The Biology and Ultrastructure of Phagotrophy in *Ochromonas danica* (Chrysophyceae: Chrysomonadida)

By S. AARONSON

Biology Department, Queens College, City University of New York,
Flushing, N.Y. 11367, U.S.A.

(Received 25 September 1973; revised 5 February 1974)

SUMMARY

Ochromonas danica can obtain energy and required nutrients by eating several different bacteria or brewer's yeast. The phagocytic process includes aggregation of bacteria into clumps which contain and are surrounded by *Ochromonas*.

Bacteria were phagocytized too fast to be seen while yeast took at least 5 min to be engulfed. 2,4-Dinitrophenol or sodium azide, but not sodium fluoride or iodoacetic acid, inhibited the phagocytic process; aerobic energy metabolism was necessary for phagocytosis. Food organisms were engulfed and the food vacuole migrated to the posterior end of the organism behind the leucosin vacuole. The bacteria were digested mainly in the food vacuole although secondary lysosomes were formed. Digestion of bacteria resulted in the proliferation of vesicles and membranes from the food vacuole membrane and the proliferation of membrane from the bacteria being digested.

INTRODUCTION

The biology and ultrastructure of the phagotrophic process (endocytosis) has been studied in relatively few protozoa (Pitelka, 1963; Müller, 1967; Aaronson, 1973*a*). Recently attention has been paid to phagotrophy in ciliates: Elliott & Clemmons (1966) studied cytochemical ultrastructure in *Tetrahymena pyriformis* while the biology of *Tetrahymena* has been examined by many (Müller, 1967; Ricketts, 1970, 1971*a, b*; Nilsson, 1972). The ultrastructure of phagocytosis in *Colpoda* was examined by Rudzinska, Jackson & Tuffran (1966) and in *Tokophrya* by Rudzinska (1970). Amoebae have also received their share of attention (Chapman-Andresen, 1971), with ultrastructural studies of phagocytosis in *Pelomyxa* (Roth, 1960) and *Entamoeba* (Rosenbaum & Wittner, 1970) and biological studies in *Acanthamoeba* (Weisman & Moore, 1969; Rabinovitch & DeStefano, 1971). To my knowledge there is little or no recent work on phagotrophy in flagellates particularly the phytoflagellates (Aaronson, 1973*a*); hence *Ochromonas danica* was chosen for study.

Ochromonas danica is capable of phagotrophy, heterotrophy, photoheterotrophy and photoautotrophy (Pringsheim, 1955; Aaronson & Baker, 1959). Earlier papers have presented information on the nutrition (Aaronson & Baker, 1959) and (briefly) the ultrastructure of phagotrophy (Schuster, Hershenov & Aaronson, 1968), while the chemical composition of photoheterotrophic and photophagotrophic *O. danica* were compared by Aaronson (1972). Further details of the biology and ultrastructure of phagotrophy in *O. danica* are presented in this paper.

Table 1. *Phagotrophic mineral medium (PMM)*

Nitrilotriacetic acid	0.2 g	Mn (as $\text{MnSO}_4 \cdot \text{H}_2\text{O}$)	5.0 mg
KH_2PO_4	0.3 g	Cu (as $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$)	0.1 mg
MgCO_3 (basic)	0.4 g	Co (as $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$)	0.1 mg
CaCO_3	0.05 g	B (as H_3BO_3)	0.1 mg
NH_4Cl	0.5 g	Mo [as $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$]	0.05 mg
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	1.0 g	V [as $\text{Na}_3\text{VO}_4 \cdot 16\text{H}_2\text{O}$]	0.05 mg
Fe [as $\text{Fe}(\text{NH}_4)_2(\text{SO}_4)_2 \cdot 6\text{H}_2\text{O}$]	13.0 mg	Distilled water	1 l
Zn (as $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$)	8.0 mg	pH 5.0	

METHODS

Cultures of *O. danica* Pringsheim (strain 933/2; Culture Centre of Algae and Protozoa, Cambridge) for inoculation were maintained axenically in a chemically defined medium (Aaronson & Baker, 1959) in white fluorescent light at 25 °C in a refrigerated incubator. For phagotrophic experiments cultures were grown axenically in phagotrophic medium (PMM) (Table 1) for periods of up to two weeks with 125 to 150 ft-candle of white fluorescent light as above. As PMM contained no carbon source for energy and no required vitamin, photoautotrophy or heterotrophy was impossible. Bacteria were grown in 500 ml BBL nutrient broth for 3 to 4 days at 37 °C, harvested at 4300 g in a Sorval RC-2B refrigerated centrifuge at 4 °C and washed twice with 30 to 40 ml distilled water before being suspended in distilled water or PMM for use as a bacterial suspension. Brewer's yeast was suspended in distilled water for 1 h in the refrigerator; only the smaller yeast cells which remained suspended were used. Carbon particle phagotrophy was studied by suspending *O. danica* in indian ink (Higgins) diluted with distilled water to yield an absorbance of about 0.6 to 0.8 at 410 nm in a Beckman DB spectrophotometer.

Cell multiplication was determined by taking a culture sample, killing the cells with a drop of 0.125 % HgCl_2 , and counting them with a haemocytometer. For determining the kinetics and energy metabolism of phagotrophy, a yeast suspension was inoculated with *O. danica*, incubated, and samples taken out at appropriate time intervals; the samples were killed with HgCl_2 and the numbers of *O. danica* containing yeast in samples of 100 were counted with a light microscope and recorded as the percentage of organisms eating.

Organisms for ultrastructural studies were centrifuged at 860 g in a Sorval angle centrifuge for 5 min; pellets were fixed for 1 h in 2.5 % (w/v) glutaraldehyde containing a trace of CaCl_2 and buffered with sodium cacodylate (0.1 M) at pH 7.4. After two rinses in cacodylate buffer the organisms were postfixed for 1 h in 1 % (w/v) osmic acid buffered with sodium cacodylate (0.1 M) at pH 7.4. During fixation pellets were placed on crushed ice. After the osmic acid was removed, the pellets were stained in 1 % (w/v) aqueous uranyl acetate for 20 min at room temperature and then dehydrated in a graded alcohol series. After clearing in propylene oxide, the organisms were embedded in Epon 812 which was polymerized at 60 °C for 48 h. Sections, cut with a diamond knife on an MT-2 Porter-Blum ultramicrotome, were stained with uranyl acetate and lead citrate. Micrographs were taken with a Philips 300 or Jeolco T7 electron microscope.

All chemicals were reagent grade obtained commercially and used without further purification.

RESULTS

Biology and gross morphology of phagotrophy

Earlier results (Aaronson, 1972) indicated that *O. danica* was able to grow and multiply on *Aerobacter aerogenes*, the classic organism for phagotrophic studies. Several other

Table 2. *Ability of micro-organisms to support O. danica multiplication*

Organism (10 mg/100 ml)	$10^{-6} \times$ No. of cells/ml after 1 week*
<i>Escherichia coli</i>	20.9
<i>Proteus mirabilis</i>	13.8
<i>Aerobacter aerogenes</i>	11.7
<i>Bacillus megaterium</i>	6.0
<i>Sarcina lutea</i>	10.8
<i>Serratia marcescens</i>	6.4
Brewer's yeast	4.9

* Inoculum = 20000 cells/ml

Table 3. *Clearance of particles in aqueous suspension by O. danica in 48 h**

Particle	Absorbance at 410 nm	
	Without <i>O. danica</i>	With <i>O. danica</i>
Indian ink	0.87	0.46
<i>Aerobacter aerogenes</i>	0.35	0.15
<i>Pseudomonas aeruginosa</i>	0.22	0.11

* Each tube contained 5 ml of particle suspension inoculated with approx. 3.6×10^6 light-grown, 6-day-old cells.

bacteria and a yeast were found to support *O. danica* multiplication in PMM (Table 2). All micro-organisms tested were able to support the multiplication of *O. danica*, though some were more effective than others.

When *A. aerogenes* (2.0 mg/ml) was suspended in 500 ml PMM in a 1 l Erlenmeyer flask with or without a small inoculum (20000 cells/ml) of light-grown *O. danica*, the cell suspension appeared white. After 3 to 4 days of incubation in the light at 25 °C the bacterial cell suspension containing *Ochromonas* began to aggregate into tiny clumps. By the 7th day, the suspension was much clearer with a light greenish-yellow colour and larger clumps or aggregates suspended throughout the medium; the number of *O. danica* had increased about 17-fold (20000 to 336000 cells/ml). The control flask without added *Ochromonas* remained as a white bacterial suspension for 2 weeks of incubation. Microscopic examination of the clumps revealed aggregations of bacteria surrounded and occasionally invaded by *Ochromonas*. The *Ochromonas* were immobile in the clump but swam about actively when the clump was broken or disturbed.

The capture of food, one of the first steps in phagocytosis, in *O. danica* involved the flow of the liquid medium toward and over the surface of the organism, induced by flagellar action (Aaronson, 1973*b*). This flow led to the trapping of suspended particles on or near the cell surface followed by particle engulfment (Table 3). Small particles (carbon or bacteria) were engulfed more rapidly than the eye could follow in the light microscope (Pringsheim, 1955). Yeast were engulfed more slowly; almost five min were required before they were inside *Ochromonas*. Mixing *Ochromonas* with a suspension of carbon or bacteria led to aggregation of the particles. This was attributed to the membranous vesicles and other substances secreted by *Ochromonas* into its environment (Aaronson, 1971; Aaronson, Behrens, Orner & Haines, 1971; Aaronson, 1973*b*). These vesicles and associated substances were believed to act like 'flypaper', causing particles to stick to the *Ochromonas* surface or to each other until groups of aggregated particles became large enough to be seen with the

Table 4. *Kinetics of phagocytosis of yeast cells by O. danica*

<i>O. danica</i> containing one or more yeast cells (%)		<i>O. danica</i> containing one or more yeast cells (%)	
Time (min)	Mean $\pm$ S.D.	Time (min)	Mean $\pm$ S.D.
5	8 $\pm$ 6	35	56 $\pm$ 10
10	17 $\pm$ 5	40	56 $\pm$ 4
15	23 $\pm$ 1	45	66 $\pm$ 9
20	32 $\pm$ 3	50	64 $\pm$ 9
25	47 $\pm$ 8	55	67 $\pm$ 8
30	51 $\pm$ 8	60	58 $\pm$ 11

Table 5. *Effect of metabolic poisons on the phagotrophy of yeast cells by O. danica*

Concn (M)	Phagotrophy (as % of control)*			
	Metabolic poisons			
	Sodium azide	Dnp	Iodoacetic acid	NaF
0	100	100	100	100
1 $\times$ 10 ⁻⁶	94	84	81	78
5 $\times$ 10 ⁻⁶	85	40	80	74
1 $\times$ 10 ⁻⁵	24	8	88	98
5 $\times$ 10 ⁻⁵	4	0	66	98
1 $\times$ 10 ⁻⁴	0	0	42	83
5 $\times$ 10 ⁻⁴	0	0	9	84
1 $\times$ 10 ⁻³	0	0	0	89

* Average of three trials. Similar results were obtained by several people in many trials.

unaided eye or to precipitate (Aaronson, 1973*b*). Clumping of bacteria by protozoan predators was also seen by Hardin (1943) to occur with the flagellate *Oikomonas*, and by H. M. Tsuchiya (personal communication) with the ciliates *Tetrahymena*, *Dictyostelium* and *Colpoda*.

Kinetics and energy metabolism of phagotrophy

A suspension of brewer's yeast cells was made in PMM or distilled water and inoculated with *O. danica*; portions were successively removed and examined for phagotrophy. *Ochromonas danica* ate yeast within 5 min, and more than 50 % of the *Ochromonas* were eating within 30 min (Table 4). At no time were 100 % of the organisms eating, although sometimes as many as 75 % were doing so.

Attempts were made to determine if phagotrophy required aerobic, anaerobic or both forms of energy metabolism. Ten million yeast cells and an equal number of *O. danica* cells were incubated in 5 ml PMM in a tube for 1 h in 170 ft-candle of white light at 25 °C in the presence of appropriate concentrations of metabolic poisons. Samples were removed and the cells killed and counted (Table 5). It is clear that *O. danica* phagotrophy was more dependent on aerobic than anaerobic energy metabolism, as the uncoupler of aerobic oxidative phosphorylation (2,4-Dnp) and the inhibitor of electron transport (sodium azide) were more effective inhibitors of phagotrophy than inhibitors of glycolysis (NaF and iodoacetic acid). That phagotrophy was dependent on metabolic activity may also be seen from Table 6, which shows that eating increased with increasing temperature. Short term (1 h) incubation at 25 °C in the light (18 % eating) was not much different from dark incubation (20 % eating).

Table 6. *Effect of temperature on O. danica phagotrophy*

Temperature (°C)*	Cells showing phagotrophy in 1 h (%)†
10 (15)	6 ± 2
15 (25)	8 ± 3
20 (25)	14 ± 3
25 (25)	19 ± 3
30 (28)	21 ± 4

* No. of trials given in parentheses.

† ± S.D.

Ultrastructure of phagotrophy and digestion

The appearance of phagotrophic organisms during eating and digestion was studied by examining *O. danica* periodically in an autoclaved bacterial or yeast suspension over a period of 90 min and then (with bacteria) for up to 9 days. Attempts to examine the fine structure of the phagocytosis of yeast were not successful because the yeast tended to break out of thin sections. The few intact sections examined indicated that the yeast were taken into large food vacuoles at the sides of the organism; these food vacuoles then seemed to migrate to the posterior part of the *Ochromonas* behind the leucosin vacuole. Food vacuoles containing bacteria seemed to form within what appeared to be seconds of contact at the sides and front of *Ochromonas*; they were then seen by light microscopy to migrate toward the rear of the organism. Similar observations were made by Pringsheim (1952, 1955). The appearance of the bacteria after incubation in PMM without *O. danica* for 2 days (Fig. 1) showed relatively little physical change; the bacterial cell envelope lifted off the cell mass and there was some decrease in density of the bacterial cytoplasm, but no membranous vesicle was seen in the medium or attached to the bacterial cells. The appearance after 9 days was almost identical. Membranous vesicles were found only at the surface of or associated with the presence of *O. danica* (Fig. 3). Short term (minute or hour) incubation of bacteria and *Ochromonas* did not seem to clarify further the phagotrophic or digestive process; longer incubations were therefore undertaken. After two days of incubation in PMM containing *O. danica*, bacteria were found throughout the *Ochromonas* cytoplasm in food vacuoles (Fig. 2), which tended to coalesce at the posterior part of the organism to form larger vacuoles containing bacteria in various stages of digestion (Fig. 3). The surface membranes of the large food vacuoles produced small vesicles (Figs. 3, 6) which presumably contained digestive enzymes, although there was no direct evidence for this. After four days of incubation the food vacuoles appeared enormous, occupying a great part of the cell volume and compressing the large leucosin vacuole; bacteria in various stages of digestion were also to be seen (Fig. 3). The smaller migrating food vacuoles seemed to empty into the enlarged posterior food vacuole (Fig. 3). After six days the food vacuoles contained many small vesicles, which were seen at all times but not in such large numbers. The small vesicles reported elsewhere (Aaronson *et al.* 1971) were also seen in the extracellular environment attached to or near bacteria. After nine days, relatively few *Ochromonas* contained bacteria, although bacteria were still present in the medium. This may be due to an inhibition of phagotrophy by the accumulation of extracellular nutrients resulting from the phagotrophic and digestive processes or extracellular digestion (Aaronson, 1973*a*), although there is no specific evidence for a mechanism.

Details of the digestion of bacteria in the food vacuole may be seen in Figs. 4 and 6.

The bacterial surface seemed to ripple and form membrane (Fig. 6) which was joined by a proliferation of membranes and vesicles from the food vacuole membrane (Fig. 6). Smaller vacuoles containing electron-dense material which may be secondary lysosomes are shown in Figs. 5 and 6. *Ochromonas* as well as bacteria were sometimes found in the same food vacuole (Fig. 5).

DISCUSSION

Ochromonas danica is capable of surviving and multiplying by phagocytizing one or several bacterial species, a yeast and as reported by Daley, Morris & Brown (1973), a blue-green alga. *Ochromonas* resembles some other protozoa which are quite unselective in their ability to use a variety of food organisms; other protozoa are more selective, able to grow and multiply on only a limited number of food organisms (Hall, 1965); some protozoa eat a variety of organisms but will select specific species if given a choice (Rapport, Berger & Reid, 1972).

The chemical composition of other protozoa grown on soluble nutrients and on bacteria has not to my knowledge been reported. *Ochromonas danica* grown photophagotrophically on *A. aerogenes* produced fewer cells because there was less energy and organic material available in the bacteria; they contained less protein and carbohydrate and more total lipids than cells grown under the same physical conditions photoheterotrophically on a chemically defined medium (Aaronson, 1972).

Few attempts have been made to study the energy metabolism of the phagotrophic process in protozoa; some have used metabolic poisons such as inhibitors of glycolysis (fluoride and iodoacetate), of aerobic respiration (cyanide and azide) and an uncoupler of oxidative phosphorylation (2,4-dinitrophenol). Cyanide, azide and Dnp, but not fluoride or iodoacetate, inhibited phagocytosis in *Acanthamoeba* sp. (Weisman & Korn, 1967). *Ochromonas danica* were more sensitive to some of these inhibitors than were the amoebae; azide and Dnp at about 10^{-5} M were very inhibitory. Iodoacetate was appreciably less inhibitory, and fluoride was not inhibitory at the tested concentrations. Hill (1972) noted that food vacuole formation in *Tetrahymena* was stopped by Dnp, but offered no data. It is apparent that the three aerobic protozoa described above depend on aerobic oxidative metabolism for energy for phagocytosis. Mammalian macrophages seem to be more variable; peritoneal macrophages tend to be facultative anaerobes while alveolar macrophages seem to be facultative aerobes (Pearsall & Weiser, 1970).

Fig. 1. Appearance of *Aerobacter aerogenes* in suspension in PMM for 2 days with no *Ochromonas* present. Note absence of membranous vesicles in medium around bacteria.

Fig. 2. *Ochromonas danica* after 2 days of incubation in a bacterial suspension. Food vacuoles at cell surface (upper right) and in cytoplasm at anterior end of organism.

Fig. 3. *Ochromonas danica* after 2 days of incubation with bacteria. Note membranous vesicles outside the *Ochromonas* or at its surface (arrows) and compressed leucosin vacuole. Large food vacuoles with many bacteria in various stages of digestion at the posterior end (right).

Fig. 4. Food vacuole with a bacterium almost completely digested; only membrane and bacterial perimeter remain.

Fig. 5. Food vacuole containing an *Ochromonas* and bacteria. Secondary lysosomes appear at top of figure.

Fig. 6. Food vacuole with a partially digested bacterium from which membranes seem to be proliferating. Secondary lysosomes are seen near the surface of the food vacuole at the top of the figure.

Symbols: C, chloroplast; F, flagellum; L, leucosin vacuole; N, nucleus.

The microscopic and ultrastructural details of bacterial phagocytosis and digestion indicate that bacteria were engulfed and food vacuoles formed in the anterior end or at the sides of the organism, and the food vacuoles migrated posteriorly behind rather than into the leucosin vacuole as claimed by Daley *et al.* (1973). Here the food vacuoles fused to form a large food vacuole at the expense of the leucosin vacuole which was compressed. The food vacuole membrane produced small vesicles and membrane, which presumably were associated with the secretion of hydrolases into the food vacuole and formed secondary lysosomes for the further cytoplasmic digestion of the material from the food vacuole. The ultrastructure of digestion showed features similar to those described in other phagocytic protozoa, including accumulation of vesicles (presumably containing hydrolytic enzymes) at the inner surface of the food vacuole, as seen in the amoeba *Pelomyxa* (Roth, 1960) and in the ciliates *Tokophrya* (Rudzinska, 1970) and *Colpoda* (Rudzinska *et al.* 1966). The digested bacteria seemed to produce large quantities of membrane which accumulated in the food vacuole, as in amoebae (Gezelius, 1959, 1961; Mercer & Shaffer, 1960; Vickerman, 1960; Schuster, 1964; Spies & Elbers, 1972), the amoeba-flagellate *Naegleria* (Schuster, 1963) and the ciliate *Colpoda* (Rudzinska *et al.* 1966).

The phagotrophic process of *O. danica* offers several features not previously described in protozoa; these include particle aggregation, fluid flow induced by the flagella carrying food organisms to the anterior end, the 'flypaper' effect of the *Ochromonas* surface, and the flow of food vacuoles to the posterior end and their fusion there into a large food vacuole independent of the leucosin vacuole.

The author is indebted to Ms U. Behrens for the electron micrographs, to L. Ardois and S. Friedman for some of the phagotrophic metabolism experiments, and to J. Bodnaruk of the Department of Chemical Engineering, City College, City University of New York, for the use of the electron microscope. This work was done with the support of grants GB7833 and GB20825 from the National Science Foundation.

REFERENCES

- AARONSON, A. (1971). The synthesis of extracellular molecules and membranes by a population of the phytoflagellate *Ochromonas danica*. *Limnology and Oceanography* **16**, 1-9.
- AARONSON, S. (1972). A new source of single-cell protein and other nutrients. *Archiv für Hydrobiologie* **41**, 108-115.
- AARONSON, S. (1973*a*). Digestion in flagellates. In *Lysosomes in Biology and Medicine*, vol. 3, pp. 18-27. Edited by J. T. Dingle. Amsterdam: North-Holland Publishing.
- AARONSON, S. (1973*b*). Particle aggregation and phagotrophy by *Ochromonas*. *Archiv für Mikrobiologie* **92**, 39-44.
- AARONSON, S. & BAKER, H. (1959). A comparative biochemical study of two species of *Ochromonas*. *Journal of Protozoology* **6**, 282-284.
- AARONSON, S., BEHRENS, U., ORNER, R. & HAINES, T. H. (1971). Ultrastructure of intracellular and extracellular vesicles, membranes, and myelin figures produced by *Ochromonas danica*. *Journal of Ultrastructure Research* **35**, 418-430.
- CHAPMAN-ANDRESEN, C. (1971). Biology of the large amoebae. *Annual Review of Microbiology* **25**, 27-48.
- DALEY, R. J., MORRIS, G. P. & BROWN, S. R. (1973). Phagotrophic ingestion of a blue-green alga by *Ochromonas*. *Journal of Protozoology* **20**, 58-61.
- ELLIOTT, A. M. & CLEMMONS, G. L. (1966). An ultrastructural study of ingestion and digestion in *Tetrahymena pyriformis*. *Journal of Protozoology* **13**, 311-323.
- GEZELIUS, K. (1959). The ultrastructure of cells and cellulose membranes in acrasiae. *Experimental Cell Research* **18**, 425-453.
- GEZELIUS, K. (1961). Further studies in the ultrastructure of acrasiae. *Experimental Cell Research* **23**, 300-310.

- HALL, R. P. (1965). *Protozoan Nutrition*, pp. 31–32. New York: Blaisdell Publishing Co.
- HARDIN, G. (1943). Flocculation of bacteria by protozoa. *Nature, London* **151**, 642.
- HILL, D. L. (1972). *The Biochemistry and Physiology of Tetrahymena*, p. 14. New York: Academic Press.
- MERCER, E. H. & SHAFFER, B. M. (1960). Electron microscopy of solitary and aggregated slime mold cells. *Journal of Biophysical and Biochemical Cytology* **7**, 353–356.
- MÜLLER, M. (1967). Digestion. In *Chemical Zoology*, vol. 1, *The Protozoa*, pp. 351–380. Edited by M. Florkin and B. T. Scheer. New York: Academic Press.
- NILSSON, J. R. (1972). Further studies on vacuole formation in *Tetrahymena pyriformis* GL. *Compte rendu des travaux du Laboratoire Carlsberg* **39**, 83–110.
- PEARSALL, N. N. & WEISER, R. S. (1970). *The Macrophage*, pp. 42–43. Philadelphia: Lea & Febiger.
- PITELKA, D. R. (1963). *Electron-microscopic Structure of Protozoa*. Oxford: Pergamon Press.
- PRINGSHEIM, E. G. (1952). On the nutrition of *Ochromonas*. *Quarterly Journal of Microscopical Science* **93**, 71–96.
- PRINGSHEIM, E. G. (1955). Über *Ochromonas danica* n. sp. und andere Arten der Gattung. *Archiv für Mikrobiologie* **23**, 181–192.
- RABINOVITCH, M. & DE STEFANO, M. J. (1971). Phagocytosis of erythrocytes by *Acanthamoeba* sp. *Experimental Cell Research* **64**, 275–284.
- RAPPORT, D. J., BERGER, J. & REID, D. B. W. (1972). Determination of food preference of *Stentor coeruleus*. *Biological Bulletin* **142**, 103–109.
- RICKETTS, T. R. (1970). Effect of endocytosis upon acid phosphatase activity of *Tetrahymena pyriformis*. *Protoplasma* **71**, 127–137.
- RICKETTS, T. R. (1971a). Periodicity of endocytosis in *Tetrahymena pyriformis*. *Protoplasma* **73**, 387–396.
- RICKETTS, T. R. (1971b). Endocytosis in *Tetrahymena pyriformis*. *Experimental Cell Research* **66**, 49–58.
- ROSENBAUM, R. M. & WITTNER, M. (1970). Ultrastructure of bacterized and axenic trophozoites of *Entamoeba histolytica* with particular reference to helical bodies. *Journal of Cell Biology* **45**, 367–382.
- ROTH, L. E. (1960). Electron microscopy of pinocytosis and food vacuoles in *Pelomyxa*. *Journal of Protozoology* **7**, 176–185.
- RUDZINSKA, M. A. (1970). The mechanism of food intake in *Tokophrya infusionum* and ultrastructural changes in food vacuoles during digestion. *Journal of Protozoology* **17**, 626–641.
- RUDZINSKA, M. A., JACKSON, G. J. & TUFFRAN, M. (1966). The fine structure of *Colpoda maupasi* with special emphasis on food vacuoles. *Journal of Protozoology* **13**, 440–459.
- SCHUSTER, F. (1963). An electron microscope study of the amoeboflagellate, *Naegleria grüberi* (Schardinger). I. The amoeboid and flagellate stages. *Journal of Protozoology* **10**, 297–313.
- SCHUSTER, F. (1964). Electron microscope observations on spore formation in the true slime mold *Didymium nigripes*. *Journal of Protozoology* **11**, 207–216.
- SCHUSTER, F. L., HERSHENOV, B. & AARONSON, S. (1968). Ultrastructural observations on aging of stationary cultures and feeding in *Ochromonas*. *Journal of Protozoology* **15**, 335–346.
- SPIES, F. & ELBERS, P. F. (1972). Axenic mass cultivation of *Entamoeba invadens* and cell membrane isolation. *Journal of Protozoology* **19**, 102–107.
- VICKERMAN, K. (1960). Patterns of cellular organization in *limax* amoebae. *Experimental Cell Research* **26**, 497–519.
- WEISMAN, R. A. & KORN, E. D. (1967). Phagocytosis of latex beads by *Acanthamoeba*. I. Biochemical properties. *Biochemistry* **6**, 485–497.
- WEISMAN, R. A. & MOORE, M. O. (1969). Bead uptake as a tool for studying differentiation in *Acanthamoeba*. *Experimental Cell Research* **54**, 17–22.