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Estudio experimental de un Oxytricha amicronucleado.

I. Estudio del animal normal con una descripción de su canibalismo.

Durante 289 generaciones se ha cultivado Oxytricha hymenostoma Stokes—desde el 10 de Julio de 1917 hasta el 17 de Noviembre de 1917— y el autor conserva aun vivos cultivos en masa de los descendientes de estos cultivos. No ha visto un micronúcleo en ningún estado de los diversos animales, durante la historia de los cultivos. No ha habido singamia, si i bien el autor cree que el organismo en cuestión ha pasado frecuentemente por un estado físico semejante al en que tiene lugar la singamia. En este momento: a) los animales se fusionan por parejas a semejanza de la unión de los conjugantes, y, b) tiene lugar el canibalismo. Cuando acaece la fusión en parejas, los animales o bien permanecen fusionados hasta su muerte o se separan. En caso de separación los organismos en cuestión continúan reproduciéndose y no presentan síntoma alguno de estar en una condición de depresión. Cuando el canibalismo tiene lugar los animales ingeridos son digeridos rápidamente y los que sobreviven vuelven a adquirir el tamaño y estructura típicos. El canibalismo produce como efecto el aumentar ligeramente la cantidad de divisiones durante un corto tiempo. No se ha observado enquistamiento. Esta raza amicronucleada de Oxytricha puede vivir indefinidamente, en apariencia, bajo condiciones ambientes favorables sin conjugación, autogamia o endomixis.

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AN EXPERIMENTAL STUDY OF AN AMICRONUCLEATE OXYTRICHA

I. STUDY OF THE NORMAL ANIMAL, WITH AN ACCOUNT OF CANNIBALISM

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TWENTY FIGURES

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1. INTRODUCTION

The presence of a macronucleus and a micronucleus has long been recognized as one of the striking features which aids in marking off the Infusoria from the rest of the Protozoa, and there is abundant evidence tending to prove that the micronucleus is an indispensable part of the organism during both conjugation and endomixis. In all the ciliates where the micronucleus has seemed to be absent in the vegetative stages of the life history, a careful study has invariably revealed its presence at the onset of sexual phases.

Thus, Neresheimer ('07) and Metcalf ('09) found in Opalina, a parasitic ciliate which has but one kind of nucleus, that a certain type of syngamy took place by the combination of uninucleate gametes, the nuclei of which contained chromatin comparable to that of a micronucleus. In Trachelocerca phenicopterus, a free-living holotrichous ciliate, micronucleus and macronucleus are merged completely in the vegetative state, but the micronucleus appears during conjugation (Lebedew, '08). Ichthyophthirius multifiliis, a parasitic holotrichous infusorian has a separate macronucleus and micronucleus in certain stages, but in others the micronucleus is contained within the former (Neresheimer, '08, and Buschkiel, '11). Calkins ('12) found in Blepharisma undulans, a free-living heterotrichous ciliate, that the micronucleus is contained within the macronuclear membrane in the ordinary vegetative stages, but becomes separated from the macronucleus during conjugation.

In the course of experiments on certain hypotrichous ciliates the writer obtained a species of Oxytricha in which, upon staining, no definitive micronucleus was visible. Recalling the work of previous investigators on apparent amicronucleate ciliates where a micronucleus manifested itself during conjugation, it was decided to breed the organism and attempt to obtain conjugation. In addition, a culture was carried by the daily isolation method to ascertain the ability of this species to live indefinitely, reproducing by division without conjugation. Although careful cytological studies have been made throughout the entire course of the experiments, no definitive micronucleus has at any time been observed, but certain interesting and suggestive results have been obtained. Under conditions favorable to conjugation, the reactions of this organism closely resembled those of a conjugant, but instead of completing the normal process of fusion, it was found that the animal invariably became cannibalistic, and that there was a strong tendency for pairs to fuse dorsally, thus forming a double animal or 'twin.'

An account of the investigation of these phenomena will be presented as follows:

PART I. 1. Study of the normal animal with attempts to induce conjugation. 2. Cannibalism.

PART II. The formation of double animals or 'twins.'

I take pleasure in acknowledging my great indebtedness to Prof. Lorande L. Woodruff, at whose suggestion this investigation was begun, for his advice and criticism throughout the entire course of this study. I also wish to express my thanks to Prof. Alexander Petrunkevitch for assistance in making the microphotographs and to Dr. Rhoda Erdmann for valuable suggestions in technique.

2. MATERIAL AND METHODS

The hypotrich used for these experiments was obtained originally in the fall of 1915 in some débris, from a pond in the vicinity of New Haven, which had been placed in a laboratory aquarium. A pedigreed culture was carried from February 22 to June 21, 1916, when it was discontinued. Since this time stock from the same culture has been in the laboratory aquaria, and an individual of this species which was isolated from an aquarium in the Osborn Zoological Laboratory on July 10, 1917, has supplied all the animals used in this experimental study.

The species has been found to agree very closely with the description given by Stokes ('88) for Oxytricha hymenostoma. Systematic works do not usually describe specifically the macronucleus or micronucleus, more stress being laid on the external structures for classification. As the absence of a micronucleus and the slight differences shown by this form from Oxytricha hymenostoma Stokes were not assumed to be of specific value, no attempt is at present made to establish a new species, the hypotrich being identified provisionally as Oxytricha hymenostoma.

On July 11th the animal, which was isolated on the previous day, had divided twice, and the four resulting organisms were isolated on depression slides in about five drops of culture medium to form the four lines of culture A. In this culture and in all

subcultures carried during the work the animals have been isolated daily by means of a capillary pipette and a Zeiss binocular, oculars 2 and objectives F55. Separate pipettes have been used for each culture, and these have been carefully sterilized before using by thorough boiling or by heating in a small gas flame. 'Stock' cultures of the animals remaining in each line after the daily isolation have been kept on the depression slides for a day or two, so that an animal could be replaced in case of accident. Both line and stock cultures have been kept in moist chambers. For special purposes animals have been allowed to multiply on these stock slides, thus forming small mass cultures. Small Petri dishes (35 cc. capacity) have been used for growing mass cultures, and this method has been found to have several advantages, since a Petri dish of this size may be quickly and accurately examined with the binocular microscope, while the cover, although fitting loosely, prevents contamination and permits but very slow evaporation.

The culture medium used was varied as much as possible. The basis of the medium was hay boiled thoroughly in tap-water. To this was added from time to time various decaying vegetable and animal material from laboratory aquaria and ponds. Occasionally living snails were finely chopped and added to the medium. In all cases contamination was prevented by thorough boiling—the medium being usually allowed to stand overnight before using. Thus an attempt was made to supply the animals with as varied an environment as possible, since such a 'varied environment' (Woodruff, '08, '11) has been used for the continued maintenance of Paramecium aurelia in pedigreed cultures.

Attempts to obtain conjugation were made, following in general the methods used by Maupas ('89), Calkins and Cull ('07), Jennings ('10), and Woodruff ('14), and others. Stock cultures were also frequently allowed to multiply on depression slides and in small Petri dishes for the same purpose. In all cases the result was the same, i.e., invariably animals were obtained which made what have been interpreted as abortive attempts to conjugate.

Specimens for cytological study were fixed in corrosive sublimate (saturated solution) with 1 per cent acetic acid and in Schaudinn's sublimate-alcohol (strong). Various stains were tried, including Heidenhain's iron hematoxylin, Delafield's haematoxylin, borax carmine and picro-carmine. Differentiation was made with acidulated alcohol (70 per cent alcohol and 0.01 per cent HCl). In general Delafield's hematoxylin has been found to give the best results. Eosin and tetra-brom-fluorescic acid were used as counterstains, the latter being especially good for staining external structures as cilia, cirri, and membranelles.

Single animals were fixed and stained on depression slides. The animal was kept under observation during the whole process except for the period necessary for staining, when the slide containing the specimen was placed in a moist chamber. The various fluids used in the process were drawn off with a capillary pipette or filter-paper, the only transfers being when the animal was isolated for fixation and when the same animal, now ready for mounting, was placed on a thin glass slide. Specimens were cleared and mounted in cedar oil or in xylol and damar. Total mounts thus made were prevented from being crushed by placing pieces of hair of suitable lengths under the cover-slips. At various times smears of large numbers of animals were made for general study. Sections, 4 μ in thickness, have been prepared, but it has been found that study of these has added nothing to the cytological details which on account of the thinness of the animal may be studied thoroughly in total mounts.

3. STUDY OF THE NORMAL ANIMAL

Although hypotrichous forms have been carefully investigated, no worker has as yet been able to breed these ciliates indefinitely by the daily isolation method without conjugation. Maupas ('88) carried cultures of Stylonychia pustulata without conjugation for 316 generations; Stylonychia mytilus, similarly, for 319 generations; Onychodromus grandis and Oxytricha for 320 to 330 generations. He concluded that in all these cases death was due to old age, no opportunity having been allowed for 'rejuvenation' by conjugation. Joukowsky ('98) kept one of four

cultures of Pleurotricha lanceolata for 458 generations without conjugation or degeneration. Woodruff ('05), working with Oxytricha, Pleurotricha, and Gastrostyla, found that the number of generations in the life cycles of these species was not at all constant. The culture of Oxytricha was longest lived (860 generations) and its life was prolonged by artificial stimulation. The same author ('13), referring to his early culture of Oxytricha, stated that he believed "that if an entirely suitable environment had been secured this culture would have given evidence of unlimited power of reproduction by division without conjugation as my present P. aurelia has done." Popoff ('07) kept a culture of Stylonychia mytilus for three and a half months, at the end of which time it died during a deep 'depression period.' He concluded that the cause of depression was not accidental changes in environment, but lay in the organism itself. Enriques ('03, '05, etc.), working with Oxytricha and Stylonychia, concluded that the cause of depression and subsequent death was prolonged poisoning with bacterial poisons, and that "agamic reproduction can be continued as long as one likes if the technique is good and bacteria are not too numerous. No change of food is necessary." Baitsell ('12) kept Stylonychia pustulata for 572 generations. He did not observe any "appearance of abnormal or degenerating animals," but the fission rate of the cultures gradually declined until death occurred. This he interpreted as due to unsuitable cultural conditions. In later work ('14) he succeeded in keeping a culture of Pleurotricha in a beef-extract medium for 943 generations. He also carried mass cultures of the same organism in test-tubes and without conjugation for twenty-two months. From this he concluded that Pleurotricha would "apparently live indefinitely without conjugation or artificial stimulation."

The evidence from these investigators proves that, if a life 'cycle' exists in hypotrichs, it is subject to great variation, depending largely, if not entirely, upon the environment. Artificial stimulation has been shown to have the effect of prolonging the life 'cycle,' and the most recent work indicates that this 'cycle' may be prolonged indefinitely if the organism is provided

with suitable environmental conditions, i.e., the 'cycle' is merely an artifact resulting from unfavorable conditions.

1. History of culture A

This culture was begun on July 11, 1917, from the four descendants of the Oxytricha isolated on the previous day. The culture was carried by daily isolations until November 17, 1917, when division stopped in all the four lines and the animals, after living one to two weeks without fission, finally died. An attempt was made to continue the race by isolating individuals from the stock cultures of this date, but this proved unsuccessful. However, animals taken from stock cultures on November 2nd have been kept living in small mass cultures (without conjugation) and are still reproducing (April 12, 1918). This fact, reviewed in the light of the work of Baitsell ('14) on Pleurotricha, seems to indicate that if suitable cultural conditions are given, there is a strong possibility of unlimited asexual reproduction.

The curve (fig. 1) showing the average division rate of culture A and hence the general physiological condition of the animals during the life of the culture has been plotted for five-day periods. This was done, according to the usual method, by averaging the divisions of the four lines together and then averaging the result for five-day periods. A study of this curve shows that the division rate during the first month was much higher than at any other time during the course of the experiments. The highest point was reached between July 31st and August 5th, with an average rate of 4.65 divisions per day. From this time on there were fluctuations[1] in the division rate which showed a

[1] A 'rhythm' was originally defined by Woodruff ('05) as "a minor periodic rise and fall of the fission rate, from which recovery is autonomous." More recently Woodruff and Erdmann ('14) have shown that there is a causal relation between endomixis and rhythms in Paramecium. Since endomixis has not been demonstrated in hypotrichs and, further, since it apparently does not occur in this form, it has been deemed inadvisable to apply the term 'rhythms' to the fluctuations in the division rate of Oxytricha hymenostoma. It should be noted that Fermor ('13) described an internal nuclear reorganization process in Stylonychia during encystment. Here the macronuclei degenerated; the micronuclei fused and later by division produced new micronuclei and

steady downward trend until the period September 9th to Sept. 14th, which had an average of 1.3 divisions per day. From this time on there occurred twice a general rise and fall in the division rate, and at the conclusion of the second drop in the rate the four lines of culture A died out.[2]

Although it has not been found possible to continue the race by the daily isolation method, it has been kept alive to date (April 12th) without conjugation and, to all appearance, in a good physiological condition in the Petri-dish cultures. The method of carrying these cultures was as follows. Several animals selected from stock slides were placed in a small Petri-dish in about 20 cc. of the same kind of medium used for culture A. This culture was examined carefully each day to preclude the possibility of unobserved conjugation. When the organisms had multiplied for a few days, isolation of several individuals was made to a fresh culture of the same kind, and the process has been repeated to date. On account of the ease with which accurate observations on these cultures may be made, it is believed that no ex-conjugants (assuming conjugation is possible in this species) have been isolated for the continuance of the race.

The result of this experiment on Oxytricha hymenostoma is very similar to that obtained by Baitsell ('14) with Pleurotricha, the life cycle of which, he found, could be prolonged indefinitely by use of a hay-infusion medium in test-tube cultures. It thus

macronuclei. Fermor states that encystment took place in apparently quite normal cultures and regards the process just described as a substitute for conjugation, since the latter process did not occur in the cultures of Stylonychia. Thus the possibility is indicated that in hypotrichous forms during encystment a process occurs which is, in a way, analogous to endomixis (Woodruff and Erdmann, '14) in Paramecium. It should be emphasized, however, that the process described by Fermor involves a fusion of micronuclei and therefore is autogamy rather than endomixis as defined by Woodruff and Erdmann in their paramecium work, since they found no micronuclear fusion. In the organism studied in this work I have observed no encystment, and it is difficult to conceive how a similar nuclear reorganization process might occur in Oxytricha hymenostoma, since no definitive micronucleus is present.

[2] A culture of Oxytricha sp. carried in the same medium and under absolutely the same conditions reached 400 generations, outliving the culture (A) by two and a half months, thus showing that the medium was apparently more suitable for other species of hypotrichs.

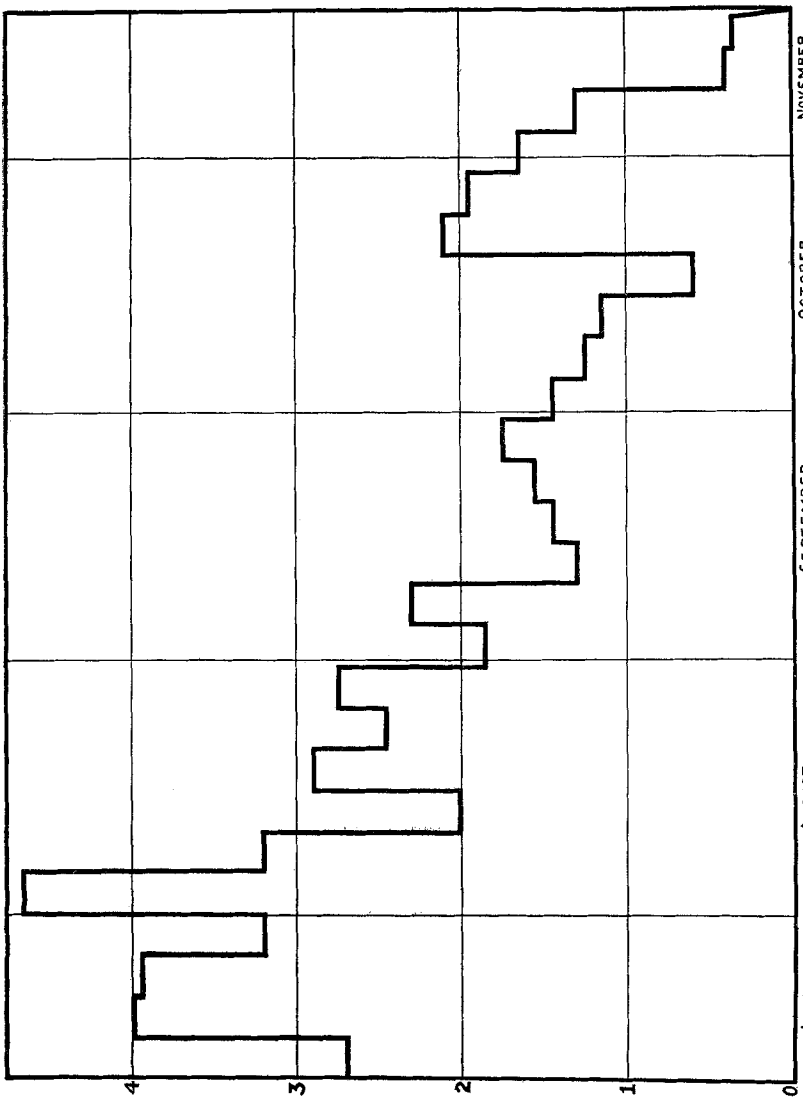

Fig. 1 Complete history of Oxytricha hymenostoma, Culture A, from July 11, 1917, to November 17, 1917. Rate of division averaged for five-day periods. The ordinates (continuous line) represent the average daily rate of division of the four lines of the culture.

is apparent that environmental conditions are responsible for many of the so-called life 'cycles' of certain infusorians, and that there is no reason to believe that, given suitable cultural conditions, this 'cycle' may not be indefinitely prolonged, or, in other words, that a 'cycle' exists at all.

2. Attempts to obtain conjugation

As is well known, in the nuclear phenomena during syngamy in all hypotrichida as yet studied, the micronucleus plays the chief part—the macronucleus invariably degenerating and being formed anew from the micronuclei resulting from the division of the synkaryon. It therefore naturally was concluded that a study of this process in Oxytricha hymenostoma would be the crucial test in the attempt to determine the presence or absence of a micronucleus.

It was soon found that stock animals, if allowed to multiply freely in Petri-dish cultures or on stock slides, after a longer or shorter period of rapid multiplication almost invariably would give evidence of being in the protoplasmic state always observed in conjugating ciliates, aptly described by Calkins ('02) as 'miscible.' In such cultures numerous pairs have been observed, fused by the oral surfaces in a manner very similar to that of normal conjugation. In the first stages this fusion was apparently limited to the peristomial membranelles, although many animals, seemingly with very slight attachment, could nevertheless be fixed and carried through the various processes incident upon staining without being separated. In many cases this fusion was not limited to the pellicle, but showed plainly a definite cytoplasmic connection between the members of a pair (fig. 6), but this connection never became quite so extensive as is usual in normal conjugants of other species of hypotrichs. Occasionally pairs have been observed united by the oral surfaces with the bodies of the two animals somewhat twisted around each other. In figure 5 a pair is shown united by the anterior ends in a manner closely resembling that figured by Maupas ('89, fig. 1, pl. 18) as an initial stage in the conjugation of Onychodromus grandis.

From time to time, as these pairs appeared, numbers were isolated for study; in all 100 pairs have been studied. Of these, 3 pairs separated within a few hours after isolation, while the remaining 97 pairs never separated and died in from six to forty-eight hours. Of the animals which separated, one of each pair was stained and the other was transferred to fresh medium on a depression slide. Study of the stained specimens showed a typical condition of the macronucleus in all cases and there was not the slightest sign of any structure which could be interpreted as a definitive micronucleus. The animals which were carried on depression slides continued to divide, thus indicating that whatever may have been the condition of these apparent conjugants, at any rate, it was not pathological.

The appearance and behavior of the animals in cultures, in which pairs were occurring in large numbers, was not different from that of typical, healthy animals. In order to determine definitely if this phenomenon was due to a depressed or pathological state, numbers of single animals were isolated and carried in lines by daily isolations. The division rate of these subcultures indicated that the organisms were in a good physiological condition. Pairs which had been attached for a few hours were separated by forcibly ejecting them from a capillary pipette. Here, again, the division rate did not show that the animals were in a pathological state. It is therefore clear that some other explanation must be sought, both for the onset of the tendency to pair and for the high mortality in permanent pairs.

3. Cytology of Oxytricha hymenostoma

In view of the fact that the presence of the micronucleus in the hypotrichous ciliates has been insisted upon so strenuously by recent investigators, and since there is an ever-present possibility of overlooking this important structure, every effort has been made in this study to determine its presence. Care has been taken to secure specimens in as many different stages of the life-history as possible, and these have been treated with a large variety of stains, as already mentioned. In addition to total

mounts of single animals and of large numbers at one time by smear preparations, sections have been prepared and studied.

The typical cytoplasmic structure of Oxytricha hymenostoma is alveolar throughout, as is so generally found in hypotrichous forms. A greater or less degree of vacuolization occurs at times, particularly when the vitality is low, as shown by the division rate. The macronucleus consists of two more or less ellipsoidal portions connected by a very delicate strand or commissure. This connection may be seen to best advantage in the living animal, as it almost invariably disappears during fixation. The macronuclei (as the two portions of the macronucleus are frequently called) are enclosed in a membrane and consist of chromatinic and achromatinic elements. The latter do not stain, and if the chromatin is overstained, as frequently occurs, are obscured. Proper differentiation, however, will always show these two elements. A 'Kernspalt' is almost invariably seen, most frequently seeming to bisect each portion of the macronucleus, but not uncommonly is found near one end (fig. 8). A typical specimen of Oxytricha hymenostoma is shown in figure 4. The cytoplasm here is seen to be somewhat vacuolated while the macronuclei have stained deeply. Both nuclei present a somewhat knobby appearance caused by small projecting portions of chromatin. As this condition has occurred frequently, such macronuclei have been most carefully studied in order to make sure that these projections were macronuclear chromatin. The result has been to establish beyond question of doubt that none of these appearances are micronuclei. A diagrammatically typical macronuclear structure is shown in figure 8.

Throughout the course of the experiments a certain amount of macronuclear fusion has been found, especially in animals in early stages of fission and also at other times. On the other hand, the macronucleus has been observed to be more or less fragmented. This fragmentation has been found to occur more frequently when the division rate was comparatively low. As Woodruff ('05) pointed out, fragmentation of the macronucleus is not necessarily an 'abnormal' condition, since this is a common occurrence during certain stages in the life-history of hypo-

trichous forms and may be succeeded by the entire state, usual at times of high reproductive activity. Essentially the same cytoplasmic and nuclear conditions are revealed by a study of the two pairs shown in figures 5 and 6. In figure 5 the animals are united by the peristomial membranelles, while in figure 6 is shown the typical method of fusion closely resembling an early stage of conjugation. An evident cytoplasmic connection is seen between the two animals.

Pathological specimens, an example of which is shown in figure 7, were obtained when culture A was dying and also in old mass cultures. In these animals both cytoplasm and nuclei stained more lightly than in the typical animal. Except for a slight vacuolization of the macronuclei, there is little difference between the structure of this specimen and of those described previously.

In regard to the question of the presence or absence of micronuclei, a most thorough and long-continued examination of many specimens has been made. As already mentioned, these specimens were prepared during all phases of the life-history of the cultures; during the early period of culture A, when the high division rate indicated that the animal was in good physiological condition; in cultures where there was no doubt that the organisms were in a depressed state, and during the different stages when numerous cases of attempted conjugation occurred. In none of these specimens have any structures been seen which could be interpreted as micronuclei.

4. CANNIBALISM

From the beginning of the experiments on O. hymenostoma the appearance of giant specimens in stock and mass cultures was noted. Stained preparations of these giants suggested that such forms had arisen as a result of one animal devouring its sister cells, or, in view of the observations of previous writers, what may be termed cannibalism.

Apparently the earliest account of cannibalism in ciliates was given by Haime ('53) in a short paper on the so-called 'Metamorphosis of Trichoda lynceus.' He figured an Oxytricha of

'medium size' which contained in the 'digestive cavity' an infusorian of the same species but in 'another stage of development.' Thirty-five years later Maupas ('88) stated that Onychodromus grandis under conditions of hunger was cannibalistic, while Joukowsky ('98) described the appearance, in a starved culture, of a large specimen of Pleurotricha lanceolata which he believed had attained its great size by eating its own relatives. Later, in cultures of Onychodromus grandis, under similar conditions, he saw large specimens, which, upon staining, showed within the cell the nuclear structure of what he interpreted as the remains of ingested animals of the same species.

As the occurrence of giant forms was so universal in mass cultures of Oxytricha hymenostoma, and as previous investigators had only referred incidentally to this phenomenon—all evidence submitted to date being largely inferential—it was decided to make a detailed study of this phase of the life-history of Oxytricha hymenostoma. In order, if possible, to relate these phenomena to characteristic physiological changes in the life of the cultures, the following study has been made:

1. Observations on the process of swallowing.
2. Digestion of the swallowing animals.
3. A study of the effect of the culture medium on cannibalism.
4. The physiological effect on the animal as shown by the division rate as compared with that of the typical animals.

1. Observations on the process of swallowing

The actual swallowing of one Oxytricha by another was suspected a considerable time before it was actually seen. During the experiment recorded in section 3 many instances were afforded of this act, since it took place on a large scale and under the most favorable conditions for observation, i.e., a large number of animals in the proper state for cannibalism were confined in a relatively small space. Not only has the process been witnessed in the case of cannibals which had already ingested one, two, or more of their fellows, but I have observed frequently the act of one animal swallowing another for the first time.

On January 1, 1918, the following observations were made on a depression-slide culture (2B, see p. 494) in which already many instances of a cannibal swallowing other animals of its species had been seen. One Oxytricha, apparently typical, seized a smaller animal by the posterior end (10.55 p.m.). At the end of five minutes its peristomial membranelles could be plainly seen, vibrating in the mouth of the larger animal. At the end of seven minutes (11.02) no sign of the smaller animal was visible. The cannibal was carefully watched for five minutes more, former experience having shown that any attempt to isolate it would probably be followed by egestion of the swallowed animal. The larger animal was then isolated, fixed and stained. Examination of the stained specimen showed the body of the ingested animal somewhat rounded off but still intact, with such structures as the peristomial membranelles and anal cirri distinctly visible. Figure 9 shows a cannibal on which a similar observation had been made, but which had some time previously swallowed another animal. It will be noted in this case that the peristomial membranelles of the ingested animal can still be seen as well as the macronuclei. The posterior macronucleus of the swallowed animal lies just under the anterior end of the posterior nucleus of the cannibal. The remains of the animal swallowed previously are visible as a large faintly staining vacuole in an advanced stage of digestion. Figure 11 shows a cannibal which has just swallowed a fused pair of smaller animals, the macronuclei of which are distinctly visible.

Many cases have been noted of apparently successful attempts to swallow followed by the disgorging of the swallowed animal, which usually seemed none the worse for the experience. Figure 13 shows such a cannibal which had already swallowed five animals and had succeeded in taking in the Oxytricha shown in figure 14, but during the operation of isolation the smaller animal was disgorged and swam about actively. A comparison of the size of these animals shows what was invariably found in all observations on swallowing, i.e., the ingested animal, when it is the first animal to be swallowed by a cannibal, is always smaller than the latter. When a culture is in a state favorable

for cannibalism, there has already taken place a rapid increase in the number of organisms in the culture. Consequently, the mean size of such animals has been found to be slightly less than that of the average normal animal. Among these animals which are below average size considerable variation in size occurs and, although the stimulus calling forth the swallowing reaction is present in most of the forms in the culture, the actual swallowing is only successfully accomplished when a relatively large Oxytricha attempts to swallow a smaller one.

Cannibalism has been invariably found to occur, throughout the whole course of the study, at the time when numerous pairs are present, attached in the manner so strikingly suggestive of the phenomenon of conjugation. Thus, cannibalism may be interpreted, in a way, as an abortive attempt at conjugation, since it occurs when the organisms are in the physical condition always found in conjugating animals. When the protoplasm of the animals studied in this work is in the 'miscible' condition and individuals happen to unite by the anterior ends, they fuse, and usually remain fused until death occurs. If the anterior end of one animal happens to come in contact with the posterior end of another, swallowing may take place.

It has happened that cannibals have been found whose length was less than that of the average length of the typical animal. The most striking increase is in the breadth of the animal—the effect being comparable to that obtained by the full inflation of an ordinary rubber hot-water bottle. In appearance the cannibal is much darker than the typical single animal, and when several animals have been ingested the dorsal surface often presents a knobby appearance where the pellicle is bulged out by the contained bodies. The pellicle in such a case is stretched almost to the breaking point and considerable care has to be used in handling these cannibals with the capillary pipette to avoid loss of the specimen by bursting.

Table 1 gives a series of measurements made on various cannibals.

It will be noted from a study of the above table that although animals at the inception of the process of cannibalism have a

smaller mean size than usual, there is an increase in both the length and the width of the cannibals depending on the number of organisms ingested. The size of cannibals after the digestion of from two to six bodies is slightly greater than that of the average size of typical animals from cultures.

In the case of the cannibals which underwent fission before digestion of the swallowed animals, the relatively great width was maintained. While the table given above shows a small increase in length and a greater increase in breadth in proportion to the number of bodies contained, great variation has,

TABLE 1

	NUMBER MEASURED	AVERAGE LENGTH IN μ	AVERAGE WIDTH IN μ
Normal Oxytricha	30	84.0	38.0
Oxytricha from culture in which cannibals were forming	20	60.0	24.0
Cannibals with 1 body	20	84.0	43.5
Cannibals with 2 bodies	20	86.4	45.4
Cannibals with 3 bodies	10	86.5	54.0
Cannibals with 4 bodies	4	88.5	55.5
Cannibals with 5 bodies	7	96.4	55.5
Cannibals with 6 bodies	3	94.0	63.0
Cannibals with 12 bodies	1	114.0	66.0
Cannibals with 15 bodies	1	135.0	72.0
Cannibals before fission and after digestion of from two to six bodies	8	87.3	39.0

nevertheless, been found, e.g., of two cannibals which had swallowed one animal each, one measured 54 x 30 μ, the other 120 x 51 μ.

One interesting feature in the occurrence of cannibals was the fact that usually when such a form was first seen it had already eaten as many of its fellows as it could hold, while the greatest patience was necessary to witness the process of swallowing the first animal. The rapidity with which cannibals eat considerable numbers of their own species after the initial act of swallowing seems to indicate that the 'swallowing reaction' persists until it is a physical impossibility to ingest any more. Further,

after the first Oxytricha has been swallowed, it is a much more simple process to swallow others, since the peristome then is considerably distended (figs. 9, 11, 13, and 16), and accordingly the process goes on rapidly until the cannibal is literally gorged with the bodies of its relatives (fig. 15).

2. Digestion of the ingested animals

For the purpose of studying the time necessary for the digestion of the ingested animals, thirteen cannibals were isolated from culture 2 B (p. 494) at 8 A.M., December 31st. All had become cannibals since 1 A.M. of the same day, i.e., at approximately the same time. In these cannibals the bodies of the ingested animals could be plainly seen and an accurate count made by using a binocular microscope with a lens combination giving a magnification of about 50 diameters. Of the thirteen animals thus isolated, 3 contained 3 bodies each; 5, 4 bodies each; 2, 5 bodies each; 1, 6 bodies; 1, 7 bodies, and 1 had 8 bodies. Observations were made on these animals for the next fifteen hours and specimens were stained from time to time until a fairly complete series illustrating the whole process of digestion was obtained. A series of measurements was also made during the experiment which shows that the offspring of the cannibals are only slightly above normal size at the end of the second generation.

Cannibals containing three bodies. No. 1. At the end of 2 hours the three food vacuoles containing the ingested animals were still distinctly seen. At the end of 3 hours no trace of any of the bodies could be seen, although the animal was still distinctly larger than normal (120 x 51 μ). It was then stained. The stained preparation showed remains of one body with the macronuclei still present, but stained very faintly.

No. 2. Little change in the appearance of the bodies was noted during the first 2 hours. Gradual disappearance of the outlines of these was noted during the next hour, at the end of which the animal was fixed and stained. The preparation showed the presence of two large vacuoles faintly stained, one

of which contained a chromatin body. The cannibal now measured 105 x 54 μ.

No. 3. At the end of 3 hours and 40 minutes little change in appearance of the bodies was noted. At the end of nine hours only one body could be seen, and this had disappeared at the end of 13 hours. The size of the animal now was 81 x 42 μ. Study of the stained preparation showed a typical appearance of cytoplasm and macronuclei.

Cannibals containing four bodies. No. 4. In 1 hour and 47 minutes the animal had divided by fission with three bodies in the anterior daughter cell,[3] 4a, and one in the posterior, 4p. 4a at the end of 3 hours (from beginning of experiment) showed two bodies distinctly visible. The animal, measuring 93 x 54 μ, was then fixed and stained. A glance at figure 16 will show that in the preparation the three food vacuoles are intact, one containing the macronuclei of a swallowed animal, the remaining two staining faintly and containing no chromatin. 4p contained one body which was barely visible at the end of 2 hours. In 3 hours this disappeared completely. The animal was fixed and stained one-half hour later and gave the appearance as of a normal animal except that its size (93 x 54 μ) was slightly greater than that of the average normal.

No. 5. At the end of 1 hour and 40 minutes four bodies were still distinctly visible. These became much less distinct in $2\frac{1}{2}$ hours. In $3\frac{1}{2}$ hours two bodies only could be seen and these were indistinct. At the end of $4\frac{1}{2}$ hours all bodies were apparently digested. The animal was then stained and had the typical structure of an ordinary vegetative animal, except for two large, faintly staining vacuoles in which no chromatin was visible.

No. 6. In $1\frac{1}{2}$ hours this animal divided by fission, two bodies passing to each daughter cell. 6a (90 x 57 μ) was stained at the end of $2\frac{1}{4}$ hours. Here one body showed the nuclear structure of the ingested animal; the other was smaller, stained faintly, and showed no chromatin. 6p showed presence of both bodies

[3] 'a' denotes that the daughter cell came from the anterior part of the parent and 'p' denotes the daughter cell from the posterior part.

for 5 hours. At the end of 7 hours they could no longer be seen. This animal was kept for 11 hours and 40 minutes, when fission took place. 6pa (75 x 30 μ) was fixed and stained and was normal in cytological structure (fig. 10). 6pp was stained at the end of $11\frac{1}{4}$ hours and examination showed that complete digestion of all the ingested animals had taken place. The size of this animal, 84 x 36 μ, was practically the same as that of the average noncannibalistic animal.

No. 7. In $2\frac{1}{2}$ hours the contained bodies could still be distinctly seen. In 2 hours and 57 minutes fission had taken place. 7a, with one body, was kept for 9 hours, at which time the body was still visible. The preparation showed plainly the nuclear structure of the swallowed animal. Size, 93 x 45 μ. 7p showed the presence of the three bodies for 9 hours, when they could no longer be seen. The animal was kept for 11 hours, at the end of which it seemed, except for its slightly greater size (90 x 56 μ), like a typical specimen. The stained preparation made at this time is shown in figure 12. Here the only indication of the ingested animal is the presence of two faintly stained chromatin bodies to the left of the posterior macronucleus of the cannibal. (The specimen is mounted ventral surface up.)

No. 8. At the end of 2 hours only two bodies could be seen distinctly. These gradually disappeared until little sign of any bodies could be seen at the end of 6 hours. In 7 hours fission occurred. 8a was stained and showed details of normal structure. Size, 75 x 42 μ. 8p was stained at the end of 14 hours and showed the presence of a vacuole staining faintly and containing no chromatin. Size, 95 x 45 μ (fig. 17).

Cannibals with five bodies. No. 9. Little change could be observed at the end of 1 hour and 20 minutes. In $2\frac{1}{4}$ hours only three bodies could be distinctly seen. In 3 hours one body remained visible. The stained specimen showed that this body still preserved its nuclear structure, while remains of two of the other bodies were present as fairly large faintly staining vacuoles containing no chromatin. Size, 108 x 51 μ.

No. 10. Bodies remained distinctly visible with little change for 3 hours. These gradually became less distinct until, at the

end of 9 hours, fission took place. 10a, with two bodies still visible in the living animal, showed upon staining that these were in an advanced stage of digestion. 10p. Examination of the living animal showed apparently normal structure. This was verified later by a study of the stained preparation. Size, 90 x 45 μ.

Cannibals with six bodies. No. 11. Fission occurred in 1 hour and 25 minutes. In each daughter cell two bodies only could be seen. In 12a, at the end of 2 hours and 10 minutes, the bodies were much less distinctly seen. The stained preparation showed that the bodies were nearly digested. Size, 102 x 45 μ. 12p was stained at the end of 3 hours. Here digestion had not proceeded so far and the nuclear structure of the swallowed animals could be distinctly seen.

Cannibals with seven bodies. No. 12. Little change could be seen at the end of 2 hours and 12 minutes. Fission then began and was completed in 25 minutes. 14a received four bodies and 14p, three. In 12 hours 14a had completely digested the four bodies and was in a prefission stage. 14p. At the end of 9 hours only two bodies could be seen. In 12 hours one body was barely visible. Fission occurred at the end of 13 hours. Half an hour after fission 14pa was stained. Examination of the specimen showed that complete digestion of the ingested animals had taken place (fig. 18). 14pp, stained at the same time, showed a similar result.

Cannibals with eight bodies. No. 13 was observed to be in fission 15 minutes after isolation and was stained and mounted. Study of figures 19 and 20 (showing two views of the specimen) reveals the presence of eight bodies, two of which are in an advanced stage of digestion. The anterior daughter cell contains five of these, the remaining three are in the posterior daughter cell. The widths of these daughter cells, 54 and 66 μ, respectively, are considerably greater than that of the average normal animal, although the length of the specimen, 165 μ, does not indicate that the cells which would have resulted, if fission had been completed would have been longer than an average non-cannibal animal.

All the observations on cannibals have shown that the process of digestion of the eaten animals is a normal one and proceeds rapidly, although there is considerable variation in the time in which equal numbers of swallowed animals are digested by different cannibals. A cannibal usually divides before the bodies of swallowed animals are fully digested. In the second generation complete digestion takes place, as all animals in the third generation which have been examined have been devoid of visible remnants of digestion, although they have a slightly greater size. The result of this experiment shows that the complete history of a cannibal from the first act of swallowing to the almost complete resumption of normal size and appearance may be carried out in time varying from ten to twenty-one hours.

3. The effect of the culture medium

Four typical animals were isolated on December 20, 1917, from a mass culture coming from stock of culture A. When each had divided twice, four subcultures, 1, 2, 3, and 4, were begun and carried on depression slides, seven drops of medium being used in each case. The animals of subculture 1—composed of four mass cultures, 1A, 1B, 1C, and 1D—were carried in the culture medium and allowed to multiply, the medium not being changed.

The second subculture, composed of four mass cultures—2A, 2B, 2C, and 2D—was carried in the same manner, but the medium was drawn off frequently and fresh medium added.

The animals in the third subculture, composed of four lines—3A, 3B, 3C, and 3D—were kept in the same medium and all the daughter cells but one in each line were removed daily.

The animals in the fourth subculture, composed of four lines—4A, 4B, 4C, and 4D—were carried in the usual way, one animal in each line being transferred to fresh culture daily.

a. History of the sub-cultures, 1, 2, 3, and 4. A summary of the history of the first two subcultures is given in tables 2 and 3.

It should be noted that for the first four days there was no essential difference in the division rate of the animals whether

TABLE 2

History of depression slide mass cultures 1A, 1B, 1C, and 1D of subculture 1. No fresh supply of medium was added. The number of animals observed on each slide is indicated in the columns under the date. Where large numbers were present the closest possible approximation is given. a = animals moving about in the medium more actively than usual. c = a cannibal. pr. att. = two animals fused in a manner similar to that of conjugating animals.

MASS CULTURE	NUMBER OF ANIMALS											
	Dec. 20	Dec. 21	Dec. 22	Dec. 23.	Dec. 24	Dec. 25	Dec. 26	Dec. 27	Dec. 28	Dec. 29	Dec. 30	Dec. 31
1A	1	4	16	48±	80± a	60± less a	45 Smaller darker fairly a	45± 4 C	30 n. 3 C	24	32± 2 pr. 1 C	30
1B	1	4	16	30	50±	60± very a	65±	60± 2 C	40±	50±	50±	50±
1C	1	4	8	50±	30± 2 C	30± 2 C	30±	40± 5 pr. att.	35±	26 5 pr. att.	40±	30±
1D	1	4	8	28	50±	50± less a	45±	45±	40± 2 C	45±	40±	38±
Total single non-cannibals	4	16	48	156±	210±	200±	185±	190±	145±	145±	162±	148±
Total cannibals					2 C	2 C	—	6 C	5 C	—	1 C	—

TABLE 3

History of depression-slide mass cultures 2A, 2B, 2C, and 2D of subculture 2. The medium here was drawn off daily and fresh medium was added. Other details as in table 2

MASS CULTURE	NUMBER OF ANIMALS												
	Dec. 20	Dec. 21	Dec. 22	Dec. 23	Dec. 24	Dec. 25	Dec. 26	Dec. 27	Dec. 28	Dec. 29	Dec. 30	Dec. 31	Jan. 1
2A	1	4	16	48±	60±	50± 3 C	80±	80± 2 C	30±	25±	36± 3 C (w. 1 body each)	40± 3 C	
2B	1	4	15	45±	60±	100± 3 C	100±	100± 1 C 18 pr. att.	100± 5 C 8 pr. att.	200± 1 C	150± 13 C 40 pr. att.	100± 25 C	70± 22 C None att.
2C	1	4	8	36±	60±	90± 1 C	100± 1 C	90± 12 pr. att.	90± 3 C 10 pr. att.	100± 4 C 20 pr. att.	180± 10 C 2 pr. att.	120± 10 C	
2D	1	4	8	24±	40± 1 C containing 1 body	60± 1 C	70	70±	18± (many dead)	12	14 2 C	16	
Total single non-cannibals	4	16	47	163±	220±	300±	350±	340±	238±	347±	380±	276	
Total cannibals					10	8	1	3	8	17	28	38	

supplied with fresh culture medium or not. From this time on, however, since no fresh medium was added, both the supply of food became scanty and the excretion products of the animals accumulated, a balanced relation of the animals and their environment was reached, and little or no further multiplication by fission took place. Just at the time this condition was reached, pairs were observed sticking together and cannibals began to appear. In all cases pairs and cannibals were isolated as soon as observed. In the second subculture cannibals appeared on the same day.

It will be seen that the appearance of cannibals in both the first and second subcultures is due essentially to the same cause, since in the latter case the medium was changed daily and the removal of waste products plus the added food supply gave a more rapid increase of the animals. This rapid increase, which in turn tended to bring about a state of balance rapidly by the using up of the food supply and the increase of excretion products, is most marked in mass cultures 2B and 2C. In mass culture 2A an accident on the seventh day led to the loss of over half the animals. The sudden drop in division rate in mass culture 2D I am at a loss to explain. Mass cultures 2B and 2C were most instructive. In these cultures there was an initial rapid multiplication. Just after this period of multiplication had reached its climax, but before a state of equilibrium had been reached, for numerous divisions by fission were still taking place, cannibalism occurred and the animals showed also a strong tendency to become united in pairs. Addition of fresh culture medium prevented a permanent balanced state such as that in the first set of cultures with the result that cannibal formation proceeded steadily until, as in the case of 2B, 20 per cent of the animals on the slide were cannibals.

The divison rate for subcultures 3 (see curve on p. 501) and 4 show that in the former set for the first five-day period reproduction took place more rapidly. This seemed somewhat surprising in view of the results obtained by Woodruff ('11) in studying the effect of excretion products on the division rate of Paramecium. Evidently in the present study excretion products

did not become a factor influencing the divison rate until after the first five days. During the second and third five-day period the divison rate of subculture 3 is distinctly lower than that of subculture 4. Here, no doubt, the increasing excretion products had a depressing effect.

In each line of subculture 4 the animals seemed at all times perfectly typical. In mass culture of subculture 3, however, on December 26th, two animals were present on the slide when it was examined. One, as usual, was removed. On the 27th, upon examination it was found that this animal had divided to give two and that these were united peristome to peristome. On the 29th they were more firmly joined and died that night. In this case, since all animals but one had been removed daily it would hardly seem that lack of food had brought on this 'miscible' state, since animals in set 1 were much more numerous on the depression slides and were still dividing, though slowly, but rather that the excretion products of the animals themselves were the underlying factor.

This experiment indicates clearly that cannibalism does not take place while the culture medium is comparatively fresh and also that the greatest amount of cannibalism does not occur in a medium in which the food supply is much depleted, but in one in which the scarcity of food is just beginning to be felt. Thus it seems probable that the accumulation of excretion products plays a part in inciting cannibalism. Jennings ('10) stated that the cause of conjugation was "a decline in the nutritive conditions after a period of exceptional richness that has induced rapid growth and multiplication." These conditions have in this case been duplicated with the result that instead of conjugation cannibalism occurred.

4. The physiological effect of cannibalism

On December 10, 1917, three apparently typical animals were isolated from three separate stock cultures coming from the original culture A. On December 11th, each animal had divided twice, giving four animals, and these were isolated to form the

four lines of subcultures, 1A, 2A, and 3A. These subcultures were carried with daily isolations until December 26th. On this date two new subcultures, C2 and C3, of four lines each were begun from three cannibals isolated from five-day-old stock cultures of 2A and 3A, respectively. On December 31st two cultures, C1 and CN1, of four lines each were added. The ancestors of these cultures were a cannibal and a non-cannibal, respectively, isolated on the preceding day from a six-day-old stock culture of subculture 1A. As the curves, averaged for five-day periods, showing the division rates of the non-cannibal subcultures (1A, 2A, and 3A) and of the cannibal subcultures (C1, C2, and C3) were essentially identical in each set, they have been combined in figure 2. The curve shows that the division rate of non-cannibal subcultures (1A, 2A, and 3A) was highest during the first three five-day periods and that there was a considerable drop in the division rate, i.e., from 2.4 divisions to 1.4 divisions per day, during the next period, while the average division rate for the cannibal subcultures was considerably higher, i.e., 2.2 divisions. These cannibal subcultures maintained a consistently higher division rate until January 20th to 25th, when it dropped below that of the non-cannibal subcultures for the first time. From this time on till the end of the cultures the division rates are very similar. It should be noted that 3A and 2A died out on February 18th and February 23rd, respectively, whereas subcultures C1, C2, C3, and 3A lived until shortly after March 1st. Subcultures CN1 between January 10th and January 20th had a higher division rate than any of the others, but this rapidly dropped until February 9th, when the subculture died.

The result of this experiment on cannibalism seemed plainly to indicate that animals descended from cannibal ancestors have a higher initial division rate as compared with animals descended from non-cannibal ancestors. This experiment also indicated the possibility, since their life in cultures was somewhat longer, that cannibal progeny are more hardy than progeny of non-cannibal animals.

A second experiment was carried out in a somewhat similar manner. On January 1, 1918, from the three existing lines (3A, 3C, 3D) of subculture 3 (see former history of this subculture on pp 494, 497) were spread out to form three new subcultures, 3A, 3C and 3D, of four lines each and, similarly, from the four lines of subculture 4 (pp. 494, 497) four new subcultures, 4A, 4B, 4C, and 4D, were begun. These subcultures were carried on with daily isolations until January 22nd. The division rates are shown graphically in figure 3.

No cannibalism had occurred in any of the lines of subcultures 3 and 4 of which the subcultures now being considered are lineal

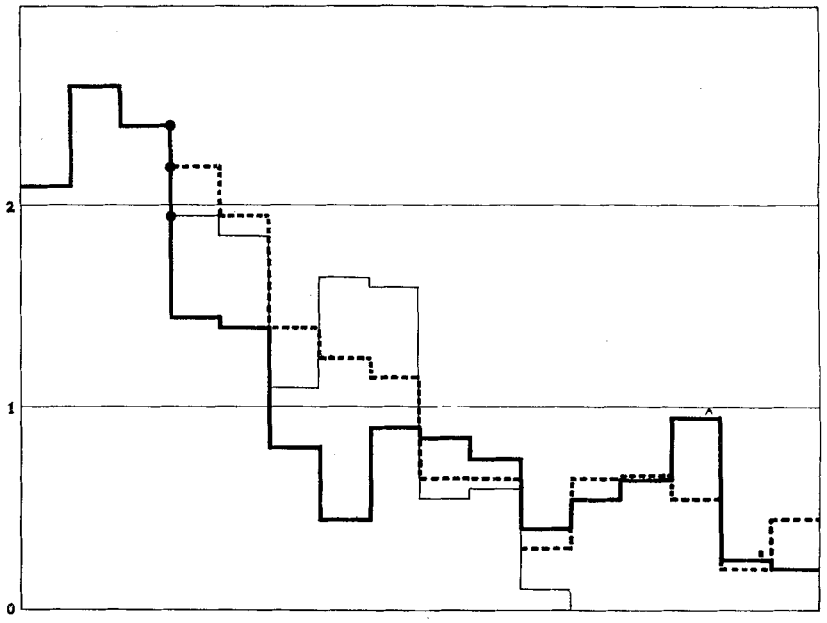

Fig. 2 Comparison of cannibal (broken line) and non-cannibal (continuous heavy and light lines) subcultures. December 12, 1917, to March 1, 1918. Continuous heavy line is a combination curve showing, in five-day periods, the average division rate for non-cannibal subcultures (1 A, 2 A, and 3 A). Dotted line shows a similar curve for cannibal subcultures (C1, C2, and C3) beginning December 26, 1917. Light full line shows the division rate, averaged for five-day periods, for the four lines of the non-cannibal subculture CN1 which died on February 4, 1918. At A the four lines of subculture C3 died (February 19). At B (February 23) the four lines of subculture C2 died. Methods of plotting same as in figure 1.

descendants, and it did not occur on any of the slides of the present subcultures in which daily isolations were made. Cannibalism did occur, however, in stock slides of four or more days standing in each of the twenty-eight lines of these subcultures.

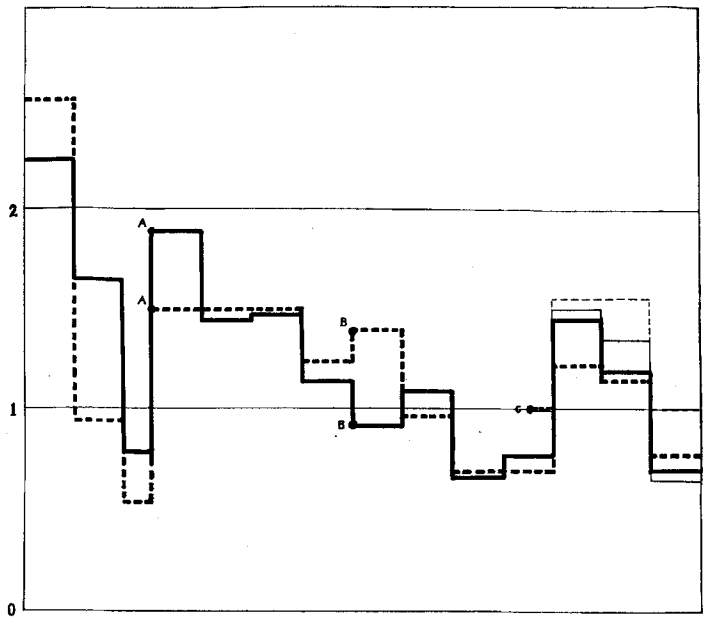

Fig. 3 Comparisons of cannibal (broken line) and non-cannibal (continuous line) subcultures begin at points B and C in this figure, which represents graphically the complete history (December 20, 1917, to February 26, 1918) of subcultures 3 and 4 (section 3) and of all subcultures of the second experiment in this section. The curves in this figure, with the exception of those drawn with light continuous and broken lines, show combined division rates of several subcultures. The broken line from beginning to A (January 2, 1918) represents the division rate of subculture 3; from A to B (January 22, 1918), the averaged division rates of subcultures 3A, 3C, and 3D; from B to the end of the curve, the division rates of the cannibal subcultures 4A–C, 4A–1C, 4B–C, and 3C–C. The continuous line to A shows division rates of subcultures 4; from A to B, the averaged division rates of subcultures 4A, 4B, 4C, and 4D; from B to the end of the curve the same for the non-cannibal subcultures 4A–N, 4A–1N, 4B–N, and 3C–N. The broken line (light) from C (February 9, 1918) to the end of the curve gives the average division rate for subculture 4A–C1, while the continuous line (light) from C to the end of the curve shows the same for subculture 4A–N1. Methods of plotting the same as in figure 1.

For the purpose now of making a comparison of cultures of cannibal and non-cannibal animals, eight new subcultures were begun from these subcultures as follows. On January 22nd from stock slides of four days standing four cannibals were selected—two from subculture 4A to begin subculture 4A-C and 4A-1C; one from subculture 4B to begin subculture 4B-C, and one from subculture 3C to begin subculture 3C-C. In all cases as soon as the animal used to begin the respective subcultures had divided twice, four lines in each subculture were established. Non-cannibal animals, from the same slides from which the cannibals were taken, were selected to form the four lines each of subcultures 4A-N, 4A-1N, 4B-N, and 3C-N. The curve shows that the average division rate for the cannibal cultures (heavy dotted line beginning at B) was higher, 1.40 as compared with .93, than the average division rate for the non-cannibal cultures (continuous line beginning at B) for the first five-day period, but that otherwise little difference can be noted.

On February 9th (continuous and dotted lines beginning at pt. C. in fig. 3) two subcultures, 4A-C1 and 4A-N1, were begun from a cannibal and a non-cannibal, respectively, selected on the previous day from a six-day-old stock culture of 4A-C. The difference in division rate here is not so striking as in the earlier part of the experiment, but the cannibal line is slightly higher during the first full five-day period, and this difference increases in the favor of the cannibal line during the rest of the life of the culture. On February 26th these series of subcultures were discontinued.

The general result of these experiments shows that the effect of cannibalism is to produce an initial higher division rate in the cannibal lines. This higher fission rate may be due merely to the extra nutrition supplied by the ingested animals, in view of the fact that Joukowsky ('98) also found a distinctly higher division rate in cultures of Pleurotricha fed on the ciliate, Uronema, in hay infusion, than in those kept in infusions of hay, flour, or albumin alone. Since, in this case, however, animals of the same species formed the food in question, there is a possibility, which should not be overlooked, that a more fundamental meta-

bolic change occurs than is implied by mere nutrition, considering that, in the present case, there is an addition of elements derived from similar cytoplasmic and nuclear constituents.

5. DISCUSSION

The most recent researches have seemed to show conclusively that, though ciliates may exist in an apparent amicronucleate state at certain periods of their life-history, sooner or later a micronucleus or chromatin equivalent to that contained by a micronucleus takes on a definitive form. Studies on the ciliates, Opalina, Trachelocerca, Ichthyophthirius, and Blepharisma, have revealed that their amicronucleate condition exists only during part of the life-history. However, in all the Hypotrichida studied to date a micronucleus has invariably been found as a normal cell constituent, although both temporary or permanent disappearance of this organelle have been described under certain conditions. Thus Maupas ('88) stated that the micronucleus in pathological cultures of Onychodromus grandis disappeared entirely. R. Hertwig ('89) questioned this statement and suggested that Maupas might possibly have overlooked the presence of the micronucleus, since he himself had often found great difficulty in observing micronuclei in Paramecium.

Calkins ('02) made the positive statement, based on his study of Paramecium, that he believed the micronucleus was present at all times in the hypotrichous ciliates. Woodruff ('05) found that in a culture of Oxytricha one of the two micronuclei—the posterior—was not present in indiviuals from the 361st to the 369th generation. On the other hand, Popff ('07), working with Stylonychia, found that during depression periods the micronuclei increased in number. Lewin ('11), working with the same form in regeneration, also found an increase in the number of micronuclei. Baitsell ('12) carried a culture of Stylonychia for 572 generations and found that micronuclei were present in non-conjugants, conjugants, and ex-conjugants at all stages.

The production of an amicronucleate race of Paramecium has been claimed by Lewin ('10), who obtained, by cutting, an amicronucleate fragment which regenerated and produced the 'amicronucleate race.' This race reproduced by fission for nearly two months and was normal in every respect except for the absence of a micronucleus. It is also interesting to note that LeDantec ('97) stated that, in the case of a ciliate (unnamed, with an elongated macronucleus and a single micronucleus, a merozoite containing a portion of the macronucleus only regenerated a micronucleus. The results of both these investigators were published in short preliminary papers and, pending more substantial proof or confirmation by other workers, must be regarded as interesting possibilities rather than as established facts.

The form described in this paper differs from all previously described hypotrichous ciliates in that it has never at any stage in its life-history, since it has been under observation, contained a definitive micronucleus. This statement is made as a result of long-continued and careful cytological study of many preparations of this form throughout the various stages of its life-history during a period of over two years.

As already stated, in other groups than the hypotrichs in which no micronucleus is visible during the vegetative stages it was invariably, during sexual phases of the life-history, found to be present in more or less close connection with the macronucleus, so that the usual dimorphic nucleus was represented apparently during the vegetative condition by a macronucleus alone, i.e., the macronucleus thus was an amphinucleus containing both tropho- and idiochromatin. In this form, therefore, since obvious but abortive attempts to conjugate occurred frequently, there is ground for considering that the nucleus is an amphinucleus, representing both the tropho- and idiochromatic phases.

It is believed by the recent investigators (Woodruff, '05, '13; Baitsell, '14) who have done extensive experimental work on the life-history of the hypotrichida, that these forms will live indefinitely without conjugation or artificial stimulation, pro-

vided an entirely suitable environment be secured. The amicronucleate species, Oxytricha hymenostoma, has lived continuously under laboratory conditions for over two years. During a considerable portion of this time (July 10, 1917, to April 30, 1918) it has been under continuous observation either on depression slides or in small mass cultures in Petri dishes with the possibility of unobserved conjugation or cannibalism precluded. At the present time (April 30, 1918) the animals in these cultures give every evidence of being able to live indefinitely. The conclusion, therefore, in this case again, is that the only requisites for the continued existence of this form are favorable environmental conditions.

The same conclusion is reached from experiments in which every effort was made to induce conjugation. Although no true process of conjugation has been obtained, there is every reason to believe that the organisms have frequently been in a general physiological condition similar to that of conjugating animals. The entire absence of any of the usual nuclear phenomena attendant upon conjugation has confirmed the belief that this form not only possesses no micronucleus, but also is apparently lacking in the chromatinic material necessary for carrying out the process of syngamy. The continued existence of such an organism indicates, therefore, that conjugation though usually taking place in all other hypotrichous forms, may be entirely dispensed with without loss of viability.

In cases where syngamy has not been observed there are three abstract possibilities (Minchin, '12): first, that it occurs, but has not been seen; secondly, that it is in abeyance; thirdly, that it is primarily absent, i.e., has never occurred in the life-history of the form. The fact that syngamy is of such general occurrence in the protozoa renders the first of these possibilities the most probable. In this case, however, there is every reason to believe that syngamy has been repeatedly attempted, but has never been carried out for the obvious reason that this form lacks the nuclear constitution necessary for the process. The fact that periodic attempts to conjugate occur indicates the probability that it has occurred in the past history of this organ-

ism. It is probable that this form at some time in its history contained a micronucleus, though it is of little value to speculate concerning the manner in which the present amicronucleate condition arose, since there are no data at hand. Such a condition might conceivably arise by some abnormal process during conjugation which resulted in suppression of differentiation between macro- and micronuclear material.

Although the significance of the process of conjugation has been exhaustively studied since Maupas' time, a complete solution of the problem has not as yet been obtained. The existence of a form which not only apparently may live indefinitely without conjugation, autogamy, or endomixis (assuming the possibility of the latter phenomenon in an hypotrichous form), but also apparently does not possess the ability to undergo any of these phenomena, brings to light an entirely new possibility in the life-history of ciliates. It has been proved quite conclusively (Woodruff, '14) that in forms which ordinarily conjugate, the continued prevention of this process brings about no loss of viability if a favorable environment be provided. However, in the organism under consideration there is apparently no possibility not only of conjugation or endomixis, but also of autogamy, and thus we have from another source crucial evidence that none of these phenomena is an indispensable factor in the life-history of this hypotrichous form.

6. SUMMARY

1. A pedigreed culture of Oxytricha hymenostoma has been carried for 289 generations, from July 10, 1917, to November 17, 1917, and since that time to date (April 30, 1918) by means of small mass cultures in petri dishes.

2. A micronucleus has not been seen at any time in any of the animals during the history of the cultures.

3. Syngamy has not occurred during the course of the experiments, although it is believed that the animal has frequently been in a physical state similar to that in which syngamy takes place.

4. While in this state, *a*) animals fuse in pairs in a manner similar to that of conjugating animals; *b*) cannibalism takes place.

5. Animals, fused as described in 4a, either remain fused until death occurs or separate. In the latter case the organisms continue to reproduce and give no signs of being in a depressed condition.

6. When cannibalism has occurred, digestion of the ingested animals proceeds rapidly and a return to the typical size and structure soon takes place.

7. Cannibalism has the effect of raising the division rate somewhat for a short time.

8. This amicronucleate race of Oxytricha hymenostoma apparently can live indefinitely under favorable environmental conditions without conjugation, autogamy, or endomixis. Indeed its amicronucleate condition seems to preclude the possibility of the occurrence of these phenomena.

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PLATES

All of the figures in plates 1 and 2 are microphotographs of permanent preparations (total mounts) stained with Delafield's hematoxylin and in most cases counterstained with tetra-brom-fluorescic acid. The use of other counterstains is denoted in the explanation of figures. The same magnification (525 diameters) was used in all cases with the exception of figures 8, 15, 18, and 19, which are magnified 375 diameters.

PLATE 1

EXPLANATION OF FIGURES

4 A typical normal individual from culture A in the fiftieth generation. July 24, 1917 (dorsal view).

5 Typical pair from stock of culture A fused in the peristomial regions.

6 Typical pair from stock of culture A. Appearance closely simulating that of conjugating animals (Del. hem.).

7 Pathological specimen from stock of culture A in 283rd generation, November 6, 1917. Preparation made at the end of the fourteenth day without division.

8 A typical cannibal with remains of five ingested animals, two of which are in an advanced stage of digestion. A kernspalt is present in each macronucleus of the cannibal (dorsal view).

9 A cannibal which has just ingested an animal, the peristome of which is visible. The posterior macronucleus of the ingested animal is partially obscured by the macronucleus of the larger animal. A large vacuole containing the remains of an individual now almost completely digested is at the left of the posterior macronucleus of the cannibal (ventral view).

10 An individual, descendant of a cannibal in the second generation (No. 6, p. 491).

11 A cannibal which has just ingested a pair of animals which were fused as if conjugating. The peristome is much distended.

12 An individual, descendant of a cannibal in the first generation, which shows almost complete digestion of ingested animals. The remains of the macronuclei of an ingested animal are visible as two chromatin bodies on the left of the posterior macronucleus (ventral view) (p. 492).

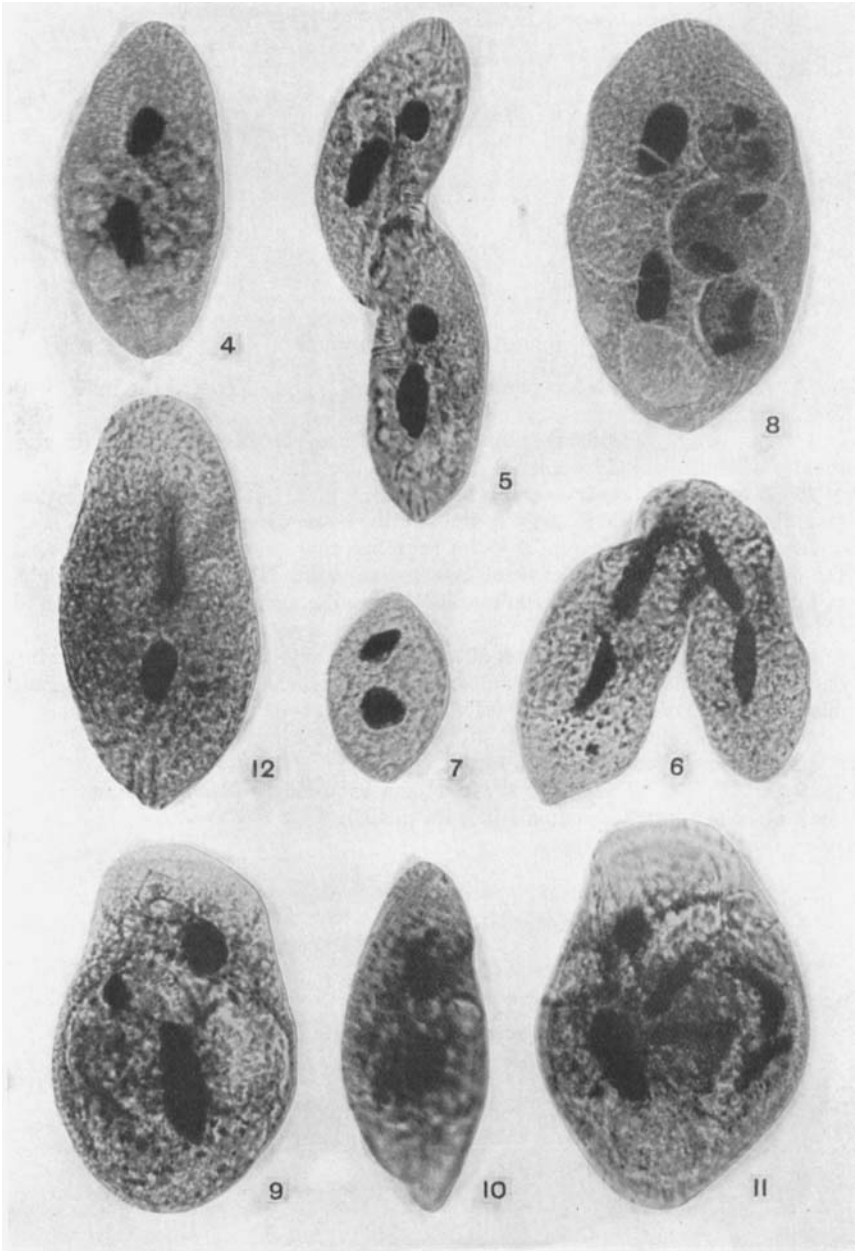
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PLATE 2

EXPLANATION OF FIGURES

13 A cannibal which has ingested five animals and disgorged the individual shown in figure 14.

14 Individual considerably under normal size which was swallowed and shortly after disgorged by animal shown in figure 13.

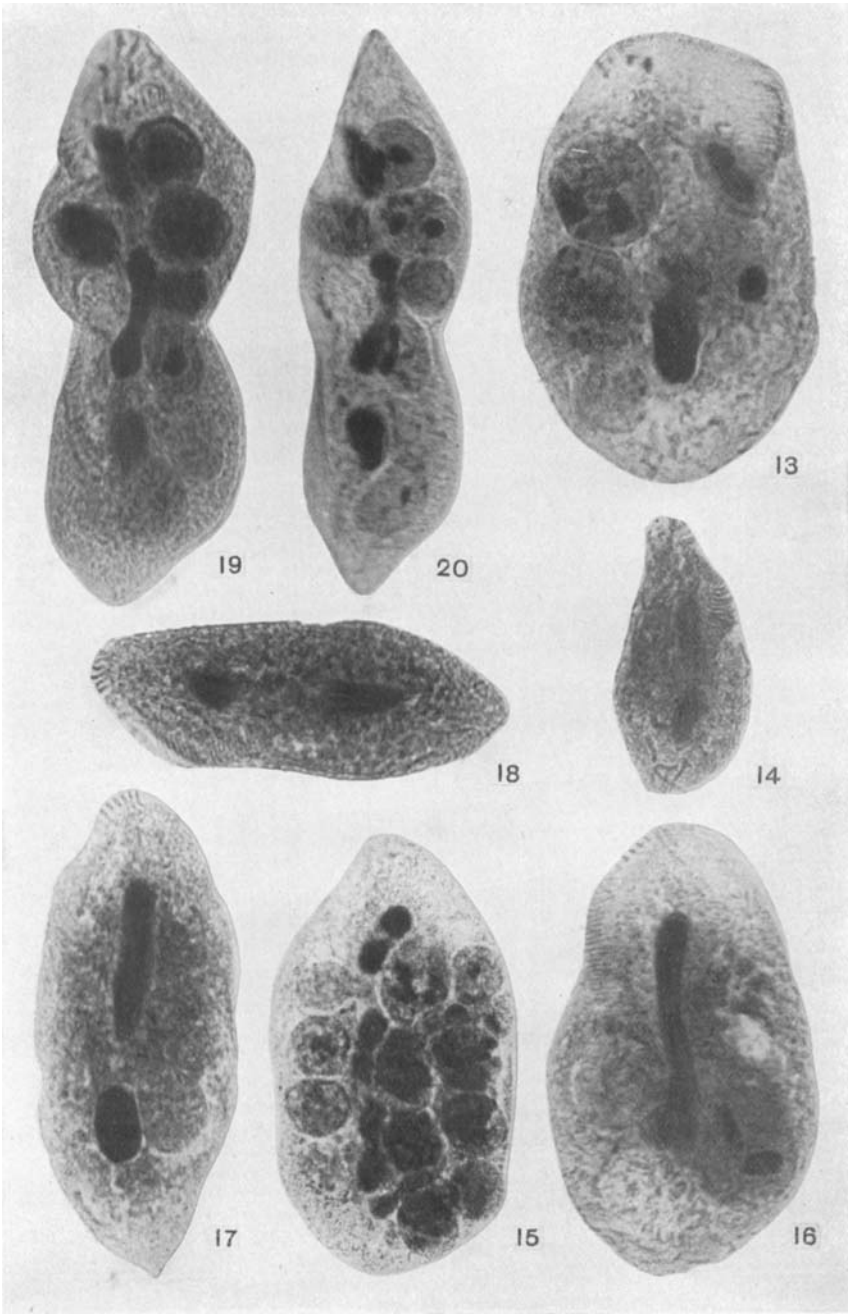
15 A cannibal in early fission stage containing the bodies of sixteen ingested animals, thirteen of which show in the figure (dorsal view) (Del. hem. only).

16 An individual, descendant of a cannibal in the first generation, showing the presence of the bodies of three ingested animals. The vacuole to the right of the fused macronucleus of the cannibal shows the macronuclei of one ingested animal (dorsal view) (p. 491).

17 An individual, descendant of a cannibal in the first generation, showing one body (to the right of the posterior macronucleus of the cannibal) in an advanced stage of digestion (dorsal view) (p. 492).

18 An individual, descendant of a cannibal, showing complete digestion of ingested animals (compare with fig. 4).

19 and 20 Dorsal and lateral views of a cannibal in fission containing the bodies of eight ingested animals (no. 13, p. 493).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