Sucrose Uptake by Pinocytosis in *Amoeba proteus* and the Influence of External Calcium

ROBERT D. PRUSCH and JO ANN HANNAFIN

From the Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912 and the Department of Biological Sciences, Dartmouth College, Hanover, New Hampshire 03755. Dr. Prusch's present address is Department of Biology, Rhode Island College, Providence, Rhode Island 02908; Ms. Hannafin's present address is Department of Biological Sciences, Dartmouth College.

ABSTRACT   The relationship between Ca^{++} and pinocytosis was investigated in *Amoeba proteus*. Pinocytosis was induced with 0.01% alcian blue, a large molecular weight dye which binds irreversibly to the cell surface. The time-course and intensity of pinocytosis was monitored by following the uptake of [^{3}H]sucrose. When the cells are exposed to 0.01% alcian blue, there is an immediate uptake of sucrose. The cells take up $\sim$ 10% of their initial volume during the time-course of pinocytosis. The duration of pinocytosis in the amoeba is $\sim$50 min, with maximum sucrose uptake occurring 15 min after the induction of pinocytosis. The pinocytotic uptake of sucrose is reversibly blocked at 3°C and a decrease in pH increases the uptake of sucrose by pinocytosis. The process of pinocytosis is also dependent upon the concentration of the inducer in the external medium. The association between Ca^{++} and pinocytosis in *A. proteus* was investigated initially by determining the effect of the external Ca^{++} concentration on sucrose uptake induced by alcian blue. In Ca^{++}-free medium, no sucrose uptake is observed in the presence of 0.01% alcian blue. As the Ca^{++} concentration is increased, up to a maximum of 0.1 mM, pinocytotic sucrose uptake is also increased. Increases in the external Ca^{++} concentration above 0.1 mM brings about a decrease in sucrose uptake. Further investigations into the association between Ca^{++} and pinocytosis demonstrated that the inducer of pinocytosis displaces surface calcium in the amoeba. It is suggested that Ca^{++} is involved in two separate stages in the process of pinocytosis; an initial displacement of surface calcium by the inducer which may increase the permeability of the membrane to solutes and a subsequent Ca^{++} influx bringing about localized increases in cytoplasmic Ca^{++} ion activity.

INTRODUCTION

The process of pinocytosis, as originally described in mammalian macrophages by Lewis (1931), involves the uptake of both extracellular fluid and solute particles by a process of membrane infolding as opposed to carrier-mediated

transport Processes or diffusion. This basic cellular process has been observed in one form or another in practically all cell types which have been examined (Silverstein et al., 1977). Mast and Doyle (1934) first described pinocytosis in freshwater amoeba and a considerable amount of work has been done since then with these cells in an attempt to elucidate the underlying causative mechanism of pinocytosis.

The first step in the initiation of pinocytosis is the binding of the pinocytotic inducer to the cell surface (Brandt, 1958; Schumaker, 1958). A great number of substances elicit pinocytosis in the amoeba and these have been broken down into three different categories essentially on the basis of how well they bind to the surface of the amoeba (Chapman-Andresen, 1962). The feature that these diverse inducers have in common is that they must possess a net positive charge. Addition of an inducer to the external medium of the amoeba brings about an inhibition of streaming and amoeboid movement (Nachmias, 1968), development of surface projections, and the generation of channels leading from the tips of these surface projections into the cytoplasm. Vesicles are then "pinched" off the cytoplasmic end of these channels, and the contents of these vesicles are incorporated into the cytoplasm.

On the basis of previous investigations, it is becoming increasingly apparent that the Ca^{++} ion is involved in some stage(s) of pinocytosis. For example, a minimal amount of external calcium is necessary for channel formation, and elevated external levels of Ca^{++} increase the amount of inducer which must be added to the external medium in order to elicit pinocytosis (Josefsson, 1968; Josefsson et al., 1975). In addition, external Ca^{++} controls the overall solute permeability of the plasmalemma of *Amoeba proteus* (Prusch and Dunham, 1972). Based on this and other indirect evidence, several investigators have suggested that Ca^{++} movements are associated with pinocytosis in amoeba (Cooper, 1968; Josefsson, 1976; Prusch, 1977). Allison and Davies (1974) have also implicated the Ca^{++} ion in both endocytosis and exocytosis in a variety of cell systems. This study was undertaken to investigate more directly the relationship between Ca^{++} and pinocytosis in *Amoeba proteus.*

MATERIALS AND METHODS

The copper phthalocyanine dye, alcian blue (Polysciences, Inc., Warrington, Pa.) was used throughout this study to induce pinocytosis in *Amoeba proteus.* Alcian blue is a relatively high molecular weight dye (~1,300) with a net charge of +4 (Scott et al., 1964), the co-ion in this case being $SO_4^=$. The advantages of using alcian blue are that it induces pinocytosis at very low concentrations (on the order of 8 × 10^{-5} M) and binds irreversibly to the surface of the amoeba (Chapman-Andresen, 1962). Unless otherwise indicated, 0.01% (0.08 mM) alcian blue was used throughout this study to induce pinocytosis in *Amoeba proteus.* This eliminated the problem of significant changes in external osmolality and ionic strength encountered with the use of some other types of inducers and permits experimental manipulation of the cell without removing the inducer from the cell surface. An additional advantage in using alcian blue as a pinocytotic inducer is that it allows for easy visualization of the pinocytotic process.

Quantification of the process of pinocytosis in the past has relied for the most part on observations of channel numbers in amoebae (Chapman-Andresen, 1962). That is,

when an inducer of pinocytosis is added to the external medium, channels form from the surface of the cell into the cytoplasm. These channels can be observed with phase contrast light microscopy in living cells, and apparently the intensity or degree of pinocytosis is positively correlated with the number of channels. There are several problems with this technique for quantitating pinocytosis. First of all, a certain amount of subjectivity is associated with simply counting channels observed through a microscope and secondly, and more seriously, even though a pinocytotic channel may have been formed by the cell in response to a specific inducer, this does not necessarily imply that the observed channel is functional, i.e., participating in the uptake of external solute. In addition, the technique of counting channels in cells is not feasible in experiments involving large numbers of cells.

In this investigation of pinocytosis in *Amoeba proteus*, duration and intensity of pinocytosis was determined by observing [³H]sucrose uptake (New England Nuclear, Boston, Mass.). Under control conditions, the cell surface is impermeable to sucrose and sucrose itself does not induce pinocytosis (Chapman-Andresen and Holter, 1955). Sucrose uptake was monitored by adding [³H]sucrose to a suspension of cells (2 μCi/ ml) which contained 1 mM unlabelled sucrose in the control medium (Prescott and James, 1955). Cell aliquots were taken at various time intervals, before and after the induction of pinocytosis with alcian blue, and the amount of sucrose taken up was determined. Inasmuch as sample volume and thus the approximate number of cells per sample were constant throughout each experiment, changes in the wet weight of the packed cell pellets were at least roughly proportional to changes in cell volume during various experimental procedures (Prusch and Dunham, 1972). The Ca^{++} content of the external medium under these various experimental conditions was measured with an Eppendorf flame photometer (Eppendorf Gerätebau, Netheler & Hinz GmbH, Hamburg, West Germany). The cells were cultured and harvested and isotopic exchange was followed as described previously (Prusch and Hannafin, 1979). Unless otherwise indicated, experiments were performed at room temperature and the results are presented as the mean ± standard error of the mean and the number of determinations.

RESULTS

Light Microscope Observations

Amoeba proteus was observed at ×200 using a Wild microscope with phase contrast optics (Wild Heerbrugg Instruments Inc., Farmingdale, N. Y.). Since these cells had been starved for 24 h before any experimental procedures were performed, the amoebae were actively streaming and, for the most part, were in the monopodial configuration. Application of alcian blue to the external medium (0.01%) brought about an almost immediate cessation of cytoplasmic streaming, generally within 1–2 min after the addition of the inducer. At the same time, the blue dye can be seen to accumulate on the surface of the cells, although the distribution of the stain is not uniform over the entire cell surface. Channel formation is observed within 5–10 min after the initiation of pinocytosis; the cells during this period assume a roughly spherical shape. Within 15 min, the interior accumulation of the blue dye can be observed. After 30 min, the cells are still roughly spherical in shape with a great number of surface projections and a large central contractile vacuole is present in almost all of the cells. If the cells are only briefly exposed to alcian blue and

are then washed in fresh control medium, they go through the same sequence of events and begin to stream actively 60 min after the initial dye exposure and otherwise appear normal.

Pinocytosis

The process of pinocytosis involves the uptake of the inducer, which binds to the cell surface, and any other solute present in the external medium which is nonselectively accumulated as the external medium is engulfed. In this study, pinocytosis was induced with 0.01% alcian blue in the external medium and followed by monitoring the uptake of [^{3}H]sucrose. That is, the bulk or fluid-phase of pinocytosis is being followed in the amoeba and it is assumed at the present time that factors influencing fluid-phase pinocytosis will also influence
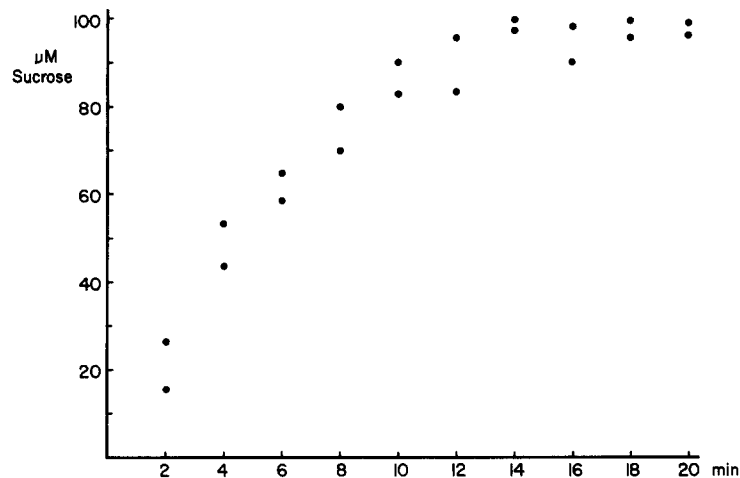

FIGURE 1.   Pinocytotic uptake of sucrose by *Amoeba proteus*. Cells were equilibrated in control medium (0.03 mM Ca^{++}, pH 6.5) with 1 mM sucrose and [^{3}H] sucrose (2 μCi/ml); pinocytosis was initiated by adding alcian blue (0.01%) to the external medium at t = 0, each point represents a single determination from one experiment.

at least qualitatively adsorptive pinocytosis. Before the induction of pinocytosis, the cells were equilibrated for variable time periods in control medium with 1 mM sucrose and [^{3}H]sucrose. Although the cell surface of *Amoeba proteus* is impermeable to sucrose, a background level of labelled sucrose was found to be associated with the cell pellets. This represented a measurement of the extracellular space of the cell pellets, which under these conditions was 27 ± 0.79 (17) % of the total pellet weight. The amount of sucrose taken up subsequently by pinocytosis was corrected for this background or extracellular sucrose.

Sucrose uptake induced by alcian blue, as is shown in Fig. 1, begins immediately after the addition of the inducer. When the external level of sucrose is 1 mM, pinocytotic sucrose uptake is ∿100 μM after 10–15 min. This

would suggest that the cells ingest 10% of their volume during the time-course of pinocytosis and there was a corresponding increase in the wet weight of the cell pellets during this period of ∿11%. It should be recalled that the contractile vacuole is inoperative during this time so that the cell is incapable of regulating its volume. Chapman-Andresen and Holter (1964) reported that *A. proteus* took up only 2% of the cells volume during a 30-min period of pinocytosis, whereas Bowers and Olszewski (1972) reported that *Acanthamoeba* ingests ∿21% of its cell volume in 1 h by pinocytosis. Earlier studies suggested that the process of pinocytosis was temperature dependent (Schumaker, 1958; DeTerra and Rustad, 1959). In this study it was found that cells held at 3°C failed to take up sucrose when the inducer was added to the external medium (Fig. 2),

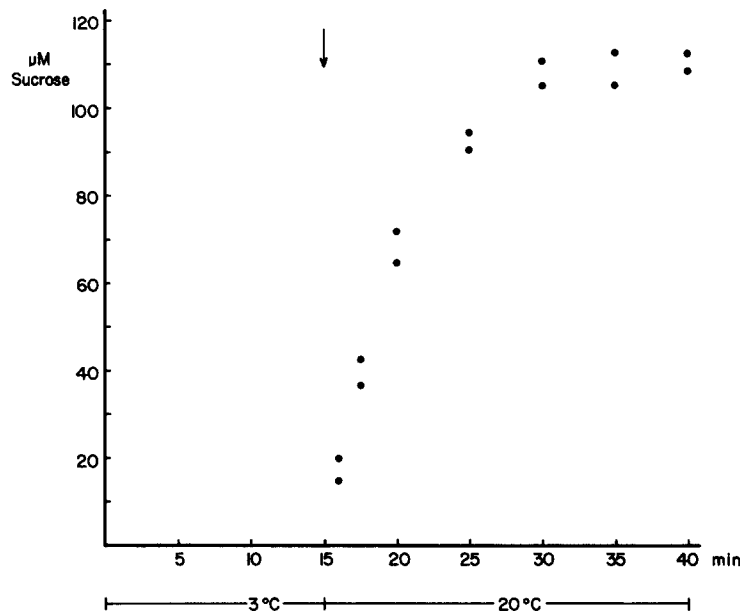

Figure 2. Effect of temperature on pinocytotic sucrose uptake. Cells were equilibrated in control medium (0.03 mM Ca^{++}, pH 6.5) with 1 mM sucrose and [^{3}H]sucrose (2 μCi/ml), initially at 3°C. Alcian blue (0.01%) was added to the external medium at $t = 0$ and sucrose uptake by the cells followed for 15 min. After the 15-min equilibration period at 3°C, the temperature of the cell suspension was quickly raised to 20°C (arrow). Each point represents a single determination from one experiment.

but when the cell suspension was quickly brought up to room temperature, the cells responded by accumulating sucrose. In this case, preincubation of the cells with alcian blue at 3°C apparently increased the rate and amount of sucrose uptake when the cells were brought up to room temperature, as compared with control conditions. This may indicate that incubation of the cells at 3°C allows more inducer to bind to the cell surface before initiation of pinocytosis.

The time-course of pinocytosis was determined by adding alcian blue to a suspension of cells at 3°C. The inducer was then removed from the external medium after a 5-min incubation period by washing the cells with fresh medium at 3°C. The cell suspension was then brought up to room temperature. Sucrose uptake was measured as before in cell aliquots removed from the cell suspension at various time intervals after the initiation of pinocytosis by the temperature increase and exposure for a 5-min period to labelled sucrose. As is shown in Fig. 3, the uptake of sucrose begins quickly after the initiation of pinocytosis, reaching a maximum uptake 15–20 min after the initiation of pinocytosis and then steadily falls off and ceases ∿50 min after the start of the induced sucrose uptake.
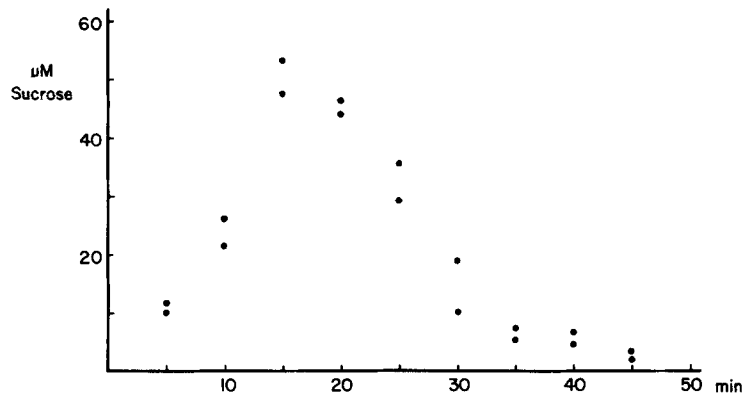

FIGURE 3.   Pinocytosis time-course in *Amoeba proteus*. Cells were exposed to 0.01% alcian blue for 5 min at 3°C and then washed twice in fresh, cold control medium. The cell suspension was then brought up to 20°C, this point being taken as $t = 0$ and the initiation of pinocytosis. Cell aliquots were taken at various time intervals after the initiation of pinocytosis and exposed to 1 mM sucrose and [^{3}H]sucrose (2 μCi/ml) for 5 min. Each point represents a single determination.

The process of pinocytosis is not an "all-or-nothing" physiological process. For a given pinocytotic inducer in amoebae, the intensity of pinocytosis apparently increases with increasing concentration of the inducer, generally up to some maximum or optimum value (Chapman-Andresen, 1962). In *Amoeba proteus* the induction and intensity of pinocytosis are also dependent upon the concentration of the inducer, alcian blue. As the amount of alcian blue in the external medium is increased from 0.001% the amount of pinocytotically induced sucrose uptake also increases (Fig. 4), with corresponding increases in cell volume. Over the relatively wide range of alcian blue concentrations used in this particular experiment, the amount of induced sucrose uptake did not appear to reach saturation.

Another environmental factor known to influence pinocytosis in amoebae is pH (Chapman-Andresen, 1962). The effects of pH on alcian blue-induced pinocytosis in *Amoeba proteus* were investigated in the pH range of 3.5–8 by

adjusting the pH of the control medium with 0.1 N NaOH or HCl. As the pH of the medium is decreased below the control medium pH of 6.5, pinocytotic uptake of sucrose increases, whereas an increase in pH above the control pH decreases sucrose uptake (Fig. 5). Previous observations demonstrated that pinocytosis is not brought about by an increase in the hydrogen ion concentration of the external medium (Brandt, 1958). This observed effect of pH on pinocytosis could be brought about by changes in the charge density of the cell surface with a subsequent change in the degree of binding of the inducer to the cell surface.
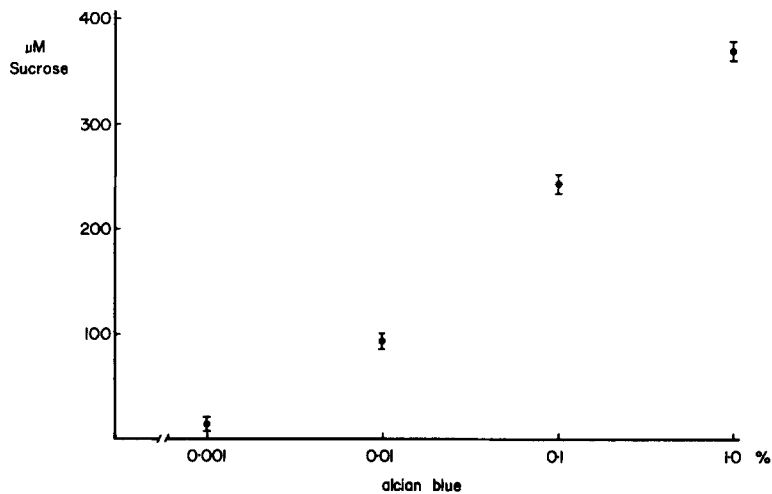

Figure 4.   Pinocytotic sucrose uptake as a function of the external alcian blue concentration. Cell aliquots were equilibrated in control medium (0.03 mM Ca^{++}, pH 6.5) with 1 mM sucrose and [^3H]sucrose (2 $\mu Ci/ml$) and varying amounts of alcian blue for 15 min. Each point represents the mean of six determinations and the vertical bar indicates $\pm$ 1 SEM.

Calcium and Pinocytosis

A large fraction of total cellular calcium in *Amoeba proteus* is associated with the cell surface (Prusch and Hannafin, 1979) and it has been previously suggested that the initiation of pinocytosis in amoeba may in part involve the displacement of surface bound calcium by the inducer (Cooper, 1968; Josefsson, 1976; Prusch, 1977). This association between calcium and pinocytosis in *Amoeba proteus* was investigated initially by observing the effect of external Ca^{++} levels on pinocytotically induced sucrose uptake. As is shown in Fig. 6, sucrose uptake induced by 0.01% alcian blue demonstrated a dependence upon external Ca^{++}; when no Ca^{++} is present in the external medium, confirmed by flame photometry, addition of alcian blue does not elicit sucrose uptake, but increasing external Ca^{++} up to a maximum level of 0.1 mM increases the amount of sucrose uptake. Increases in the external Ca^{++} level above 0.1 mM decreases the amount of sucrose uptake. Two points should be emphasized here: first of all, when no Ca^{++} is present in the external medium,

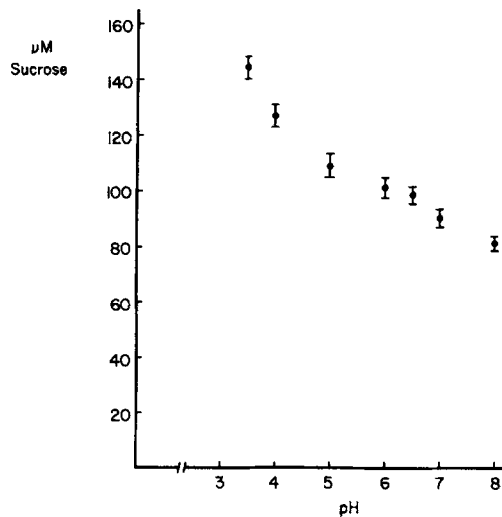FIGURE 5. Effect of pH on pinocytotic sucrose uptake. Cell aliquots were equilibrated in control medium (0.03 mM Ca^{++}) with 1 mM sucrose and [^{3}H] sucrose (2 μCi/ml) in which the pH was varied with either 0.1 N NaOH or HCl. Each point represents the mean of five determinations and the vertical bar indicates ± 1 SEM.
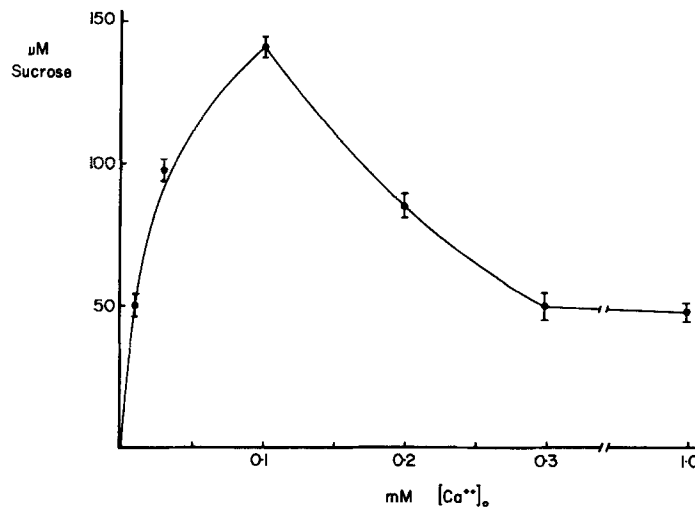

FIGURE 6. Pinocytotic sucrose uptake as a function of the external Ca^{++} concentration. Cell aliquots were equilibrated in control medium with 1 mM sucrose and [^{3}H]sucrose (2 μCi/ml) with varying Ca^{++} concentrations, as CaCl$_2$, and 0.01% alcian blue for 15 min. Each point represents the mean of six determinations and the vertical bar represents ± 1 SEM.

the cell is apparently incapable of pinocytosis and no sucrose is taken up from the external medium when alcian blue is present; and secondly, there is an optimal level of Ca^{++} in the external medium associated with the uptake of sucrose by pinocytosis in *Amoeba proteus*.

Calcium movements during the actual process of pinocytosis were determined with labelled Ca^{++}. Calcium-45 was added to a suspension of cells in the control medium, which has a Ca^{++} concentration of 0.03 mM, and its uptake followed as described previously (Prusch and Hannafin, 1979). As observed in previous studies, there is an immediate and rapid exchange of Ca^{++} within 1 min or less and then very little exchange thereafter. When alcian blue (0.01%) is added to the external medium, with ^{45}Ca still present, there is an initial displacement of labelled calcium from the cell (arrow, Fig. 7). The amount of calcium displaced from the cell is small under these conditions, maximally 0.1 mmol/kg of cells, but this observation is consistently reproducible. The observed influx of Ca^{++} into the cell following the initial
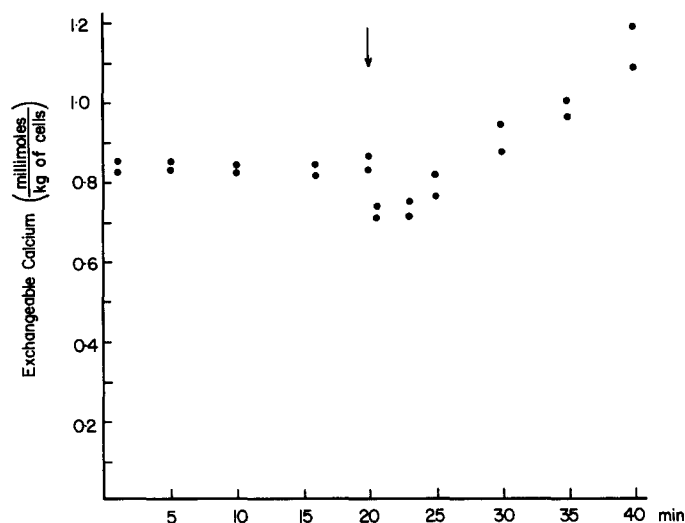

FIGURE 7. Pinocytosis and Ca^{++} exchange. Ca^{++} exchange was followed by adding ^{45}Ca (2 $\mu Ci/ml$) to a suspension of cells in control medium (0.03 mM Ca^{++}, pH 6.5). Alcian blue (0.01%) was added after a 20-min equilibration period (arrow) with ^{45}Ca still present in the external medium. Each point represents a single determination.

displacement of surface calcium by the inducer is most likely due to overall solute uptake initiated by pinocytosis. This pinocytotic uptake of Ca^{++} does not follow the same time-course of induced sucrose uptake under essentially the same experimental conditions (Fig. 1). The influx of Ca^{++} is apparently slower and continues for a longer period of time than does the influx of sucrose. The reason for this apparent discrepancy is unknown but may in part be related to the fact that the Ca^{++} concentration (0.03 mM) is lower than the sucrose concentration (1.0 mM) in the external medium, and that although Ca^{++} may be taken up along with the bulk medium during pinocytosis, calcium also binds to the amoeba surface (Prusch and Hannafin, 1979) which may complicate the Ca^{++} influx kinetics during pinocytosis.

In order to separate or uncouple the pinocytosis binding stimulus and Ca^{++} movements from the following active solute uptake by pinocytosis, including

the uptake of external Ca^{++}, Ca^{++} exchange and pinocytosis were followed at 3°C (Marshall and Nachmias, 1965). The binding of the inducer to the cell surface is temperature independent (DeTerra and Rustad, 1959), whereas the subsequent uptake of solute by pinocytosis is temperature dependent (Fig. 2). Cell suspensions were kept at 3°C, ^{45}Ca was added (2 $\mu Ci/ml$), and the cells were allowed to equilibrate for 15 min; then alcian blue (0.001–1.0%) was added to the cell suspensions. Addition of a pinocytotic inducer to the cells under these conditions, i.e., inhibition of the active steps of pinocytosis, displaces calcium from the cell surface and the amount of calcium displaced increases with the amount of inducer added to the external medium (Fig. 8). Addition of 0.001% alcian blue displaces approximately 0.3 mmol calcium/kg of cells from the cell and brings about a very small uptake of sucrose (Fig. 4), but as the alcian blue concentration is increased up to 1%, and most of the previously exchanged calcium is displaced from the cell, sucrose uptake is considerably increased.
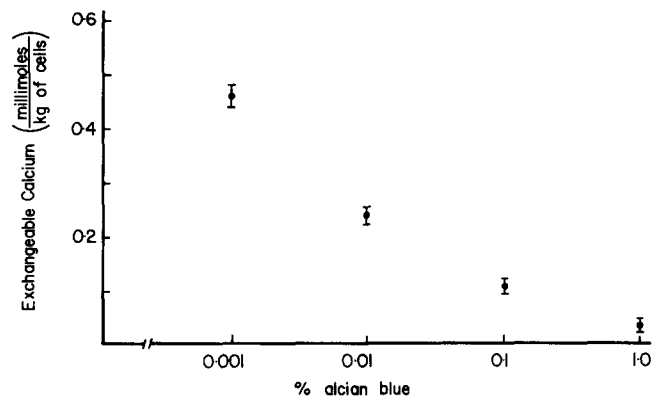

FIGURE 8. Ca^{++} exchange as a function of the external alcian blue concentration at 3°C. Cell aliquots were equilibrated in the control medium (0.03 mM Ca^{++}, pH 6.5) with ^{45}Ca (2 $\mu Ci/ml$) and varying alcian blue concentrations for 15 min at 3°C. Each point represents the mean of six determinations and the vertical bar indicated ± 1 SEM.

DISCUSSION

Pinocytosis involves the uptake by a cell of both membrane bound solute and extracellular solute present in the external medium by a process of membrane infolding and vesiculation. In freshwater amoebae pinocytosis is initiated or induced by a large variety of substances, all of which have a net positive charge and bind to the cell surface. In this present study of pinocytosis in *Amoeba proteus*, the bulk or fluid phase of pinocytosis was followed by observing the uptake of labelled surcrose when pinocytosis was initiated with alcian blue.

The results obtained from this investigation indicate very strongly that calcium is associated with the mechanism of pinocytosis in amoebae. First of all, the amount of sucrose taken up during pinocytosis is influenced by external Ca^{++} (Fig. 4). In the absence of external Ca^{++}, little or no sucrose uptake is

observed in the presence of alcian blue, but as the concentration of Ca^{++} in the external medium is increased, up to a maximal level of 0.1 mM, pinocytotic sucrose uptake is also increased. Increases in external Ca^{++} above 0.1 mM produces a decrease in pinocytotic sucrose uptake. It is interesting to note that Josefsson (1976) obtained similar results by observing channel formation in *Amoeba proteus* induced with alcian blue. That is, the number of pinocytotic channels formed in response to 0.01% alcian blue increased as the Ca^{++} concentration of the external medium was increased from 0.01 up to 0.1 or 1.0 mM, with further increases in external Ca^{++} decreasing the observed number of channels. This reduction in pinocytotic sucrose uptake and channel formation, by increases in external Ca^{++} levels above a certain value, may be due to, among other things, a decrease in the amoeba plasmalemma solute permeability (Prusch and Dunham, 1972), or simply to a mass action effect between the inducer and surface calcium.

Secondly, an association between Ca^{++} and pinocytosis is indicated from the observations of the effect of the pinocytotic inducer, in this case alcian blue, on both sucrose uptake and displacement of surface calcium. As the concentration of the inducer in the external medium is increased, the intensity of pinocytosis, as determined by sucrose uptake, increases (Fig. 4). Concomittantly, this increase in external inducer concentration increases the amount of calcium displaced from the cell (Fig. 8). In that the inducer of pinocytosis physically binds to the surface of the cell (Brandt and Pappas, 1960), release of surface calcium in the amoeba (Prusch and Hannafin, 1979) by the inducer could be brought about by a competition of the positively charged inducer ions for sites normally occupied by Ca^{++}, or the inducer could bind to surface sites normally not occupied by Ca^{++} and alter the affinity of the primary Ca^{++}-binding sites. Alternatively, physical binding of the inducer to the cell surface could decrease the negative charge density of the cell surface and decrease the amount of Ca^{++} normally associated with the diffuse double layer.

Associated with the onset of pinocytosis, as demonstrated in several previous studies, is an increase in the membrane permeability or conductance. For example, Brandt and Freeman (1967) and Josefsson (1968) showed that the resistance of *Amoeba proteus* plasmalemma decreases under conditions which elicit pinocytosis. In addition, Brandt and Hendil (1970) found a 10-fold increase in membrane conductance when pinocytosis was induced in amoeba. Since Ca^{++} is known to control the overall solute permeability of the amoeba plasmalemma (Prusch and Dunham, 1972), removal of surface calcium could conceivably be associated with the above observed changes in membrane permeability during the onset of pinocytosis. An increase in plasmalemma solute permeability could in turn lead to increased solute movements, including Ca^{++}, across the cell surface and bring about transitory changes in cytoplasmic ion activities. If this was found to be the case, it would suggest then that the Ca^{++} ion may be associated with at least two different steps in the overall process of pinocytosis: surface calcium displaced by the inducer and subsequent Ca^{++} influx. If the induction of pinocytosis was initiated simply by the removal of surface calcium, then pinocytosis should be induced

by eliminating Ca^{++} from the external medium, or at least the ease with which pinocytosis is elicited should be increased in the absence of external Ca^{++}. Since it has already been demonstrated in this study that a minimal external Ca^{++} level is required in order to initiate pinocytosis with alcian blue (Fig. 6), then this cannot be the case. This then suggests further that specific sites on the cell surface are occupied by Ca^{++} which may control membrane permeability or structural integrity, and that the displacement of Ca^{++} from these sites is involved at least in part with the initiation of pinocytosis. The second stage of pinocytosis requiring the presence of external Ca^{++} is involved with the possible inward movement of the Ca^{++} ion in response to the initial displacement of surface associated calcium by the inducer. Both Nachmias (1968) and Josefsson (1976) have hypothesized an inward movement of Ca^{++} during pinocytosis which may trigger contractile elements beneath the cell surface.

The process of pinocytosis is used for a variety of cellular physiological functions, ranging from providing a mechanism for feeding in cultured *Acanthamoeba castellanii* (Bowers and Olszewski, 1972) to providing a means for protein uptake in higher cell types (Ryser, 1968). Although pinocytosis may be utilized for a variety of physiological roles, the underlying mechanism may be similar in the different cell types which exhibit this phenomenon. It is even conjectured that the processes of phagocytosis and pinocytosis, which differ greatly in their quantitative aspects, are brought about by similar mechanisms (Jacques, 1969). On the basis of this particular study, it can be speculated that as in the amoeba, the Ca^{++} ion most likely plays a pivotal role in the mechanism of pinocytosis in these other cell types. Allison and Davies (1974) have implicated Ca^{++} in the mechanism of pinocytosis in mammalian cells, and Douglas (1974) claims that Ca^{++} is involved in the process of exocytosis. As is the case with amoebae, initiation of pinocytosis apparently elicits a depolarization of the membrane potential in mammalian macrophages (Kouri, as quoted by Allison and Davies, 1974). This depolarization of the membrane could again be associated with a general increase in membrane permeability caused by a displacement of surface calcium by the inducer. Although this study has demonstrated that Ca^{++} is associated with the process of pinocytosis in *Amoeba proteus*, a considerable amount of work is required to further elucidate the mechanism of pinocytosis in the amoeba and other cell types.

REFERENCES

ALLISON, A. C., and P. DAVIES. 1974. Mechanisms of endocytosis and exocytosis. *Symp. Soc. Exp. Biol.* **28:**419–446.

BOWERS, B., and T. E. OLSZEWSKI. 1972. Pinocytosis in *Acanthamoeba castellanii.* Kinetics and morphology. *J. Cell Biol.* **53:**681–694.

BRANDT, P. W. 1958. A study of the mechanism of pinocytosis. *Exp. Cell Res.* **15:**300–313.

BRANDT, P. W., and A. R. FREEMAN. 1967. Plasma membrane: substructural changes correlated with electric resistance and pinocytosis. *Science (Wash. D.C.).* **155:**582–585.

BRANDT, P. W., and K. B. HENDIL. 1970. Properties of the plasma membrane of amoeba. *Adv. Exp. Med. Biol.* **7:**323–335.

BRANDT, P. W., and G. W. PAPPAS. 1960. An electron microscope study of pinocytosis in amoeba. I. The surface attachment phase. *J. Biophys. Biochem. Cytol.* **8:**675–687.

CHAPMAN-ANDRESEN, C. 1962. Studies on pinocytosis in amoebae. *C. R. Trav. Lab. Carlsberg.* **33:** 73–264.

CHAPMAN-ANDRESEN, C., and H. HOLTER. 1955. Studies on the ingestion of ^{14}C glucose by pinocytosis in the amoeba *Chaos chaos. Exp. Cell Res.* **3:**52–63.

CHAPMAN-ANDRESEN, C., and H. HOLTER. 1964. Differential uptake of protein and glucose by pinocytosis in *Amoeba proteus. C. R. Trav. Lab. Carlsberg.* **34:**211–226.

COOPER, B. A. 1968. Quantitative studies of pinocytosis induced in *Amoeba proteus* by simple cations. *C. R. Trav. Lab. Carlsberg.* **36:**385–403.

DE TERRA, N., and R. C. RUSTAD. 1959. The dependence of pinocytosis on temperature and aerobic respiration. *Exp. Cell Res.* **17:**191–195.

DOUGLAS, W. W. 1974. Involvement of calcium in exocytosis and the exocytosis-vesiculation sequence. *Biochem. Soc. Symp.* **39:**1–28.

JACQUES, P. J. 1969. Endocytosis. *In* Lysosomes in Biology and Pathology. J. T. Dingle and H. B. Fell, editors. North-Holland Publishing Co., Amsterdam. 395–420.

JOSEFSSON, J. O. 1968. Induction and inhibition of pinocytosis in *Amoeba proteus. Acta Physiol. Scand.* **73:**481–490.

JOSEFSSON, J. O. 1976. Studies on the mechanism of induction of pinocytosis in *Amoeba proteus. Acta Physiol. Scand. Suppl.* **432:**1–65.

JOSEFSSON, J. O., N. G. HOLMER, and S. E. HANSSON. 1975. Membrane potential and conductance during pinocytosis induced in *Amoeba proteus* with alkali metal ions. *Acta Physiol. Scand.* **94:**278–288.

LEWIS, W. H. 1931. Pinocytosis. *Bull. Johns Hopkins Hosp.* **49:**17–27.

MARSHALL, J. M., and V. T. NACHMIAS. 1965. Cell surface and pinocytosis. *J. Histochem. Cytochem.* **13:**92–104.

MAST, S. O., and W. L. DOYLE. 1934. Ingestion of fluids by *Amoeba. Protoplasma.* **20:**555–560.

NACHMIAS, V. T. 1968. Inhibition of streaming in amoeba by pinocytosis inducers. *Exp. Cell Res.* **51:**347–361.

PRESCOTT, D. M., and T. W. JAMES. 1955. Culturing of *Amoeba proteus* on *Tetrahymena. Exp. Cell Res.* **8:**256–258.

PRUSCH, R. D. 1977. Protozoan osmotic and ionic regulation. *In* Transport of Ions and Water in Animals. B. L. Gupta, R. B. Moreton, J. L. Oschman, and B. J. Wall, editors. Academic Press, Inc., New York. 363–377.

PRUSCH, R. D., and P. B. DUNHAM. 1972. Ionic distribution in *Amoeba proteus. J. Exp. Biol.* **56:** 551–563.

PRUSCH, R. D., and J. HANNAFIN. 1979. Calcium distribution in *Amoeba proteus. J. Gen. Physiol.* **74:**511–521.

RYSER, H. J. P. 1968. Uptake of protein by mammalian cells: an undeveloped area. *Science (Wash. D.C.)* **159:**390–396.

SCHUMAKER, V. N. 1958. Uptake of protein from solution by *Amoeba proteus. Exp. Cell Res.* **15:** 314–331.

SCOTT, J. E., G. QUINTARELLI, and M. C. DELLOVA. 1964. The chemical and histochemical properties of alcian blue. I. The mechanism of alcian blue staining. *Histochemie.* **4:**73–85.

SILVERSTEIN, S. C., R. M. STEINMAN, and Z. A. COHN. 1977. Endocytosis. *Annu. Rev. Biochem.* **46:** 669–722.