Phagocytosis and Pinocytosis in *Acanthamoeba castellanii*

By J. A. CHAMBERS AND J. E. THOMPSON

*Department of Biology, University of Waterloo,
Waterloo, Ontario, N2L 3G1, Canada*

(Received 23 January 1975; revised 10 June 1975)

SUMMARY

Endocytotic activity of *Acanthamoeba* trophozoites attenuates once the cells enter stationary phase in liquid culture. Phagocytosis, monitored by the ingestion of polystyrene latex beads, essentially ceases and the uptake of [3 H]inulin, known to be mediated by pinocytosis, is reduced by about half. The reduced pinocytotic activity of stationary-phase cells remains sensitive to respiratory inhibitors. Pre-incubation of stationary-phase cells in fresh growth medium for 1.5 h before the initiation of endocytosis has no effect on phagocytosis and only marginally increases pinocytosis. This impairment of ingestion, particularly of pinocytosis, may account for the reduced contractile vacuole activity known to characterize stationary-phase cells of this organism. The unequal responses of phagocytosis and pinocytosis to the onset of stationary-phase growth suggest that they are independent processes subject to different controls.

INTRODUCTION

Acanthamoeba castellanii is a small soil amoeba which can be grown axenically in a chemically-defined medium (Neff *et al.*, 1964). Under optimum conditions the amoebae grow exponentially with a mean generation time of 7 h to a population density of about 10^8 cells/ml. Thereafter, the growth rate progressively decreases and growth stops completely at population densities from 2×10^8 to 3.5×10^8 cells/ml (Wilkins & Thompson, 1974). Cessation of growth does not mark the initiation of encystment, for by 7 days after the termination of mitosis cultures still show less than 1 % encystment (Wilkins & Thompson, 1974). It thus appears to reflect a true stationary phase.

The transition to stationary phase is accompanied by a marked decrease in the incidence of water expulsion from the cells. Microscopic examination of contractile vacuole activity revealed that the discharge of water from stationary-phase amoebae occurs at only about half the rate characteristic of exponentially-growing cells (Pal, 1972). This reduced need to eliminate water could simply reflect a decline in the osmotic pressure of the cytoplasm. However, it could also reflect fundamental changes in those properties of the plasma membrane which regulate water flux. One such specialized property of *Acanthamoeba* is the capacity for endocytosis. The amoeba are able to phagocytize particles (Goodall & Thompson, 1971) and they take in carbohydrates and amino acids by pinocytosis (Bowers & Olszewski, 1972). We have investigated whether curtailed endocytosis gives rise to a reduced need for elimination of intracellular water in stationary-phase cells.

METHODS

Culturing. Trophozoites of *Acanthamoeba castellanii* (Neff) were cultured axenically in 2 l Erlenmeyer flasks containing 1 l medium (Schultz & Thompson, 1969). The flasks were inoculated with 2 ml of stationary-phase culture and incubated on a rotary shaker (100 rev./min) at 30 °C, for 2 days if exponentially-growing cells were required or for 7 days for stationary-phase cells.

Measurements of phagocytosis and pinocytosis. Phagocytic activity was monitored for both exponential- and stationary-phase cells by measuring the ingestion of polystyrene latex beads essentially as described by Weisman & Korn (1967), except that phagocytosis was carried out in growth medium rather than in medium containing only proteose peptone in phosphate buffer. Cells were harvested by centrifuging at 800 *g* for 2 min and then resuspended in the same medium at a population density of 3.4×10^6 /ml. Phagocytosis reaction mixtures were run in duplicate in 50 ml Erlenmeyer flasks for 30 min at 30 °C in a reciprocating water bath operating at 80 strokes/min. For each reaction, 8 ml of cell suspension and 1 ml of growth medium containing 33 mg of polystyrene latex beads (1.01 μ m diam.) were equilibrated separately at 30 °C for 15 min and then mixed in a 50 ml Erlenmeyer flask to initiate phagocytosis. At specified intervals during the reaction period 1 ml samples were withdrawn and levels of ingested latex were determined as described by Weisman & Korn (1967). The number of ingested beads was estimated by examining wet mounts of the cells with a phase-contrast microscope.

Pinocytotic activity of the amoebae was measured by monitoring the uptake of [³H]inulin (Amersham Searle) essentially by the procedure of Bowers & Olszewski (1972). Cells of either exponential- or stationary-phase cultures were harvested by centrifuging at 800 *g* for 5 min and then resuspended in the same medium at a population density of 1.2×10^6 cells/ml. Determinations were run in duplicate in 50 ml Erlenmeyer flasks for 60 min at 30 °C in a reciprocating water bath set at 80 strokes/min. For each reaction 10 ml of cell suspension and 2 ml of growth medium containing 5.6×10^7 d.p.m. of [³H]inulin (1.5×10^{15} d.p.m./mol) were equilibrated separately at 30 °C for 15 min and then mixed in a 50 ml Erlenmeyer flask to initiate pinocytosis of the labelled compound. In some of the experiments inhibitors were mixed with the cells at the beginning of the equilibration period which preceded the initiation of pinocytosis. Dinitrophenol was added at a final concentration 0.2 mM, potassium cyanide at 20 mM or sodium azide at 2 mM. At specified intervals during the incubation 2 ml samples were withdrawn and mixed with 10 ml of ice-cold 0.1 M-sodium phosphate buffer pH 6.8. The cells were sedimented by centrifuging at 1800 *g* for 2 min and washed three times with 5 ml of phosphate buffer. The final pellet of washed cells was solubilized in 0.6 ml of NCS solubilizer (Amersham Searle) overnight at room temperature. Completely solubilized samples were then quantitatively transferred to scintillation vials with 10 ml of 7 % 2,5-diphenyloxazole (PPO) in toluene and counted in a Beckman LS-2 scintillation counter. The efficiency of counting was consistently 52 to 58 %.

In some of the phagocytosis and pinocytosis experiments with stationary-phase cells the amoebae were resuspended in fresh growth medium, rather than in the medium from which they had just been harvested, and then preincubated for 1 to 4 h before the initiation of endocytosis. Such experiments were otherwise identical to those outlined above.

Fig. 1

Fig. 2

Fig. 1. Phagocytosis of polystyrene latex beads by exponential- and stationary-phase cells. The amoebae were resuspended at a density of 3.4×10^6 cells/ml in the growth medium from which they had been harvested. $\circ$, Exponential-phase cells; $\triangle$, stationary-phase cells. Standard errors of the means are indicated; $n = 4$.

Fig. 2. Pinocytosis of [^3H]inulin by exponential- and stationary-phase cells. The amoebae were resuspended at a density of 1.2×10^6 cells/ml in the growth medium from which they had been harvested. $\circ$, Exponential-phase cells; $\triangle$, stationary-phase cells. Standard errors of the means are indicated; $n = 3$ or 4.

Table 1. *The effects of respiratory inhibitors on pinocytosis of [^3H]inulin by exponential- and stationary-phase cells*

Inhibitor	Percentage inhibition after 1 h of ingestion	
	Exponential-phase cells	Stationary-phase cells
Dinitrophenol (0.2 mM)	99.0	96.8
Potassium cyanide (20 mM)	92.2	98.4
Sodium azide (2 mM)	96.9	98.6

RESULTS

Comparisons of phagocytic activity revealed that exponentially growing *Acanthamoeba* ingest latex beads as a function of time, but that this stops once the cells achieve stationary phase (Fig. 1). Dioxane extracts of stationary-phase cells incubated in the presence of latex beads contained no detectable latex. This observation was confirmed by examining wet mounts of the cells with a phase-contrast microscope at the end of the incubation period; exponential-phase cells were packed with at least 100 beads, whereas stationary-phase cells regularly contained only 2 to 8 beads/cell. The dioxane extraction procedure is presumably insensitive to such small amounts of latex.

A comparison of rates of pinocytosis for exponential- and stationary-phase cells revealed that ingestion by this mode of [^3H]inulin was reduced in the latter cells by about one-half (Fig. 2).

Phagocytosis of latex beads by this organism is 97 to 100 % inhibited by 20 mM-cyanide,

Fig. 3. The effect of resuspending stationary-phase cells in fresh growth medium on pinocytosis. ▲, Stationary-phase cells resuspended at 1.2×10^6 cells/ml in the medium from which they had been harvested; △, stationary-phase cells resuspended at 1.2×10^6 cells/ml in fresh growth medium. Pinocytosis was initiated 1.5 h after the cells had been resuspended. The solid lines and the broken lines represent two separate experiments.

2 mM-azide or 0.2 mM-dinitrophenol (Weisman & Korn, 1967). Pinocytosis by both exponential- and stationary-phase cells is susceptible to the same concentrations of these compounds (Table 1). The ingestion of [^3H]inulin was consistently reduced by 92 to 99 % in the presence of any one of the inhibitors.

Preincubation of the stationary-phase cells in fresh growth medium for up to 1.5 h before the addition of latex beads had no detectable effect on phagocytosis. By contrast such treatment consistently increased the rate of pinocytosis by about 6 % relative to controls not treated with fresh medium (Fig. 3). However, this difference, while reproducible, is probably not relevant because there was no further increase in pinocytosis when the preincubation period was extended to as much as 4 h.

DISCUSSION

Trophozoites of *A. castellanii* phagocytize bacteria in their native soil habitat (Neff, 1957). Amoebae in suspension ingest latex beads in a manner that simulates naturally-occurring phagocytosis (Weisman & Korn, 1967), and accumulate solute molecules from liquid growth medium by pinocytosis (Bowers & Olszewski, 1972). We have shown that both modes of ingestion markedly attenuate as cells in culture make the transition to stationary phase. This diminished ingestion explains previous observations of a reduced need for stationary-phase cells to discharge cytoplasmic water (Pal, 1972; Wilkins & Thompson, 1974). *Acanthamoeba* ingests liquid growth medium by pinocytosis. Were it not so, the organism, which feeds predominantly by phagocytosis in its native environment could probably not be cultured axenically in chemically-defined liquid medium. This ingestion of fluid is counterbalanced by excretion of cellular water by the contractile vacuole (Vickerman, 1962). Because the method of ingestion of latex beads virtually excludes entry of liquid medium (Weisman & Korn, 1967), it is probably the alteration in pinocytosis rather than phagocytosis that lessens the requirement of stationary-phase cells for water excretion. The reduction in contractile vacuole activity of stationary-phase cells is of much the same magnitude as the decrease in pinocytosis.

The waning endocytotic activity of stationary-phase cells appears to be more a reflexion of altered conditions within the cells than of alteration in the growth medium, since simply replenishing the medium of stationary-phase cells had no detectable stimulating influence on phagocytosis, and only marginally improved pinocytosis. Had the impairment of endocytosis been due only to an impoverished growth medium, such deficiencies should have been surmounted after a period of up to 4 h in fresh medium, particularly as stationary-phase cells retain more than 50 % of exponential-phase pinocytotic activity.

There are indications from other systems that pinocytosis and phagocytosis are basically different ingestion processes. For example, in peritoneal macrophages the two can be distinguished by their differential responses to cytochalasin B, which inhibits phagocytosis (Klaus, 1973) but has no effect on pinocytosis (Wills *et al.* 1972). However, this distinction is not definitive, for there are also reports that cytochalasin B suppresses pinocytosis (Wagner, Rosenberg & Estensen, 1971; Taylor *et al.* 1971; Allison, Davies & de Petris, 1971). A further distinction between the two modes of ingestion in macrophages is the possibility that the energy for phagocytosis is generated principally from glycolysis whereas pinocytosis is largely dependent upon energy produced by oxidative phosphorylation (Klaus, 1973). This does not appear to be universal, for in *Acanthamoeba* both pinocytosis and phagocytosis are sensitive to inhibitors of oxidative phosphorylation. Our finding that the two modes of ingestion in *Acanthamoeba* respond differently to the onset of the stationary phase in culture, supports the view that pinocytosis and phagocytosis are at least partially independent processes, subject to different controls or perhaps responding differently to the same controls.

This work was supported by a grant from the National Research Council of Canada.

REFERENCES

- ALLISON, A. C., DAVIES, P. & DE PETRIS, S. (1971). Role of contractile microfilaments in macrophage movement and endocytosis. *Nature New Biology* **232**, 153–155.
- BOWERS, B. & OLSZEWSKI, T. E. (1972). Pinocytosis in *Acanthamoeba castellanii*. *Journal of Cell Biology* **53**, 681–694.
- GOODALL, R. J. & THOMPSON, J. E. (1971). A scanning electron microscopic study of phagocytosis. *Experimental Cell Research* **64**, 1–8.
- KLAUS, G. G. (1973). Cytochalasin B. Dissociation of pinocytosis and phagocytosis by peritoneal macrophages. *Experimental Cell Research* **79**, 73–78.
- NEFF, R. J. (1957). Purification, axenic cultivation and description of a soil amoeba *Acanthamoeba* sp. *Journal of Protozoology* **4**, 176–182.
- NEFF, R. J., RAY, S. A., BENTON, W. F. & WILBORN, M. (1964). Induction of synchronous encystment in *Acanthamoeba* sp. In *Methods in Cell Physiology*, pp. 55–83. Edited by D. M. Prescott. New York: Academic Press.
- PAL, R. A. (1972). The osmoregulatory system of the amoeba *Acanthamoeba castellanii*. *Journal of Experimental Biology* **57**, 55–76.
- SCHULTZ, T. M. G. & THOMPSON, J. E. (1969). Enrichment of 5'-nucleotidase in membrane fragments isolated from *Acanthamoeba* sp. *Biochimica et biophysica acta* **193**, 203–211.
- TAYLOR, R. B., DUFFUS, P. H., RAFF, M. C. & DE PETRIS, S. (1971). Redistribution and pinocytosis of lymphocyte surface immunoglobulin molecules reduced by anti-immunoglobulin antibody. *Nature New Biology* **233**, 225–229.
- VICKERMAN, K. (1962). Patterns of cellular organization in limax amoebae. *Experimental Cell Research* **26**, 497–519.
- WAGNER, R., ROSENBERG, M. & ESTENSEN, R. (1971). Endocytosis in Chang liver cells. *Journal of Cell Biology* **50**, 804–817.
- WEISMAN, R. A. & KORN, E. D. (1967). Phagocytosis of latex beads by *Acanthamoeba*. I. Biochemical properties. *Biochemistry* **6**, 485–497.
- WILKINS, J. A. & THOMPSON, J. E. (1974). The effects of cell population density on the plasma membrane of *Acanthamoeba castellanii*. *Experimental Cell Research* **89**, 143–153.
- WILLS, E. J., DAVIES, P., ALLISON, A. C. & HASWELL, A. D. (1972). *Nature New Biology* **240**, 58–60.