THE FEEDING APPARATUS AND THE FOOD VACUOLE IN PARAMECIUM MULTIMICRONUCLEATUM

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THE FEEDING APPARATUS AND THE FOOD VACUOLE IN PARAMECIUM MULTIMICRONUCLEATUM

By

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The author wishes to dedicate this manuscript to the memory of his father, who was always encouraging and assisting. Also, to his mother, whose spirit is always his inspiration to achievement.

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CURRICULUM VITAE

The author, Salah El Dareer, was born November 4, 1926, in Tanta, Egypt, where he completed his primary and secondary school education. He entered the Veterinary College of Fouad Ist. University, Giza, Egypt, in 1942, and obtained his B.V.Sc. from the same college in 1947. After working for the Egyptian Ministry of Agriculture for two months, during which he took part in combating the cholera epidemic that flared in Egypt in 1947, he was appointed to work as an instructor at the Veterinary College of Fouad Ist. University. In 1949 he was awarded an Egyptian Government Scholarship to study Zoology in the United States. Since his arrival at this country he has been engaged in postgraduate work in the Department of Zoology at Michigan State College. After completing the requirements for his M.S. degree he plans to work towards the Ph.D. degree in the same department.

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I. INTRODUCTION

Paramecium multimicronucleatum was used to study the protozoan feeding apparatus, formation of the food vacuole, its separation from the pharynx and also for its movement through the body. Frisch (1937) maintains that the mouth of Paramecium is a narrow slit bounded by raised and thickened folds which are sometimes closed. Mast (1947) and others maintain that the mouth is a fixed oval opening.

Lund (1933, 1941) holds that there are long fibers attached to the pharynx in paramecium and that they are not fixed, but they are attached near the distal end of the pharynx on all sides. He further maintains that there are also "five or more heavy fibers" which extend from their attachment at the anterior edge of the opening at the distal end of the pharynx to the posterior edge where each ends in a "large granule" which, he thinks, make part of the neuromotor system.

Bragg (1935, 1936) and others conclude that contraction of the distal end of the pharynx and cyclosis are involved in separation of the food vacuole from the distal end of the pharynx. Kalmus (1931) and others seem to think that surface tension plays an important role in the formation of the food vacuoles. Mast (1947)

believes that Lund's postulations reasonably account for all that has been seen. Yet, he further maintains that those "post esophageal fibrils" could not be identified.

Furthermore, several workers have put into consideration different factors affecting the frequencies of ingestion in Paramecium and other unicellular animals. Metalnikow (1907, 1912) maintains that ingestion of food is decreased in specimens of Paramecium by low temperatures, high temperatures, weak alkaline solutions and old culture solutions. On the other hand, ingestion is increased by moderate temperatures, weak acid solutions, alcohols and arsenious compounds. He found that the nature of the food particles is an important factor in the frequency of ingestion, i.e., digestible substances are more easily ingested than indigestibles. Bragg (1936, 1939) and others support the above conclusions of Metalnikow, regarding selection between various particles by paramecia. Nelson (1933) holds that selection between various particles in at least some ciliates, is dependent upon the chemical properties.

Trager (1937) shows that liver extract, killed yeast and fresh kidney are all essential to the growth of Paramecium (sp. caudatum and sp. multimicronucleatum) in the absence of other micro-organisms. He did not show the effect of either living or dead yeast alone on the growth of Paramecium.

Meanwhile, considerable observations have been made concerning the determining factors in other protozoa. Schaeffer (1910) made a thorough study of feeding in Stentor caeruleus. He believes that hunger accelerates and satiety retards frequency of ingestion in this species, and that there was evidence that stentors take some organisms more readily than others. Schaeffer (1916) maintains that the most important single factor in inducing feeding in Amoeba is movement of the food, but that the character of food particles taken in is also involved. He says that in general, soluble particles are more readily taken than insoluble ones. However, uric acid grains are ingested less readily than carmine. Ectoplasm and endoplasm of the cell react to carmine in opposite ways, i.e., the former attracts while the latter repels it.

Mast and Hahnert (1935) came to the following conclusion:

> Amoeba proteus does not feed when it is attached to the substratum, therefore, anything which facilitates attachment facilitates feeding. It has been repeatedly observed that feeding increased greatly if amoebae were transferred from a solution in which attachment was weak, to one in which it was strong, e.g., fresh Chalkley solution. It was repeatedly observed that feeding increased if amoebae were agitated. This was particularly marked in specimens transferred from ordinary culture dishes to slides. Starvation facilitates feeding, but not if it continues until the amoebae no longer attach firmly. Specimens kept without food five to eight days usually do not feed again no matter how they are treated although they live ten days or longer.

Hargitt and Fray (1917) in their study of nutrition in paramecia found that mixed species of bacteria would maintain growth

and reproduction for a longer period of time than when a single species of bacteria was used as the food organism. Phillips (1922) seems to support the contentions of Hargitt and Fray.

II. MATERIAL AND METHODS

Reagent grade chemicals were used exclusively in all experiments. Double pyrex glass-distilled water was used for the preparation of the different solutions. All glassware was washed first with soap and water, then with cleaning solution, and rinsed 15 times with tap water, followed by one rinsing with distilled water. Before use of any piece of glassware, it was rinsed with the solution to be used in the experiment. The solutions were kept at room temperature, ranging between 20° and 22° C.

The formula of Jones (1932) was used as a culture medium for Paramecia:

Timothy Hay	1.000 g.
White Flour	0.100 g.
Lettuce	3-5 g.
Distilled Water)	
or)	700 c.c.
Spring Water)	

Thyroid culture media (0.800 g. Armour's dessicated sheep thyroids in 1,000 c.c. Chalkley solution) were used in some experiments. These above mixtures were innoculated with 3 to 5 c.c. of culture media from a culture in which paramecia were abundant.

Organisms used for study of ingestion were obtained from stock cultures in which paramecia were abundant. Specimens were collected for experimentation by centrifugation in an angle-head

centrifuge. One-tenth c.c. of paramecia was obtained from each fifteen c.c. portion of culture fluid in the following manner: Fifteen c.c. of fluid were added to a graduated 15 c.c. centrifuge tube and centrifuged at a moderate speed for 5 minutes. Then the tube was removed from the centrifuge and the supernatant was poured off. This procedure was repeated until the desired quantity of organisms was obtained for the experiment.

Specimens of paramecia were then transferred to Chalkley solution by the following method: The volume of paramecia (0.1 c.c.) was made up to 15 c.c. with Chalkley solution. Resuspension of paramecia in Chalkley solution was accomplished by gently agitating the centrifuge tube. Organisms were again concentrated at the bottom of the tube by centrifugation at a moderate speed for 5 minutes. The tube was removed from the centrifuge, the supernatant was poured off, and the procedure was repeated two additional times. Organisms were then starved by leaving them in a centrifuge tube suspended in Chalkley solution for 24 to 48 hours. Mannitol (in Chalkley solution) of the desired concentration was then added to the tube until the volume was made up to 15 c.c. The tube was centrifuged at a moderate speed for 5 minutes, and then all but 0.2 c.c. of the supernatant fluid was removed with a pipette. This procedure was repeated two additional times. Control organisms

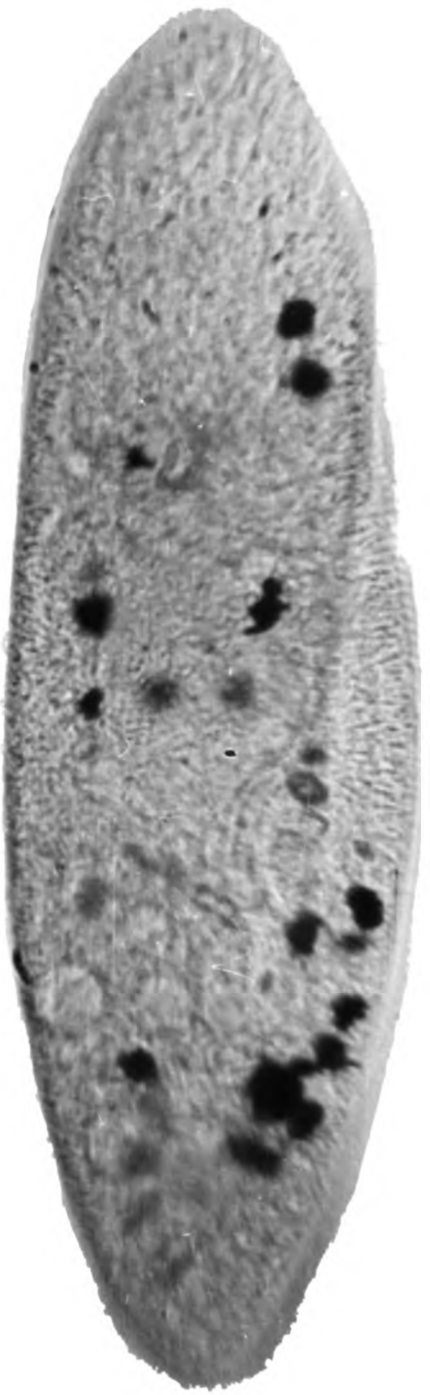
were treated in essentially the same manner except that Mannitol was omitted from the Chalkley solution.

Paramecia to be used for experimentation, and also control organisms, were transferred to small dishes, and congo-red yeast emulsion was added to each dish (0.2 c.c. of Chalkley solution). Congo-red emulsion was prepared in the following manner: Three grams of yeast, and 1.3 grams of congo-red in 10 c.c. of Chalkley solution were boiled moderately for approximately 10 minutes. The suspension was then cooled under the tap.

Fifteen minutes after the addition of the congo-red yeast emulsion to the culture dishes, a few drops of fluid were transferred to a glass slide. Evaporation of the fluid was retarded by encircling the culture medium with vaseline. The culture fluid was then covered with a cover slip and the number of food vacuoles in the cytoplasm of 10 to 20 paramecia were counted and recorded. Figure 1 shows food vacuoles within the cytoplasm of a Paramecium. This procedure was repeated at 30, 60, and 90 minutes after addition of the congo-red yeast suspension to the culture solution. Organisms in the central solution were treated in essentially the same manner.

All observations for the purpose of studying the courses and sizes of the food vacuoles were on preparations made as follows: A drop of Chalkley solution containing numerous paramecia were mounted within a small vaseline ring on each of several slides.

Figure 1. Photomicrograph showing food vacuoles in the cytoplasm of a Paramecium multimicronucleatum after feeding for 15 minutes in Chalkley solution containing lamp-black.



0.1 MM

Yeast cells stained with congo-red were added to some slides and lampblack suspensions were added to others. Then all were covered with cover glasses.

After a short period of time, the paramecia in these preparations were quieted to an extent considered satisfactory for observations. Some individuals in some preparations were so quiet that it was easy to study them continuously for several hours.

Dow Methocel was used in studying the effects of viscosity of the solution on the rate of ingestion in Paramecium multimicronucleatum.

Properties of Methocel. It is a cellulose ether manufactured in the form of white fibers. It is soluble in cold water, insoluble in hot water, saturated salt solutions and most organic solvents, and is unaffected by oily or greasy materials of animal, vegetable or mineral origin. It is stable in alkalies and dilute acids. Aqueous solutions are stable and ordinarily do not require a preservative.

Methocel (15 centipose) was prepared by thoroughly mixing 40 grams of the material with 500 c.c. of Chalkley solution at a temperature of about 95° C. It was left to soak for 30 minutes and then a four per cent solution was made by adding 500 c.c. of Chalkley solution to the mixture. This solution was cooled at room temperature until smooth.

On analysis of the Methocel viscosity concentration chart, it can be seen that with a solution of 15 centipose Methocel, the viscosity of the experimental solutions used varies considerably, reaching about 5.8 centipose in a one per cent Methocel solution by weight. Since water has a viscosity of one centipose, the addition of even a 0.1 per cent solution will raise the viscosity considerably. As the concentration in percentage by weight increases, the viscosity increases markedly, so that in a 15 centipose Methocel solution at a concentration of four per cent the viscosity increases to 80 centipose at a temperature of 20° C. The viscosity of Methocel solution in centipose is based on the viscosity of a twenty per cent aqueous solution at 20° C.

Mannitol (mannite) highest purity $CH_2OH(CHOH)_4\ CH_2OH$, was supplied by Fisher Scientific Company. It has a molecular weight of 182.17, with a melting point of 166-168°, soluble in both cold and hot water.

The molecular weight of this reagent (182.17 grams) was dissolved in 1,000 c.c. of sterile Chalkley solution at room temperature. Then different concentrations from the one M mannitol solution were made by diluting to the desired concentration with sterile Chalkley solution instead of distilled water. By this, we could have different concentrations of this reagent possessing different corresponding osmotic concentrations.

Yeast cakes were freshly supplied for immediate use. Congo-red was supplied by the Chemistry Department of Michigan State College.

III. RESULTS

1. Morphology of Paramecium multimicronucleatum

For convenience, the following description will be used when referring to different structures in various parts of the cell:

Paramecium multimicronucleatum is a unicellular animal, elongated and oval in shape. Its anterior end is rounded, while its posterior end is conical in shape. It has a thickness that varies from the anterior to the posterior ends. The animal has four surfaces; one ventral, one dorsal and two lateral. The side of the animal which exhibits the mouth opening is the ventral surface (Figure 2). The lateral lobes, right and left, are differentiated by a fairly deep longitudinal depression called the oral groove, which can be seen along the anterior two-thirds of the ventral surface. The two lobes are connected dorsally along the whole length of the body. Each lobe extends from the anterior end of the animal to about its posterior one-third. The posterior end of the oral groove marks the posterior end of both lobes. The posterior end of the body is conical in shape and tapers toward the posterior end. A cross-section at the level of the middle of the anterior two-thirds would resemble a "U" that has a rather uniform thickness.

Figure 2. Camera outline of Paramecium multimicronucleatum when observed from the ventro-lateral surface. A, oral groove; B, right and left lateral lobes; C, pharynx; D, vestibulum; E, food vacuoles; F, position where the food vacuole is formed; and G, contractile vacuole.

2. The Feeding Apparatus

The feeding apparatus of Paramecium multimicronucleatum consists of an oral groove. The groove is deeper in adult animals than in younger growing animals. It extends from the anterior end to about the posterior one-third of the body. At the posterior end of this groove, there is a ciliated depression, the vestibulum, which leads to a ciliated tube (pharynx) extending from an opening in the floor of the depression (the mouth) posteriorly into the body.

The tube is called pharynx by some authors, cytopharynx, gullet or esophagus by others, and a portion of it pharynx and the rest esophagus still by others. For convenience, in this paper the tube will be called the "pharynx."

The pharynx can be seen very distinctly only in living specimens. It extends from the mouth opening, which is oval in shape, toward the center of the body, posteriorly. Then it turns slightly to the left and posteriorly to proceed parallel with the surface of the body and more to the left side for some distance. Finally, it turns at an angle slightly to the right side of the body and ends leading into the forming food vacuole.

The feeding apparatus has been described by Mast (1947) and others who hold that it is essentially the same in different species of Paramecium. Observations in preparation of this paper

agree with previous work done, and briefly maintain that it consists of the oral groove, the vestibulum, the mouth, and a tube (pharynx) leading from the mouth and extending posteriorly into the depth of the animal protoplasm. This tube is frequently referred to as the gullet, cytopharynx or esophagus. Mast (1947) concluded from his observations on paramecia that the mouth is a fixed oval opening. But Frisch (1937) holds that it is a narrow slit bounded by a raised, thickened border, and that it is sometimes closed. He made his observations on an exconjugant individual continuing for one and one-half hours immediately after it had separated from its mate.

The gullet was established in the following manner: The anterior cell in a dividing individual was observed. At 3:00 p.m. the two halves of the animal were separated into two freely swimming individuals. Each individual had its nuclei which appeared to be darker greenish than the rest of the animal protoplasm. The individual which developed from the anterior half was observed to ascertain how the newly formed paramecium developed a gullet. The oral groove extended almost to the posterior tip of the cell. The animal moved rapidly, but at various intervals of time (maximum 73 seconds) it anchored itself to the substratum. Meanwhile the cilia in the area of gullet development beat rapidly and then a small cone-shaped structure appeared which differentiated into the adult gullet. Twelve minutes after the animal had divided, the posterior

end of the oral groove was 20 microns away from the animal's posterior end.

The animal continued moving fast in a whirling fashion. Meanwhile, there were protoplasmic movements which were seen to be pushed against the wall of the posterior end of the animal which resulted first in an irregular shape different from the usual nearly rounded posterior end.

At 3:45 p.m. the Paramecium showed distinct and well-developed lobes, and the gullet exhibited numerous vibrating cilia, which forced suspended particles and fluid into the developing gullet. At that time the posterior two-third of the left lobe was larger than the right lobe. At 3:50 p.m. the animal was essentially like an adult Paramecium, but, during the developmental period (50 minutes) no food was ingested. The first food vacuole started at 4:05 p.m. The Paramecium was feeding on lampblack particles in Chalkley solution.

3. The Formation of the Food Vacuole

Observations were made on many specimens of paramecia while they were feeding on yeast cells and on others feeding on lampblack. An attempt was made to reveal more about how the food vacuole was formed and also to get a better understanding of the factors causing its separation from the end of the gullet.

Paramecia ingested food after cessation of movement or while they were moving very slightly. Some individuals stopped beside clumps of food particles for ingestion of food. That habit of Paramecium made it fairly convenient to observe the food vacuole while it was forming. During feeding there was a current of suspended food particles moving into the oral groove and into the vestibulum. Some of these particles in fluid passed through the mouth into the pharynx, while the others were immediately forced out again. Some of the particles upon entrance into the pharynx were rejected while other particles and some fluid continued through the pharynx and into the esophageal sac. There, the particles suspended in fluid were seen to rotate rapidly while additional particles were added to the esophogeal sac. Vigorous vibrations of the particles were observed during the rotation process. The sac, as the process continued, increased in size. When the sac reached a certain size, it pulled away from the end of the pharynx as a food vacuole and passed into the protoplasmic substance of the organism towards its posterior end.

4. The Separation of the Food Vacuole From the Pharynx

During the process of formation of the food vacuole, it was found that a current was created at the oral groove by ciliary activity. Culture fluid, with particles in suspension, was forced into

the pharynx, which ended blindly. The material was pushed against the membranous-like closed end of the pharynx. That membrane could be expanded inside the protoplasmic substance by the pressure created against its elastic wall from the outside. In that response, it was similar to a balloon that can be expanded by blowing air into it, but the membranous-like closed end of the pharynx could only be seen when it expanded as a sac. It seemed to be made of a coalescent substance because as soon as the edges of the end of the pharynx came together after the food vacuole was separated, there was no more opening left until another vacuole started forming—indicated by the new expanded sac formed in the same manner as mentioned before. While that sac was enlarging, some large particles or granules were observed gathering around the anterior surface and around the connection between the expanding sac and the distal end of the pharynx.

It was observed that these granules were coming from the posterior end of the organism towards the lateral surfaces of the forming food vacuole floating inside the protoplasm.

There were also protoplasmic currents occurring in the area of the enlarged sac forcing the latter gently away from the pharynx. These regional currents also seemed to exert some pressure on the cytoplasm adjacent to the vacuole. Cytoplasmic granules appeared to facilitate release of the food vacuole from the

pharynx. They resembled the other granules floating within the protoplasmic substance of the organism, and were seen scattered all over the length of the body inside the cytoplasm during cyclosis.

Granules within the cytoplasm were responsible in some way for cytoplasmic currents, i.e., cyclosis. This conclusion was reached following observations made on an animal in which most of its granules were accumulated at the anterior end. The animal was forming food vacuoles which were concentrated in the posterior portion of the body. The flow of the protoplasm was very slow and looked as if its stopped completely. In animals in which the granules were scattered throughout the protoplasm, cyclosis was maintained and the flow of the protoplasm was considerably fast.

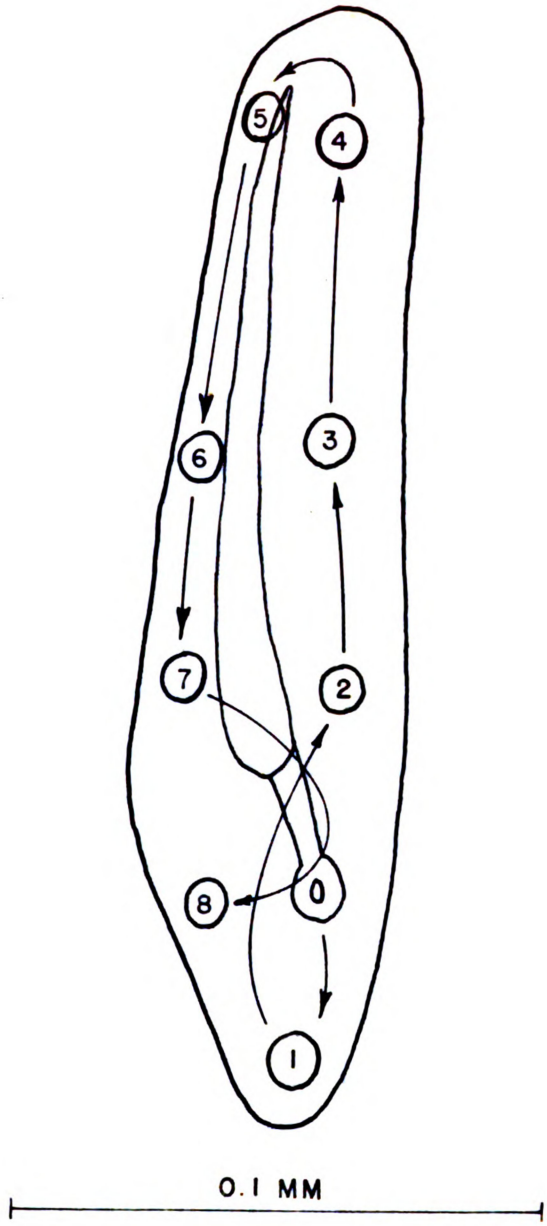
5. The Course of the Food Vacuole

Paramecia were observed closely to determine the course of the food vacuole within the cytoplasm. It required an average time of 45 seconds from the time the food vacuole first appeared until it was released at the end of the gullet. Then it was immediately thrown in a rotating movement towards the posterior end of the animal. An average time of 5 seconds was required for the food vacuole to travel from the place where it was formed at position 0 to position 1—the rear end of the animal.

It should be borne in mind that positions 0, 1, 2, 3, 4, 5, 6, 7 and 8 are reference points which mark the positions of food vacuoles from the time they leave the pharynx until they are discharged outside the animal body through the anal pore. If the animal is observed from the dorsal surface, position 0 will be the area where the food vacuole is formed. Position 1 will be at the rear of the Paramecium and positions 2, 3 and 4 will be at the posterior, middle and anterior ends of the left lobe, respectively. On the other hand, positions 5, 6 and 7 will be at the anterior, middle and posterior ends of the right lobe. Lastly, position 8 will be at the area adjacent to the anal pore.

After the food vacuole had reached position 1 at the posterior end of the Paramecium, it was directed anteriorly and laterally towards the left lobe. Then it moved anteriorly for the entire length of the organism to reach the anterior end of the animal, and, in doing so it passed positions 2, 3 and 4. From there the food vacuole passed around the convexity of the anterior end of the oral groove to be directed posteriorly into the right lateral lobe, bypassing positions 5, 6 and 7 to reach position 8 where it coalesced with other vacuoles opposite to the anal pore (Figure 3). After several vacuoles coalesced to form an enlarged mass, the contents were evacuated. The anal opening was observed only during excretion and appeared at variable intervals of time (5 to 10 minutes).

Figure 3. Camera outline of Paramecium multimicronucleatum when observed from the ventral surface. The circles represent positions of food vacuoles at various intervals of time subsequent to ingestion. The arrows indicate the direction of the course of the food vacuoles.


5
4
6
3
7
2
8
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1
0.1 MM

It is evident in Table I that an average time of five seconds was required for the food vacuole to move from position 0 to 1, and that it required 260 seconds to travel from the posterior end to the anterior end, while on the other hand, 480 seconds were required for the vacuole to move from the anterior end to near the posterior end opposite to the anal pore. Specimens of Paramecium multimicronucleatum fed on yeast cells exhibited vacuoles which moved from position 0 to 8 in 765 seconds.

Table I also shows that when other specimens of paramecia were fed on lampblack particles, the food vacuole traveled a similar distance in 860 seconds. On one hand it was carried from the posterior to the anterior end in 335 seconds, while on the other hand it traveled from the anterior end to the anal pore in 490 seconds.

Furthermore, the food vacuole of both yeast and lampblack traveled the first half of its course faster than the second half. Nevertheless, when lampblack was used instead of yeast, the food vacuole required more time to travel throughout its course.

Table II shows that in paramecia fed on yeast the food vacuole required an average of 45 seconds to be formed, while on the other hand, the time increased to 55 seconds when they were fed on lampblack. This suggests strongly that the nature of the food affects the rate of formation of the food vacuoles. These results

TABLE I

AVERAGE TIME REQUIRED FOR CONGO-RED STAINED YEAST AND LAMPBLACK FOOD VACUOLES TO MOVE FROM THE MOUTH TO THE ANAL PORE OF PARAMECIUM MULTIMICRONUCLEATUM

Type of Food Used	Average Time (in seconds) Required to Reach Different Positions								Total Time for Cycle
	1	2	3	4	5	6	7	8	
Yeast cells stained with congo-red	5	125	55	80	20	135	185	160	765
Lampblack particles	5	180	65	90	30	120	145	225	860

TABLE II

AVERAGE TIME REQUIRED FOR FORMATION OF FOOD VACUOLES BY PARAMECIUM MULTIMICRONUCLEATUM WHEN FED ON CONGO-RED STAINED YEAST AND LAMPBLACK

Type of Food Used	Time (in seconds) Required for the Formation of the Food Vacuole		
	Average	Minimum	Maximum
Yeast cells stained with congo-red	45	25	75
Lampblack particles	55	30	80

are in accord with the contentions of Metalnikow (1912) and they appear to support his implication that digestible substances are more easily ingested than indigestible ones.

Further, he contends that the rate of formation of the food vacuoles is affected by the hydrogen-ion concentration, and the temperature of the surrounding fluid—but that it is not affected by the number of the particles suspended in it. Frisch (1937) asserts that the time required for the formation of the food-vacuoles varied from 17 to 365 seconds, and he maintains that the time required for the formation of the food vacuole is not dependent upon the size of the vacuole but that it is largely dependent upon the quality and the quantity of the food present. He also strongly holds that the time varies inversely with the quantity of bacteria and the extent of their usefulness as food. However, no definite views have been expressed as to how the factors involved act in the control of the rate of formation of food vacuoles.

It has been found in observations made with paramecia, in a culture fluid containing yeast cells, that the time required for formation of food vacuoles varied from 25 to 75 seconds, while formation of food vacuoles in culture solutions containing lamp-black particles required from 30 to 80 seconds. Thus there was a change in the rate of formation of the food vacuoles depending upon

the quality of food, which agrees with Metalnikow and Frisch in this respect.

6. The Size of the Food Vacuole

It is evident (Table III) that in specimens of Paramecium multimicronucleatum, fed with yeast cells, the average diameter of the food vacuoles in various individuals varied from 8.6 to 14.6 microns. The diameter of the largest and smallest vacuoles were 24.57 and 6.4 microns, respectively.

Table III shows that when lampblack was substituted for yeast, the food vacuoles which were formed decreased in diameter to an average varying from 6.2 to 12.8 microns. The largest vacuole measured 24.0 microns and the smallest 4.5 microns.

On the other hand, it is also shown in Table III that the average diameter of the food vacuoles formed by Paramecium multimicronucleatum increased in four per cent Methocel from 15.60 to 21.0 microns and from 12.45 to 19.25 microns in 0.005 M Mannitol. The largest food vacuoles measured were 30.50 and 31.0 microns for Methocel and Mannitol solutions respectively, while the smallest food vacuoles measured were likewise 9.50 microns and 7.25 microns.

Table IV shows that paramecia varied in size from 220.71 to 346.30 microns (length) and from 54.59 to 63.21 microns (width).

TABLE III

DIAMETER OF FOOD VACUOLES IN PARAMECIUM MULTIMICRONUCLEATUM

Type of Culture Solution	Size in Microns		
	Average	Smallest	Largest
Chalkley solution plus yeast cells	8.6 -14.6	6.40	24.57
Chalkley solution plus lampblack	6.2 -12.8	4.50	24.00
0.005 M Mannitol plus yeast cells	12.45-19.25	7.25	31.00
4% Methocel plus yeast cells	15.60-21.00	9.50	39.50

TABLE IV

SIZE OF PARAMECIUM MULTIMICRONUCLEATUM

Average Length in Microns	Average Width in Microns
220.71-346.30	54.59-63.21

It is consequently evident that the size of the food vacuoles formed varied with the type of food and with the condition of the solution in which the paramecia were swimming. The food vacuoles decreased in size when the animal was fed on lampblack, which is an indigestible substance, while on the other hand, size of vacuoles was increased by increasing the viscosity and pressure of culture solutions. These observations strongly suggest that the size of vacuoles is closely correlated with the osmotic pressure and viscosity of the culture solution.

It is well established that the size of food vacuoles in paramecium varies greatly in various individuals. Metalnikow (1912) maintains that in solutions which contain only indigestible substances (carmine, Chinese ink, etc.) the food vacuoles formed are abnormally small. Frisch (1937) came to the conclusion that in well-fed paramecia, the size of the food vacuoles is not closely correlated with the size of the body. He maintains, however, that during the early stage of population growth the average diameter of the food vacuoles in different individuals varied from 17.25 microns to 25 microns. In aged cultures average diameter of the food vacuoles decreased to 3.45 microns. The results obtained by Mast (1947) were in full accord with those obtained by Metalnikow and Frisch. He further maintains that if the viscosity of the fluid became high enough to retard locomotion, but not high enough to

inhibit it, the paramecia ingested the fluid rapidly, formed unusually large food vacuoles and soon became filled with them. The results obtained in this paper seem to be in full accord with Mast's findings.

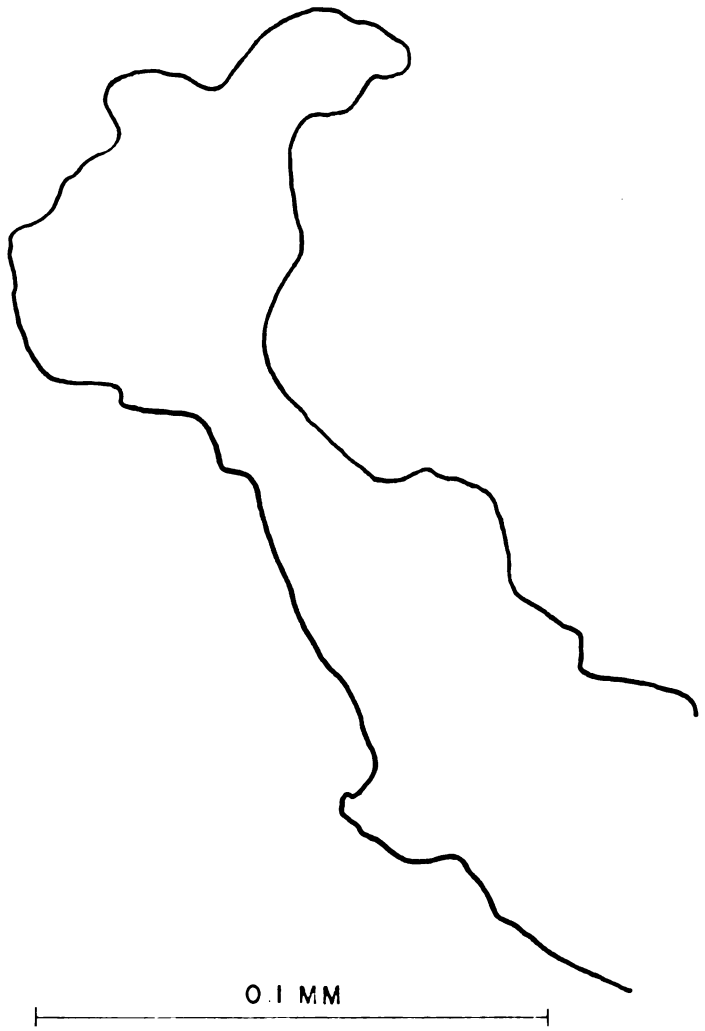
Mast and Bowen (1944) postulate that the primary factors involved in controlling the size of the food vacuoles are: (1) the quantitative rate of ingestion of fluid and solid particles; (2) the quantitative rate of passage of fluid from the esophageal sac into the cytoplasm; and (3) the length of the intervals between consecutive constrictive actions of the esophageal fibers. This hypothesis appears to account for all that is known concerning this phenomenon in Paramecium. However, the third factor mentioned above needs to be investigated in greater detail since these fibers could not be identified in any of the specimens observed during the course of this investigation. The large cytoplasmic granules that travel posteriorly to form aggregates in the vicinity of the developing food vacuole seem to play a rather important role in determination of the size and rate of formation of the food vacuoles. It could be well assumed that these large granules exert their action in combination with other factors through their rate of aggregation around the food vacuole.

Moreover, the results obtained suggest strongly that the size of the formed food vacuoles is not dependent upon the time consumed for their formation.

7. Behavioral Characteristics of *Paramecia*

Owing to the interest of these observations, they will be reported in considerable detail. An experiment was made for the purpose of studying the course of the food vacuoles. On looking at the prepared slide under the microscope, a paramecium was found to be captured in a trap made with vaseline and some aggregated yeast particles. The trap was irregular in shape, as shown in Figure 4. The animal's anterior escape route was blocked by clumped particles of yeast. In moving anteriorly, the cell entered a small chamber of the trap, and under these conditions the cell became amoeboid. Its anterior end was thrown into irregular folds in a fashion similar to the pseudopods of an *Amoeba*. The animal then followed the walls of the trap which were in contact with the body surface. The cilia were moving slowly. The outer layer of its body looked as if it were muscular, and by its contraction the animal was able to simulate the locomotor movements of an earthworm. When the animal could not move any farther, the anterior part of the body was contracted to force protoplasmic materials against the walls of the posterior portion of the body. In this fashion it could push its body into the narrow passages of the trap. In all the efforts made by the animal to move, whether forwards or backwards, the shape of the body was altered, and it did

Figure 4. Camera outline of the trap in which the Paramecium was captured.


0.1 MM

not look at all like the familiar shape of Paramecium. It exhibited projections when it was pushing its body against the excavations in the trap, but they were always rounded. The various morphological features of the animal, while it was in the trap, are shown in Figure 5. The animal moved forwards and backwards until it finally succeeded in leaving the trap by repeated backward movements.

Figure 6 shows the shape of the animal after its escape from the trap.

8. The Frequency of Ingestion of Yeast Cells by Paramecium multimicronucleatum in Various Concentrations of Mannitol

It is evident in Table V and Figure 7 that the number of food vacuoles formed by Paramecium multimicronucleatum in 90 minutes increased from 36 in 0.00005 M Mannitol solution to a maximum of 40 in 0.0002 M Mannitol; and then the number of food vacuoles formed decreased to a minimum of 14 as the concentration of Mannitol was increased to 0.035 M. It is also evident in Table V that the number of food vacuoles formed in 15, 30, 60 and 90 minutes likewise increased to a maximum and then decreased to a minimum.

Table V also shows that about 44 per cent of the vacuoles were formed in 30 minutes in 0.00005 M Mannitol. On the other hand more than 50 per cent of the food vacuoles were formed during

Figure 5. Camera outlines of Paramecium multimicronucleatum showing its different shapes while it was in the trap. The circles are food vacuoles.

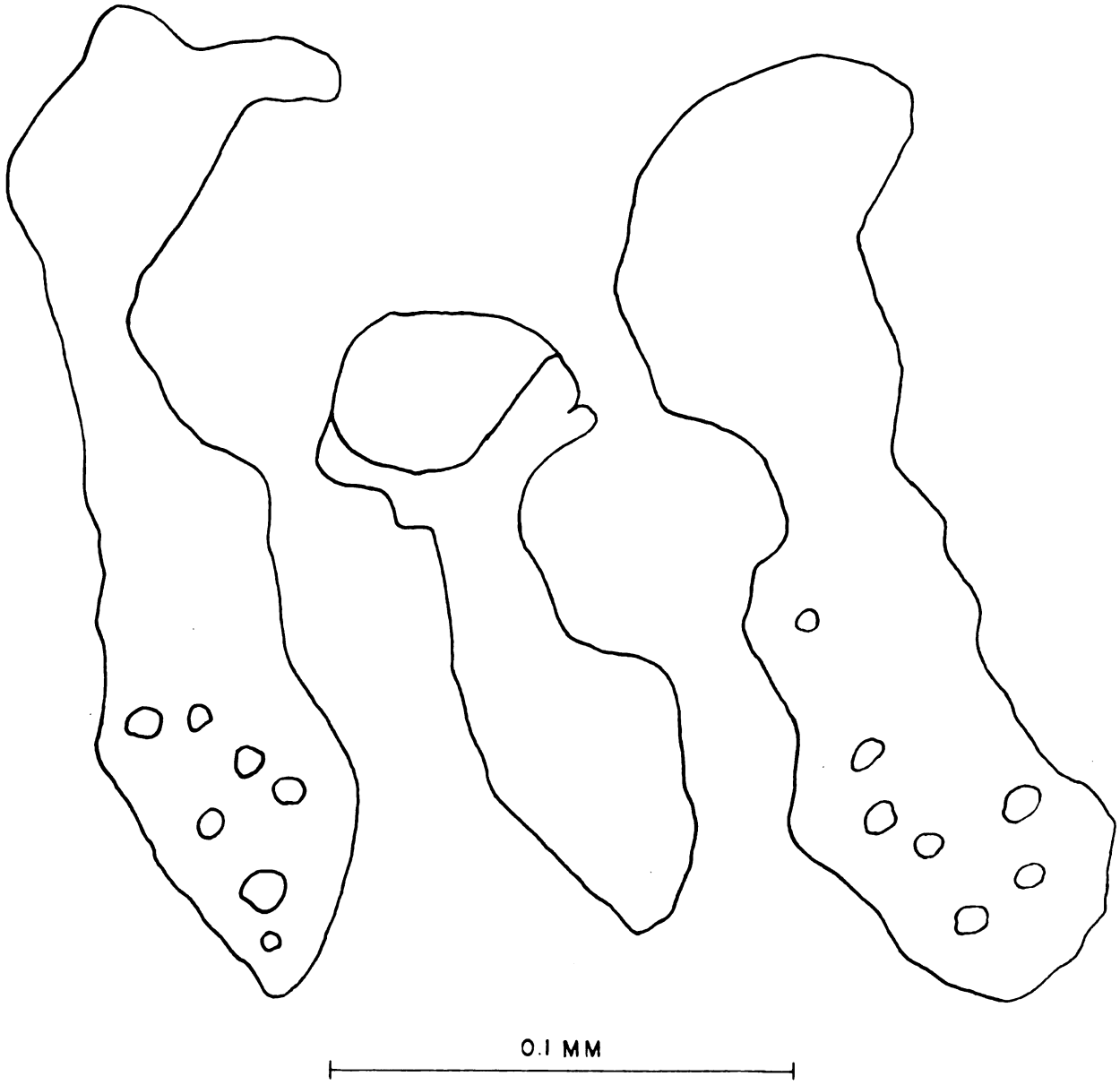

0.1 MM

Figure 6. Camera outline of Paramecium multimicronucleatum showing its shape after it had escaped from the trap.

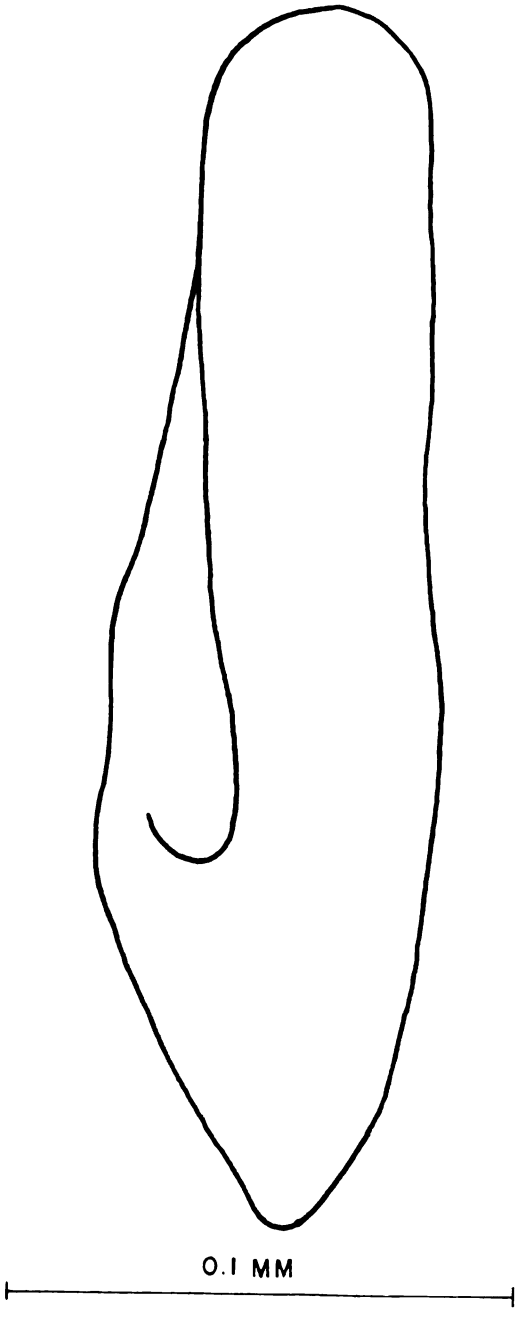
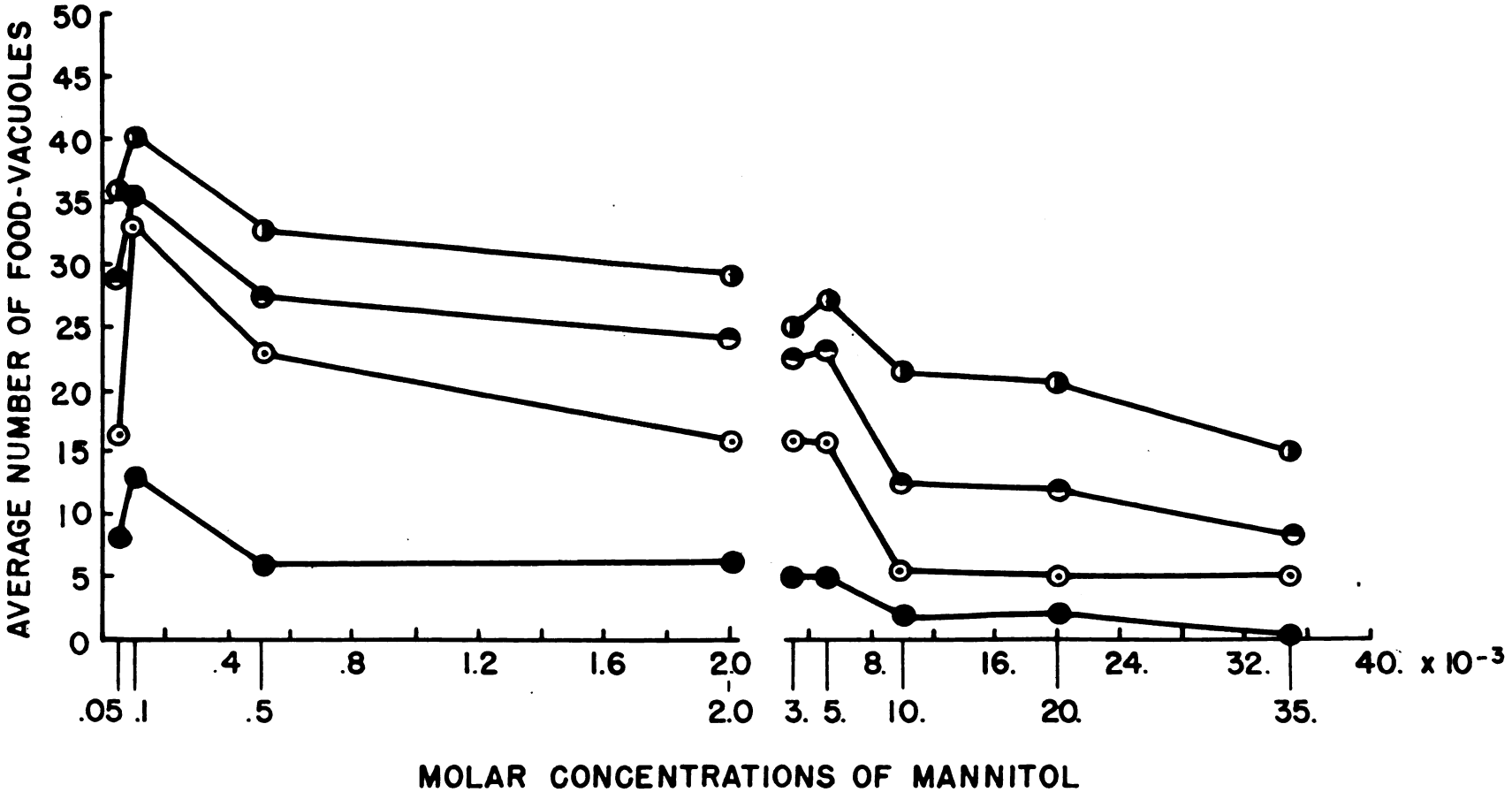

0.1 MM

TABLE V

THE RELATION BETWEEN OSMOTIC CONCENTRATION AND FREQUENCY OF INGESTION IN PARAMECIUM MULTIMICRONUCLEATUM

Molar Concentration of Mannitol Solution	Average Number of Food Vacuoles Formed per Individual				Number of Food Vacuoles Formed per Individual in 90 Minutes	
	Minutes				Lowest	Highest
	15	30	60	90		
0.00005 M Mannitol	8	16	28	36	27	45
0.0001 M Mannitol	13	33	36	40	35	47
0.0005 M Mannitol	6	22	27	33	28	38
0.002 M Mannitol	6	16	24	28	24	31
0.003 M Mannitol	5	15	22	24	16	36
0.005 M Mannitol	5	15	23	27	17	34
0.01 M Mannitol	2	5	12	21	17	24
0.02 M Mannitol	2	4	12	19	11	23
0.035 M Mannitol	0.4	4	8	14	3	33

Figure 7. The relation between frequency of ingestion of congo-red stained yeast cells by Paramecium multimicronucleatum and osmotic pressure. Ordinate, number of food vacuoles formed; abscissae, molar concentration of Mannitol solutions. ● number ingested in 15 minutes; ⊙ 30 minutes; ◓ 60 minutes; ◑ 90 minutes.


AVERAGE NUMBER OF FOOD-VACUOLES
50
45
40
35
30
25
20
15
10
5
0
.4
.8
1.2
1.6
2.0
.05
.1
.5
2.0
3.
5.
10.
20.
35.
8.
16.
24.
32.
40. x 10-3
MOLAR CONCENTRATIONS OF MANNITOL

the first 30 minutes of the experiment in concentrations of Mannitol varying between 0.0001 and 0.005 M, and in solutions in which the concentrations increased from 0.01 to 0.035 M the majority of the vacuoles were formed during the last 60 minutes of the experiment.

Figure 7 shows that in all concentrations of Mannitol there was a gradual increase in the number of the food vacuoles ingested as time passed, and that the frequency of ingestion varies inversely with increased concentrations from 0.0001 to 0.035 M Mannitol.

It becomes evident from the results obtained that there is a close relationship between the frequencies of ingestion and the osmotic pressure of the culture solution. In all concentrations used, it is assumed that any effect of the solvent (Chalkley solution) was essentially the same in all experiments. Changes that occurred in the frequencies of ingestion must have been due to the presence of either the solute or to the solute-Mannitol. In either case, the effect seemed to be more likely due to the presence of the Mannitol reagent, i.e., the effect was due to osmotic pressure of the culture solution. As has been mentioned before, that effect was a decrease in the frequencies of ingestion as the osmotic concentration increased. But there seemed to be a certain level at which the maximum increase in the frequencies of ingestion occurred. When a concentration of 0.00005 M Mannitol was used, there was a marked drop

in the number of food vacuoles ingested. Mast and Fennell (1938) maintain that the effect of salts on ingestion is not entirely due to their osmotic action in Amoeba. Thus it is suggested that there is a critical dilution for the Mannitol reagent, beyond which its effect upon the frequencies of ingestion became negligible and in which case the frequencies of ingestion were entirely controlled by the solvent. On the other hand, any higher concentration beyond that same dilution will result in a decrease in the frequencies of ingestion. In such case, the decrease was directly correlated with the amount of the solute-Mannitol present in the same solvent.

9. The Relation Between Viscosity and Frequency of Ingestion in Paramecium multimicronucleatum

Table VI shows that 57.14 per cent of the food vacuoles in Paramecium multimicronucleatum were formed in 30 minutes in 0.001 per cent Methocel and that in the same period of time 60.23 per cent of the food vacuoles were formed in 0.025 per cent Methocel. It is also evident in Table VI that the percentage of food vacuoles formed in 30 minutes increased to 82.95, 72.70 and 86.96 per cent as the concentration of Methocel increased to 0.05, 1 and 4 per cent respectively.

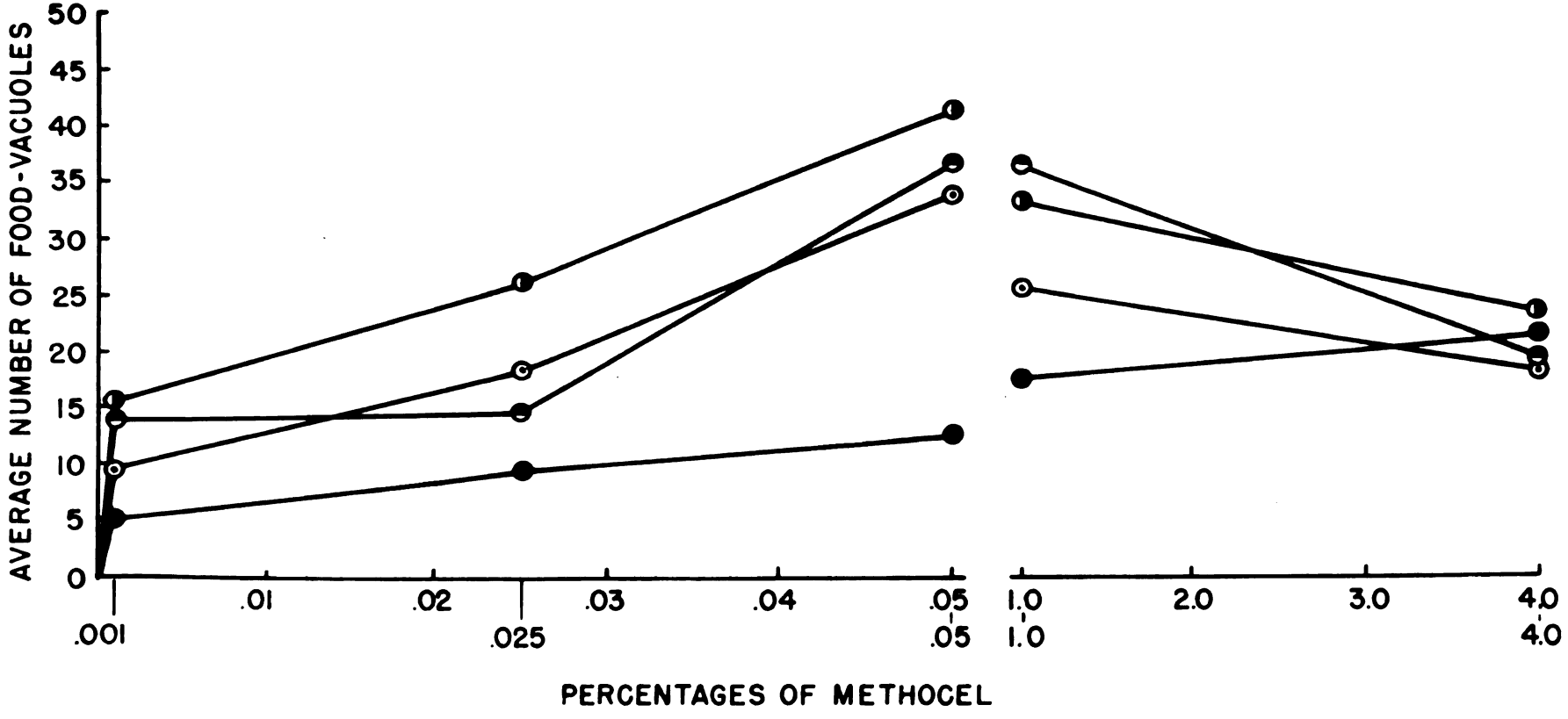
It is also shown in Table VI and Figure 8 that the number of the food vacuoles increased from 8 in 30 minutes to 13 in 60

TABLE VI

THE RELATION BETWEEN VISCOSITY AND FREQUENCY OF INGESTION IN PARAMECIUM MULTIMICRONUCLEATUM

Concentration of Methocel Solution (percentages)	Average Number of Food Vacuoles Formed per Individual				Number of Food Vacuoles Formed per Individual in 90 Minutes	
	Minutes					
	15	30	60	90	Lowest	Highest
0.001%	4	8	13	14	9	25
0.025%	8	18	15	26	21	31
0.05 %	12	34	36	41	35	47
1.0 %	17	25	36	33	24	45
4.0 %	21	20	20	23	11	44
Controls			27	31	18	44

Figure 8. The relation between frequency of ingestion of congo-red stained yeast cells by Paramecium multimicronucleatum and viscosity in Chalkley solutions containing Methocel as the viscosity agent. ● number ingested in 15 minutes; ⊙ 30 minutes; ◒ 60 minutes; ◑ 90 minutes. Lowest and highest number of food vacuoles formed per individual in 90 minutes is 9 and 25, respectively, at 0.001 per cent Methocel; 21 and 31 at 0.025 per cent Methocel, the optimum Methocel concentration. Similar individual variation occurred in all other tests. Ordinate, number of food-vacuoles formed; abscissae, percentages of Methocel in Chalkley solutions.


AVERAGE NUMBER OF FOOD-VACUOLES
50
45
40
35
30
25
20
15
10
5
0
.001
.01
.02
.025
.03
.04
.05
.05
1.0
1.0
2.0
3.0
4.0
4.0
PERCENTAGES OF METHOCEL

minutes in 0.001 per cent Methocel, while on the other hand the number of the food vacuoles decreased from an average of 18 in 30 minutes to an average of 15 in 60 minutes in 0.025 per cent Methocel. In 0.05 the average number of the food vacuoles formed increased from 34 in 30 minutes to 36 in 60 minutes. Again, the average number of the food vacuoles formed in a concentration of one per cent Methocel increased from 25 in 30 minutes to 36 in 60 minutes, while in four per cent Methocel there was no increase in the number of the food vacuoles during the second thirty minutes.

Table VI shows that in concentrations of 0.001 per cent, 0.05 per cent and 4 per cent Methocel the average number of food vacuoles formed increased but little in the last thirty minutes, while in 0.025 per cent Methocel they increased from an average of 15 in 60 minutes to an average of 26 in 90 minutes and in one per cent Methocel they decreased from 36 in 60 minutes to 33 in 90 minutes.

In the control solution 87.09 per cent of the formed food vacuoles in 90 minutes were formed in the first 60 minutes while only 12.91 per cent were formed in the last 30 minutes. From the results obtained it can be maintained that in concentrations of Methocel between 0.001 per cent and 0.05 per cent, the frequency of ingestion increased directly with the increase in viscosity. In concentrations between 0.05 per cent and 4 per cent Methocel the

frequency of ingestion was in inverse relationship with increased viscosity.

In high viscosity solutions, e.g., four per cent Methocel, it was evident that the frequency of ingestion reached its maximum in 15 minutes. This seems to agree with the contentions of Mast (1947) in which he maintains that in viscosities high enough to retard locomotion but not to inhibit it, the paramecia formed unusually large vacuoles and soon became filled with them. It was also observed that in paramecia in which the frequency of ingestion was lowered by higher concentrations of Methocel, the food vacuoles formed were larger than usual, while in the control solution the food vacuoles were smaller than those formed in any other concentration of Methocel.

Stiles (1947) maintains that as the viscosity of the culture solution increases, the rate of locomotion decreases. It has been observed in the work presented in this paper that it was distinctly noticeable that in one per cent and four per cent Methocel concentrations, the paramecia moved remarkably slower than in lower concentrations.

In all concentrations of Methocel and in the control solutions the frequency of ingestion seemed to be closely correlated with time.

The results reported indicate that there is much variation in different individuals under the same conditions and in the same individual under different conditions.

IV. DISCUSSION

1. The Formation of the Food Vacuole

It was shown in the preceding pages that starved Paramecium ingested both lampblack and congo-red stained yeast. Cilia on the oral groove moved rapidly to force the suspended particles into the pharynx through the mouth opening at the floor of the vestibulum. Those currents were forced against the thin membrane at the posterior end of the pharynx to form a sac-like structure representing the forming food vacuole. During the time the food vacuole was forming, particles of suspended food material became more or less concentrated and the food vacuole became enlarged. At certain intervals, varying with the type of food and its surroundings, the food vacuole separated from the posterior end of the pharynx to travel into the cell protoplasm in a definite course. Metalnikow (1907, 1912) concludes that paramecia ingest all sorts of small particles, but they take digestible substances more readily than indigestibles. This conclusion is in accord with the results presented in this paper and has been abundantly confirmed by Bozler (1924), Losina-Losinsky (1931), Bragg (1936, 1939), Mast (1947) and others.

Horning (1926) maintains that the content of the pharynx is in direct contact with the cytoplasm, that there is no membrane intervening at the distal opening, and that the vacuoles which pass from the pharynx into the cytoplasm are surrounded merely by a surface film. Gelei (1934) concludes that a definite membrane separates the content of the pharynx from the cytoplasm. Further, Mast (1947) maintains that a portion of the esophageal sac remains as a membrane over the distal opening of the pharynx, when a food-vacuole is separated. He further holds that this membrane bulges slightly into the cytoplasm, forming a new esophageal sac. It has been observed that the cilia in the pharynx forced fluid with particles in suspension into the esophageal sac and that it was this that caused the enlargement of the sac, as Bozler and others maintained. And since no solid particles left during the time of formation of the food vacuole, it becomes obvious that a membrane was surrounding the enlarging sac. Moreover, as the esophageal sac enlarged, the membrane at its surface was continuously stretched, and as it was stretched, it must have been continuously built up by the interaction between materials in the sac and the adjoining cytoplasm so as to prevent rupture.

2. The Separation of the Food Vacuole From the Pharynx and Its Course Through the Body

It has been shown by many investigators that after the food vacuole has reached the posterior end of the body, it moved throughout the rest of its course in the cytoplasmic stream, i.e., by cyclosis, but there is marked diversity of opinion concerning the processes involved in the separation of the food vacuole from the pharynx.

Bragg (1935, 1936) holds that contraction of the distal end of the pharynx and cyclosis are involved but Mast (1947) concludes that none of these factors is essential.

Lund (1941) says (p. 564):

> With the growth of the vacuole the postesophageal fibrils contract about its base, the vacuole is pressed posteriorly, and once with considerable rapidity. Their concerned action produces an effect somewhat resembling that produced by a peristaltic wave in the esophagus of higher vertebrae.

Mast (1947) contends that neither Lund nor himself did actually see the process described above, though he believes that his postulations reasonably account for nearly all that has been seen.

In the experiment carried in this paper concerning the problem of separation of the food vacuole from the pharynx, the following phenomenon has been observed:

The cilia in the pharynx forced fluid and yeast cells in suspension against a membrane over the distal end of the pharynx

producing an enlarging sac with its free end bulging into the cell protoplasm. As the sac enlarged, some granules that seemed to be specialized, gathered around its anterior and lateral surfaces. At the same time currents of protoplasm occurred more pronounced in the area of the enlarging sac. Those specialized granules together with the intensified currents were gently pushing the sac away from the distal end of the pharynx. Finally the granules became more concentrated around the anterior end of the sac, enough to constrict it off from the pharynx and carry it with the rapid currents directing the newly formed food vacuole in a fast movement to reach the posterior end of the animal. As soon as the food vacuole was separated from the pharynx more currents of fluid with suspended particles were forced again against the membrane sealing the distal end of the pharynx resulting in a newly enlarged sac. Meanwhile, more granules came from the posterior and postero-lateral parts of the body to concentrate around the antero-lateral surface of the newly forming food vacuole. Thus it can be well maintained that the forced substances into the esophageal sac, the vigorous cytoplasmic currents and some specialized granules are involved in the process of separation of the food vacuole from the distal end of the pharynx. The fact that in each food vacuole formed, there was seen a concentration of some granules around its connection with the pharynx, suggests strongly that these are specialized

granules which are essential in some way in the separation of the food vacuole. Probably they are concerned too with secreting a substance that seals off the distal end of the pharynx which is distended by substances forced by the pharyngeal cilia, thus substituting it for the detached portion of the esophageal sac surrounding the vacuolar elements which became the vacuolar membrane. On the other hand, it is quite possible that those specialized granules also control the size and rate of formation of the food vacuole; in which case they can be considered as a portion of the nervous system in the Paramecium.

In further steps in this paper concerning the course of the food vacuole after it has been separated from the distal end of the pharynx, strikingly enough, it has been observed that every food vacuole was accompanied by some granules in the flow of cytoplasm. Also, as soon as the food vacuole was separated, it was carried rapidly towards the posterior end of the paramecium, accompanied by those granules which had concentrated around it during its formation. Furthermore, it has been observed that in an individual paramecium where most of the granules gathered at its anterior end the flow of the cytoplasm was very slow and apparently stopped. In that particular animal the food vacuoles formed were packed in its posterior one-third. This brings up a conclusion of major importance regarding some functions of the granules in the cytoplasm.

It can be strongly held that one of the functions of the granules in specimens of Paramecium is concerned with the movements of the food vacuole, either by pushing or by inducing cyclosis when normally scattered throughout the cytoplasm. Furthermore, it was obviously seen that each food vacuole was accompanied by some granules, but none of them was seen to have entered inside any of the food vacuoles. This leads to the conclusion that these granules are probably concerned with digestive functions.

Koehring (1930) made extensive observations on neutral red granules in various protozoa. She concludes that they are concerned with enzymic functions, but that they do not enter the food-vacuoles. Referring to Paramecium caudatum she says (p. 67):

> As the new vacuole is being formed, they (the neutral-red granules) gather at the membrane, bombarding it like hailstones, but making no impression on the firm surface. Then as this vacuole flows away . . . some of the granules leave this vacuole and return to the next, which is already in the process of formation. Those left continue bombardment of the vacuole as the pink color slowly forms within.

Dunihue (1931) maintains that in Paramecium caudatum the surface of the forming food vacuoles becomes "closely packed" with neutral-red staining globules and that they do not enter the vacuoles. Mast (1947) concludes that the ventral-red granules found in the cytoplasm in Paramecium do not enter the food vacuoles but that they are probably involved in digestion. Hall and Dunihue (1931) and Mast and Bowen (1944) observed that in Vorticella and other

Peritricha the neutral red granules do not aggregate at the surface of the food vacuoles, and Mast (1926) found that in Amoeba there are none. Mast (1947) further concludes that the function of these granules in digestion in Paramecium is negligible.

Horning (1926a) holds that in Amoeba sp. janus-green staining bodies (mitochondria) aggregate among particles of food is the cytoplasm; then a membrane forms and encloses the food and the mitochondria in a vacuole. He also asserts that in Paramecium sp. mitochondria are extruded from the cell protoplasm through the vacuolar membrane into the food vacuoles during the alkaline phase. Thus, he concludes, the mitochondria in these protozoa function in carrying digestive enzymes to the ingested food. On the other hand, Volkonsky (1934) maintains that the mitochondria do not enter the food-vacuoles and that they do not take part in digestion in protozoa.

Thus the evidence in support of the contention that the mitochondria and the neutral-red granules in Paramecium are enzyme carriers is therefore not well accepted.

After the food vacuole had become free it was carried rapidly to reach the posterior end of the animal. Then it was directed anteriorly in a rotary movement towards the left lateral lobe where it continued its movement towards the anterior end of the Paramecium. There it turned around the convexity of the oral groove to reach the

right lateral lobe where it was directed posteriorly throughout its entire length. Finally, it reached a position opposite to the anal opening where it coalesced with other vacuoles to form a larger food vacuole in which the undigested substances were more concentrated. At various intervals of time the contents of this large vacuole were discharged through the anal pore. The time required for the food vacuole to reach the final position adjacent to the anal opening seemed to be dependent upon the nature of food ingested. It was clearly shown in this experiment carried for this purpose, that the food vacuole required less time to travel the entire course when paramecia were given more digestible food (yeast cells) than when fed on indigestible food (lampblack). Yet in either case the food vacuoles always followed a definite course and were carried in the cytoplasmic stream.

3. Size and Rate of Formation of the Food-Vacuole

It is well accepted that the food-vacuoles formed in Paramecium vary widely in size and rate of formation in different individuals.

Metalnikow (1912) found that when paramecia were transferred from a solution which was poor to one which was rich in digestible substance (e.g., bacteria, milk, egg-yolk, etc.) the first vacuole formed was always huge, nearly 40 times larger than

normal vacuoles. He also showed that in solutions which contained only indigestible particles (carmine, Chinese ink, etc.) the food vacuoles formed were abnormally small. Mast (1947) found that the size of the food vacuole was not at all closely correlated with the composition of the surrounding media.

It was shown previously in this paper that the size of the food vacuoles was dependent upon the type of food ingested and the composition of the surrounding medium. Judging from the figures given, it can be asserted that the size of the vacuoles decreased when paramecia were fed on lampblack (indigestible substance) instead of yeast cells (more digestible substance). This indicates clearly that the size of the food vacuole is closely correlated with the type of food presented.

On the other hand, when the type of food was maintained the same and the nature of the culture solution was changed, it was found that the size of the vacuoles increased with increased osmotic pressure or viscosity, of the solutions. The food vacuoles formed in high viscosity solutions were much larger than those formed in any other solution experimented upon. Some of the vacuoles measured five times larger than those formed in Chalkley solution. In high osmotic pressure solutions the vacuoles were definitely larger than those formed in Chalkley solutions, but not quite as large as those in high viscosity solutions. Thus, it becomes

evident that the size of the food vacuole is also correlated with the nature of the surrounding medium.

Cosmovici (1931) asserts that specimens of Colpidium colpoda in culture fluid containing amylodextrine forms tubular food-vacuoles, some of which extend from the pharynx to the anus. He seems to think that in ciliates, there is a very complicated closed capillary digestive system, through which substance is moved by waves of cytoplasmic contraction, and that cyclosis is an optical illusion, due to this movement. Mast (1947) maintains that when he repeated Cosmovici's observations using paramecia in place of colpidia, he obtained no evidence at all to support his contentions.

During experimentation, one Paramecium was seen to be captured in a trap and behaved in a way similar to Amoeba, contracting itself in different odd shapes so that the cytoplasm was all mixed up and showed no cyclosis. The food vacuoles were being forced forwards and backwards irregularly according to the efforts made by the cell during its earthworm-like contractions. Figure 5 shows clearly how the animal looked with the food vacuoles scattered irregularly inside its protoplasm. This gives strong evidence against Cosmovici's conclusions referring to the presence of a capillary digestive system in Ciliates. These results also support Mast's contentions in that respect. It is obvious that cyclosis is the essential factor for the movement of the food vacuole.

Frisch (1937) made extensive measurements on the food-vacuoles in specimens of Paramecium multimicronucleatum from given cultures on successive days for more than three weeks. He concluded that in well-fed paramecia the size of the food vacuole is not closely correlated with the size of the body and that the observed decrease in the size of the vacuoles during the declining period of the cultures was largely, if not entirely, due to decrease in quantity and quality of food, i.e., bacteria. The results obtained in this work seem to be generally in full accord with those obtained by Frisch.

It has also been found that the size of the food vacuoles depends upon the osmotic pressure and viscosity of the surrounding medium. There has been a pronounced increase in the size of the food vacuoles in both cases. These results are in accord with Mast (1947) in regard to his contentions in this matter.

Lee (1942) holds that the size of the food-vacuoles in Paramecium is independent of the hydrogen-ion concentration of the surrounding medium. Mast (1947) concludes that there are at least four environmental factors which are involved in the control of the size of the food vacuoles, namely, the quantity and quality of the particles in suspension, and the chemical composition and viscosity of the surrounding fluid. His conclusions are much supported with evidence presented previously in this paper about the

effects of such factors as quality of food (whether digestible or indigestible), the osmotic pressures, and the viscosity of the surrounding culture fluid.

In reference to the rate of formation of the food vacuoles, Metalnikow (1912) contends that it is dependent upon the hydrogen-ion concentration and upon the temperature of the surrounding fluid but that it is independent of the number of particles suspended in it.

Bozler (1924) and Frisch (1937) seem to agree that there is a correlation between the concentration of particles and the rate of formation of food vacuoles. Frisch further maintains that the time required for the formation of the food vacuole is not dependent upon its size, but is mainly dependent upon the quality and quantity of the food present. It has been concluded from the results obtained that the time required for the formation of the food vacuoles varied inversely with the extent of usefulness of the particles as food. These results consequently support obtained by Frisch.

Lee (1942) maintains that the rate of formation of the food vacuole is directly proportional to the activity of the cilia in the vestibulum ("peristome") and that this is correlated with acidity, temperature, etc. But Mast (1947) asserts that he has repeatedly seen paramecia in which the cilia in the vestibulum were very active and many particles entered the vestibulum, but none passed

into the pharynx, all being thrown out. Moreover, Lee's conclusions do not count for the increased frequencies of ingestion of congo-red stained yeast cells in high viscosity solutions in which the cilia were considerably less active than when in Chalkley solutions.

Not only that, but also the food vacuoles were on the average larger in size and even contained more particles. This indicates, as Mast (1947) says (p. 49):

> . . . that the amount of substance which enters the pharynx depends upon the nature of the activity of the cilia in the vestibulum quite as much as upon the magnitude of their activity . . .

but not directly proportional to the activity of the cilia of the vestibulum.

As mentioned before, each forming food vacuole was always accompanied by some specialized granules that concentrated at its anterior end so if these concentrations occurred at regular intervals, then the rate of formation of the food vacuoles must depend upon the length of these intervals, and the effect on the rate of formation produced by other factors (e.g., quality of food, viscosity, osmotic pressure, acidity, temperature, physiological states, etc.) must be due to alternations exerted by them, in the length of intervals. Moreover, if this holds true, then those specialized granules are more likely to be portion of the nervous system in

Paramecium and these changes in the culture medium could then be sensed by them and the cell cytoplasm respond accordingly.

This above hypothesis seems to be more reasonable to tie up all conclusions derived from and supported by several strong evidences presented before.

V. SUMMARY AND CONCLUSIONS

1. Paramecium exhibits ventral (location of the oral groove), dorsal and lateral surfaces, and right and left lobes. The anterior end of the animal is rounded and the posterior end is conical.

2. The feeding apparatus in Paramecium multimicronucleatum consists of a shallow ciliated oral groove which extends from the anterior end to slightly beyond the middle of the body, and a ciliated depression (the vestibulum) posterior to the oral groove. The pharynx is a ciliated tube which extends from the floor of the vestibulum (the mouth) backward into the body. Numerous granules concerned with the formation of the food vacuoles encircle the vestibulum.

3. During the formation of food vacuoles cilia in the pharynx push fluid and suspended particles in toward the membrane over the posterior end of the pharynx to form food vacuoles.

4. Paramecia can differentiate between various small particles. As the food vacuole increases in size, granules collect on its anterior and lateral surfaces and protoplasmic cyclosis becomes intensified.

5. The food vacuole, after release into the cytoplasm, moves to the posterior end of the cell then it passes anteriorly beneath the left lateral lobe to reach the anterior end of the cell. From there it passes around the anterior end of the oral groove into the right lateral lobe to reach the anal opening where it coalesces with other vacuoles before discharge of its contents into the culture medium.

6. In the course of the food vacuole, the time required for its complete course was more when paramecia were fed on lampblack than when fed on yeast cells.

7. The size of the food vacuole varies widely from animal to animal and in individual paramecia. This is correlated with the quality of the particles suspended in the surrounding fluid and the chemical composition of this fluid and the rate of ingestion.

8. The frequency of ingestion is correlated with the quality of the food, and the condition of the fluid in which the paramecia are feeding.

9. The granules floating in the animal protoplasm are concerned with the formation of the food vacuole, its size, its course in the body and cyclosis.

10. The frequency of ingestion is related to the viscosity of the solution. The frequency of ingestion is increased as the viscosity increases from 0.001 per cent to 0.05 per cent, then it gradually decreases as the viscosity further increases to four per cent Methocel.

11. In very high viscosity solutions as in four per cent Methocel the food vacuoles formed almost reached the maximum in number in the first 15 minutes and they were very large.

12. The frequency of ingestion is also correlated with the osmotic concentration of the fluid. It is inversely related with increased concentrations of Mannitol from 0.0001 M Mannitol to 0.035 M Mannitol.

13. The frequency of ingestion increases directly as the time of feeding is increased.

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