flagella that are retained for a short time after encystment. The axostyles are indistinguishable, but the nuclei usually can be recognized, and small vacuoles can be faintly discerned in some cases. The cysts usually stain a deep brown when treated with iodine, due to the presence of glycogen, which occurs in the form of fine granules or, in some cases, in irregular masses. This affords an easy method of demonstrating the presence of cysts, which stand out conspicuously against the more lightly stained background. It is of interest to note that ordinarily no trace of glycogen can be seen in the flagellated forms, but in one instance, when the cysts were exceptionally abundant, numerous flagellates were observed, which contained varying amounts of glycogen in the posterior half of the body, where it was evidently being stored in preparation for encystment. The cysts are usually about 7 microns wide by 10 microns long, but may reach a length of 12 microns with a corresponding decrease in the width.

Figure 15 shows a newly formed cyst in which the paired nuclei, blepharoplasts, flagella, and axostyles can be distinguished, but the texture of the cytoplasm is much looser than before encystment. This is characteristic of the cysts and is probably due to extensive formation of glycogen, which, of course, disappears as a result of the treatment to which the cysts are subjected. The flagella disappear

shortly after encystment, followed by the division of the nuclei, blepharoplasts, and axostyles (fig. 16). Since this occurs so quickly after encystment, the great majority of the cysts always contain two pairs of nuclei, either close together or some distance apart (figs. 17 and 18). Later the cysts become more elongate, with a pair of nuclei at each end (fig. 19). It is probable that the contents of the cyst is always divided into two distinct individuals at this time, but they are so closely crowded together that in most cases it is impossible to determine this with certainty. An exceptional case is shown in Figure 20, where two individuals can be clearly distinguished owing to the fact that they do not entirely fill the cavity of the cyst, as is usually the case.

Advanced stages of the cysts, such as are shown in Figures 19 and 20, are found only in the extreme posterior end of the intestine. They evidently pass out with the excrement at this stage, and their further development has not been followed. Presumably they are accidentally ingested by another fish and in this way start a new infection. It is very probable that this is the usual method by which the parasite gains entrance to a new host, although when the fish are crowded closely together it is not impossible that the flagellated forms may set up a new infection. Experiments have shown that the flagellates can live outside the host for 15 to 30 minutes, but that they are unable to survive in water for a much longer period. However, even should the flagellates gain entrance to a new host while still in active condition, it is by no means certain that they could survive in the stomach for any length of time.

INTRACELLULAR STAGES

In addition to the flagellates that are found only in the lumen of the intestine, intracellular stages are always present in the intestinal epithelium of infected fish. Ordinarily these forms are comparatively rare, but under certain conditions may become very abundant. They occur only in the epithelial lining of the pyloric cæca and the anterior end of the intestine.

The earliest stage of the intracellular parasite appears as a small rounded cell containing a relatively large deeply staining nucleus (figs. 21 to 24). They are usually found in the distal end of the cells, as shown in Figures 21 and 23, but in some cases may lie in the proximal portion of the cell below the nucleus. The parasite is almost invariably surrounded by a distinct vacuole, which is probably, in large part at least, the result of shrinkage. They grow rapidly to many times their original size (figs. 25 to 30), causing hypertrophy of the host cell, which in probably all cases is eventually destroyed. At first the nucleus is filled with a diffuse chromatic network (figs. 24 to 26), but as the organism grows there is a tendency for the chromatin to become aggregated in several large masses on the nuclear membrane (figs. 27 to 30). At first there are five or six large chromatic masses, but at a little later stage these bodies are smaller and more numerous (figs. 31 and 32). A careful study of a number of cells at this stage shows that there are always about 12 of these masses of chromatin in each nucleus. It is believed that they are formed by the division of the larger chromatic bodies that were present at an earlier stage and that they are, in reality, chromosomes that have divided pre-

cociously in advance of the division of the cells, which occurs somewhat later. At this stage the organism is usually somewhat elongated, with the nucleus at one end (fig. 31). Occasionally the host cell may disintegrate at this stage, the parasite passing into the intestinal lumen. In such cases it is apparently unable to complete its development, since, although this stage of the organism is sometimes not uncommon in the intestine, they invariably appear to be in a moribund condition and later stages have not been found.

Division of the full-grown parasite or schizont is accomplished by a very primitive form of mitosis shown in Figures 33 to 36, but which is very difficult to follow in detail. The daughter cells formed by this division (figs. 37 and 38) are unsymmetrical, and instead of lying side by side, as in ordinary division, one is partially inclosed by the other. This peculiar relation of the two cells is very characteristic; sometimes the outer cell is much flattened and almost entirely incloses the other. Often the nucleus of the enveloping cell has a quite different structure from that of the inner cell (figs. 39 and 40), and in some cases, at least, this cell may eventually degenerate. Several divisions of the inner cells follow in rapid succession until as a result of this process numbers of small spores or merozoites are formed (figs. 41 to 47), which then make their way into uninfected cells and repeat the cycle. As stated above, the enveloping cell often degenerates during the process of schizogony, but whether this is always the case is uncertain. In Figures 43 and 44 it is very evident that the cell is gradually distintegrating, but in many other instances, such as shown in Figure 41, no trace of a degenerating cell could be found. There appears to be considerable variation in the behavior of the cells during schizogony, and it is probable that many of the parasites are destroyed during the process. In many cases entire groups of cells in various stages of schizogony do not stain normally and are apparently moribund. In fact, the whole process gives one the impression that in this species the intracellular cycle is a comparatively recent development and that the organism has not yet become completely adjusted to such an environment.

It is obvious that schizogony furnishes a rapid means of multiplication, since the entire process requires but a short time, probably not more than 24 to 48 hours under favorable conditions. It has often been noticed that infection of the epithelial cells is not uniform but that here and there are groups of cells in which nearly every one is infected, while between such areas there may be only an occasional infected cell. Such a condition is evidently due to infection by merozoites that invade the surrounding epithelium, causing an intensive infestation of the cells in the immediate vicinity and in many cases the complete destruction of the epithelium.

In addition to the stages just described, other intracellular forms are sometimes met with which do not fit into the cycle of schizogonous development. A number of these forms are shown in Figures 48 to 56. It is evident that this series shows a gradual transition from the typical intracellular parasite to the flagellated form in the lumen of the intestine. In the early stages these cells differ from those previously described only in the fact that nearly all the chromatin is collected in two large masses on opposite sides of the nucleus (figs. 48 and 49) instead of being uniformly distributed, as in the majority of parasites at this stage. However, the writer is by no means convinced that all cells showing such an aggrega-

tion of chromatin develop directly into flagellates, since there is apparently a tendency for the chromatin to collect on opposite sides of the nucleus in many cells that are evidently in the schizogenic cycle. Later, as shown in Figures 50 to 53, the cells become elongated, with the nucleus at one end containing the paired chromatin masses symmetrically arranged, one at each side. Between the chromatic bodies there is always some residual chromatin, which gradually loses its affinity for stains. At a somewhat later stage the outline of the original nucleus has disappeared, and each of the chromatic bodies now forms the karyosome of a distinct nucleus (figs. 54 to 56). These nuclei show a striking resemblance to the nuclei of the flagellates, each karyosome being surrounded by a membrane separated from it by a clear area. In addition, there is usually a deeply staining body, evidently the blepharoplast, between the paired nuclei. The position of the blepharoplast suggests that it is derived from the residual chromatin left in the nucleus after the formation of the karyosomes. It has been impossible to distinguish flagella on the intracellular parasites at any stage, but in a number of instances there were structures that appeared to be developing axostyles, although in no case were they very distinct. However, in spite of the fact that no clear case of the presence of axostyles or flagella has been demonstrated, it is nevertheless believed that such a series as shown in Figures 48 to 56 can be explained only on the assumption that they represent a gradual metamorphosis of the intracellular parasites into the free-swimming flagellates.

Since no intracellular stage has previously been attributed to any flagellate at all closely related to Octomitus, it may not be out of place to briefly review at this time the evidence for the assumption that the intracellular parasites and flagellates are simply different stages in the life cycle of a single species. Undoubtedly the strongest evidence in favor of such a belief is the fact that various stages can be found among the intracellular parasites which, when arranged in series, show a gradual transition from the typical intracellular form to the fully developed flagellate. While it is true that such stages are not common, they can scarcely be regarded as exceptional, since several can often be found on the same slide.

Furthermore, it has been found that the intracellular parasites and flagellates always occur together. In no case have flagellates been found in fish in which the intestinal epithelium did not contain at least a few of the intracellular forms. It is true that the latter have been found in fish when no flagellates could be demonstrated in the intestine, but in all such cases flagellates were found in other trout from the same locality. When we take into consideration the wide distribution of Octomitus, the constant association of the flagellates and intracellular parasites becomes all the more significant. It is difficult to explain such a relation except on the ground of specific indentity.

Of special significance is the fact that an undescribed species of Octomitus occurs in the fan-tailed darter (*Etheostoma flabellare*) at White Sulphur Springs, W. Va., which, like *O. salmonis*, lives in the anterior end of the intestine. Sections of the cæca of infected fish show the presence of a few intracellular parasites (fig. 57) that are evidently distinct from those in the trout. Presumably these are the intracellular stages of the species of Octomitus peculiar to the darter.

PATHOGENESIS

The effects of the parasite on the host undoubtedly vary greatly under different conditions, and there is still much to learn in this connection. The evidence at hand is quite contradictory in some respects, but it is believed that much of this apparent discrepancy can be explained on the basis of the two cycles of development within the host. Since the extracellular and intracellular stages develop under such different conditions, it is to be expected that the effects on the host will show corresponding variations.

There can be no doubt that fish may harbor large numbers of flagellates in the intestine without exhibiting any noticeable injurious effects. In fact, it has been a source of wonder to the writer that fish on occasion may contain such numbers of flagellates and still appear to be in a healthy, vigorous condition. However, this appears to be largely a matter of age, and probably also of acquired immunity on the part of the host. Ordinarily Octomitus does not cause serious injury after the first summer, and it is a common occurrence to find yearling fish in a healthy condition in spite of the fact that they are infested with large numbers of flagellates. Since at most hatcheries practically all the fish become infected during the fingerling stage, it is reasonable to assume that these yearlings have developed more or less immunity to the effects of the parasite. However, the writer does not mean to imply that under such circumstances the parasites are harmless, for they probably interfere with the growth of the host—possibly to a greater extent than is realized.

Among the fingerlings the effects of a severe infestation by the flagellates are usually quite marked. The fish lose their appetite and become thin and emaciated, the large head and attenuated body suggesting the term "pinhead," by which they are commonly known among fish-culturists. Such fish are usually weak and listless and in late stages of the disease may become too feeble to fight the current, being finally carried against the screen at the lower end of the trough, where they soon expire.

This chronic form of octomitiasis is usually most prevalent during the spring and early summer when the fingerlings are about 2 to 2½ inches long. While the mortality is usually not very heavy at any one time, this condition may persist for several weeks or months, with the result that the total loss may be considerable, sometimes reaching as high as 50 or 75 per cent.

Of course it is not contended that all "pinheads" are due to Octomitus, since anything that will interfere with nutrition will tend to have the same effect. However, the examination of a large number of "pinheads" at several hatcheries has shown that such fish are almost invariably infected with the flagellate stage of *Octomitus salmonis*, and, furthermore, that on the average the number of flagellates present is much greater in the "pinheads" than in other fish from the same lot that are not so emaciated.

No doubt there are a number of environmental factors that tend to bring on the chronic form of octomitiasis. Among these a rise in temperature, even though comparatively slight, and overcrowding are believed to be especially important. There is also reason to think that the character of the food may exert considerable

influence on the abundance of the flagellates. We have found in a recent feeding experiment that rainbow trout on a sheep-liver diet contained, on the average, a smaller number of flagellates than trout of the same age kept under similar conditions but fed beef heart instead of liver. This is shown in the accompanying table, which indicates the comparative numbers of flagellates in the intestines of 40 rainbow fingerlings at the White Sulphur Springs hatchery. These fish were examined on August 7, 1924, and the relative abundance of flagellates indicated as correctly as possible by the familiar plus-sign method. All of the fish examined were in a fairly vigorous condition at the time, and the mortality was not excessive.

Kind	+	++	+++	++++	+++++	Total number of fish
Liver-fed fish	4	7	7	2	0	20
Heart-fed fish	3	3	3	4	7	20

While, as previously stated, the flagellates are ordinarily not a serious problem in the yearling fish, a notable exception to this rule has come to the writer's attention. The Manchester (Iowa) station has for a number of years experienced considerable difficulty with both brook and rainbow trout during the second summer when the fish are 1½ years old. These fish are stunted, grow slowly, and may develop a "pot-belly," while the mortality is considerably higher than is usually the case with fish of this age. It was found that, almost without exception, the yearlings at this station are heavily infested with flagellates; so much so, in fact, that the intestinal contents is often a squirming, seething mass of these parasites. Remarkably enough, the fingerlings of both rainbow and brook trout are not infected, and little difficulty is experienced with these fish, which are healthy and vigorous and show a rapid growth with a comparatively low mortality. Just why the fingerlings at this station do not become infected is not entirely clear. Possibly it may be due to the fact that the water supply is derived from a covered spring and there is no possibility of its becoming contaminated by fish before reaching the hatchery. The greater susceptibility of the yearling fish to the effects of Octomitus is probably due to the fact that they have had no opportunity to acquire an immunity. When the fingerlings are infected, those that survive doubtless develop a more or less complete immunity by the end of the first summer, which protects them from serious injury the following year.

While the chronic, wasting disease just described is undoubtedly the most common result of infection by *Octomitus salmonis* and is prevalent to a greater or less extent at nearly all trout hatcheries, there is good reason to believe that under certain conditions the disease may manifest itself in a quite different manner. The writer's attention was first called to this infection in the spring of 1922 by reports that the rainbow fingerlings from the White Sulphur Springs (W. Va.) hatchery did not stand up well during shipment. The loss during the first 24 hours was not excessive, but if the fish were in transit for a much longer period the mortality was exceptionally heavy, sometimes reaching 75 per cent, or even more. In an attempt to discover the cause of the trouble, a number of the fish were shipped to

Washington, and it was found that in many instances the anterior end of the intestine was badly inflamed. When sectioned, the congested portion of the intestine and cæca were found to contain intracellular parasites in such numbers that a large proportion of the epithelial cells was destroyed, which was undoubtedly the cause of the inflammation. In striking contrast to the chronic form of octomitiasis, we have here an acute disease that results in a heavy mortality within two or three days. Evidently the changed conditions to which the fish are subjected during distribution result in a sudden and rapid increase in the intracellular stages of Octomitus. That this is the case has been shown by a number of experiments in which fish have been taken directly from the hatchery troughs and placed in cans (such as are used in distribution) for 24 to 48 hours. In every case this has resulted in a noticeable increase in the number of intracellular parasites, the great majority of which were in various stages of schizogony. The picture presented in sections of such material is in striking contrast to that ordinarily found in sections from infected fish in which the intracellular parasites are not abundant and division stages are the exception.

There is still some uncertainty as to the factors involved in stimulating the development of the intracellular stages, but undoubtedly a rise in temperature, deficiency of oxygen, and cessation of feeding are among the more important, although it is not necessary for all of the factors to be present to cause increased activity among these forms. For instance, when the fish are held in cans placed in running water at the hatchery there is usually an increase in the intracellular stages although the temperature is constant. However, the increase is not as rapid as when the temperature is allowed to rise slightly. That the practice of withholding food from the fish during distribution is also a factor is indicated by some observations during the past summer. In the course of some feeding experiments at the White Sulphur Springs station it was necessary on two occasions to allow the fish to go two or three days without food, owing to delay in the usual shipments of heart and liver. In both instances there was an increased mortality after the first day, and in practically every instance an examination of the cæca of the dead fish showed that large numbers of intracellular parasites were present in an actively growing condition. Strangely enough, the mortality was greatest among fish fed on liver, although, as stated above, the flagellates were more numerous in heart-fed fish.

In addition to the mortality during the distribution of fingerlings there is reason to believe that severe epidemics, accompanied by heavy mortality, may occur under certain conditions while the fish are still at the hatchery. Such epidemics occur only sporadically and are not of regular recurrence year after year, as in the case of the common chronic form of octomitiasis. While the writer has not had an opportunity to conduct a personal investigation of such an epidemic, there is evidence that several heavy mortalities at the bureau's hatcheries in recent years were primarily due to this cause, and Dr. Emmeline Moore (1923 and 1924) states that a number of severe epidemics in the New York State hatcheries have been caused by Octomitus. Such a heavy mortality in a comparatively short time suggests that the intracellular stages may have been primarily responsible, although it is by no means certain that in some instances, at least, another organism in addi-

tion to Octomitus may not have been involved. Theoretically it is not improbable that under certain conditions the intracellular forms may exhibit a sudden and rapid increase while the fish are still in the hatchery troughs which would result in a severe mortality. Fortunately, under ordinary circumstances there appears to be little danger of such an outbreak; otherwise it is difficult to account for the fact that at many hatcheries in which the fingerlings are known to be thoroughly infected with Octomitus there has been no sudden excessive mortality for a number of years. However, given the proper conditions there would seem to be no reason why the intracellular forms might not develop as rapidly at the hatchery as in cans during distribution.

Although it is believed that there are thus two quite different forms of octomitiasis produced by different phases in the life history of the parasite, and that, as the result of environmental conditions, one or the other form may be more prominent, it is nevertheless true that in the great majority of cases we have to deal with a combination of the two types. In some instances the injurious effects appear to be due almost entirely to the flagellated stage, while in others it is the intracellular stages that are primarily responsible. Obviously the character and severity of the disease will depend on which stage of the parasite is the more abundant, although in most cases both stages are not without their influence. It is very probable that even in the chronic, wasting type of octomitiasis the mortality may to a considerable extent be due to the intracellular parasites, and a comparison of the dying fish with emaciated but fairly vigorous fish from the same lot has shown that in most instances the intracellular stages were distinctly more numerous in the former than in the latter.

As previously pointed out, fish are sometimes infected with the intracellular stages when no flagellates can be found in the intestine. This is usually true only of an occasional fish, but in one notable instance a lot of brook-trout fingerlings 1 to 1½ inches long were observed in which there was a heavy mortality, although no flagellates could be found in the intestine. The explanation of this was not apparent until it was found that the intracellular stages were abundant in the cæcal epithelium.

While apparently all species of trout and salmon may be infected with Octomitus, not all species are equally susceptible to the injurious effects of the parasite. At the bureau's hatcheries Octomitus appears to be more injurious to the rainbow trout than to other species, although according to Dr. Emmeline Moore in the New York State hatcheries it is the brook trout that are the principal sufferers. The explanation of this fact is not evident, although it is suggested that since the bureau's hatcheries at which most of the investigations on this parasite have been carried on are primarily rainbow hatcheries, it may be that a strain of Octomitus has been developed which is especially adapted to this species. The fact that the brood stock at these hatcheries consists entirely of rainbow trout may be significant in this connection.

No evidence is at hand regarding the effects of *Octomitus salmonis* on young salmon, but since considerable losses sometimes occur among the fingerlings in early spring, at about the time its effects are most noticeable in trout, it is not improbable that Octomitus may be responsible.

CONTROL MEASURES

Since *Octomitus salmonis* is so widely distributed and occurs in fish of all ages, it is believed that it will be impracticable to eliminate the parasite from the hatcheries. It is probable that many, if not all, adult fish in infected hatcheries harbor small numbers of the parasite and are thus carriers of the disease. Even when no flagellates can be found in the intestine a few intracellular forms can usually be discovered if the search is sufficiently prolonged. It is very doubtful if these parasites can be reached by any drug administered in the food, so that in order to free a hatchery from infection it would be necessary to remove all the fish and throughly disinfect the entire plant. Even were this done, it would probably not be practicable to restock with uninfected fish.

It is even doubtful if drugs could be successfully used in getting rid of the flagellates. While no experiments have been conducted along this line, the fact that attempts to get rid of Giardia (a closely related parasite in man) by this means has so far resulted in failure affords little encouragement in that direction, especially when we consider the many practical difficulties in attempting to administer drugs to trout 1 to 2 inches long.

In view of these facts it is felt that efforts to control the disease must be based almost entirely on prophylactic measures. Fortunately there is good reason to believe that by the adoption of such methods the ravages of the disease can be reduced to a point where it will no longer be a serious menace to trout culture. In this connection the fact that Octomitus is apparently not injurious to trout under natural conditions is especially significant. All the evidence at hand points clearly to the conclusion that the ravages of octomitiasis are in inverse ratio to the suitability of the environment, and that if the fish can be kept in a strong, vigorous condition they will be able to resist the attacks of the parasite.

The factors that appear to be most conducive to the development of Octomitus are overcrowding, an unsuitable water supply, especially a deficiency of oxygen and improper food. Owing to the inherent defects of artificial propagation, it is almost impossible to entirely avoid the unfavorable effects of these factors, but certainly in most cases much can be done to improve present conditions. There are, for instance, probably few hatcheries where the fish are not overcrowded at certain seasons. It is a natural tendency to attempt to increase the output by overloading the equipment. In some cases this may be successful for a time, but in others it is attended with disaster. That such a practice is ever advisable in the long run is more than questionable, since, even if not attended by heavy mortality, the fish reared under such conditions are likely to be weak and underdeveloped.

It is very probable that part of the injurious effects of overcrowding are due to an insufficient supply of oxygen, and this is, of course, especially noticeable when the water is not properly aerated before entering the troughs. With rare exceptions the water from springs is deficient in oxygen and should always be made to flow over an efficient aerating device before entering the troughs or ponds.

The third factor, that of improper food, is less easy to correct. Undoubtedly no substitute can equal natural food, but it will probably never be practicable to supply this in sufficient quantities to meet the demands of the hatcheries. To

attempt to rear trout on natural food alone would in most cases increase the cost of artificial propagation beyond all reason; but it may be possible to effect a compromise whereby the fish can be given some natural food in addition to the usual hatchery diet. Experiments have shown that such a diet will give much better results than the straight heart or liver diet commonly used.

In connection with the investigations on Octomitus we have carried on some experiments on the additions of vitamins to trout foods with very encouraging results. In these experiments cod-liver oil and dried brewer's yeast were added to the ordinary heart and liver diet to correct any deficiency of these foods in vitamins A and B. The results to date indicate that fish fed on a diet containing small percentages of oil and yeast show a greatly decreased mortality and increased growth over those fed a straight heart diet.[1]

While our experiments have not yet been sufficiently extensive to justify any general conclusions as to the effectiveness of cod-liver oil and yeast in the control of octomitiasis, it is believed that improvement in the food, either through the addition of vitamins in oil and yeast or the addition of some natural food to the diet, affords one of the most important methods of combating the disease.

It has been found that the excessive loss which frequently occurred when infected fish were held in cans for more than 24 hours during distribution can be entirely prevented by avoiding overcrowding and keeping the fish at a temperature considerably below that at which they were formerly carried. When infected fish are held at temperatures of 40 to 45° F. and given an abundant supply of oxygen, there is very little loss, even though they may be several days in transit.

SUMMARY

1. *Octomitus salmonis* is widely distributed throughout the country and probably occurs in all species of trout and salmon.

2. The parasite has two phases of development within the host—one intracellular, the other extracellular.

3. The intracellular stage is found in the epithelium of the pyloric cæca and the anterior end of the intestine. This stage reproduces by a process of schizogony, resulting in the formation of a number of merozoites, which in turn invade neighboring epithelial cells and repeat the process.

4. After a time some of the intracellular forms cease to divide and become metamorphosed into the flagellated form.

5. The extracellular or flagellated stage occurs in the lumen of the intestine and multiplies by a complicated process of binary fission.

6. The flagellates form cysts, within which they divide once by binary fission. These cysts serve to spread the infection from one fish to another.

7. Severe infestation by the parasite may result in serious injury to the fingerling fish. Excessive numbers of the flagellates may cause the fingerlings to lose their appetite and become greatly emaciated. Rapid multiplication of the intracellular stage may result in an acute form of the disease accompanied by heavy mortality.

[1] See Davis and James (1925) for further details regarding these experiments.

8. While the parasites are ordinarily most abundant in fish less than one year old, small numbers are usually present in the older fish, which become carriers of the disease.

9. It is believed that control of the disease must be based primarily on prophylactic measures. This can best be accomplished by keeping the fish under as favorable environmental conditions as possible.

BIBLIOGRAPHY

Alexeieff, A.
1910. Sur les Flagellés intestinaux des Poissons marins. Archives de Zoologie expérimentale et générale. Notes et Revue. 5e Série, Tome VI (whole series Vol. XLVI), 1910, No. 1, pp. I–XX, figs. 1–12. Paris.
1911. Notes sur les Flagellés. Archives de Zoologie expérimentale et générale, 5e Série, Tome VI (whole series Vol. XLVI), Numéro 14, 1910–1911, pp. 491–527, figs. 1–15. Paris.
1914. Notes protistologiques. Zoologischer Anzeiger, Band XLIV, Nr. 5, May 12, 1914, pp. 193–213. Leipzig und Berlin.

Davis, H. S.
1923. Observations on an intestinal flagellate of trout. Journal of Parasitology, Vol. 9, March, 1923, pp. 153–160, pls. XII–XIII. London.
1925. The intestinal Protozoa of trout. Transactions of the American Fisheries Society, Vol. LIV, 1924 (1925), pp. 57–63.

Davis, H. S., and M. C. James.
1925. Some experiments on the addition of vitamins to trout foods. Transactions of the American Fisheries Society, Vol. LIV, 1924 (1925), pp. 77–85.

Dobell, C. Clifford.
1909. Researches on the intestinal Protozoa of frogs and toads. Quarterly Journal of Microscopical Science, Vol. 53, new series, 1909, pp. 201–277, pls. 2–5. London.

Moore, Emmeline.
1922. *Octomitus salmonis*, a new species of intestinal parasite in trout. Transactions of the American Fisheries Society, Vol. LII, 1922–23, pp. 74–97, Pls. I–II. Washington.
1923. Diseases of fish in State hatcheries. Twelfth Annual Report, New York Conservation Commission, 1922 (1923), pp. 66–76. Albany.
1923a. A report of progress on the study of trout diseases. Transactions of the American Fisheries Society, Vol. LIII, 1923, pp. 74–94, 2 figs. Hartford.
1924. Diseases of fish in State waters. Thirteenth Annual Report, New York Conservation Commission, 1923 (1924), pp. 49–61. Albany.
1925. The transmission of *Octomitus salmonis* in the egg of trout. Transactions of the American Fisheries Society, Vol. LIV, 1924 (1925), pp. 54–56.
1925a. Fish diseases. Fourteenth Annual Report, New York Conservation Commission, 1924 (1925), pp. 83–97. Albany.

Moroff, Theodor.
1904. Beitrag zur Kenntnis einiger Flagellaten. Archiv für Protistenkunde, Band 3, Heft 1 (1904), pp. 69–106, Pls. VII–VIII. Jena.

Schmidt, Wilhelm.
1920. Untersuchungen über *Octomitus intestinalis truttæ*. Archiv für Protistenkunde, Band 40, Heft 3, 1920, pp. 253–289, pls. 20–21. Jena.

Swezy, Olive.
1915. Binary and multiple fission in *Hexamitus*. University of California Publications in Zoology, Vol. 16, No. 6, pp. 71–88, pls. 9–11. Berkeley.

von Prowazek, S.
1911. Zur Kenntnis der Flagellaten des Darmtraktus. Archiv für Protistenkunde, Band 23, 1911, pp. 96–100, figs. 1–16. Jena.

Wenyon, C. M.
1907. Observations on the Protozoa in the intestine of mice. Archiv für Protistenkunde, Supplement I (1907), pp. 169–201, Pls. X–XII. Jena.

EXPLANATION OF FIGURES

FIG. 1.—View of a typical flagellate. ×3150.
FIG. 2.—Similar to Figure 1, but more strongly decolorized. ×3150.
FIG. 3.—Flagellate with anterior end restricted, causing flattening of nuclei along the side. ×3150.
FIG. 4.—Flagellate viewed at a different angle from Figures 1 to 3 to show attachment of anterior flagella. Very strongly decolorized. ×3150.
FIG. 5.—Flagellate viewed at right angles to Figures 1 to 3. Only one nucleus is shown, the other lying directly underneath. ×3150.
FIG. 6.—View of flagellate from anterior end to show origin of flagella. ×3150.
FIG. 7.—View of flagellate from posterior end. Note openings of small funnel-shaped organs. ×3150.
FIG. 8.—Flagellate with anterior end abnormally retracted. Note the deeply staining envelope around the base of the flagella. ×3150.
FIGS. 9 to 14.—Successive stages in the division of the flagellates. Figure 9, prophase; 10, metaphase; 11, anaphase; 12 to 14, telophase. ×3150.
FIG. 15.—Early stage of cyst in which the nuclei are preparing for division. ×2400.
FIG. 16.—Slightly later stage of cyst. Nuclei beginning to divide. ×2400.
FIGS. 17 and 18.—Cysts at a somewhat later stage with two pairs of nuclei. ×2400.
FIGS. 19 and 20.—Cysts at a still later stage of development with contents divided into two distinct individuals. ×2400.
FIG. 21.—Epithelial cell from cæcum of trout containing early stage of intracellular parasite. ×1640.
FIG. 22.—Portion of an epithelial cell containing parasite at about same stage as Figure 21. ×2400.
FIG. 23.—Epithelial cell containing early stage of parasite. ×1640.
FIG. 24.—Intracellular parasite at about same stage as Figure 23, but more highly magnified. ×2400.
FIG. 25.—Slightly later stage than Figure 24. ×2400.
FIGS. 26 to 28.—Epithelial cells containing successively later stages of the parasite. ×1640.
FIG. 29.—Intracellular parasite showing tendency of the chromatin to collect in large masses on the nuclear wall. ×2400.
FIG. 30.—Fully grown intracellular parasite or schizont. ×2400.
FIGS. 31 and 32.—Slightly later stages of schizont just before division. ×2400.
FIGS. 33 to 36.—Successive stages in the first division of the schizont. ×2400.
FIGS. 37 to 40.—Daughter cells formed by division of schizont. In Figures 39 and 40 the nucleus of the enveloping cell has a very different structure from that of the inner cell. ×2400.
FIG. 41.—Epithelial cell containing parasite, which is undergoing schizogony. ×1640.
FIG. 42.—Intracellular parasite at about same stage as Figure 41. Note that nucleus of enveloping cell shows evidences of disintegration. ×2400.
FIG. 43.—Slightly later stages in schizogony. Enveloping cells disintegrating. ×2400.
FIG. 45.—Somewhat later stage in schizogony. ×2400.
FIGS. 46 and 47.—Late stage in schizogony, showing formation of merozoites. ×2400.
FIG. 48.—Epithelial cell containing parasite with chromatin in two masses at opposite sides of nucleus. ×1640.
FIG. 49.—Portions of two adjoining epithelial cells containing parasites at a somewhat earlier stage than Figure 48. ×2400.
FIG. 50.—Epithelial cell with parasite at a later stage of development than Figure 48. ×1640.
FIGS. 51 to 56.—Series of stages showing transition from the typical intracellular form to the flagellate. ×2400.
FIG. 57.—Epithelial cell from the cæcum of the fantailed darter (*Etheostoma flabellare*), containing intracellular stage of Octomitus sp. ×2400.