GROWTH STUDIES ON CILIATES

IV. The Influence of Food on the Structure and Growth of Glaucoma vorax sp. nov.

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Morphological variations which may be correlated with physiological condition have been noted in many different protozoa. It is well recognized that well fed protozoa are usually proportionately large while starved specimens may be quite small. Monsters of various types may result from certain cultural conditions such as certain bacterial diets (Kidder and Stuart, 1939) or from less well known influences during population decline. Among the hypotrichous ciliates one sees a marked change in size taking place when these forms become cannibalistic (Geise and Alden, 1938), but this increase in size is seldom accompanied by drastic morphological variations. The limits of morphological variation under varied conditions of food and other environmental factors must be recognized in order that the taxonomic validity of any species may be established.

The following report is based on the study of a hitherto undescribed species of *Glaucoma* (herein designated as *G. vorax sp. nov.*) and the experiments carried out were designed to gain some understanding of the food requirements, the mechanism of feeding and the factors influencing the profound structural changes of this interesting ciliate.

Material and Methods

The Ciliate

Glaucoma vorax was discovered in samples brought in from a small fresh water pond located in the vicinity of Providence, Rhode Island. In these samples large numbers of *Colpidium campylum* were also present. After remaining in the laboratory for a few days it was noted that the *Colpidium* were decreasing in number and a very large (150–250 μ) ciliate was becoming quite numerous. Upon isolation this ciliate, after two to three divisions, reverted to a slim, tailed *Glaucoma,* very similar in general appearance to *G. frontata.* These tailed forms were then

placed in a suspension of our pure line *Colpidium campylum* and, within 24 hours, large forms were again present. It was apparent that the tailed *Glaucoma* had become carnivorous and were using the *Colpidium* for food. One of these large carnivores was selected for sterilization and it is from this organism that all of our stock lines have been derived.

Sterilization

The method of sterilization employed was a modification of that used by Parpart (1928) but the changes in manipulation appear to be worthy of description. Petri dishes were replaced by Syracuse watch glasses. Ten of these were placed individually in cellophane bags,[1] the open ends of the bags folded over several times and the whole sterilized in the autoclave. After these had cooled, 5 ml. of sterile distilled water was placed in one of the watch glasses, the bag being opened only enough to permit the entrance of the flamed lip of the tube containing the water. Following a few preliminary washes, the single large ciliate was placed in the watch glass by inserting the micropipette into the open end of the cellophane bag. The ciliate was allowed to swim about in the distilled water for ten minutes and was then transferred to a second similarly prepared dish.

The advantage of the cellophane bag as protection for the washing fluid is that the fluid or the watch glass is never exposed to the air from above, all manipulations being carried on from the side. Another important advantage is that the swimming organism can be watched under the dissecting microscope at all times because there is no condensation of water on the cellophane as there is on the cover of a Petri dish.

After the ciliate had been carried through five such transfers it was left in the fifth dish for twelve hours, a precaution against ingested, but viable spores. To this dish, however, had been added a loop of sterile *Colpidium campylum* taken from a proteose-peptone agar slant. This departure from the usual method is applicable only to carnivores, but it does obviate the difficulty of finishing with a starved ciliate. In this case the experimental ciliate fed and two divisions resulted, so that four large carnivores were present at the end of the twelve hours.

One of these four carnivores was carried through the remaining five dishes in the same manner as before. By the time the tenth dish was reached all of the *Colpidium* had been left behind. Yeast extract (1 per cent) was substituted for the distilled water in the tenth dish and the ciliate was left in this medium for forty-eight hours. Unlike *Colpidium*

[1] The cellophane bags have been successfully used by one of us (D. M. L.) during the sterilization of a number of hypotrichous ciliates for nutritional studies. The results of these studies will appear shortly.

striatum (Elliott, 1933) and *Glaucoma ficaria* (Johnson, 1935), this ciliate did not appear to require any "acclimatization" to the broth but was able to utilize the dissolved proteins at once. After size reduction (which will be described later) it began to multiply, so that many ciliates were present in the dish after forty-eight hours.

A number of *Glaucoma* were transferred aseptically from the tenth dish into tubes of yeast extract and flourishing cultures resulted. From these tubes all of the regular checks for sterility were conducted (see Kidder and Stuart, 1939) with negative results. Agar plates have been poured from time to time, some incubated at 37° C. and some at room temperature, and all kept for a minimum of two weeks. There has been no indication of bacterial contamination in any of our stock lines.

Food Organisms

The bacteria-free ciliates and flagellates used as experimental food were obtained as follows: *Colpidium campylum* was sent to us by Dr. Robert H. Hall and is the strain investigated by him for oxygen consumption (Hall, 1938). *Colpidium striatum* was sent to us by Dr. A. M. Elliott and is the strain isolated by him in 1933. *Glaucoma pyriformis* was sent through the courtesy of Dr. Austin Phelps and is the strain investigated by him (Phelps, 1936). *Glaucoma ficaria* was isolated bacteria free in this laboratory, as was *Glaucoma scintillans* (the method employed in these isolations together with their growth characteristics will be reported later). *Astasia klebsii* in bacteria-free culture was obtained through the courtesy of Dr. Herman Von Dach. *Chilomonas paramecium* was supplied by Dr. J. B. Loefer and is the strain investigated by him in regard to the utilisation of dextrose (Loefer, 1938). *Euglena gracilis* was sterilized and established in culture in this laboratory. The yeast used was obtained by streaking from a Fleischmann yeast-cake onto proteose-peptone-dextrose agar plates and selecting isolated colonies for pure culture.

Techniques

For a study of the structure of *Glaucoma vorax* various preparations were made. The usual preparations (haematoxylin and the Feulgen reaction) for nuclei proved very satisfactory. For details of the cilia, ciliary lines, and mouth parts nigrosin and opal blue preparations gave beautiful results. A ciliate prepared by either of these relief methods is transparent to a degree so that the ciliary lines can be studied on both sides of the animal by focusing down from the top to the bottom surface. The silver line system was demonstrated in a satisfactory manner by using the dry method of Klein (1926).

Living material was observed and studied in hanging drop cultures. Under these conditions the ciliates remain healthy in appearance, feed, grow and multiply for many days. The hanging drops were prepared in the same manner as has been previously described (Kidder and Stuart, 1939*a*, 1939*b*) and were free from bacterial contamination.

Observations

Having at hand an unlimited supply of sterile saprozoic *Glaucoma vorax*, it was possible to test their ability to utilize certain common types of foods and to study the relative effects of these foods on structure and growth. The following account is largely qualitative, but observations on division rates have been made and will be indicated pending a more complete report to be given at a future date.

Bacteria

Microscopic examination of the ciliates from the wild had demonstrated the fact that bacteria were taken into the food vacuoles. We have made no attempt to determine what degree of species selectivity is practiced in nature, but have rather tested the influence of a single strain of living bacteria (*Aerobacter cloacae*) on the structure and growth of our experimental ciliate.

When washed, broth-grown ciliates are placed in a suspension of *Aerobacter* in distilled water they began to feed at once, small vacuoles form and the organisms resemble in every detail the thin tailed organisms found in nature (Fig. 1, *A*). These ciliates range from 50 μ to 75 μ in length and from 10 μ to 18 μ in width. Well fed ciliates appear quite granular, but as the bacteria are eaten out of the culture they become clear and refractive. Division of the tailed forms occurs and the daughter ciliates are also tailed. During the early periods of growth when adequate food organisms are present the interdivisional time is about six hours at 25° C.

Living bacteria as food have been considered first because this type of food is the one utilized by the ciliates in nature, at least to a large extent.

Fig. 1. Illustrations of *Glaucoma vorax* from life. × 500. *A*. Tailed form, bacteria-feeder. *B*. Broth-grown saprozoic form, during early period of growth. *C*. Small saprozoic form during the decline period of a broth culture. *D*. *Glaucoma* which has fed on dead *Colpidium*. *E*. Starved form from a dead *Colpidium* culture. *F–I*. Progressive form changes of saprozoic form (*B*) in the presence of living *Colpidium*. Note the formation of the preparatory vacuole and the size increase of the cell. *J*. A young carnivore removed to living yeast.

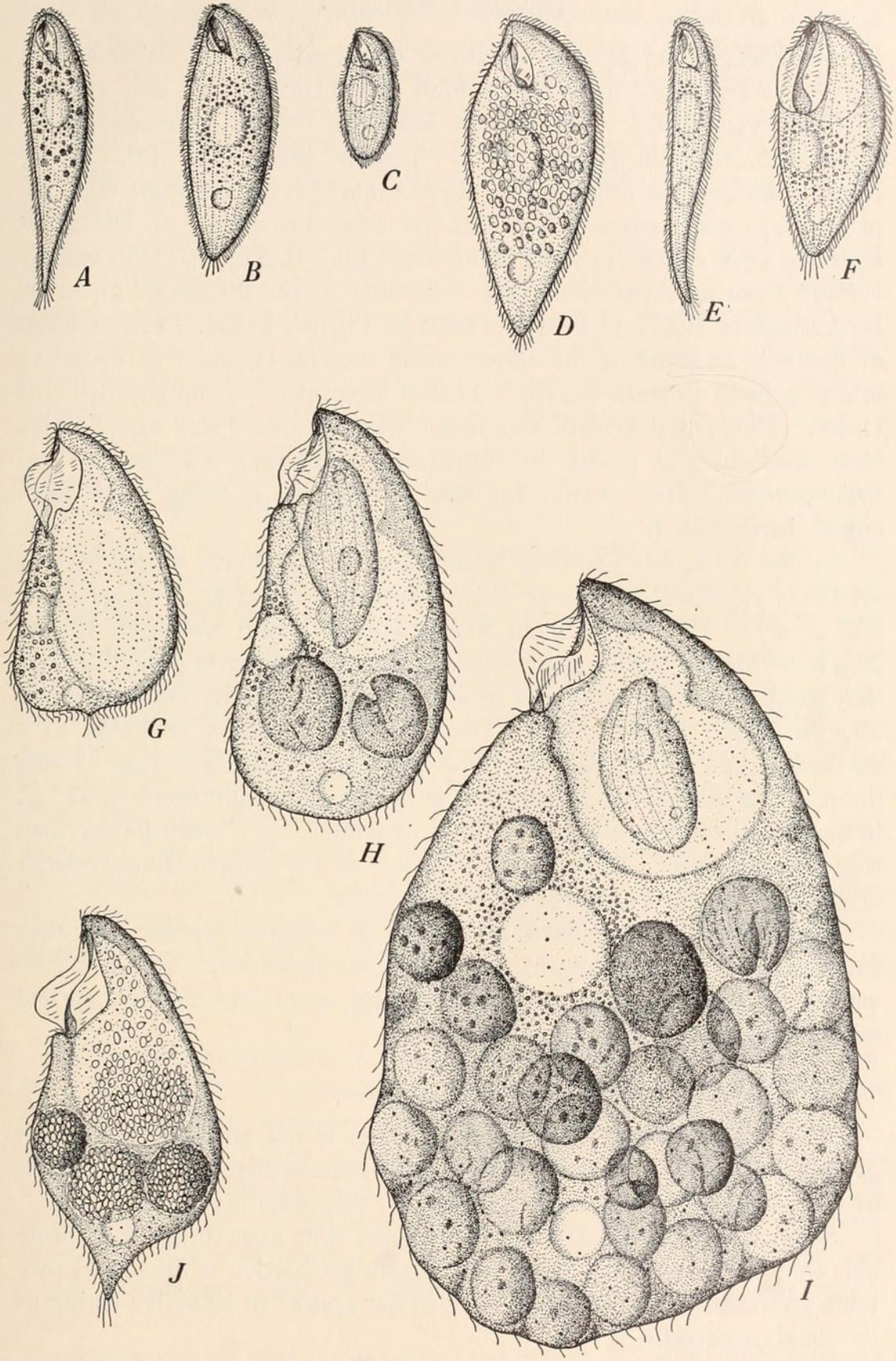

FIG. 1.

Dissolved Proteins

Glaucoma vorax is able to utilize dissolved proteins as food and thereby to reproduce to relatively high concentrations per unit volume. We have grown this ciliate on the following media: proteose-peptone broth (0.5, 1.0, 2.0, 4.0 per cents); peptone broth (0.5, 1.0, 2.0 per cents); silk peptone broth (0.5, 1.0 per cents); tryptone broth (0.5, 1.0 per cents); yeast extract (0.5, 1.0 per cents by weight of Difco dehydrated yeast extract); yeast autolysate (1.0, 2.0, 5.0, 10.0 per cents by volume from a preparation made according to the directions given by Hetherington, 1932 and 1933 and used by Phelps, 1936). The addition of dextrose to some of the above media was made, but was found to inhibit growth to some degree. This is in contrast to the statement of Loefer (1938) that growth was better when dextrose was added to his basic medium and tested on *Colpidium, Glaucoma, Chilomonas* and *Chlorogonium.* The reason for this discrepancy is being more thoroughly investigated.

Sterile *Glaucoma* growing in any one of the above media become relatively clear and lose their distinctive tailed appearance (Fig. 1, *B;* Fig. 2, *A*). They range from 50 μ to 70 μ in length and from 15 μ to 22 μ in width, during the early stages of growth. After a period of days they gradually become reduced in size until the majority are only about 30 μ in length (Fig. 1, *C*). These saprozoic ciliates resemble to a marked degree *Colpidium campylum* growing in the same type of medium. The interdivisional period is approximately eight hours at 25° C. in most of the above media. This is, of course, very much longer than that of *Colpidium.* More detailed studies of the growth characteristics are being conducted and will be reported later.

When broth-grown ciliates are placed on proteose-peptone agar they form colonies and multiply to very high concentrations. This is a convenient way to carry stock cultures as a single agar slant may be kept for months and viable ciliates recovered.

Particulate Proteins

Autoclaved *Aerobacter* in distilled water is not adequate to support growth in *Glaucoma vorax.* The ciliates remain alive for long periods of time, but do not increase in size nor do they multiply.

Suspensions of autoclaved yeast support slow growth, but it is probable that the ciliates are taking in a certain proportion of dissolved proteins from the yeast cells as there does not appear to be active eating of the dead yeast cells.

Yeast Harris [2] (dehydrated) in 1 per cent by weight concentrations

[2] Brewers' Yeast—Harris. Pasteurized, dehydrated yeast cells prepared by the Harris Laboratories, Tuckahoe, New York.

supports fairly good growth. The interdivisional period is approximately ten hours at 25° C., but the cultures remain in good condition for weeks. The maximum concentration in tubes of Yeast Harris eventually equals that of the proteose-peptone of yeast extract. The form of the ciliate in Yeast Harris is the same as the bacteria-feeder, tailed, and small food vacuoles containing the particles are scattered throughout the cytoplasm.

Autoclaved *Colpidium campylum* (loops of colpidia taken from an agar slant, suspended in distilled water and autoclaved) and *C. campylum* shaken with sterile sand and not autoclaved serve as excellent food for the *Glaucoma*. The form changes are quite characteristic under these conditions and are identical for both autoclaved and fresh (sand-shaken) colpidia. The *Glaucoma* became proportionately much broader (30 μ to 40 μ). The pointed posterior end is evident though not so tail-like as in the bacteria-feeders and the cytoplasm becomes densely granular and quite dark (Fig. 1, *D*). Microscopic examination shows that the pieces of colpidia are taken into food vacuoles and become distributed throughout the cell, giving it its dark appearance. Interdivisional time is approximately 5.5 hours during the early period of growth when an adequate number of colpidia particles are present. Division of these darkly granular forms results in rounded daughter ciliates which rapidly assume the shape and size of the parent cell. After the particles of colpidia are depleted from the medium form changes take place in which the ciliates lose their granular appearance and become extremely thin (Fig. 1, *E*). These thin ciliates will persist in a tube for weeks without losing their activity. The concentration of living organisms gradually falls, but even after three months many viable forms may be recovered from the tube. It is probable that these remaining ciliates have been subsisting on the dead bodies of their sister cells.

Living Protozoa

If a sterile suspension of *Colpidium campylum* is prepared by placing a loopful from an agar slant in sterile distilled water or balanced salt solution and a few *Glaucoma vorax* are inoculated into this tube, within twenty-four hours large carnivores have made their appearance. The interesting form changes which occur can be followed best in hanging-drop preparations. In these latter preparations a single small *Glaucoma* placed in a heavy suspension of living colpidia may be followed by frequent observations under the medium powers of the compound microscope.

Within a few hours after the preparation is made the *Glaucoma* begins to broaden and the mouth becomes larger and more open. Back of the mouth a large clear space appears which is continuous with the

outside through the mouth opening (Fig. 1, *F*). This is the start of the formation of the "preparatory vacuole" and probably represents the small vacuole of the tailed form which becomes enlarged and fused with the gullet. The "preparatory vacuole" increases in size until it nearly reaches the posterior end of the ciliate. At this period the vacuole occupies the greatest volume of the cell with the *Glaucoma* protoplasm surrounding it in a thin film (Fig. 1, *G*). Only after the completion of the formation of the "preparatory vacuole" can the *Glaucoma* feed on the live *Colpidium*. The first meal appears to be the most difficult to accomplish. As the mouth is open and large the membranes create strong currents into the "preparatory vacuole," eventually a *Colpidium* is drawn in. The *Glaucoma* immediately becomes quite active and swims in circles with the mouth directed toward the inner part of the circle. This first prey swims about in the vacuole in an entirely normal manner and may eventually swim out of the mouth and escape. Sometimes two or more colpidia are drawn in together (Fig. 2, *E*). Eventually the *Glaucoma* protoplasm closes down until the "preparatory vacuole," with its trapped *Colpidium,* is cut off from the mouth region. The enclosed prey continues to swim about, but the fluid content of the vacuole decreases slowly and the protoplasms of the carnivore and prey come to lie close together and the motion of the latter is restricted. After about twenty minutes the prey has lost all activity and digestion is under way. As digestion proceeds the *Glaucoma* increases in size and from that time on it is able to capture colpidia much more rapidly (Fig. 1, *H*, 2, *B–E*). Within the space of an hour 40 to 50 colpidia may be captured and the resulting growth of the *Glaucoma* may bring its length up to 250 μ and its width to as much as 150 μ (Fig. 1, *I*). These large carnivores eventually divide transversely (Fig. 2, *F*), each daughter ciliate carrying over about half of the food inclusions. After separation the daughter ciliates redifferentiate, feed, grow in size and again divide. This process is repeated until the colpidia in the medium become scarce. The *Glaucoma* continue to divide, but each daughter cell is smaller until the typical tailed form is again attained. As long as there are any colpidia present, however, the "preparatory vacuole" is retained in at

FIG. 2. Photomicrographs of living *Glaucoma vorax*. These pictures were (with the exception of *A*) made from a hanging drop preparation in which, forty-eight hours previous to photographing, one small saprozoic *Glaucoma* was transferred aseptically to a suspension of living *Colpidium*. *A*. Saprozoic ciliate. × 320. *B*. Carnivore showing enlarged mouth and rounded food inclusions (*Colpidium*). × 180. *C*. Same, but with many more food inclusions. × 180. *D*. One carnivore with a few inclusions to show the relative size of the *Glaucoma* and the prey. × 60. *E*. Carnivore showing the preparatory vacuole in which two colpidia are trapped. × 250. *F*. Division of a carnivore. × 180.

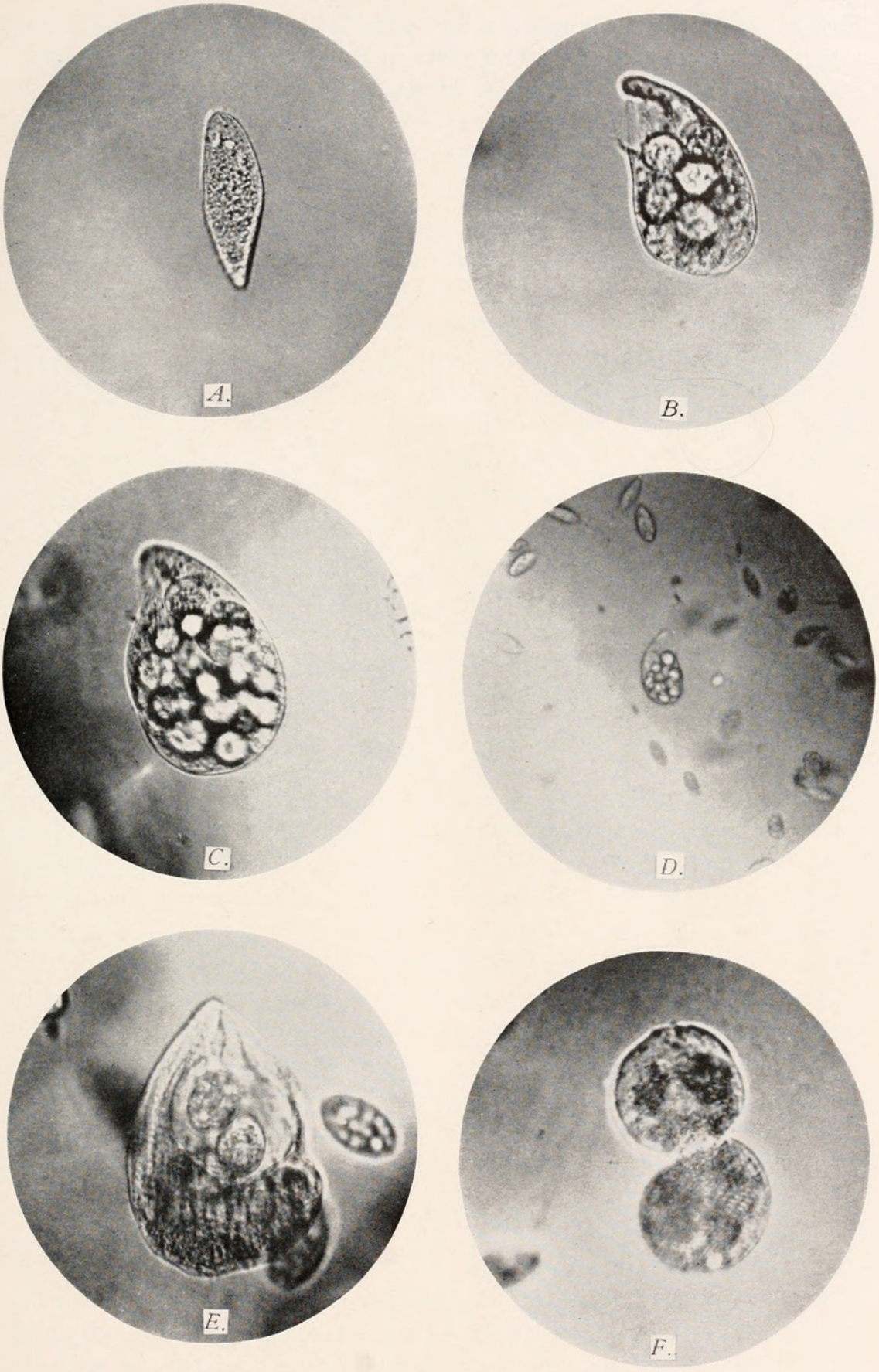

FIG. 2.

least some of the *Glaucoma* so that eventually all of the colpidia are eaten. We have recorded many cases in hanging drops where the ratio of *Colpidium* to *Glaucoma* at the start was about 2000:1 and after four to five days many *Glaucoma* were present and no *Colpidium*. Likewise the *Glaucoma* are able to clear out the colpidia from a tube until none can be found after five to six days.

These large carnivores are the type which we encountered in our original pond sample, one of which was used for sterilization.

The formation of the preparatory vacuole is not dependent upon anything peculiar to *Colpidium campylum*, however, as exactly the same response is evoked by the presence of all of the ciliates tried. These were *Colpidium striatum* (Elliott strain), *Glaucoma pyriformis* (Phelps strain), *Glaucoma ficaria* (our strain), *Glaucoma scintillans* (our strain) and *Colpoda* (our (Kidder and Stuart, 1939) strain grown on *Aerobacter*). Cannibalism also occurs, especially in broth cultures. After the concentration of a pure culture of *Glaucoma vorax* has reached a value somewhere between 15,000 and 25,000 ciliates per ml. a few organisms assume the form described for the *Colpidium*-feeders and begin eating members of their own species. The proportion of cannibals to non-cannibals is always very low, however, for after one or two divisions these forms revert to the typical saprozoic types.

Living Yeast

If saprozoic ciliates are placed in a suspension of living yeast they appear to be unable to ingest the yeast cells. They live for a week or more but finally disappear from the culture. No form change ensues except for a decrease in size due to starvation. If, however, a carnivore is placed in a like suspension it is able to eat the yeast cells. The yeast is concentrated in the preparatory vacuole in large balls containing hundreds of cells and then the vacuole is separated from the mouth region in the same manner as previously described for colpidia-feeders. These yeast-filled *Glaucoma* (Fig. 1, *J*) grow slowly and divide, the daughter ciliates again feeding as had the parent. The yeast-feeders never reach the large size of the carnivores and reversion to a tailed condition usually takes place long before the food is exhausted.

Living Flagellates

We have tested the effect of the following bacteria-free flagellates on *Glaucoma vorax*: *Astasia klebsii, Chilomonas paramecium* and *Euglena gracilis*. Saprozoic *Glaucoma* placed in a washed suspension of any one of the above flagellates slowly starve and disappear from the culture. As with the yeast cells, there is no alteration of form. However, pre-

formed carnivores feed on the flagellates. Both the feeding mechanism and the reproduction of the *Glaucoma* are slowed down under these conditions. The flagellates are ingested sparingly and the ciliates do not increase appreciably in size, even after many hours. One, or sometimes two, divisions occur and reversion to the tailed condition ensues. It is possible that this type of food is entirely insufficient for reproduction and the one or two divisions noted result from a carry over of nutrient materials within the ciliates from their previous environment.

Discussion

Glaucoma vorax exhibits the most varied food-taking habits of any known species of the genus. Like a number of other species within the genus it may be a bacteria-feeder or, when deprived of its associated bacteria, utilize dissolved proteins. On the other hand, it resembles to a marked degree *Leucophrys patula* in that it can ingest and digest relatively large ciliates. In order to do this, however, drastic form changes must take place. In *Leucophrys* the large receiving vacuole appears to be a permanent structure (M. G. Brown, unpublished observation) and when the food ciliates are exhausted from the medium there is no change in form but simply a diminution of size. *Leucophrys* appears to be more strictly an obligate carnivore.

One of the most significant facts about *Glaucoma vorax* is the formation of the preparatory vacuole previous to the ingestion of living protozoa. This formation appears to be stimulated by the presence within the medium of living ciliates. We have made a number of preliminary experiments in an attempt to determine what factor or combination of factors may be operating. Extracts from *Colpidium* were placed in the medium. These extracts were prepared by cutting up the colpidia by shaking with sand and passing the fluid through a Seitz filter. The filtrate was entirely without effect on the form of the saprozoic *Glaucoma.* The dead bodies of *Colpidium,* although eaten by the *Glaucoma,* fail to evoke the vacuole formation. Pieces of fresh *Colpidium,* shaken with sterile sand, were eaten but did not cause vacuole formation. Live flagellates and yeast failed to evoke the vacuole formation although a pre-formed carnivore was capable of ingesting these organisms. Although investigations which are now being carried on may clear up our understanding of the factors involved in this important cell mechanism, we can only say at the present that the stimulus necessary for the preparatory vacuole formation and the accompanying form changes appears to reside in the living, actively moving ciliate. It may be that the stimulus is of a mechanical nature and that any cell possessing the right size and degree of activity will evoke the response.

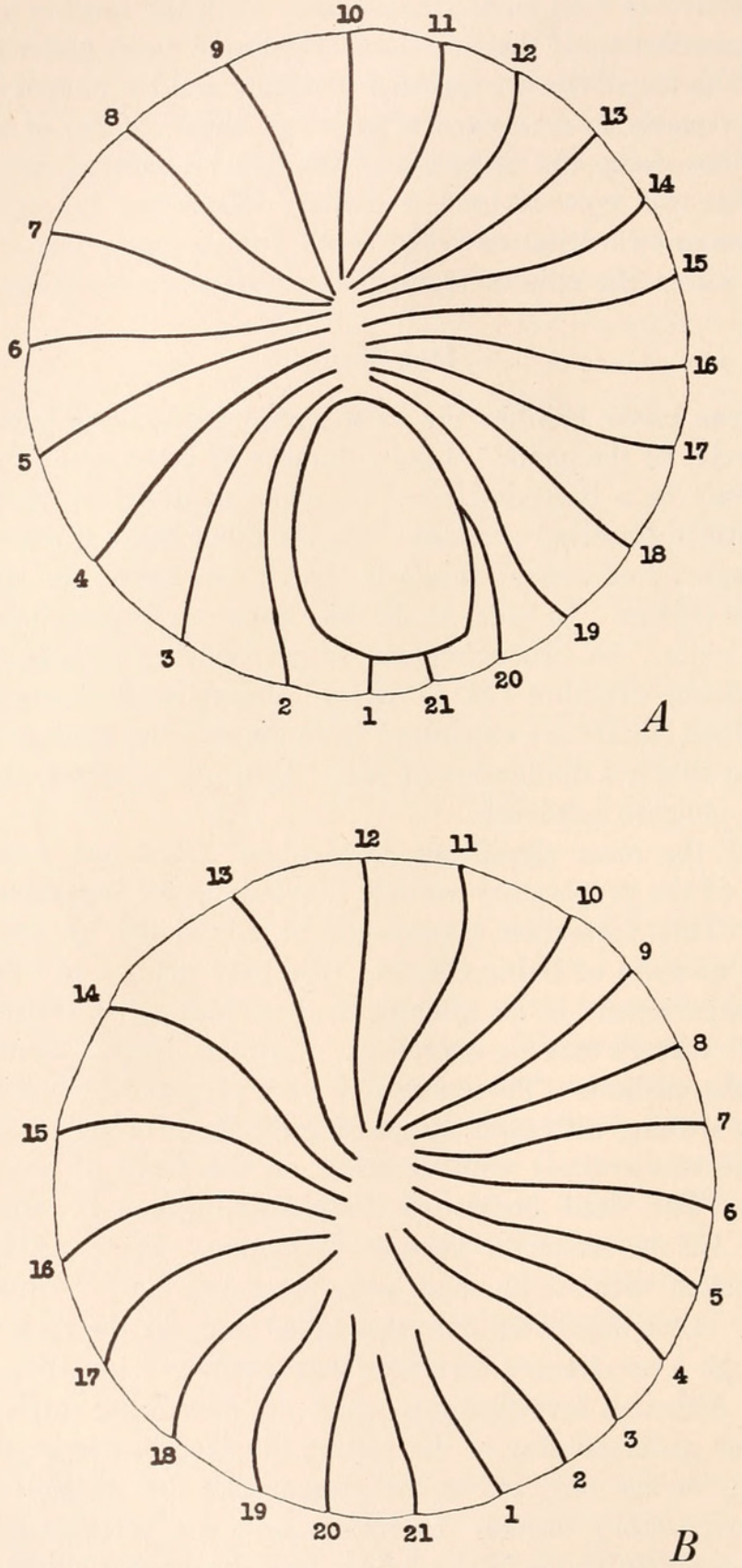

FIG. 3. Diagrammatic representation of the ciliary lines in *Glaucoma vorax*. *A*. Anterior view showing the mouth and the origin of 21 rows of cilia. *B*. Posterior view showing the general region of termination of the 21 rows.

Glaucoma vorax seems to offer exceptional opportunities for investigations into the mechanisms of feeding, specific food requirements including accessory factors, the growth characteristics under varied conditions and the further effects of associated organisms on structure and metabolism.

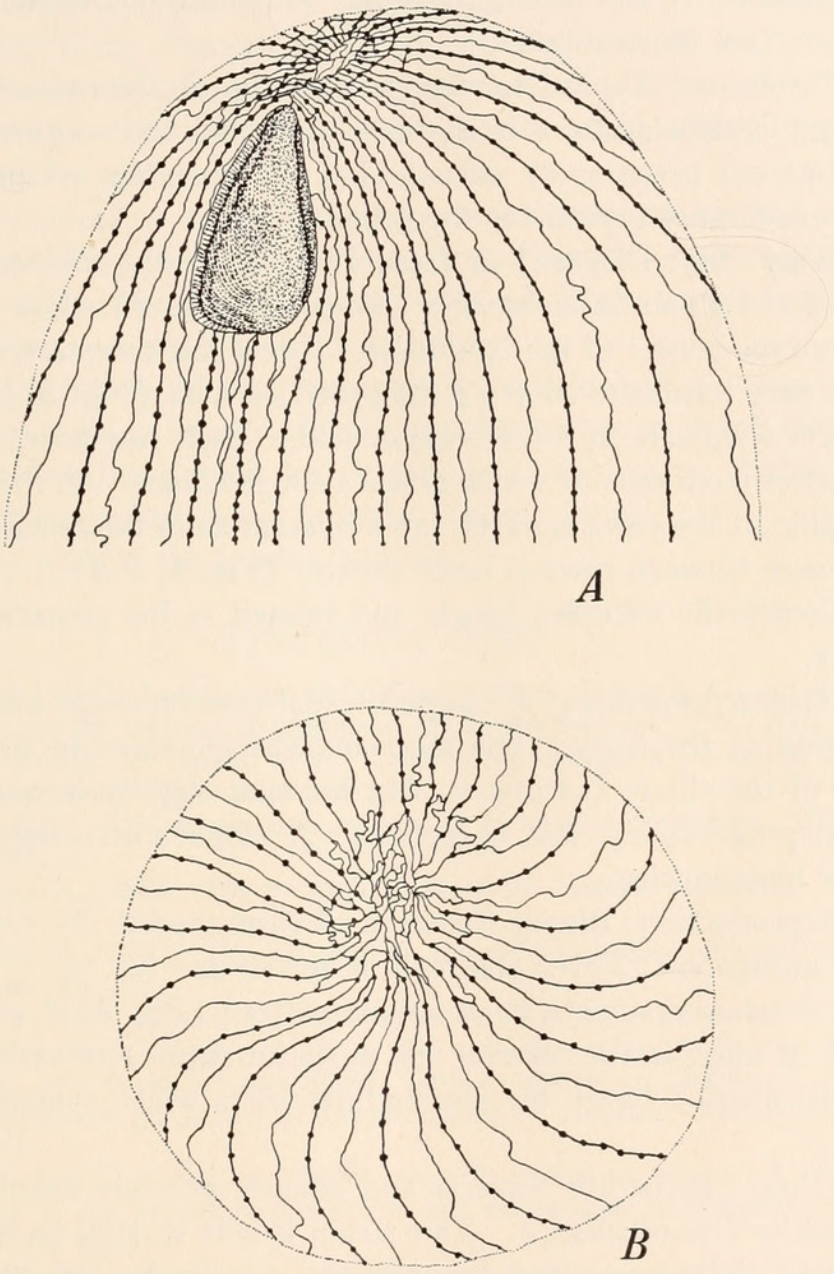

FIG. 4. Silverline system of *Glaucoma vorax*. *A*. Anterior end showing mouth and anterior suture. Note interstrial fibers. *B*. Posterior end showing network of fibers in the posterior suture.

SPECIES DIAGNOSIS OF GLAUCOMA VORAX SP. NOV.

1. Sizes: Bacteria-feeders 50 μ to 75 μ; saprozoic forms 30 μ to 70 μ, the size decreasing with the age of the culture; sterile particle-feeders

(autoclaved or otherwise killed ciliates) 60 μ to 80 μ; carnivores and cannibals 100 μ to 250 μ, depending upon the amount of food taken.

2. Shape: Varies enormously depending upon the amount and type of food taken. Bacteria-feeders elongate with a distinct tail process; saprozoic forms spindle-shaped to ovoid; carnivores and cannibals distended posteriorly and usually irregular in outline due to the large food inclusions (see illustrations).

3. Cytostome: Typical for the genus except in the case of the carnivores and cannibals where it is vastly distended and continuous with a large, internal preparatory vacuole for the reception of prey. Cytostomal membranes typical for the genus.

4. Body cilia: Disposed in from 19 to 21 rows. Sixteen to 18 of these rows originate in an anterior suture (Fig. 3, *A*) while three originate from the border of the cytostome. These last three are the shortest rows as they terminate short of the posterior field (Fig. 3, *B*). All of the others terminate in the posterior field. Each row bears a series of basal bodies from each of which arises a single cilium. In the carnivores the spacing of the rows is in the same relation as in the tailed forms, but the distance between rows is much greater (Fig. 1, *F–I*).

5. Contractile vacuole: Single and located in the posterior third of the body.

6. Nuclear apparatus: A single ovoidal macronucleus located in the mid-region of the body. The macronucleus increases in size with the growth of the ciliate (carnivore) but becomes slightly irregular in outline and progressively less basophilic. A single micronucleus, located near the macronucleus.

7. Reproduction: Binary fission in all form types.

8. Conjugation: Never observed in our strain.

9. Silverline system: Characteristic pattern (Fig. 4, *A* and *B*) with network of fibers in the anterior and posterior sutures and with irregular interstrial fibers between the longitudinal fibers which connect the basal bodies.

10. Food: In nature bacteria and ciliates, possibly members of the same species (cannibalistic). Our strain is able to feed on a variety of particulate and non-particulate proteins. Carnivores produced in the presence of living ciliates of any one of a number of different species. Live yeast and certain flagellates may serve poorly as food provided they can be ingested.

11. Type strain: May be procured bacteria-free from our laboratory.

Summary

1. *Glaucoma vorax* sp. nov. was isolated from pond water in the vicinity of Providence, R. I.

2. It was sterilized and established in broth culture by a modification of the transfer washing method in which the containers were enclosed in cellophane bags before autoclaving.

3. Various form changes were correlated with types of food; bacteria-feeders are tailed, saprozoic organisms are spindle-shaped to ovoidal while carnivores and cannibals are irregularly ovoid with the greatest width at the posterior end.

4. When saprozoic forms are placed in a culture or washed suspension of other ciliates (*Colpidium, Glaucoma, Colpoda*) they change their form preparatory to becoming carnivorous. A large vacuole forms back of and continuous with the mouth to receive the prey.

5. The formation of the preparatory vacuole and the concomitant form changes seem to be evoked only in the presence of living ciliates, as killed ciliates (either autoclaved or freshly sand-shaken), living flagellates, bacteria or yeasts do not stimulate its formation.

6. A diagnosis of the species is given.

LITERATURE CITED

Elliott, A. M., 1933. Isolation of Colpidium striatum Stokes in bacteria-free cultures and the relation of growth to pH of the medium. *Biol. Bull.*, **65**: 45.

Giese, A. C., and R. H. Alden, 1938. Cannibalism and giant formation in Stylonychia. *Jour. Exper. Zoöl.*, **78**: 117.

Hall, R. H., 1938. The oxygen-consumption of Colpidium campylum. *Biol. Bull.*, **75**: 395.

Hetherington, A., 1932. The constant culture of Stentor coeruleus. *Arch. f. Protist.*, **76**: 118.

Hetherington, A., 1933. The culture of some holotrichous ciliates. *Arch. f. Protist.*, **80**: 255.

Johnson, D. F., 1935. Isolation of Glaucoma ficaria Kahl in bacteria-free cultures and growth in relation to pH of the medium. *Arch. f. Protist.*, **86**: 263.

Kidder, G. W., and C. A. Stuart, 1939*a*. Growth studies on ciliates. I. The role of bacteria in the growth and reproduction of Colpoda. *Physiol. Zoöl.* (in press).

Kidder, G. W., and C. A. Stuart, 1939*b*. Growth studies on ciliates. II. The food factor in the growth, reproduction and encystment of Colpoda. *Physiol. Zoöl.* (in press).

Klein, B. M., 1926. Ergebnisse mit einer Silbermethode methode bei Ciliaten. *Arch. f. Protist.*, **56**: 243.

Loefer, J. B., 1938. Utilization of dextrose by Colpidium, Glaucoma, Chilomonas and Chlorogonium in bacteria-free cultures. *Jour. Exper. Zoöl.*, **79**: 167.

Parpart, A. K., 1928. The bacteriological sterilization of Paramecium. *Biol. Bull.*, **55**: 113.

Phelps, A., 1936. Growth of protozoa in pure culture. II. Effect upon the growth curve of different concentrations of nutrient materials. *Jour. Exper. Zoöl.*, **72**: 479.