Binding Sites for Bacterial Flagella at the Surface of the Soil Amoeba *Acanthamoeba*

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(Received 22 September 1983; revised 7 December 1983)

Acanthamoeba castellanii (CCAP 1534/3) was found to bind avidly the common soil bacterium *Pseudomonas fluorescens*. This adhesion was mediated not by pili nor by the general bacterial surface but by the polar flagella. Because of the nature of the flagellar rotary motor, the cell bodies of the attached bacteria could be seen rotating clearly. While initially bacterial binding occurred uniformly over the cell membrane of *Acanthamoeba*, the bacteria were soon swept posteriorly to form a cap and either endocytosed or sloughed off, still agglutinated by their flagella. Such capped amoebae would not bind *Pseudomonas* if challenged immediately, indicating a depletion of flagella-binding sites. The bacteria could not bind to amoebae pre-treated with concanavalin A (Con A) even after the lectin had been capped to the uroid. However, capping of flagella-binding sites did not co-cap all the Con A-binding sites on the surface of the amoeba. The flagella-binding sites were not affected by pre-treatment with Pronase (1 mg ml⁻¹) or anti-*Acanthamoeba* surface antibody. *Proteus mirabilis* also bound avidly by its flagella to *Acanthamoeba* and, furthermore, competition experiments suggested that *Proteus* and *Pseudomonas* adhere to a common surface site on the amoeba. The presence of sites on the cell membrane of *A. castellanii* that are specific for flagellin would enhance strongly the adsorption of motile bacteria prior to endocytosis. This would represent an excellent feeding strategy for a soil-dwelling phagotroph.

INTRODUCTION

It is widely appreciated that in metazoan organisms cell surfaces play a key role in molecular recognition phenomena that occur, for example, during embryogenesis. Amoeboid Protozoa, with rare exceptions (*Dictyostelium*, for example), do not indulge in social behaviour and so any surface recognition ability they might have would be expected to be directed towards their feeding behaviour. Although some amoebae may be expressly carnivorous (*Amoeba*, *Chaos*) and others herbivorous (*Pelomyxa*) nothing is known of the mechanism of this selectivity. In certain circumstances the control of food preferences may have considerable repercussions on the stability of a host–parasite relationship, e.g. in previously asymptomatic human infections with *Entamoeba histolytica* a switch in the diet of the trophozoites from gut flora to host cells precipitates the onset of amoebic dysentery.

Ray (1951) described the ability of *Hartmannella* (= *Acanthamoeba*) to accumulate large numbers of motile bacteria at its periphery. These agglutinated bacteria were endocytosed after being swept backwards into what she termed a ‘cap’. The wider significance of this finding was not appreciated, and it is ironic that it was 20 years later that what is generally considered to be the first description of ‘capping’ was made. This was an account of an analogous process, namely the redistribution of externally cross-linked cell-surface receptors on mammalian lymphocytes (Taylor *et al.*, 1971).

We have investigated the nature of this binding property displayed by *Acanthamoeba* not only for its intrinsic interest but also because there is currently much discussion about the adherence of bacteria to eukaryotic cells (see Beachey, 1980 for a recent review). In many instances the

Abbreviations: Con A, concanavalin A; FITC, fluorescein isothiocyanate.

adhesiveness of bacteria is conferred by the presence of filamentous, proteinaceous appendages termed pili. There is persuasive evidence that these pili can recognize particular chemical groups on the surface, for example, of epithelial cells and thus, through a stereospecific interaction, secure adherence to the eukaryote as a first step in colonization. It is known that the pili of *Escherichia coli* display lectin-like behaviour towards D-mannose (Ofek *et al.*, 1977). On the other hand, there is little information concerning bacterial adhesion to biological surfaces by means of their flagella alone, although some strains of *E. coli* carrying mutations in the structural gene for flagellin (*hag*) produce flagella which show an increased stickiness to glass (Icho & Iino, 1978).

In this paper we show that several species of *Acanthamoeba* have the capacity to bind motile bacteria to their surface by means of the bacterial flagella.

METHODS

Protozoa. Samples of the following stocks of amoebae were obtained from the Culture Centre of Algae and Protozoa, Cambridge, UK: *Acanthamoeba astronyxis* (1534/1), *A. castellanii* (1534/3) and (1501/1), *Dictyostelium mucoroides* (1515/1), *Naegleria gruberi* (1518/1c), *Paratetramitus jugosus* (1588/3f) and *Tetramitus rostratus* (1581/1). *Acanthamoeba* spp. were grown axenically as monolayers in tissue culture flasks at 25 °C in a liquid medium (Korn, 1963). Cultures were harvested in the exponential growth phase. First unattached cells were removed by decanting the growth medium. Adhesive cells were then removed into 50 mM-NaCl by vigorous shaking of the flask and then concentrated by gentle centrifugation. After a repeat of this cycle, the amoebae were resuspended in 50 mM-NaCl at 10^7 cells ml⁻¹. The other amoebae were maintained at 25 °C in monoxenic culture with *Escherichia coli* on agar plates and harvested free from bacteria as described previously (Preston & King, 1978).

Bacteria. *Pseudomonas fluorescens* RIII⁶ was isolated from soil cores taken from the grounds of King's College field station, Rogate, W. Sussex, UK. Small crumbs of soil were incubated in 10 ml sodium benzoate medium (5 g NaCl, 0.2 g MgSO₄, 1 g (NH₄)₂HPO₄ and 2 g sodium benzoate l⁻¹, pH 6.8) at 25 °C for several days. Samples from any turbid supernatants were streaked onto King-Ward-Raney agar plates (20 g peptone, 1.5 g K₂HPO₄, 1.5 g MgSO₄ · 7H₂O, 10 g sodium succinate and 15 g agar l⁻¹). *Ps. fluorescens* colonies were identified by their yellow coloration and UV-excited fluorescence. Clones prepared from such colonies were subsequently maintained on nutrient agar plates. Bacteria used were from plates less than 24 h old to measure maximum motility.

Stocks of *Proteus mirabilis* and *Rhodospirillum rubrum* were generously provided by Dr D. G. Smith (Department of Botany and Microbiology, University College, London, UK). *Pr. mirabilis* swimmers were isolated by transferring cells from an established colony to the centre of a fresh nutrient agar plate with a wire loop. The swimmers, which by 24 h had migrated out radially from the central colony to a distance of 3–4 cm, could be harvested free of short forms.

Lectin and antisera. Native and fluorescein (FITC)-labelled concanavalin A (Con A) was obtained from Miles Laboratories, Stoke Poges, Slough, UK, and used at a final concentration of 100 µg ml⁻¹ with 5×10^6 cells.

Antisera to *A. castellanii* 1534/3 were prepared by intramuscular injection of suspensions of formalin-treated amoebae in Freund's complete adjuvant into rabbits with a booster dose given three weeks later. The antiserum from one rabbit (T101) had activity directed against the surface of *A. castellanii* 1534/3, as judged by immunofluorescence and by immunodiffusion tests against both water soluble and Triton X-100 extracts of unfixed amoebae. This crude antiserum was subjected to ammonium sulphate fractionation to yield the γ-globulin component. This fraction was used as the source of anti-*Acanthamoeba* antibody in the experiments reported below.

Tetramethyl rhodamine isothiocyanate-labelled rabbit antiserum to Con A was obtained from Nordic Immunological Laboratories, Maidenhead, UK. IgG fractions of goat antiserum to rabbit IgG, labelled with FITC, were obtained from Miles Laboratories.

Electron microscopy. Amoebae suspended in 50 mM-NaCl were allowed to settle for 20 min on detergent-cleaned coverslips in a humid chamber. Small amounts of a suspension of *Ps. fluorescens* in 50 mM-NaCl freshly harvested from nutrient agar plates were added to these amoebae; this was taken to be time zero. The first group of coverslips was immersed in a dialdehyde fixative at room temperature after 3 min and then the second after 20 min. The dialdehyde fixative had the following composition: 1% (w/v) glutaraldehyde, 2% (w/v) freshly prepared formaldehyde, and 2 mM-CaCl₂ in 10 mM-HEPES buffer at pH 7.3. After 15 min the coverslips in fixative were transferred to 3 °C for a further 2 h, then washed thoroughly in ice cold 10 mM-HEPES buffer at pH 7.3 and post-fixed in 1% (w/v) OsO₄ in the same buffer at 3 °C for 1 h.

Samples for scanning electron microscopy were then dehydrated directly through a graded series of ethanol/water mixtures before critical point drying. These specimens were coated with carbon then gold before

examination in a Cambridge S4-10 microscope at 10 or 30 kV. Ilford FP4 emulsion was used for photographic records.

Following postfixation in OsO_4 , samples to be processed for transmission electron microscopy were immersed in 0.5% (w/v) aqueous uranyl acetate for 30 min before dehydration through ethanol/water mixtures. After three changes in absolute ethanol the specimens were transferred via a 1:1 acetone/Araldite mixture to complete Araldite resin and then left overnight at 25 °C. The next day coverslips were transferred to fresh resin for embedding. Coverslips were removed from the polymerized blocks after these had been softened by plunging into boiling water. Thin sections of chosen groups of cells were cut parallel to the original planar glass substrate on a diamond knife mounted on a Huxley ultramicrotome. Sections collected on uncoated copper grids were stained in lead citrate and then examined in an AEI EM6B microscope operated at an accelerating voltage of 60 kV with an objective aperture of 50 μm . Images were recorded on Kodak EM4489 emulsion.

Light microscopy. Phase-contrast and fluorescence microscopy was done on a Leitz Orthoplan stand fitted routinely with Zeiss objective lenses. Photographic records were made on Kodak Tri-X or Ektachrome 400 emulsion. Recordings of cell behaviour were made by means of a videocamera connected to an inverted Zeiss Ultraphot stand and linked to a videorecorder and TV monitor (Preston & King, 1978).

Surface labelling of cells. The following procedure was adopted as a standard for labelling *Acanthamoeba* with antibody or lectin. Cell suspension (0.5 ml, 10^7 cells ml^{-1}) in 50 mM-NaCl was pipetted into a plastic centrifuge tube held in an ice bucket. Antibody or lectin was then added together with enough saline to bring the final volume to 1 ml. After 30 min incubation the cells were gently pelleted and washed twice with ice cold saline and finally resuspended in 0.5 ml of this solution. Where second layer labelling was required these resuspended cells were incubated on ice for a further 30 min with the requisite ligand, then washed thoroughly before resuspension and microscopic examination. Tests by fluorescence microscopy for non-specific absorption of rabbit or goat γ -globulin at the surface of *Acanthamoeba* proved negative.

The availability of Con A-binding sites at the cell surface of *Acanthamoeba* following adsorption of bacteria was tested as follows. Cells settled in plastic dishes were challenged with a suspension of *Ps. fluorescens* in 50 mM-NaCl and then fixed some 20 min later with 2% (w/v) formaldehyde. After extensive washing in 1% (w/v) glycine in 50 mM-NaCl, to block any reactive aldehyde groups introduced by the fixative, these substrate-attached amoebae were treated with FITC-Con A for 30 min, washed thoroughly with 50 mM-NaCl and then examined with a X40 Zeiss phase-contrast water-immersion objective lens.

RESULTS

Light microscopy

When a suspension of freshly harvested *Ps. fluorescens* was added to *A. castellanii* 1534/3 cells that had been allowed to settle for 15 min on a clean coverslip in 50 mM-NaCl, the actively swimming bacteria stuck to the amoebae on colliding with them. Within a few minutes these amoebae were entirely covered with bacteria. On close inspection the bacteria were seen to be attached solely by their polar flagella and did not appear to be stuck to each other. Since their flagella were tethered to the surface of the amoebae, the bacterial cell bodies rotated producing a jostling monolayer around each amoeba. Although they remained attached to the substratum, many amoebae had rounded up from their previous extended locomotory shape at this stage. Subsequently, the uniform bacterial layer became redistributed as each protozoon recommenced locomotion. The anterior (leading) edge of an amoeba was cleared of bacteria first, and the bound bacteria became increasingly concentrated in the posterior (uroid) region as a well-defined cap. Such capped amoebae when challenged with fresh bacteria were found to bind few (if any) of the added bacteria. By this time it was possible to see within these moving amoebae phagosomes containing varying numbers of *Ps. fluorescens*. However, not all the bacterial aggregates were endocytosed and some were shed from the uroid into the medium.

If the fixed *Acanthamoeba* were substituted for live cells bacterial binding still took place to form a uniform layer around each protozoon, but neither redistribution into a cap nor endocytosis took place.

This pattern of behaviour with *Ps. fluorescens* was not confined to *A. castellanii* 1534/3 but was demonstrated by each of the *Acanthamoeba* stocks used. On the other hand, there was no evidence of flagellar binding of this bacterium to the surface of other common soil amoebae such as *Naegleria*, *Tetramitus*, *Paratetramitus* and *Dictyostelium*.

The surface agglutination phenomenon displayed by *Acanthamoeba* was not specific for *Pseudomonas* alone since actively motile forms of the unrelated bacteria *Proteus mirabilis* and

Fig. 1. (a) Bright field micrograph of a suspension of *Acanthamoeba* cells in 50 mM-NaCl agglutinated by the addition of *Rhodospirillum rubrum*. No significant agglutination occurred in controls without *R. rubrum*. Magnification approx. $\times 350$. (b-d) Phase-contrast micrographs of stages in the process of polar binding of *R. rubrum* to the surface of *Acanthamoeba* and the subsequent endocytosis of the prokaryotes. Phagosomes containing single bacteria are particularly clear in (d). Magnification approx. $\times 1070$.

Fig. 2. (a) Scanning electron micrograph demonstrating the ease with which the peritrichous swarm cells of *Proteus mirabilis* can cross-link locomoting amoebae. Magnification approx. $\times 1800$. (b) Scanning electron micrograph showing two *Pr. mirabilis* swarm cells tightly bound to, and following the surface contours of, an individual *Acanthamoeba*. Magnification approx. $\times 7560$.

Rhodospirillum rubrum were captured with equal facility and subsequently capped. Unlike *Pseudomonas*, *Rhodospirillum* bears flagella at both poles, and it was therefore able to cause agglutination of amoebae when added to *Acanthamoeba* in suspension (Fig. 1a). *Proteus*, in contrast to *Pseudomonas* and *Rhodospirillum*, is peritrichous and was therefore not restricted simply to polar attachments on collision with *Acanthamoeba*. Indeed any region of the *Proteus* cell was able to attach to the surface of the amoeba (see Fig. 2b). In some cases a single bacterium was attached at both ends to an individual amoeba resulting in the bacterium being held in an arc-like position. In other cases it was found that one bacterial swarm cell could cross-link two amoebae (see Fig. 2a).

Whereas *Acanthamoeba* had no difficulty in endocytosing *Pseudomonas* it could engulf only the smallest representatives of populations of *Rhodospirillum* (Fig. 1b-d) and *Proteus*.

Electron microscopy

The general morphological features of the bacterial interaction with *Acanthamoeba* inferred from phase-contrast microscopy were borne out by scanning and transmission electron microscopy. Scanning electron microscopy of *Acanthamoeba* fixed 3 min after exposure to *Ps. fluorescens* revealed the initial high density of bacterial packing at the surface of the amoeba and confirmed the lack of anything but polar adhesion of the prokaryote cells (Fig. 3a). In preparations of *Acanthamoeba* fixed 20 min after exposure to bacteria, the majority of amoeba had already resumed locomotion and had redistributed bound pseudomonads into a posterior cap (Fig. 3b). There was no evidence for additional bacteria being bound anteriorly on these cells.

Despite the strength of the bacterial adhesion to *Acanthamoeba* there was always a large gap between the cell body of *Pseudomonas* and the plasmalemma of the protozoon (Fig. 4a). Only bacterial flagella were to be seen making direct contact with the cell surface of *Acanthamoeba* (Fig. 4b).

Experimental treatments aimed at blocking bacterial binding to *A. castellanii* 1534/3 amoebae

Use of anti-Acanthamoeba antibody. Amoebae treated with T101 (anti-*Acanthamoeba*) antibody followed by FITC-labelled goat anti-rabbit IgG demonstrated strong surface fluorescence. This fluorescence was originally uniform, becoming redistributed into patches which were then capped as the cells recommenced locomotion. When amoebae were labelled with this anti-*Acanthamoeba* antibody and then challenged with *Ps. fluorescens*, the bacteria attached by their flagella to the surface of the amoeba on collision. Bacteria were seen to bind on both patched and cleared areas of *Acanthamoeba* surface membrane.

Use of the lectin Con A. Amoebae exposed in the cold to FITC-Con A were uniformly coated by this lectin. If these cells were immediately challenged with suspensions of *Ps. fluorescens* the bacteria did not stick to the amoebae in spite of making numerous collisions. Even when the bacterial challenge was delayed until after the bound lectin had been capped with a second layer antibody directed against Con A, flagellar binding was still inhibited. When amoebae labelled with FITC-Con A were incubated in the corresponding hapten (methyl α -D-mannopyranoside) at 50 mM final concentration the fluorescent lectin was rapidly lost. We had previously established that at this concentration the sugar had no effect on bacterial binding to untreated *Acanthamoeba*. Therefore, immediately after amoebae had been surface-labelled with FITC-Con A they were challenged with *Ps. fluorescens* in the presence of competing sugar. Only those amoebae which had lost their fluorescence were able to bind the bacteria in an optimal manner.

A reciprocal experiment was carried out in which amoebae were challenged first with *Ps. fluorescens* and then tested to see if they could still bind FITC-Con A. Even though they had bound and, in many cases, successfully capped bacteria, the amoebae were still able to take up FITC-Con A uniformly over the surface.

In order to check whether the peripheral binding sites for bacterial flagella on *A. castellanii* 1534/3 contained exposed protein, amoebae were preincubated in Pronase before challenge with either *Ps. fluorescens* or FITC-Con A. Such proteolytic treatment did not remove the ability to bind bacteria or lectin.

Fig. 3. (a) Scanning electron micrograph of a rounded up *Acanthamoeba* with numerous *Pseudomonas fluorescens* agglutinated over its surface. The bacteria are clearly bound to the protozoon by one pole; no flagella are visible at the pole distal to the point of attachment. (b) Scanning electron micrograph of an amoeba which has resumed locomotion after having agglutinated *Ps. fluorescens* at its surface and subsequently redistributed them posteriorly into a cap at the uroid. Anterior to the cap the surface of the amoeba is free of bacteria while numerous dorsal acanthopodia are evident. Magnification approx. $\times 2988$.

Fig. 4. (a) Transmission electron micrograph of a thin section showing the considerable gap between the cell body of a trapped bacterium (*Ps. fluorescens*) and the surface of an *Acanthamoeba*. Bar, 0.25 μ m. (b) Micrograph showing a typical arrangement of flagellar filaments of bacteria attached to the surface membrane of *Acanthamoeba* (arrowed). Bar, 0.1 μ m.

DISCUSSION

The experiments reported here confirm the original report (Ray, 1951) of surface agglutination and subsequent redistribution of motile bacteria by *Hartmanella* (= *Acanthamoeba*), extend our understanding of the bacteria-amoeba interaction, and suggest the presence of surface membrane sites on amoebae which are specific for the binding of bacterial flagella. *Pseudomonas fluorescens* attaches to *Acanthamoeba* only by one pole, and then exhibits active rotation of its body in the same manner as *E. coli* tethered to a glass slide coated with specific antibody to flagellin (Silverman & Simon, 1974). This suggests strongly that adhesion is mediated via the prokaryote flagellum. Our electron microscope observations are in accord with this interpretation and also indicate that adhesion may not be confined to the flagellar tip. It should be noted that the phage $\phi\chi_7$, which attacks many motile strains of the *Proteus*-

Providence group of bacteria, initially adsorbs to the bacterial flagella but shows no restriction with regard to the region of binding (Appelbaum *et al.*, 1971).

All three stocks of *Acanthamoeba* tested bound motile bacteria equally well, suggesting that the amoebae display common components at their periphery. This was not unexpected because the most thorough immunological analysis of the plasma membranes of several *Acanthamoeba* stocks and species has shown, despite considerable heterogeneity of their surface antigens, that some cross-reaction does exist (Stevens *et al.*, 1977).

The binding of bacteria to previously fixed *Acanthamoeba* demonstrates that agglutination is not dependent on the metabolic activity of the protozoon. The persistence of agglutination of *Pseudomonas* in sloughed caps might be explained by the following. (a) Involvement of cytoskeletal proteins from the amoeba. There is good evidence from *Dictyostelium* (Condeelis, 1979) that cortical actomyosin tightly bound to the plasmalemma concentrates into the posterior caps induced by Con A-receptor complexes. Thus it is possible that such cytoskeletal elements could explain the persistence of sloughed bacterial caps shown here in *Acanthamoeba*. (b) Interactions occurring at the uroid between exocytosed vesicles and the bacteria. This situation is commonly found in caps of cationized ferritin sloughed from the uroid of the limax amoeba *Naegleria* (King & Preston, 1977). (c) Production of agglutinins by *Pseudomonas* leading to the cross-linking of bacteria (Gilboa-Garber, 1972).

The failure of capped amoebae to agglutinate further bacteria of the same or different species at their cleared leading edge suggests (i) that the surface binding sites for flagella are saturable and (ii) that there is a common flagella-binding site for all bacteria.

Since binding of motile bacteria is not affected by preincubation of *Acanthamoeba* in the broad spectrum protease Pronase but is inhibited by Con A, this suggests that the prokaryote flagella interact with carbohydrates, not protein, at the surface of the amoeba. In contrast to Con A, a polyclonal antibody, raised against whole, formalin-fixed *A. castellanii* 1534/3 and possessing activity directed against the amoebal surface, failed to block bacterial binding. Furthermore, amoebae with Con A sites capped before exposure to bacteria were then unable to bind bacteria on challenge. On the other hand, Con A was able to interact with amoebae whose bacterial flagella-binding sites had been capped. The implication therefore is that there is indeed some correspondence between *Acanthamoeba* surface binding sites for Con A and those for bacterial flagella. The simplest explanation is that the Con A-binding sites are heterogeneous and that just one class of these has the additional ability to entrap bacterial flagella.

The plasmalemma of *Acanthamoeba* is relatively simple in macromolecular composition (see Thompson & Pauls, 1980 for a recent review) with one major polypeptide accounting for 40–50% of total membrane protein and two carbohydrate-containing molecules – lipophosphoglycans (Korn & Wright, 1973). These two inositol-containing glycosphingolipids have a different sugar composition but are otherwise similar and together constitute about one-third of the plasmalemma mass (Dearborn *et al.*, 1976). There are no glycoproteins in the plasmalemma. Consequently the acidic carbohydrates visualized at the surface of intact cells (and on both faces of isolated plasmalemmas) by electron microscope cytochemistry (Bowers & Korn, 1974) suggest that some part of the lipophosphoglycan molecules is exposed to the exterior.

Bacterial flagellar filaments are considered to be composed entirely of a single protein – flagellin (Smith, 1982). As yet there is no evidence of accessory carbohydrate along the filament and we were unable to induce flagellar agglutination of *Pseudomonas* with Con A. From this it seems likely that adsorption of bacteria to the surface of *Acanthamoeba* is due to an interaction between the protein flagellin and some carbohydrate residues of amoebal lipophosphoglycan. Up to now there has been no clear function ascribed to these lipophosphoglycans in spite of their abundance in the plasmalemma. The implication of at least some of them in food capture by stereospecific interaction with a prey macromolecule would bear out an earlier suggestion (Dearborn & Korn, 1974) that these membrane components might serve functions similar to those played by glycoproteins and glycolipids at metazoan cell surfaces. There is indeed evidence that the cell surface carbohydrates of eukaryotes play a central role in recognition phenomena (Berger *et al.*, 1982).

Since the bacterial flagellum is a linear polymer of flagellin, one filament could readily

interact with several independent binding sites on the *Acanthamoeba* cell. In so doing the prokaryote would effectively cross-link these receptors and fulfil the preliminary conditions required for patching and endocytosis either immediately or subsequently at the uroid following cap formation.

It is of interest that other common naked amoebae, *Naegleria*, *Tetramitus*, *Dictyostelium* and *Paratetramitus*, often occupy the same soil ecosystem as *Acanthamoeba*. Although these organisms do not present an agglutinating surface to bacterial flagella, they nonetheless feed avidly on these microbes. The adhesive 'fly paper' method of capturing food in an aqueous environment is by its very nature non-selective. *Ps. fluorescens*, while providing an excellent model for studying flagellar adhesion to *Acanthamoeba*, will not support, unlike *E. coli*, the growth of *A. castellanii* 1534/3 on agar plates. *Acanthamoeba castellanii* has been isolated from soil and freshwater samples as a predator of both unicellular and filamentous cyanobacteria (Wright *et al.*, 1981). This clearly indicates that *in vivo* this protozoon does not feed exclusively on flagellated prokaryotes and that it may deploy a repertoire of surface recognition properties for use in feeding.

Although many classical studies on endocytosis have been carried out on the phagocytosis of latex beads by *Acanthamoeba* (Weismann & Korn, 1967), little work has been done on the possible specificity in the endocytotic reactions. However, Brown *et al.* (1975) proposed that *Acanthamoeba* might bind potential food organisms by means of an undefined surface lectin which could recognize specific carbohydrate residues in their prey.

The range of surface binding properties of *Acanthamoeba* and their expression as a function of environmental factors warrants further study, for some species are able to become opportunistic pathogens in man causing meningoencephalitis (*A. culbertsoni*; Culbertson, 1971) and keratitis (*A. polyphaga*; Visvesvara *et al.*, 1975).

We wish to thank L. G. Cooper for excellent technical assistance. SEM services were provided by the University of London Board of Studies in Zoology. This work was supported by a SERC grant to T. M. P.

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