OBSERVATIONS ON THE PHYSIOLOGY OF THE AMEBA

MAYORELLA BIGELLOA (SCHAEFFER)

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DISSERTATION

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INTRODUCTION

In the spring of 1930, in a mixed culture of Protozoa, the writer found a considerable number of amebas of medium size, all of which were evidently of the same species. A clone culture was successfully established and maintained with good results by using *Euglena gracilis* Klebs as the source of food. This ameba was identified as belonging to the species *Mayorella bigemma*, described by Schaeffer in 1918 as *Amoeba bigemma*. In 1926, the same author erected the genus *Mayorella* to which he transferred this species.

There is very little information on the species in the literature, and its validity has been questioned by Johnson (1928) and by Mast (1928). Both Johnson and Mast expressed the view that this species is identical with *Amoeba doylei* Neresheimer, described in 1905. Replying to this, Schaeffer, in 1929, again reviewed the more important specific differences between these two species. Taylor (1929) stated that stained preparations of *Mayorella bigemma* could easily be mistaken for young *Amoeba proteus* unless the cytoplasmic characters had been observed before fixation of the specimen.
The food of this species was listed by Schaeffer (1918) in his original description as "flagellates, ciliates, diatoms, rhizopods, nematodes, vegetal tissue, etc.". In a very brief paper, Botsford (1922) reported the culturing of *Mayorella bigemma* in solutions of beef extract, in which case, it is assumed by the writer that bacteria served as a source of food. Taylor (1929) stated that *Mayorella bigemma* can easily be cultivated under the same conditions as *Amoeba proteus*.

The present investigation has been concerned with observations on ingestion, digestion, and egestion, and other physiological processes in *Mayorella bigemma* while cultured on an exclusive diet of *Euglena gracilis*. This investigation was suggested by Professor W. J. Kostir, of the Department of Zoology and Entomology, Ohio State University, to whom the author is indebted for many constructive criticisms, helpful suggestions, and the general supervision of the work.
MATERIALS AND METHODS

Distilled water, well water, and a synthetic well water were used in the preparation of culture media. Cultures made up with natural well water proved to be the most satisfactory and hence were used exclusively in the latter part of the investigation. This well water was taken from a well on the campus of The Ohio State University.

Solutions of aminoids (either Beef or Milk), a commercial product of the Arlington Chemical Co., Yonkers, New York, were used in the cultivation of Euglena gracilis. A solution of 0.04% aminoids was very satisfactory and had no appreciable effect upon the amebas introduced into such cultures.

Pyrex Erlenmeyer flasks were used exclusively in culture work. The best results were obtained by using 125 cc. flasks with 75 cc. of culture medium. The medium was first made up in a liter flask and then the proper amount was poured into each of the smaller flasks. The flasks were then loosely stoppered with sterile cotton and placed on a hot plate. When the contents just came to a boil, the flasks were removed and allowed to cool. Immediately thereafter, the medium was inoculated. This procedure was very effective in eliminating contamination of the cultures by other Protozoa.
Transfer of the organisms was made with pipettes which were sterilized in boiling water before and after being used.

Stock cultures of *Euglena gracilis* were obtained from Professor W. J. Kostir, who had started a clone culture of this species in September, 1921. For the most part, cultures were inoculated with euglenas only at first, and the amebas were introduced into these cultures about four weeks later. Although it was found that the amebas could be introduced into the cultures along with the euglenas, the number of amebas produced in such cultures was never as great as it was when the euglenas were given a good start beforehand. A clone culture was established by isolating an ameba in a very small drop of water on a sterile cover-glass; after examination of the drop, to make certain that only one ameba and no other protozoan was present, the coverglass was dropped into a euglena culture. This method proved more satisfactory than transferring the isolated ameba to a culture with a pipette, because the amebas adhere very readily to glass and often remain attached to the pipette. No attempt was made to exclude bacteria from the cultures, since this was not deemed necessary and would have greatly limited the scope of the present work. There were very few bacteria present in the cultures at any one time.
Observations were made on both living and fixed and stained amebas. Living amebas were studied on glass slides with and without a coverglass and also in hanging drops in concavity slides. A good 2 mm. oil immersion lens, with 15x and 20x oculars proved very satisfactory for observations on the finer details. A good Zeiss polarizing attachment was used in the study of the crystals present in the cytoplasm of the amebas. As an aid in recording observations, a special camera was built by the author. This camera was so constructed that the magnification of all pictures made with a certain combination of lenses in the microscope would be the same. Regular 35 mm. motion picture film was used and 300 or more pictures could be taken, at the rate of twenty per minute, on one loading.

Permanent preparations were first made by placing a drop of culture material on a glass slide and leaving it undisturbed for five minutes. This allowed most of the amebas to settle to the bottom and become attached. No albumen fixative was used on the slides. The slide was then held at a slight inclination and a small stream of water was run over it, washing off the flagellae and detritus and leaving the attached amebas. Worcester's formol-sublimate-acetic mixture was used for killing and fixation. It was found to give the best results when heated almost to the boiling point. A drop of this hot solution was
placed on the amebas. This instantly killed and fixed then without appreciable distortion. The amebas were then thoroughly washed in distilled water, stained with Delafield's hematoxylin and counter-stained with eosin. The amebas were dehydrated in the following series of alcohols: 5%, 10%, 20%, 35%, 50%, 70%, 85%, 95% absolute alcohol. They were cleared with xylol and mounted in balsam. Very few of the amebas became dislodged in this procedure.

Further observations disclosed that the amebas, after being placed upon a slide, became flattened and otherwise changed their form, so that they were very different in shape from those in cultures. Upon experimentation, it was found that the amebas could be killed and fixed very satisfactorily in the cultures. After the amebas and eucaryons became settled at the bottom of a culture, as much of the liquid as possible was poured out without disturbing the organisms. The amount of culture material remaining in the flask was estimated and an equal volume of hot fixing solution was quickly poured into the flask. Five minutes later the amebas and eucaryons were washed in five changes of distilled water. A centrifuge was used to concentrate the organisms in the process of washing and dehydration. The same series of alcohols mentioned above was used in dehydration. After dehydration,
the amebas and euglenas were concentrated in a few drops of xylool. These few drops were then placed in a syracuse watch glass, and balsam was added. This was allowed to stand over night. A drop of this mixture was placed on each slide and a coverglass was placed on it. In this case the organisms were stained in bulk, Delafield's hematoxylin and eosin being used as before. This method of making permanent preparations is much more satisfactory than the one previously described in that one can be sure of having a much better representation of all the organisms present in a culture. Such preparations also enable one to study the normal form of the amebas, since very few of them are ever distorted to any appreciable extent.
DESCRIPTION OF THE AMEBA

The amebas used in the various observations and experiments recorded in this paper were all members of a single clone, isolated as above described. The amebas of this clone have all the important diagnostic characteristics of Mayorella bigemma (Schaeffer, 1918) Schaeffer, 1926. During locomotion, they range in length from 70 to 300 microns. In younger cultures, the average length of the amebas in locomotion is about 150 microns, while in old cultures, amebas as small as 25 microns in diameter are sometimes found. The latter, however, were never observed to move in any manner, except that very short pseudopods were occasionally extended and retracted.

During locomotion, the amebas are usually somewhat triangular in shape; the anterior border being broad and composed of clear hyaline protoplasm which advances somewhat irregularly in the form of tongue-like projections occurring here and there along the entire border. There are no definite pseudopods into which the endoplasm flows and the direction of locomotion is determined by the advancing anterior border of hyaline protoplasm. Small, clear, conical pseudopods are usually present during active locomotion. They are not permanent structures, but are always in the process of extension or retraction. They do not direct locomotion and always have rather blunt
tips. Locomotion is frequently jerky, especially when endoplasmic streaming is interrupted by large food vacuoles and large clear vacuoles which sometimes become lodged together in the main current of endoplasm.

The amebas may at times assume a radiosa-like form. Long, slender, tapering, but blunt-pointed pseudopods of about equal length may be somewhat regularly arranged around the spheroidal body of the ameba. However, most of the amebas in this condition are less regular in outline and the pseudopods are more irregular in size, shape, and their distribution over the surface of the ameba.

The nucleus is spherical and contains a granular endosome. In an ameba of about 150 microns in length during locomotion, the nucleus is about 12 microns in diameter and the endosome is about 7 microns in diameter. On prepared slides, the nucleus of an ameba of about 70 microns in length is about 10 microns in diameter and the endosome is about 5 microns in diameter.

The small dumb-bell-shaped or hour-glass-shaped crystals described and figured by Schaeffer as diagnostic for this species are readily demonstrated with a polariscope, both in the living amebas and in those which have been fixed and stained. They measure about 1.5 to 2.0 microns in length.
Usually, at least four or five contractile vacuoles are demonstrable, and at times twelve or more such vacuoles can be seen. They usually undergo systole when they are about 15 microns in diameter, but sometimes they attain a size of 30 microns or more.

In addition to these clear vacuoles which gradually increase in size and sooner or later undergo systole, the endoplasm of the amebas contains other clear vacuoles which may be very numerous at times. Very little, if any, change in the size of these can be detected in a period of two hours. For the most part, they are about 10 microns in diameter, but sometimes smaller, and occasionally much larger, even up to 30 microns.

A clone culture of *Mayorella bigemma* has offered the writer an excellent opportunity to make a thorough investigation of this species, and to compare its characteristics with those of other species with which it might be confused. In certain respects, *Mayorella bigemma* resembles both *Amoeba vespertilio* Fenard, 1902 and *Amoeba doeleini* Nereshaimer, 1905. However, there are constant differences between the former and the latter two species. On the basis of size, *Amoeba doeleini* (80-150 microns) might be confused with *Mayorella bigemma* (70-300 microns), though *Amoeba vespertilio* seems to be definitely smaller (70 microns). The tips of the pseudopods of *Amoeba bigemma* are always
blunt, and never sharp as described for the other two species. Both Amoeba dofleini and Amoeba vespertilio have an endosome type of nucleus, but the endosome is not granular, as it is in Mayorella bigemma. Clear, conical pseudopods of the type found in Mayorella bigemma are not mentioned for either Amoeba dofleini or Amoeba vespertilio. Penard (1902) mentioned "grains d'excrétion" in Amoeba vespertilio but gave no description of them.

The crystals in Amoeba dofleini were described by Neresheimer as being usually rod-shaped, sometimes irregular and club-shaped, but seldom thickened at both ends. Neresheimer used polarized light and made a detailed study of the crystals.

In brief, the characteristics mentioned by Schaeffer (1918) for the species Mayorella bigemma have been confirmed by the present writer, and after a careful comparison with the descriptions of Amoeba vespertilio and Amoeba dofleini, it is his opinion that Mayorella bigemma is a distinct and valid species.
INGESTION

Since the amebas were cultured on a diet of *Euglena gracilis*, observations on ingestion, for the most part, were limited to the ingestion of these organisms by the amebas.

In most cases, the process of ingestion involves the formation of a food cup or the surrounding of the food by pseudopods which come together and fuse at their outer extremities. This results in the formation of a food vacuole (Pl. II, figs. 1-6). No definite generalization can be made concerning the size of the food cup. Sometimes it is just sufficiently large to surround the prey, while at other times, it may be much larger and some liquid from the culture may be taken in along with the food.

When an ameba responds to food, the surface of the ameba nearest the food advances. The cup is formed when the edges of the projection advance more rapidly than the region in its center. When the object is non-motile, the food cup is advanced over the object and when the center of the food cup almost comes in contact with it, the advancement ceases in that region and the edges of the cup constrict and continue to advance until they come in contact with each other and fuse. If the object is not pushed forward by the food cup or is in such a position that it can be easily
surrounded, in most cases the food cup will constrict tightly against the object and very little water will be taken in. In the case of a moving object, the ameba may follow the object for some distance before capturing it. In the pursuit of the object, the edges of the food cup may continue to advance much faster than the center of the cup so that the food cup becomes much elongated. When the edges of the cup constrict and fuse, a certain amount of water will be retained in the vacuole thus formed. In one case, a large ameba was observed to follow another ameba for fifteen minutes. During this time, the pursuing ameba formed a food cup over the posterior end of the other ameba. As the two amebas moved about, the pursued ameba was able to move somewhat faster than the other and finally escaped. The edges of the food cup constricted and fused and a vacuole was formed which contained only a large amount of water. These observations are in accord with those of Schaeffer (1916). He concluded that "food cups with considerable quantities of water in them are usually formed only over moving animals; dead animals or parts of animals are surrounded by food cups only very slightly larger than these objects themselves".

Frequently amebas were observed to ingest active euglenas without the formation of food cups (Pl. IV, figs. 1-6). While under observation, many of the euglenas with
flagella grouped around masses of debris and amebas. Some of the euglenas backed up, advanced against the side of the amebas, and continued in this manner until they were finally enclosed in an invagination or channel on the side of the ameba. After the euglenas thus became imbedded, they usually remained motionless until the ameba started to constrict the opening of the channel. Some were able to move out but many of the euglenas became entrapped and were soon enclosed in a food vacuole. Becker (1926) records similar phenomena in enucleated Amoeba dubia. In his observations, however, the euglenas did not have flagella and "approached the amoebe by typical 'euglenoid movement'." Doflein (1916) describes the process of ingestion by Amoeba terricola where the food was taken in by the invagination of the ameba at the place of contact with the food. It is possible that in the process of ingestion of euglenas without the formation of food cups as observed by Becker and the writer, the invagination is not caused by the force of the euglena alone, but is also partly due to a response made by the ameba. Schaeffer (1917a) observed that isolated proteins are sometimes ingested by amebas without the formation of food cups, in which case the protoplasm "merely flows around the food particle".
The writer made a number of observations to determine whether the amebas responded differently to active euglenas, to passive euglenas, or to euglenas with a gelatinous envelope. A large number of amebas were placed on a glass slide containing a large number of euglenas in all three conditions. About equal numbers of the euglenas in each condition were used. The material was kept under continuous observation for two hours or longer. The number and condition of the euglenas ingested during this period was recorded. It was found that active and passive euglenas were ingested in about equal numbers and that euglenas with a gelatinous sheath were not as readily ingested. In any given observation, less than half of the amebas would respond positively to food. Food cups were not induced by contact with the prey, but the formation of the food cup was started when the ameba was still a few microns away.

There are probably a number of factors influencing ingestion in amebas. Since ingestion is brought about as a response or series of responses of protoplasm to some foreign object, it is reasonable to suspect that many things which might affect the physiological processes in an ameba would affect the way in which the animal would respond to food. It was noted that many amebas, when transferred from a culture and placed under the microscope for observation, became sluggish and often spheroidal.
Often as much as thirty minutes were required for the amebas to regain their normal form and to move about in the usual manner. When the illumination was very intense, for example in making photographs, a longer period of time was necessary. It is evident, therefore, that changes in the intensity of illumination affect the amebas. Schaeffer (1916) found that movement of a particle was an important factor in bringing about a positive response in the formation of a food cup. Edwards (1925) was able to induce the formation of a food cup by solutions of certain salts. It is interesting to note that in this case the stimulus was such that food vacuoles were formed which contained no solid material. Kepner and Edwards (1917) found that the "choice" of food does not depend on whether \( \text{CO}_2 \) or \( \text{O}_2 \) is given off by the material to be ingested.

As has been pointed out, not all amebas under the same external conditions respond to food. To explain this fact it is necessary to postulate physiological differences between amebas which respond and those which do not respond to food. This conclusion is in accord with that of Kepner and Taliaferro (1913) who made similar observations and believed that there were metabolic conditions, "demanding or not demanding", which determined the response that the ameba would make.
Rhumbler (1898) attempted a physical analysis of ingestion and the "selection" of food. Ingestion, he suggested, is due to the adhesion of the particle of food to the surface of the ameba. The food is drawn into the ameba in a manner comparable to the pulling inward of a small splinter of wood by a drop of water. Selection of food is determined by whether or not particular substances will adhere to the ameba. Rhumbler evidently based this conclusion on his many observations on Amoeba verrucosa where the adhesion of a particle to the ameba undoubtedly is an important factor in ingestion. His explanation, however, is not satisfactory in many other cases.
DIGESTION

It was only after more than a hundred attempts were made to trace the complete digestion of prey in any one food vacuole that this was successfully accomplished. It was desirable to begin the observations with the witnessing of the ingestion of the food so that the exact sequence of the phenomena accompanying digestion might be determined. A few major difficulties were encountered in this study. Not all amebas under observation would ingest euglenas. In many cases in which an ameba was observed to ingest a euglena, the latter was again egested before digestion was completed. In still other cases, circumstances made it necessary to interrupt the observations sooner than was desirable.

However, the earlier stages in digestion were worked over a number of times and the writer was then able to recognize these readily with some degree of accuracy whenever they were observed in other amebas. Amebas with newly formed food vacuoles were then selected and kept under observation. The complete story of digestion was obtained in this manner. This was later verified in at least ten different cases, in each of which the ingestion of the food was witnessed and the food vacuole was kept under continuous observation until digestion was traced
The time required to kill a euglena after it has been ingested by an ameba varies. Living euglenas which are inactive before and during the process of ingestion, soon become activated in the food vacuoles. Sooner or later, the activity of the euglena ceases, and the writer considers this presumptive evidence that the euglena is dead or dying. Active euglenas were observed to move about in a food vacuole as long as 45 minutes and then move about by "euglenoid movement" after they had been ejected. In other cases, the euglenas were evidently killed in about thirty minutes after they were ingested. A much longer period of time is required for the amebas to kill euglenas which are inclosed in gelatinous sheaths when they are ingested. The writer observed nine or more cases where euglenas with a gelatinous sheath were still moving after being in food vacuoles for more than one hour.

The food vacuole begins to increase in size soon after it is formed. This change in size is very slow and usually continues over a long period of time. Soon after the prey ceases to move, it gradually becomes shriveled as if it were being dehydrated. In about eight hours, only undigested paramylum grains are usually observed in the food vacuole. Sooner or later, the food vacuole begins
to decrease in size, and finally, the paraxylum grains
and any other solid particles become scattered throughout
the cytoplasm of the ameba.

The writer observed one case in which a partly
digested euglena moved through the membrane of the food
vacuole into the cytoplasm. The vacuole, now containing
only liquid, moved to the posterior end of the ameba and
its contents were expelled to the outside. This was
followed almost immediately by the egestion of the partly
digested euglena. In this particular case, the food vacuole
when first formed was about 17 microns in diameter and
contained very little liquid surrounding the euglena. At
the end of 5½ hours and just before the partly digested
euglena left the food vacuole, the vacuole had increased
in size until it was then 31 microns in diameter while the
undigested remains of the euglena were in a spherical mass
about 12 microns in diameter. A record of this observation
was made in the form of photomicrographs.

The process of digestion is not always as simple
as that described above. This is especially true when the
euglenas ingested are from a relatively young or newly
started culture. In many cases, soon after the prey has
been killed, the food vacuole containing it tends to
become constricted or distorted and sooner or later divides.
Each vacuole thus formed receives a portion of the prey
torn apart in the process. Each of these vacuoles may become further sub-divided a number of times in a like manner. Sometimes this phenomenon occurs when the ameba is somewhat spherical and is moving very slowly. In this case, one can actually observe protoplasmic projections moving in toward the prey. However, this phenomenon occurs more readily when an ameba has become elongated and constricted, and the food vacuole has become lodged in the center of the ameba where it tends to obstruct the streaming endoplasm. In this event, the food vacuole is evidently pulled apart.

Each division of the food vacuole, of course, results in smaller vacuoles which contain all of the material of the original vacuole from which they are derived. These derived vacuoles gradually increase in size and often become sub-divided. Sooner or later, the vacuoles gradually decrease in size, and finally disappear. The undigested remains then become scattered throughout the endoplasm of the ameba. In most cases, only paramylum grains are found in the vacuoles just before they disappear.

The time required to digest a euglena is appreciably lessened by the subdivision of the food vacuole. In many cases, this entire process was completed in less than three hours after the euglena was ingested.
Sometimes very large food vacuoles tend to become divided. In some of these cases, two vacuoles may be formed, one of which may receive only liquid from the original vacuole. The writer observed one case in which the ameba was almost cut in two. The vacuole was very large and was in the center of an elongated ameba. There was only a very thin layer of endoplasm between the vacuolar membrane and the plasmalemma. When the vacuole became constricted the ameba also constricted. However, complete division of the vacuole was not accomplished.

No apparent changes in paramylum grains could be observed while these were in the food vacuole. The almost continuous addition of paramylum grains to the endoplasm would lead one to suspect that possibly the paramylum grains are digested there. When amebas were immersed in a weak solution of neutral red (0.0025%) the film of protoplasm surrounding each paramylum grain became stained dark red while the rest of the endoplasm was stained light pink. Food vacuoles also became stained red. At no time was a food vacuole found to be alkaline in reaction. Yellow color sometimes present in older vacuoles is due to chlorophyll which has changed color during digestion.

That the liquid disappears from food vacuoles and that particles less easily digested become scattered and are carried about in the endoplasm of Amoeba proteus was
reported by Schaeffer (1916).

Although paramylum is not readily digested, there is some evidence that this substance may be digested very slowly. No apparent changes could be observed in paramylum grains while they were contained in food vacuoles over a period of about six hours, during which time, they remained colorless. However, about four hours later, the color of the grains became grey with a light bluish tint.

Becker (1926) observed the digestion of euglenas by enucleated ameoba. He states that "food once ingested can be digested after the fashion of normal amoebae". The following is his description of the process in an enucleated ameoba: "First the Euglena gradually loses its green color, becoming brown in an hour or so; then it is reduced in size, and finally after many hours only some brown undigested remains are left in the food vacuole. The vacuole shows the acid reaction up to the end of the process, as proved by its intense redness to a very weak neutral red solution in water". Evidently digestion was not traced further and it is possible that the undigested remains mentioned included paramylum.

Paramylum, according to Minchin (1922) is a substance allied to starch and has the same chemical composition as starch according to Doflein (1916). Weissner (1888) found that *Amoeba radiosa*, *Amoeba princes*, and *Pelomyxa palustris*
did not digest rice starch: Štoltz (1900), however, found that *Amoeba proteus* ingested and digested small amounts of wheat starch. He also found that certain granules in *Pelomyxa*, which he identified as glycogen, diminished upon starving the organism, and again appeared in normal amounts when it was fed starch or filter paper and cellulose from cotton.

It is interesting to note that in at least some of the Protozoa, digestion is a physical as well as a chemical process. The breaking up of the food by a protozoan is a mechanical process and might be compared with the trituration of the food by a higher animal. The phenomenon of the constriction of prey being ingested by *Amoeba* is not uncommon, especially in the case of *Paramecium*. Beers observed the same phenomenon when *Amoeba* ingested *Frontonia*. Blochmann (1885) described the ingestion of *Hematoema* by *Amoeba blochmanni* where the ameba squeezed off bit after bit of the prey until the latter was completely devoured. Entz (1932) observed that *Didinium* devoured *Paramecium* "in ganzen oder grossen Stücken".

The mechanical process is further illustrated by the breaking up of the food after it has been ingested. Leidy (1879) observed that an *Amoeba verrucosa* was broken up into five spherical balls while contained in an *Amoeba proteus*. Entz (1925) observed that *Cyclidium* was broken up into pieces in *Amoeba vespertilio*. Bélár, (1921) in
speaking of the digestion of food by the flagellate *Collodictyon triciliatum*, states: "Wir sind gewöhnt zu sehen, dass der aufgenommene Nahrungskörper einer zunehmenden Maceration und Substanzverminderung unterworfen wird, bis schliesslich einige bräunliche Granula als Fäces ausgestossen werden". Perard (1912) gives a very good description of the digestion of Rotifers by *Amoeba terricola*:

"L'A. terricola, on le sait, engloutit tout entiers les Rotifères, parfois presque aussi gros qu'elle-même, et la digestion s'en fait d'une manière assez curieuse: l'amibe 'coupe' sa proie, comme avec des ciseaux; en réalité cette proie s'entrelange, se divise en fragments très gros puis toujours plus petits, et après un temps variable mais qui ne dépasse guère en général les huit jours, on ne trouve guère à l'intérieur du plasma que des particules de très-faible volume, et plus tard enfin des poussières fines". Entz, in 1932, published a paper in which he discussed various references to "Nahrungszerkleinerung" in literature.
EGESTION

The contents of food vacuoles are often expelled soon after they are ingested and in all conditions of digestion. The writer observed cases where food vacuoles underwent systole after all food contained in them had been dissolved.

Schaeffer (1918) described "fecal" vacuoles in Navorella bigemra, but was not certain whether these vacuoles were formed as food vacuoles or whether fecal material was gathered into them. The writer has found that these so-called fecal vacuoles may be either food vacuoles or vacuoles formed by the fusion of apparently very small vacuoles containing a few small granules or undigested particles.

Food vacuoles move more or less freely with the streaming endoplasm and the first tendency toward the egestion of the contents of a fecal vacuole is the movement of the vacuole toward the periphery of the ameba (Plan. III, figs. 1-6). As the protoplasm streams forward, the vacuole lags behind, so that it comes to rest in the posterior end of the ameba. The vacuolar membrane comes in contact with the cell membrane, and soon the individual membranes cannot be distinguished at that point. There is a gradual disintegration or thinning of the apparently fused membranes and finally a rupture occurs. This is
followed by a fairly rapid recession of the edges of the ruptured membrane and is sometimes accompanied by a rush of the endoplasm immediately adjacent to the vacuole, and the contents are forcibly extruded. The inner surface of the vacuole then becomes a portion of the outer surface of the ameba. The surface of the ameba may become somewhat wrinkled or thrown up into fine folds at this region and a brief cessation of endoplasmic streaming ensues, but after a few minutes endoplasmic streaming again takes place and this surface may immediately become smooth, or a somewhat wrinkled and folded protuberance on the posterior end of the ameba may be carried around for a few minutes.

In cases where fecal vacuoles are formed from scattered particles in the endoplasm, the particles tend to accumulate in the posterior end of the ameba during locomotion. No definite vacuolar membrane around such a particle can be discerned. However, it is evident that each particle is surrounded by a thin layer of liquid. When these particles come together, more and more liquid is observed to surround the mass until finally a fairly large vacuole is formed. The contents of such a vacuole are then expelled in the usual manner.

Insoluble substances are occasionally expelled directly from the endoplasm and without first being contained in a definite single vacuole (Pl. III, figs. 7-9).
In most of these cases, granules and particles in the endoplasm tend to concentrate in the posterior end of the moving ameba. There may be an almost complete cessation of endoplasmic streaming for a few moments, during which time the cell membrane suddenly ruptures at the posterior end and the collected granules rush out. A small amount of the more liquid portion of the endoplasm may escape during this process and coming in contact with the water, may either be miscible with the water or, a membrane may immediately be formed around the mass. These masses frequently resemble small rounded amebas, but at no time were any signs of life manifested by these.

The factors responsible for inducing the ejection of solid materials by an ameba are not well understood. That light might be a factor was considered by Rhumbler (1898), Harrington and Leaming (1900), Schaeffer (1917b), and others. Rhumbler believed that the intense illumination used during observations not only accounted for the fewness of the cases of feeding observed, but also for a case in which he observed a partly ingested algal form to be egested by an *Amoeba verrucosa*. Harrington and Leaming (1900) found that amebas quickly become accustomed to strong light and Schaeffer (1917b) supports this view. However, sudden changes in the intensity of illumination do tend to bring about the ejection of food as has been
found by Schaeffer (1917b) and in the present investigation. But since the intensity of illumination remains practically unchanged during an observation, light is not the only factor bringing about egestion.

A few simple experiments performed by the writer seemed to indicate that heat from the source of illumination was not responsible for the frequent egestion of food that was observed. Amoebas were found to egest partly digested food even after they had been kept under observation over two hours, the temperature of the slide containing them being kept at 10 to 30 °C, and the illumination remaining constant.

That the nature of the substance itself plays an important part in determining its egestion, has been clearly shown by the experiments of Lund (1914). He found that in *Euplotes*, Chinese ink was ingested soon after it had been ingested, while fat-free yolk was retained until it was digested. This was found to be the case when the egg yolk and the ink were in separate vacuoles. However, when the ink and yolk were ingested together, the ink was retained until the yolk had been digested. Schaeffer (1916) found that raptorial amoebas egest carmine grains soon after they are ingested. Egestion of these, according to Schaeffer, is explicable on the basis that the carmine grains are actually "disagreeable" and not merely because they are (presumably) indigestible.
CONTRACTILE VACUOLES

Schaeffer (1918) described two types of clear vacuoles in *Mayorella bigemma*, namely, contractile vacuoles and "permanent" vacuoles. These latter vacuoles were so called because they were never observed to undergo systole, even while under observation for a long period of time. Both of these types of vacuoles were observed by the writer.

As described above, the writer found that in many cases, food vacuoles become subdivided, and often one of the resulting vacuoles contains little or no solid material from the original vacuole. Such vacuoles are sometimes carried about in the endoplasm as long as eight hours or more, but sooner or later, the vacuoles diminish in size and finally disappear, or they undergo systole. These vacuoles might well be considered under Schaeffer's "permanent" vacuoles. However, many small vacuoles are sometimes carried about in the endoplasm of the amebas over a long period of time and it is very difficult to ascertain whether or not these are contractile vacuoles.

The contractile vacuoles arise and grow independently of each other, and, even when two or more become pushed together, no fusion seems to occur. This observation was also recorded by Schaeffer. Before systole occurs, the vacuole moves to the periphery of the ameba, and, as
in the case of food vacuoles, the vacuolar membrane comes to lie in close contact with the cell membrane (Pl. I, figs. 1-6). There is a gradual dissolution of these combined membranes in the center of the region of contact, followed by a rupture or opening to the outside. If the vacuoles are large, there is a gradual recession of the edges of the vacuole and the pore becomes much enlarged. In general, systole of a contractile vacuole is somewhat slower than that of a fecal vacuole and the rush of endoplasm adjacent to the contractile vacuole is not so marked. The membrane of the contractile vacuole gradually contracts and thickens and is finally pushed out even with the periphery of the ameba. Occasionally, vacuoles undergoing systole were completely everted due to a rather sudden rush of the endoplasm against the contractile vacuole and a convex protuberance was thus formed, and was bounded by what had been the membrane of the vacuole. From such cases, it can be concluded that the membrane of a contractile vacuole can be substituted for cell membrane, or can be readily changed into cell membrane. On a prepared slide a specimen was found which contained two large clear vacuoles, one of which was apparently undergoing systole at the time the ameba was killed. The vacuolar membrane in this ameba was continuous with the cell membrane and was perfectly smooth and unscarred at the place of union.
Hürenberg (1838) maintained that there was a definite permanent wall around the contractile vacuoles and that the vacuoles remained in the same place and did not change in number. Claparède (1854) and Lachmann (1859) agreed with Hürenberg. Degen (1905) and Stempell (1914) found it necessary to assume that there was a differentiated and permanent vacuolar membrane in support of their hypothesis that the contractile vacuole functions in the regulation of osmotic relations to the environment.

On the other hand, the vacuolar membrane is regarded as a more or less temporary structure by a number of investigators. Among these are Dujardin (1838), Stein (1859), Haupas (1863), Rhumbler (1898), Mitschli (1887), Lankester (1909), Taylor (1923), and Howland (1924). Taylor found that the contractile vacuole in Euplotes moved to the periphery of the cell, became contiguous with the papilla pulsatoria, and a discharge pore was formed, probably, by a change from gel to sol in the contiguous boundaries of the contractile vacuole and papilla. Upon the completion of systole, "the discharge pore is closed apparently by the vestige of the gelated wall of the contractile vacuole".

In many of the protozoa, the contractile vacuole arises from the fusion of two or more small vacuoles as shown by Taylor (1923), Howland (1924), and others. Taylor
states that the coalescence of vacuoles involves the slow reversion of their contiguous boundaries from a gel to a sol condition. In Mayorella bigemna, the contractile vacuoles do come in contact with each other and are often pushed together to the extent that they become distorted, yet apparently they never coalesce. The failure of these vacuoles to fuse might be explained on the basis that the vacuolar membrane is much more viscous than in protozoa where coalescence has been observed.

Various functions have been assigned to the contractile vacuole. Ehrenberg (1838) described it as a spermatic gland and among others, Lieberkühn and Siebold (1856), Claparède (1854), and Lachmann (1859) considered it to be a rudimentary heart, and believed that it does not communicate with the outside. A respiratory function was ascribed to the contractile vacuole by Spallanzani (1776) and Dujardin (1838). The present generally accepted view that the contractile vacuole has an excretory function dates back to the work of Stein (1859). However, Jennings (1904) was the first to prove beyond doubt that a contractile vacuole actually discharges its contents to the outside.

The view that the contractile vacuole acts as a mechanism regulating osmotic pressure was advanced by Hartog (1888) and this view has apparently been substantiated by a number of investigators. Gruber (1889) was able to decrease
the vacuolation in Actinoplanae sol by placing it in salt water, and to increase the vacuolation again by transferring it to fresh water. Zuelzer (1907) obtained similar results with the contractile vacuole in Amoeba verrucosa. An interesting phenomenon accompanying digestion of prey by a cannibal was observed in two cases by the writer and is additional evidence in support of this view (Pl. V, figs. 1-9). At the time that the prey was ingested, and up to the time that the food vacuoles started to diminish in size, only from two to three contractile vacuoles could be observed at any one time. Not more than four or five systoles were observed in one hour. However, when the food vacuoles started to decrease in size, almost immediately the number of contractile vacuoles increased until from six to eight could be observed at any one time. These vacuoles arose in the vicinity of the food vacuoles and moved out to the periphery of the ameba and then underwent systole. As many as six systoles were observed in a period of fifteen minutes. Soon after the liquid content of the food vacuoles disappeared, the contractile vacuoles gradually decreased in number until only three could be observed at any one time.

The correlation between the disappearance of the liquid in the food vacuoles and the increase in the amount of liquid expelled by the contractile vacuoles shows
definitely that at least one of the functions of the contractile vacuole is that of the regulation of water in the protoplasm of the amebae. Taylor (1923) described "artificial" vacuoles in the endoplasm of *Euglena*. He found a correlation between the presence of the food vacuoles and the occurrence of these artificial vacuoles in that some of these vacuoles arose in regions especially near food vacuoles and were not found "in the area left of the cytostome where also food vacuoles never normally appear". Khainsky (1910) believed that there was a more direct connection between food vacuoles and contractile vacuoles. According to Khainsky, so-called Exkretionskörper are precursors of the contractile vacuoles and arise in the food vacuoles. During the present investigation, small vacuoles enclosing parzaylum grains were observed to increase in size and finally undergo systole. It is possible that such vacuoles are concerned with excretion; however, the solid material is apparently unchanged and the vacuoles are not different from "fecal" vacuoles.
CARNIBALLIC

At certain times when material was taken from a culture and examined, a number of peculiar "spheres" was found. These resembled small amebas in a rounded condition. While under observation, an active ameba ingested one of these spheres, and after about 15 minutes, the sphere was liberated. Upon further investigation, a number of amebas was found to contain similar spheres in vacuoles.

The question then arose as to what was the origin and significance of such spheres. Thirty cultures were on hand. They were then examined but spheres were found to be present in only three of these cultures. These three cultures were started at the same time while all others had been started at some other time. More cultures were started at various times and sooner or later spheres made their appearance in all of these cultures.

An attempt was then made to study the conditions of a culture when the spheres first made their appearance and to determine what eventually happens to the spheres. Fourteen cultures were started at the same time and maintained under identical conditions. From previous observations it was found that the condition of all the cultures would be very similar at any one time. In order to keep
from disturbing the conditions which bring about the appearance of the spheres, two cultures were examined daily for a period of one week, at the end of which time, two other cultures were examined in a similar manner. Thus two previously undisturbed cultures were examined each week, and no culture was disturbed for a period of over one week.

A gradual increase in the number of amebas present in the cultures was noted until the end of the fourth week at which time the amebas were very numerous. At this time the first spheres were found. Beginning near the middle of the second week there was a rapid decrease in the number of euglenas present at any one time. There were relatively few euglenas when the first spheres were found. Upon examination, all but three of the cultures were found to contain small spheres. Spheres, however, were found in these three when examined two days later. The first spheres were usually little more than 25 to 35 microns in diameter and were somewhat smaller than any active amebas found in cultures at this time. The number of spheres became more numerous for five days after which time there was a marked decrease in number. The size of the spheres increased during the next ten days, at the end of which time, nearly all spheres had disappeared. At any one time there was a direct correlation between the
number of spheres present in a culture and the number of amebas containing such spheres as inclusion bodies.

Many of the spheres, at least, were alive as manifested by the presence of contractile vacuoles which were seen to undergo diastole and systole when under observation for two hours or longer. Furthermore, occasionally a small blunt pseudopod was extended and retracted. However, many of the larger spheres were evidently dead. There was a distinct membrane around them, inside of which was found only the nucleus, a few granules, and sometimes, a few strands of dense material. Small active amebas were frequently observed to approach one of these "shells" and if it were ruptured, the amebas moved into it. After a short time, they would usually move out again. In one case, three small active amebas were observed to enter the same "shell". The shell became somewhat distended when the third ameba entered. The amebas continued to move about for five minutes in these close quarters, but finally a new rupture occurred in the shell and the amebas moved out through this opening.

During the time when the spheres were present in a culture, a few large and small, somewhat sluggish, amebas were found. Fifteen of these were carefully isolated in concavity slides and placed in a moist chamber. Four days later these amebas were found to be rounded and had the
same characteristics as the spheres found in the cultures. This gave some evidence that the spheres found in cultures were probably amebas which were active at one time.

Attention was given to amebas which contained these spheres. In the course of a number of observations, at certain times, large numbers of amebas were observed to ingest spheres but sooner or later, the spheres were usually egested. Over a period of an hour, one sphere was observed to be ingested and egested three consecutive times by the same ameba. After many observations, the writer witnessed the complete digestion of one of the spheres by an active ameba. In later observations, the ingestion and digestion not only of five spheres, but also of three active amebas, was witnessed.

There is no doubt that this phenomenon is true cannibalism. The spheres were found to be amebas which had become sluggish and rounded and, in most cases, were living. Grosse-Allermann in 1909 saw, in two cases, a swollen *Amoeba terricola* and thought he was dealing with multiple fission. Penard (1905) made similar observations but regarded these inclusion bodies as parasites which had developed within the ameba and were liberated after the death of the ameba. Liston and Martin (1911) described what they regarded as endogenous budding in a large ameba from liver-abscess pus. Martin found no evi-
idence from the stained material that the nucleus of the ameba played any part in the formation of the buds. The writer found no evidence in the present study that the spheres were ever formed in amebas. The spheres are often egested by amebas and this might lead one to at first conclude that such spheres actually originate inside other amebas. However, further observations show that such spheres are not only frequently ingested but also digested.
DIGESTION OF AN AMEBA BY A CANNIBAL

The time required to kill the prey undoubtedly varies with the physiological condition of both the prey and the cannibal. Living prey was observed to be in a food vacuole as long as three hours, at the end of which time it was still alive as evidenced by the activity of the contractile vacuoles and the occasional sending out of pseudopodia. In two cases, the prey was observed to egest some solid materials even while still being contained in a food vacuole after a period of two hours. Dead amebas containing spheres were sometimes found. Some spheres were ingested and egested a number of times before they were finally ingested by an ameba which also digested them. The physiological condition of such a sphere was undoubtedly affected every time it was ingested and retained for a time.

No change was manifested in the prey until its plasmalemma suddenly disrupted. Even up to this time, diastole could be seen in the contractile vacuoles of the prey and it was evident that this was about the time the ameba was killed. In cases where complete digestion of the prey was observed, the prey was in the food vacuole about four hours before the rupturing of the plasmalemma occurred.
Very little liquid was ingested with the prey. There was only a slight increase in the size of the food vacuole until the cell membrane of the prey was ruptured, after which time, the increase in size was much more rapid (Pl. V, figs. 1-9). Almost immediately after the membrane was ruptured it disappeared entirely from view. This was followed by the dispersion of the contents of the prey throughout the food vacuole. In only one case was there a slight tendency toward the coagulation of some of the contents of the prey.

In three cases, the food vacuole became somewhat distorted and a short time later constricted and became divided into two vacuoles, each of which contained some of the undigested remains of the prey. Each of these vacuoles was subdivided a number of times. In one case, thirteen food vacuoles were formed from the original food vacuole in a period of twenty minutes after the plasma-lemma of the prey disappeared. The nucleus (endosome) of the prey could still be seen and it was not until twenty minutes later that it disappeared.

One hour later, only a small amount of solid material remained in the vacuoles. This, for the most part, evidently consisted of paramylum grains which were dispersed in the endoplasm of the prey. A number of contractile vacuoles appeared in the vicinity of the food
vacuoles, at which time a gradual decrease in the size of the food vacuoles was noticeable. The rate of diastole and systole was much higher than is ordinarily found in the amebas. However, when liquid was no longer present in the food vacuoles, the contractile vacuoles decreased in number until only one or two of these could be observed. The rate of diastole and systole was normal. The solid remains of the prey became scattered throughout the endoplasm of the cannibal two hours after the plasmalemma of the prey disappeared.

In one case, the prey was rather small and the food vacuole was not subdivided as in the other cases. There was an increase in the size of the food vacuole, and after the prey was in solution and only paramyllum grains remained, the vacuole gradually decreased in size and finally disappeared. The undigested remains became scattered throughout the endoplasm of the cannibal. During the time the liquid was leaving the food vacuole, there was a marked increase in the number and rate of diastole and systole of the contractile vacuoles. In this case, the original food vacuole did not disappear until three hours after the cell membrane of the prey had been ruptured.

Doflein (1911) in his general discussion on Amoeba states that he has often observed cannibalism. Haime (1853) drew an illustration of Oxytricha in which
was contained another organism described by him as representing a form in the metamorphoses of *Oxytricha*. However, there is some doubt about the included organism as being of the same species. Kaupas (1888) stated that hungary *Onychodromus* were cannibalistic. Joukowsky (1898) observed a large *Pleurotricha* in a starved culture. He believed that it had eaten some of its own kind. In similar cultures of *Onychodromous* he believed that the nuclear structure of partly digested organisms of the same species was shown in large stained specimens. Dawson (1919) described cannibalism in *Oxytricha*. Cannibalism in *Amoeba vespertilio* was described by Lapage (1922). Lapage, however, was not certain that this was a case of cannibalism since he did not have conclusive evidence to state the origin of the "spheres" ingested, nor did he actually observe the digestion of a "sphere" by a cannibal. Gelei (1925) described cannibalism in *Stentor*. Mattes (1924) briefly mentioned cannibalism in *Amoeba terricola* and *Amoeba sphaeronucleolus* and published a much fuller account of this in 1923. Ivanic (1927) reported cannibalism in *Amoeba vespertilio*, *Amoeba verrucosa*, and *Amoeba Giorgievici*. He stated that he observed cannibalism in *Stentor* in 1910.

There seems to be some correlation between food shortage and the appearance of cannibalism in a culture in the writer's investigation. Carnibals were never found
in new cultures nor in extremely old cultures. Cannibalism always appeared in the cultures at the time when the food shortage was just beginning to be noticeable. Maupas and Joukowsky found cannibalism in starved or "hungry" cultures. Dawson found that cannibalism does not take place while the culture medium is relatively fresh and the greatest amount of cannibalism does not occur in a medium in which the food supply is much depleted, but at the time when the scarcity of food is just beginning to be felt. He states that "it seems probable that the accumulation of excretion products plays a part in inciting cannibalism". In the observations of Lepage "no trustworthy evidence was found as to the nature of the stimulus which caused the adoption of these habits". He states that "At first I was inclined to think that starvation played a part in causing the amoebae to become cannibalistic. They showed, however, few other signs of starvation. They exhibited normal activity, they multiplied abundantly, and, beyond what was probably a more marked vacuolation than is usual for the species, were in no other way abnormal" and "a possible explanation may be sought in the view that the amoebae had become so numerous in the cultures that the active ones were ingesting the rounded ones and, finding them indigestible, were extruding them again". He also
suggests since "An amoeba, in the absence of its normal
diet, will eat almost anything", and the normal food
supply in his original cultures "must have been for long
scarce", it is not surprising that the amebas "should
have turned, under the stimulus of the change of environ-
ment provided by the sub-cultures, to the ingestion, not
only of the diatoms which developed in those sub-cultures,
but also of other amebae, both of its own and of other
species". Gelei believed that cannibalism in Stentor
was a property of the animal which was hereditary. Ivanic
believed that cannibalism was due to disturbances and
finally the inhibition of cell processes caused by long
cultivation. Mattes stated that cannibalism in soil
amebas was accidental ingestion of amebas of the same
kind and was brought about by outer conditions, especially,
the quick changes in dry and wet periods.
CAUSE OF CANNIBALISM

On the basis of the few accounts of cannibalism in the literature on the protozoa, this phenomenon does not appear to be wide-spread among this group of animals. A number of factors should be considered; however, none of these are well understood.

It is not known whether the protozoa can "recognize" animals of their own kind (species). It is true that one would not conclude that this "recognition" is comparable to that in a higher organism such as man, but would be the stimulation or non-stimulation of protoplasm by similar protoplasm chemically and, perhaps, physically. If there is a recognition of the members of the same kind, it might be concluded that cannibalism is due to the "accidental" ingestion of animals of the same kind as suggested by Matte. If cannibalism is due entirely to the accidental ingestion of animals of the same kind, we would expect to find cannibals at any one time in an ameba culture. This was not the case in the writer's observations, nor in those of Haupas, Joukowsky, Dawson, Lapage, and Ivanic.

Gelei found cannibalism in certain of his cultures of Stentor, but not in all similar cultures. He concluded that cannibalism must be hereditary in Stentor. Many of the writer's cultures were inoculated with material from
cultures in which cannibalism was prevalent, but cannibals were not found until the amebas became very numerous and the food (euglenas) relatively scarce. There was, however, the possibility that the cannibals introduced into such cultures did not reproduce. Therefore eight cultures of euglenas were started and maintained under identical conditions. At the same time later, cannibals were isolated and placed in each of five cultures. In the sixth culture was placed a cannibal which contained a cannibal in a food vacuole. The included cannibal had its prey partly digested. This sixth culture was prepared to be certain that the offspring of a cannibal and not its prey would give rise to the ameba in the culture, since, in many cases, the prey is egested while still living. All amebas in such a culture would then develop from a cannibal, since the prey is also a cannibal. Two cultures were inoculated with material from the source culture of the cannibals, care being taken to at first remove all the amebas or spheres from this material. These latter cultures were intended to test the possibility that unrecognizable stages in the life history were introduced into cultures along with the cannibals, and that it was these stages instead of cannibals that gave rise to the amebas produced in the cultures. Amebas reproduced normally in the six cultures in which the cannibals had been introduced, while at no time were amebas
found in the other two cultures. Cannibalism made its appearance under the same conditions as in other cultures and there was no evidence that it was hereditary.

Dawson stated that it seems probable that the accumulation of excretion products "plays a part in inciting cannibalism" in *Oxytricha*. This does not seem to be the case in the writer's cultures. In cultures of amebas feeding on passive euglenas which are inclosed in gelatinous sheaths, the increase in the population of amebas is not nearly so rapid as in cultures of active and passive euglenas without gelatinous sheaths. Cannibalism in the latter cultures appears earlier than in the former. If waste products tend to accumulate in cultures and brings about cannibalism, one would expect to find cannibalism in all cultures after a definite period of time. As shown above, this is not the case.

To further test the hypothesis that cannibalism is due to the accumulation of waste products a culture was started in a flask equipped with a constant level siphon. Water was permitted to run into the culture at the rate of 400 cc. in 24 hours. The siphon kept the volume of material in the culture fairly constant (about 350 cc. Both well water and distilled water were used in two successive experiments, and in both cases, a few cannibals were found. Many euglenas and amebas were
washed out of the culture. The amebas at no time were very numerous in the culture, and this may account for the few cannibals observed. Furthermore, the writer has maintained one culture since February, 1930 in which only cannibalism is found at certain times. Nothing, except a small sample at times, has been removed from the culture and as the volume of the liquid decreases due to evaporation, some material from an old euglena culture is poured in. This is done on the average of once in every two or three months. The addition of the food is soon followed by an increase in the population of the amebas and sooner or later, the appearance of cannibalism. If cannibalism is caused by the accumulation of wastes, it stands to reason, that cannibalism should increase and continue in this culture, which, of course, is not the case.

Ivanic believed that cannibalism was due to disturbances and finally the inhibition of cell processes caused by long cultivation. The culture mentioned above disproves this view, at least in the amebas cultured by the writer.

Lapage's views were somewhat indefinite concerning the cause of cannibalism. However, one of his suggestions was to the effect that "an amoeba, in the absence of its normal diet, will eat almost anything" and the normal food supply in the original cultures had long been
scarce, the change in the environment brought about in making sub-cultures was the stimulus for cannibalism in his amebas. He did not believe that it was brought about by starvation since the amebas multiplied abundantly and exhibited normal activity. In the next paragraph he stated that "the amoebe had become so numerous in the cultures that the active ones were ingesting rounded ones" which raised the question in the writer's mind as to whether or not Lapage considered the rounded amebas as being "normal". In either case, if the amebas in the writer's cultures were or were not on a "normal" diet, cannibalism was not caused by the making of sub-cultures.

Cultures in syracuse watch glasses enabled the writer to study the amebas in relation to the amount of euglenas present. As in cultures made up in Erlemeyer flasks, the euglenas settled to the bottom. When the amebas became very numerous, there were certain regions on the bottom of the cultures in which there were numerous amebas, but no euglenas. Many amebas in these regions formed food cups when they approached other amebas. Sometimes two amebas with food cups met. The food cups came in contact with each other, but neither ameba ingested the other. Frequently, an ameba was pursued for some distance by another ameba. The food cup of the pursuing ameba, in this case, was usually kept around the posterior
end of the pursued ameba. Occasionally, three or more amebas moved in a cilia; the posterior of each ameba being contained in a food cup on the anterior end of the pursuing ameba. This phenomenon was not observed in other regions of the culture where the euglenas were fairly numerous.

Five days later, a few cannibals were observed in the regions where the euglenas had first become depleted. At the same time, many amebas in these regions were rather sluggish and some had become spheroidal or rounded. The amebas in the regions where euglenas were present were active and appeared to be normal in all respects. Cannibals were found in all regions of the culture when euglenas were no longer present.

From the evidence found in the present investigation, it can only be concluded that cannibalism in the writer's cultures is due to a temporary physiological condition in the amebas and this condition is brought about by the depletion of the euglenas, the normal food of the amebas during cultivation.
SUMMARY

1. A clone culture of *Mayorella bigemma* was established and was successfully maintained for four years. A clone culture of *Euglena gracilis* served as a source of nutrition for the amebas.

2. Passive euglenas are as readily ingested as active euglenas.

3. In most cases, euglenas are ingested in food cups. Active euglenas are sometimes ingested by "invagination" of the surface of the ameba.

4. In most cases, the food cups are of a fairly definite size. When very small objects are ingested, a certain amount of liquid from the culture is usually taken in at the same time. When a large, non-motile object is ingested, the food cup is just sufficiently large to enclose the latter and little or no liquid from the culture is taken in.

5. During any observation on feeding, only a certain number of the amebas were seen to ingest euglenas. There is probably a temporary physiological difference between amebas which feed and those which do not.

6. Soon after the food vacuole has been formed, it begins to increase in size. This indicates that liquid is being absorbed by the food vacuole. A vacuole may
become very large, but sooner or later it gradually decreases in size and may finally disappear entirely. Food particles remaining undissolved at this time become scattered through the endoplasm of the amebas.

7. The food vacuole becomes acid in reaction soon after it is formed and remains acid in reaction throughout the course of its existence.

8. Paramylum grains of *Euglena gracilis* are not readily digested and become scattered throughout the endoplasm of the amebas. While carried about in the endoplasm, each is surrounded by a very thin layer of liquid (or protoplasm?) which is acid in reaction. Paramylum grains contained in an ameba are at first colorless; then after a few hours they become grey with a bluish tint. It has not been possible to determine the final fate of such changed paramylum grains, but it seems possible that they may be partly or wholly digested while in the endoplasm, over a long period of time.

9. Food vacuoles and their contents may become sub-divided a number of times during digestion. Some resulting food vacuoles receive little or no solid material from the original vacuole.

10. Three methods of egestion have been observed. The undigested materials may be expelled (1) directly to the outside from the food vacuole in which they had been
ingested, (2) from a "fecal" vacuole arising from a collection of the particles carried about in the endoplasm, or, (3) directly from the endoplasm, in which case no vacuole can be seen and a small amount of the more liquid portion of the endoplasm is also extruded.

11. Sudden changes in the intensity of illumination will cause many of the amebas to egest material from the food vacuoles. Heat from a microscope lamp has no appreciable effect on egestion.

12. Systole is essentially similar in food vacuoles, fecal vacuoles, and contractile vacuoles. In each case, the membrane of the vacuole seems to fuse with the cell membrane at their point of contact. The discharge pore occurs at this point, and is probably brought about by the solution of the membranes. In each case, the membrane of the vacuole becomes continuous with the cell membrane.

13. Contractile vacuoles arise independently of each other and do not fuse, even when pushed tightly against each other. Their inability to fuse may be due to a high degree of gelation in the membrane surrounding them.

14. In two cases, when the food vacuoles began to decrease in size, there was an increase in the number of contractile vacuoles, and in the rapidity of their
growth. After the food vacuoles disappeared, the contractile vacuoles decreased in number and their rate of growth became less. This may be interpreted as evidence that one of the functions of the contractile vacuole is the regulation of liquid (water?) in an ameba.

15. Cannibalism has been observed in *Mayorella birena* and the complete digestion of the prey by a cannibal has been traced. The process of digestion is similar to the digestion of euglenas.

16. In older cultures, and under certain conditions, a number of amebas become rounded. These are often ingested by active amebas. The latter also pursue other active amebas, and occasionally capture them. The amebas pursued may be of any size, but it is only when they are definitely smaller than the pursuer that they are captured. Many of the cannibals, under conditions of observation, egest their prey soon after they have ingested it.

17. Cannibalism is apparently not a normal phenomenon, nor is it a constant phenomenon characterising only certain races of this species. It does not occur when an abundant supply of *Euglena gracilis* is available. It occurs invariably when the supply of *Euglena gracilis* has been depleted below a certain point.
13. It seems probable that cannibalism in *Mayorella bigemmata* is due to a temporary physiological change, brought about by the lack of the normal food, which alters the normal mechanism of response.
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PLATE I

Figures 1 to 6. Drawings illustrating systole of a large contractile vacuole (CV). Only a portion of the cell membrane (CM) is shown. The migration of the vacuole to the periphery of the cell is shown in figures 1 to 3. Figure 4 shows the formation of the discharge pore (DP) and the fusion of the cell membrane with the vacuolar membrane (CVm) around the discharge pore. Figure 5 shows the vacuolar membrane somewhat straightened out and in Figure 6 it has been pushed out by the endoplasm and is the outer limit of a protruberance on the side of the ameba.
PLATE II

Figures 1 to 6. Drawings illustrating the formation of a food cup and the ingestion of a non-motile euglena by an ameba.
Figures 1 to 6. Drawings illustrating the ejection of a mass of undigested food (F) from a food vacuole. Figures 1 and 2 show the migration of the food vacuole to the periphery of the cell where the membrane of the food vacuole (FVM) becomes contiguous with the cell membrane (CM). Figure 3 shows the discharge pore (DP) and the fusion of cell membrane with that of the food vacuole. Figures 4, 5, and 6 show the recession of the edges of the discharge pore and the straightening out of the vacuolar membrane.

Figures 7 to 9. Drawings illustrating the ejection of granules (G) directly from the endoplasm. Figure 7 shows the granules somewhat concentrated in the posterior end of an ameba. Figure 8 shows the discharge pore as a rupture in the cell membrane (CM) through which is passing the concentrated mass of granules along with some viscous fluid. Figure 9 shows the discharge pore closed over and the mass of extruded material surrounded by a membrane.
PLATE IV

Figures 1 to 6. Drawings illustrating the ingestion of an active euglena without the formation of a food cup by the ameba. The euglena seems to push its way into the side of the ameba.
PLATE V

Figures 1 to 6. Drawings illustrating the digestion of an ameba by a cannibal in which the food vacuole became subsequently divided a number of times. Figure 1 shows the prey inclosed in the food vacuole of the cannibal. The nucleus (N) is visible in both amebas. A small amount of liquid is present between the cell membrane (CM) of the prey and the membrane of the food vacuole (FVM) in which it is enclosed. Only two contractile vacuoles (CV) are present in the cannibal. Figure 2 shows the prey after its cell membrane has been dissolved and its contents become scattered throughout the food vacuole. Figure 3 shows the food vacuole after it has become larger. Only the endosome (E) of the nucleus of the prey can be seen. Figure 4 shows the constriction of the food vacuole by the endoplasm of the cannibal. Figure 5 shows the prey broken up into six pieces and Figure 6, nine pieces have been formed. A mass of somewhat viscous material (FM) is shown in the endoplasm. This was extruded from two of the small food vacuoles. The food vacuoles are starting to decrease in size, and at the same time there is an increase in the number of contractile vacuoles. Figure 7 shows a further
increase in the number of food vacuoles and a decrease in size of these. In figure 8 the viscous material (PM) has been broken up into three parts. There is also a further decrease in the size of the food vacuoles. In figure 9, all of the liquid has disappeared from the food vacuoles, and the undigested remains of the prey have become scattered in the endoplasm of the cannibal. The number of contractile vacuoles has become reduced and only two are found.
AUTobiography

I, John Calvin Lotze, was born in Darlington Township, Beaver County, Pennsylvania, January 11, 1906. I received all of my secondary education in the public and high schools of the City of East Palestine, Ohio; and my undergraduate education at Miami University, Oxford, Ohio, from which I received the degree of A. B. in 1929. While in residence at Miami University, I acted in the capacity of student assistant in the Department of Zoology from 1927 to 1929. In 1929, I received from The Ohio State University an appointment as graduate assistant in the Department of Zoology and Entomology, and in 1930, I received an appointment as assistant in the same department, which position I held while completing the requirements for the degree of Doctor of Philosophy.