

SCHIZAMOEBA SALMONIS, A NEW AMEBA PARASITIC IN SALMONID FISHES

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INTRODUCTION AND METHODS

During the course of an investigation on the intestinal protozoa of trout it was found that an ameba which exhibits a number of very interesting characteristics is common in these fish. Unlike the parasitic amebæ of other vertebrates, this species lives in the stomach rather than in the intestine, only the cysts being found in the latter organ. This ameba is evidently widely distributed, since it has been found in a number of localities east of the Mississippi River and on the Pacific coast. Nor is it restricted to any one species of fish, having been found in several species of trout and salmon, but it is probably confined to the Salmonidæ.

Practically all of the material for this investigation was obtained at the Bureau of Fisheries' station at White Sulphur Springs, W. Va. Since the amebæ die very quickly when removed from the stomach, nearly all my observations have been based on preserved material. For this purpose the mucus covering the epithelial lining of the stomach was scraped off with a scalpel and smeared as quickly as possible over a cover glass, which was at once dropped into a fixing solution. Worcester's formol-sublimate-acetic fluid was found to give the best results, although Schaudinn's fluid was also found to be of considerable value. Both smears and sections were stained with iron-hematoxylin, which was found to give excellent results when properly decolorized.

THE TROPHOZOITE

As stated above, the trophozoites are found only in the stomach, where the organism is often very abundant in the mucus covering the epithelial lining. The living amebæ are colorless and very transparent, showing a faintly granular structure of the protoplasm within which the nuclei are usually easily distinguishable. No

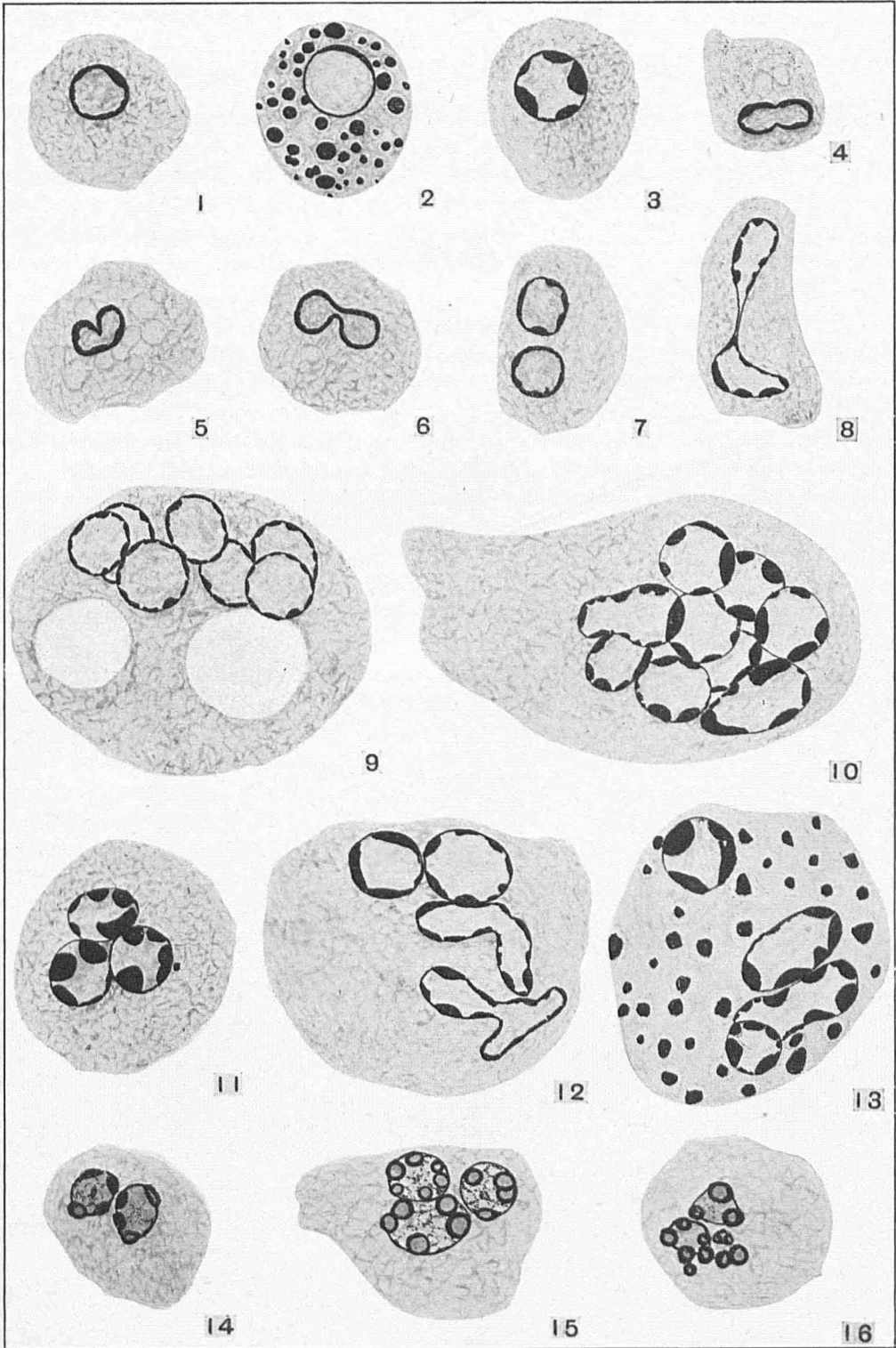
ectoplasmic layer can be distinguished, and there are usually no distinct vacuoles, although a few small fat globules are sometimes present. Treatment with iodine has failed to disclose any evidence of the presence of glycogen at this stage. No trace of ingested food particles could be found, and it is probable that the amebæ are entirely dependent on liquid food. Although somewhat irregular in shape, no ameboid movements could be distinguished, and it seems probable that even when in the stomach such movements are comparatively slow.

The appearance of the trophozoites after being stained is shown in Figures 1 to 12. They vary greatly in size, and although the majority have a diameter of about 10 to 15 microns, individuals with diameters of 20 to 25 microns are found occasionally. Usually there is only one nucleus, although multinucleate stages occur and are sometimes quite numerous. The latter are, of course, larger than the mononucleate amebæ and may contain from 2 to 10 nuclei—possibly even more, since it is very difficult to distinguish the individual nuclei when crowded closely together. However, individuals with 6 or more nuclei are very exceptional and can not be considered typical of the species. As a matter of fact, it is believed that this ameba must be looked upon as primarily mononucleate, with the occasional occurrence of multinucleate forms.

The nuclei are of the vesicular type and never possess a karyosome, the interior being filled with achromatic material that exhibits no distinct structure. The chromatin is confined to the periphery of the nucleus, where it sometimes forms a thin, nearly continuous layer attached to the nuclear membrane (figs. 1 and 2), but which in many instances can be seen to be composed of a large number of small granules (fig. 9). A similar arrangement of the chromatin has been described by several writers in other species of amebæ. Frequently, instead of forming a more or less continuous layer, the chromatin is concentrated in several large masses, as shown in Figures 3, 10, and 11. These blobs of chromatin are evidently formed by the aggregation of the granules previously referred to, and this is believed to form the first stage in the development of the cysts that will be described later.

The cytoplasm usually has a quite uniform, indistinctly vacuolated structure. Occasionally one or more large vacuoles can be distinguished (fig. 9), which in life probably contained fat globules, but such vacuoles are exceptional and in no wise characteristic. Usually a small percentage of the amebæ contain numerous chromatoidal bodies, as shown in Figures 2 and 13. These bodies usually are spherical, sometimes somewhat irregular in shape, and vary greatly in size. They are distributed uniformly throughout the cytoplasm and apparently have no relation to the nucleus. That the presence of these bodies is not due to differences in fixation or staining is shown by the fact that amebæ containing them are found side by side with individuals in which no trace of such structures can be found. It is believed that these bodies probably are composed of reserve food material, and it is an interesting fact that the protoplasm of amebæ containing chromatoidal bodies always has a dense homogeneous structure without any trace of vacuoles or a network.

Nuclear division in the ameboid stages is probably always amitotic, as is strongly suggested by Figures 4 to 8, which are by no means exceptional. In Figure 12, which is drawn from a section through a multinucleate individual, two of the nuclei



FIGS. 1 and 2.—Mononucleate amoebae showing typical arrangement of the chromatin on the inside of the nuclear membrane. In Figure 2 the cytoplasm contains numerous chromatoidal bodies. $\times 1640$
FIG. 3.—Mononucleate amoeba with the chromatin collected in large masses on the nuclear membrane in preparation for encystment. $\times 1640$
FIGS. 4 to 8.—Amoebae showing different stages of nuclear division by amitosis. Figure 8 was drawn from a section. $\times 1640$
FIG. 9.—Large multinucleate amoeba containing two large vacuoles. $\times 1640$
FIGS. 10 to 13.—Multinucleate amoebae showing chromatin collected in large masses on the nuclear membrane. Figures 12 and 13 were drawn from sections. Numerous chromatoidal bodies are present in the cytoplasm in Figure 13. $\times 1640$
FIGS. 14 to 16.—Successive stages in the development of the cyst nuclei. $\times 1640$

appear to be dividing by amitosis. That mitotic division does not occur in the ameoboid stage is also indicated by the fact that a careful search has failed to disclose any evidence of mitosis at this stage. This agrees with the observations of Taylor (1923), who was unable to find any evidence of mitotic division in the ameoboid stage of *Amœba proteus*.

STRUCTURE OF CYSTS

Usually, in addition to the ameoboid stages just described, there are numerous cysts present in the stomach which have a quite different appearance. These cysts are often more abundant than the vegetative stages, which in several instances were rare while the former were present in large numbers. On the other hand, the opposite condition may obtain, and in one noteworthy case very few cysts were found, although the ameoboid stages were extremely abundant. This would indicate that cyst formation is more rapid at certain times than at others, although little evidence has been obtained regarding the factors controlling their development. The facts at hand indicate that cyst formation may possibly be correlated in some way with the amount of food in the stomach of the host.

The cysts are spherical and vary greatly in size, the diameter usually being between 15 and 35 microns, with an average diameter of 20 to 25 microns. They are surrounded by a thin transparent membrane and usually contain numerous spherical refringent bodies, which are evidently fat globules since they stain deeply with Sudan III and are unaffected by iodine. No glycogen vacuoles could be demonstrated, but since the inner half of the cysts usually stains deeply with iodine it is believed that glycogen is present in a diffused condition. In a few instances small irregular bodies staining deeply with iodine could be distinguished near the center of the cyst. After being on the slide for some time the fat globules may be squeezed out of the cytoplasm and coalesce to form a large globule in the space between it and the cyst wall.

A study of stained preparations shows that the cysts are always multinucleate, containing from three up to a large number of nuclei (figs. 20 to 22). The structure of the nuclei is very different from that of the vegetative stages, and were it not for the fact that a complete series of transition stages can be found between the two forms it would be difficult to believe that they belong to the same species. In striking contrast to the ameoboid forms the cyst nuclei always contain a large deeply-staining karyosome near the center, while the nuclear wall is entirely free from chromatic material (fig. 20). The karyosome usually stains intensely and uniformly with chromatin stains, but in a few strongly decolorized specimens could be seen to be composed of closely crowded chromatic granules embedded in a lighter staining matrix. On one side of the karyosome, usually that turned toward the periphery of the cyst, is a mass of achromatic material extending from the karyosome to the nuclear wall. It does not, however, extend directly to the wall, but flares out in all directions a short distance from the karyosome to form a mushroom-shaped structure with its edges in contact with the nuclear membrane (fig. 20). Embedded in the achromatic material are numerous small deeply-staining granules, whose later history indicates that some of them, at least, are to be considered as homologous

with the centrioles and blepharoplasts of other protozoa. That they differ chemically from the chromatin of the karyosome is indicated by the fact that in very strongly decolorized nuclei they are unstained while the karyosomes still retain the stain. In fact, the appearance and abundance of these granules appears to be largely dependent on the stage to which the destaining process has been carried.

The arrangement of the nuclei in the cyst is characteristic. Except in the case of the small cysts with few nuclei they are usually arranged quite regularly in a single layer about one-half the distance from the center to the periphery of the cyst (figs. 20 and 22). This arrangement divides the cytoplasm into two regions, which present a quite different appearance. Within the nuclear layer the cytoplasm is strongly vacuolated, while in the outer zone between the nuclear layer and the periphery the vacuolated structure is much less noticeable and in the larger cysts may entirely disappear.

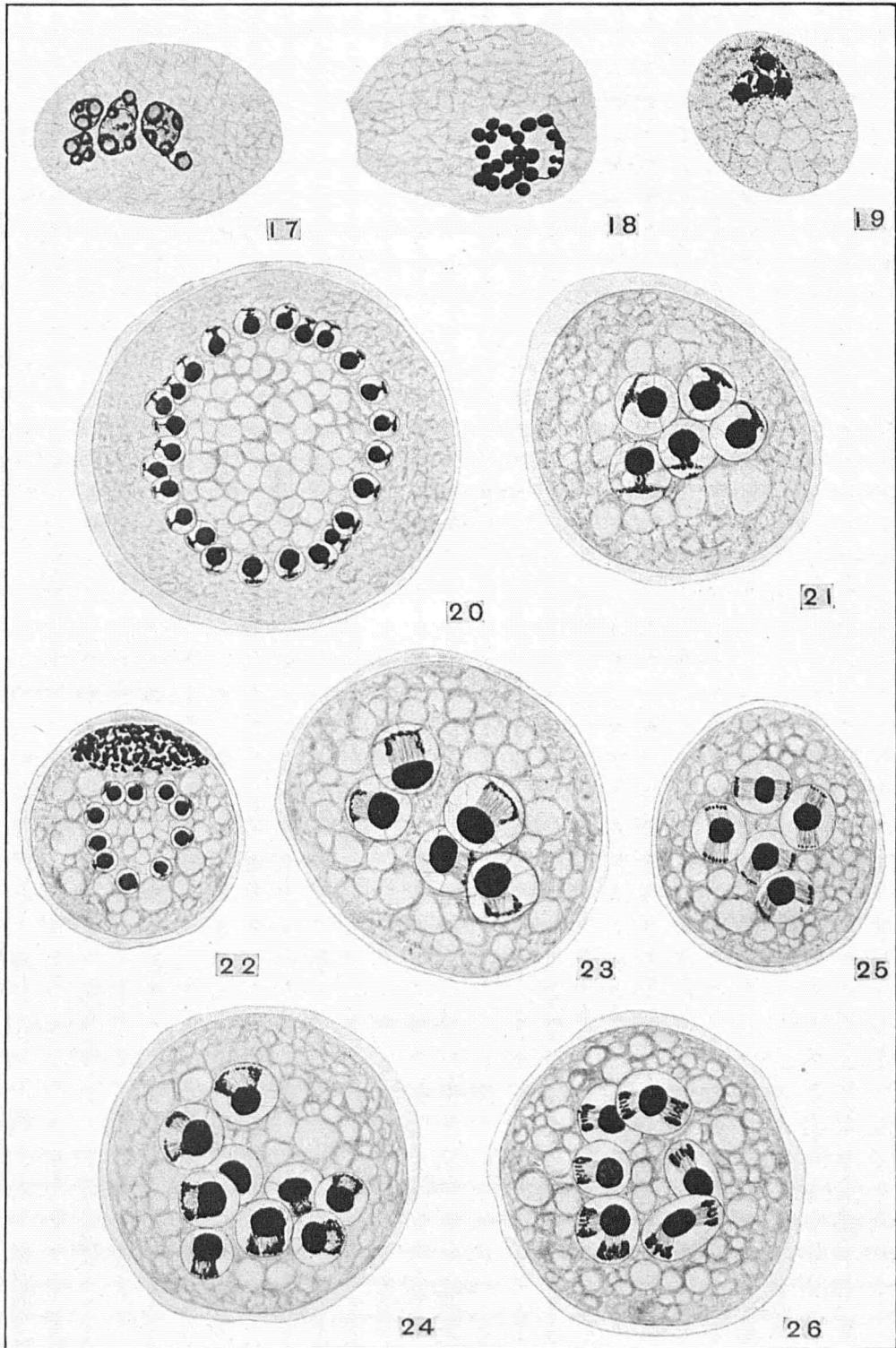
In some instances a mass of deeply staining granules is present at one side of the cyst, which is probably homologous with the chromatoidal structures of other amebæ, but in the great majority of cysts no trace of such a structure can be distinguished. The explanation of this fact is not obvious, since it can hardly be due to differences in fixation or staining.

DEVELOPMENT OF CYSTS

Perhaps one of the most interesting features in the life cycle of this species is to be found in the development of the cysts from the ameboid stage, since the process is quite different from that described for other amebæ. As previously noted, the nuclei of the amebæ frequently exhibit a quite different arrangement of the chromatin from that usually found. Instead of forming a thin layer, either continuous or composed of discrete granules on the inside of the nuclear membrane, the chromatin becomes arranged in large blobs, as shown in Figures 3 and 10 to 13. There are usually five or six of these chromatin masses in each nucleus, but the number is by no means constant. This arrangement of the chromatin is apparently the first step in cyst formation and may occur in both mononucleate and multinucleate individuals. Judging from their abundance, this stage lasts for some time, and the nuclei may even divide amitotically while in this condition. After a time the chromatic masses become rounded and usually exhibit a vesicular structure (figs. 14 to 17), the chromatin forming a peripheral layer around the less deeply staining center.¹ At this time the nuclear wall shows signs of disintegration and a little later entirely breaks down, leaving the chromatin spherules free in the cytoplasm (figs. 18 and 19). These spherules form the karyosomes of the cyst nuclei, losing their vesicular structure and each becoming surrounded by a nuclear membrane.

The nuclear changes involved in the development of the cysts are accompanied by corresponding changes in the cytoplasm. The organisms become rounded and are surrounded by a thin transparent membrane which is difficult to distinguish in stained specimens. The cytoplasm is distinctly vacuolated and contains numer-

¹ The vesicular structure is evident only in strongly decolorized individuals.



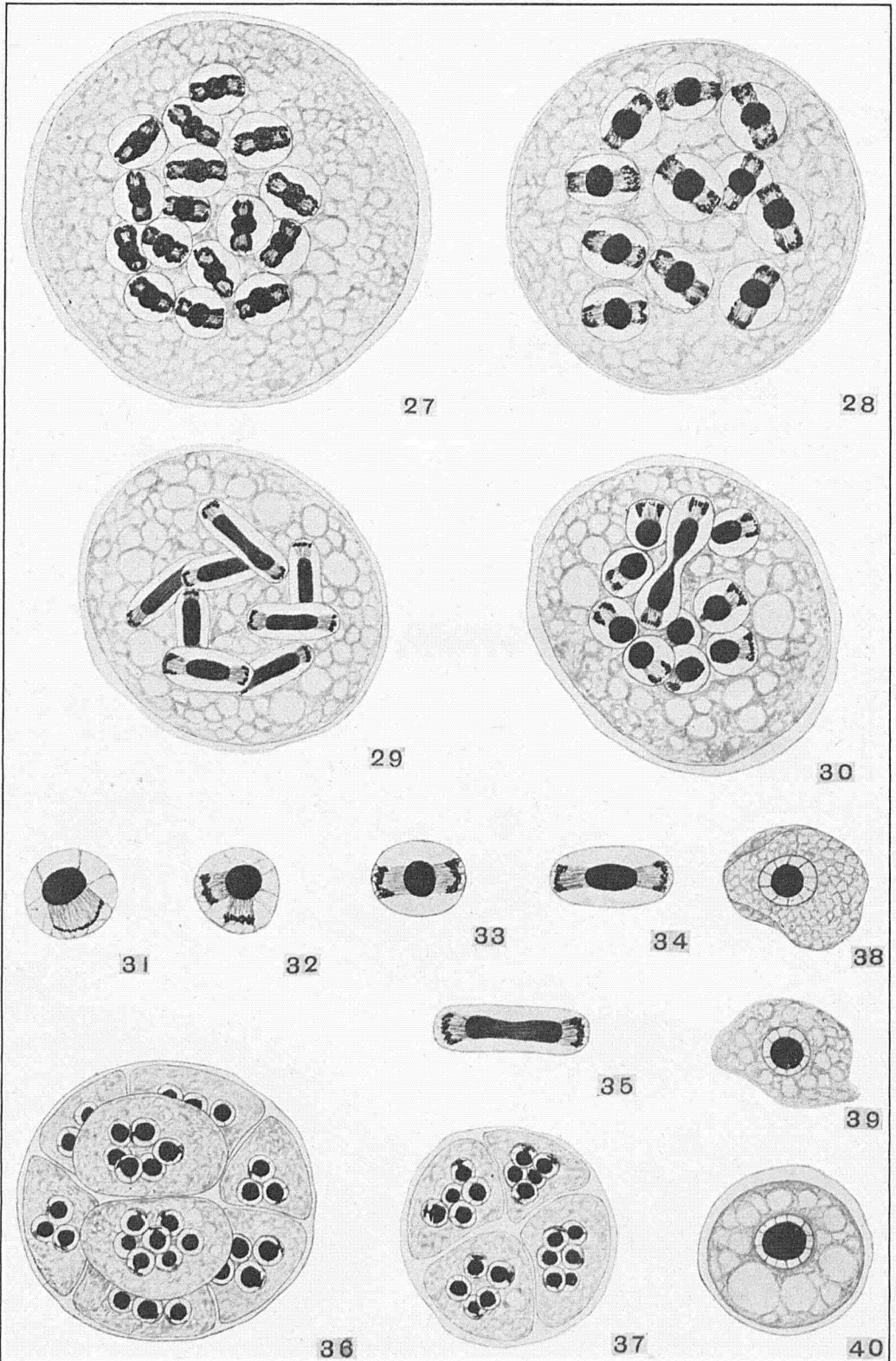
FIGS. 17 to 19.—Successive stages in the development of the cyst nuclei. The nuclear membrane is disintegrating in Figures 18 and 19. $\times 1640$

FIG. 20.—Optical section of a cyst showing typical arrangement of the nuclei. $\times 1800$

FIG. 21.—Small cyst with five nuclei drawn at a higher magnification to show details of structure. $\times 2700$

FIG. 22.—Optical section of cyst with a mass of chromatoidal bodies on one side. $\times 1800$

FIGS. 23 to 26.—Cysts showing successive stages in the division of the nuclei. Prophases. $\times 2700$



FIGS. 27 to 30.—Cysts showing successive stages in the division of the nuclei. Figures 27 and 28, metaphase; Figure 29, anaphase; Figure 30, telophase. $\times 2700$
 FIGS. 31 to 35.—Nuclei drawn at a higher magnification to show successive stages in division. $\times 3150$
 FIGS. 36 and 37.—Cysts that have recently divided into a number of approximately equal parts. The cyst shown in Figure 36 contained 11 cells, three on the lower side not being shown in the figure. $\times 1800$
 FIGS. 38 to 40.—An undescribed species of amoeba occasionally found in the digestive tract of trout. Figures 38 and 39 are the trophozoites from the stomach, while Figure 40 is the encysted stage from the intestine. $\times 1640$

ous fat globules. It also stains more deeply with iodine, indicating the formation of glycogen, which is evidently present in a diffused condition.

Since the cysts may develop from mononucleate, binucleate, or multinucleate individuals, there is obviously great variation both as regards size and number of nuclei in the newly formed cysts. In fact, cysts are formed with as few as three nuclei, while others may contain a large number. However, the later history of the cysts is such as to make it impossible to determine with any degree of accuracy whether any particular cyst is newly formed or has already gone through a course of development in the encysted stage. Their further development is of particular interest, since no similar phenomena appear to have been observed in other species of amoebæ. These changes take place both in the stomach and intestine, and all stages may be found in both organs. In fact, no difference could be detected in cysts from the stomach and intestines and, unlike the vegetative stages, they are apparently indifferent to the surrounding fluids.

While the newly formed cysts vary greatly in size, there is good reason to believe that the organisms continue to grow after encystment, since the percentage of large cysts with a correspondingly large number of nuclei is always much greater than that of the multinucleate vegetative forms. Furthermore, the division of the nuclei, followed eventually by division of the cysts themselves, also indicate growth during this stage. In striking contrast to the ameboid stage the cyst nuclei always divide by a primitive form of mitosis. The details of this process are shown in Figures 23 to 30. In preparation for division the karyosomes become enlarged and the chromatic material at one side develops a fibrous structure (figs. 23, 24, and 31). The chromatic granules, which in the resting nucleus are embedded in this material, become arranged in an irregular manner at the ends of the fibers nearest the nuclear membrane. A little later the group of fibers splits into two equal parts (figs. 25 and 32), which separate and eventually come to lie on opposite sides of the karyosome (figs. 27, 28, and 33). At this stage, which is evidently the metaphase, the mitotic figure extends across the nucleus with the karyosome in the middle of the spindle. The chromatic granules now form a deeply staining mass at the ends of the spindle fibers. Often they appear to be scattered along the sides of the spindle, but this may be due to the fact that the fibers are of unequal length. Shortly after this the karyosome becomes elongated and later constricted in the middle, as shown in Figures 29, 30, 33, and 34. Eventually the two parts separate, thus giving rise to two daughter nuclei similar in every respect to the original nucleus except in the matter of size. While this is evidently a form of promitosis no chromosomes could be detected in the karyosome, although it appears to have an indistinct fibrous structure when greatly elongated. The arrangement and behavior of the chromatic granules on the spindle fibers indicates that they should probably be considered as homologous with the blepharoplasts and centrioles of other protozoa.

The details of this process of promitosis appear to be quite different from anything described in the literature. In some respects there is a superficial resemblance to the nuclear division in the trophozoites of *Amoeba tachypodia* (Gläser, 1912; Ford, 1914) and of *Naxleria gruberi* (Wilson, 1916). However, in both species the details of the process are quite different in several respects. The peripheral chromatin, which collects at one side of the karyosome in these two species,

appears strikingly like the granules embedded in the achromatic structure joining the karyosome to the nuclear wall in *S. salmonis*. However, it is very evident that in *salmonis* these granules take no part in the formation of the chromosomes but become arranged at the poles of the spindle during the prophase. In this respect they would appear to correspond with the chromatic polar masses, which in *Amœba tachypodia* and *Nægleria gruberi* are derived from the karyosome. Obviously, further investigations are required before it will be possible to determine the homologies of the nuclear structures in the two types of amebæ.

Coincident with the division of the nuclei there is a marked increase in the size of the cysts, which may eventually reach a diameter of 35 microns and contain a large number of nuclei. Occasionally a large cyst is found in which the nuclei, instead of being distributed quite regularly around the central portion of the cyst, as previously described, are arranged in several groups separated a short distance from each other. This arrangement of the nuclei is preparatory to division of the cyst into a number of distinct parts corresponding to the number of nuclear groups. Superficially this division has a striking resemblance to the segmentation of an egg but differs in several fundamental respects. Unlike an egg, the cyst divides simultaneously into from 4 to 11 cells of approximately equal size (figs. 36 and 37). Each cell usually contains from 4 to 8 nuclei, there being considerable variation in this respect even in daughter cells from the same cyst. The disintegration of the cyst membrane, which quickly follows, allows the cells to separate, and presumably each in turn may go through a similar course of development. Dividing cysts, while not common, have been found in a number of instances in both the stomach and intestine, and there can be no doubt that this is a normal process in the life cycle of the species.

The further development of the cysts has not been followed, but it is known that they pass out of the intestine in the excrement in the condition shown in Figures 20 and 22, and presumably germinate after being ingested by another fish

SYSTEMATIC RELATIONSHIP

It is evident that this ameba differs in several essential respects from other known species. The most striking differences are found in the encysted stage, and these are believed to be of sufficient importance to justify the creation of a new genus coordinate with other genera of the Amœbida. The essential characters of this genus are as follows:

Schizamœba gen. nov.

Parasitic. Trophozoites mononucleate and multinucleate. Cyst nuclei formed by fragmentation of nuclei of trophozoite, with large central karyosome connected at one side with the nuclear membrane by an achromatic structure in which are embedded numerous chromatic granules. Cysts divide into a number of multinucleate cells, each of which forms a separate cyst.

Type species **Schizamœba salmonis** sp. nov.

Trophozoite slowly ameboid, with one to several vesicular nuclei without a karyosome, and with the chromatin arranged on the nuclear wall. During encyst-

ment the chromatin collects in several large masses on the nuclear membrane, which eventually become rounded and, following disintegration of the nuclear wall, form the karyosomes of the newly-formed cyst nuclei. The karyosomes are connected at one side with the nonchromatic nuclear wall by a mushroom-shaped achromatic structure, in which are embedded numerous chromatic granules. The cyst nuclei divide several times by promitosis, during which the karyosome is divided into two equal parts without losing its identity. Eventually the cysts divide into several multinucleate cells, which separate and form new cysts like the original. The trophozoites are found only in the stomach; the cyst in both stomach and intestine of salmonid fishes.

DISTRIBUTION

That this species is widely distributed is shown by the fact that, although it has been under observation for only a short time and but little attention has been paid to this feature, it has already been found in several species of trout, including the rainbow (*Salmo shasta*), the brook trout (*Salvelinus fontinalis*), and the brown trout (*Salmo fario*), at the White Sulphur Springs (W. Va.) and Wytheville (Va.) hatcheries. The cysts of this amoeba have also been found in the young of the chinook salmon (*Oncorhynchus tshawytscha*) and the silver salmon (*Oncorhynchus kisutch*) from several hatcheries on the Pacific coast. Dr. Emmeline Moore also reports that it occurs in trout at a number of the New York State hatcheries, where the cysts have been confused with those of *Octomitus salmonis*. It is very evident that this amoeba is widely distributed among the Salmonidæ.

RELATION TO HOST

There is, as yet, no evidence that *Endamoeba salmonis* is directly harmful to the host, although it is sometimes present in very large numbers. In fact, it has been observed in several instances that the parasites were exceptionally abundant in fish that appeared to be in excellent condition, while there were only a few amoebæ in sick fish from the same lot. This difference in the abundance of the parasites was probably due to the fact that the sick fish were taking little if any food, since it has been found that the amoebæ rarely occur in numbers in the stomachs of fish that have been without food for several days. But while apparently not directly injurious, at least under ordinary conditions, they are doubtless of considerable importance in nutrition, since when abundant they must consume appreciable quantities of food that would otherwise be utilized by the host.

OTHER AMEBÆ IN TROUT

In addition to *Endamoeba salmonis*, another amoeba has been found in the stomach and intestines of trout in several instances. As in the case of *S. salmonis*, the vegetative stages were found in the stomach while only cysts were observed in the intestine. In appearance this species is quite distinct from *S. salmonis*, especially as regards the structure of the nucleus, which always contains a prominent karyosome even in the amoeboid stage (figs. 38 and 39). The protoplasm is more

sharply defined than in *S. salmonis*, and the general appearance of this species suggests that it is a typical ameboid form of the limax type. The cyst shown in Figure 40 is mononuclear and very similar in appearance to the ameboid stage.

This species has been found in only a few instances, and then in too small numbers to permit of a detailed study, but such observations as have been made indicate that it differs in no essential respect from the orthodox type of parasitic amebæ.

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