EFFECTS OF HIGH PRESSURE ON PINOCYTOSIS IN *AMOEBA PROTEUS*

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Although pinocytosis was observed in amoebae by Edwards (1) as early as 1925, relatively little has been learned about its mechanism (2–5). Pinocytosis is a process by which materials, including relatively large molecules which ordinarily do not pass through the cell membrane, enter the interior of a cell. In amoebae, pinocytosis typically begins with the formation of small, relatively agranular pseudopodia containing channels. Small vacuoles subsequently pinch off from the bottom of each channel and migrate deeper into the cytoplasm (6). Pinocytosis has been chemically induced in amoebae following treatment with solutions of proteins, basic dyes, and hypertonic salts (5–7). The induction of pinocytosis is accompanied by the attachment of large quantities of the inducing chemical to the cell surface (5, 7–9). Both protein ingestion (8, 10) and the formation of visible channels (5, 11) are temperature dependent and may be suppressed by respiratory inhibitors. Alteration of either the cell surface or the plasmagel might be the basis for pinocytosis. Since high hydrostatic pressure is known to weaken the plasmagel by modifying the sol-gel equilibria (12, 13), it was employed for studying pinocytosis in amoeba.

MATERIALS AND METHODS

Amoeba proteus were cultured in inorganic salt solutions and fed washed *Tetrahymena geleii* according to the methods of Prescott and James (14). For each experiment, 100 to 200 amoebae were transferred through several changes of the inorganic culture solution and starved for 24 to 48 hours. Pinocytosis was induced by immersing the amoebae into ribonuclease 0.04 to 0.1 per cent (Worthington Biochemical Corp., Harrison, New Jersey) or into 1 to 2 per cent bovine albumin (Armour Pharmaceutical Company, Kankakee, Illinois) which had been dialyzed against the inorganic salt solution. An alternate method of inducing pinocytosis was to place the amoebae into 30 to 40 per cent sea water.

The temperature-pressure apparatus was patterned after one designed by Marsland (15), with certain modifications. The microscope-pressure chamber permits cells to be observed at magnifications up to ×600 while being subjected to hydrostatic pressures as high as 20,000 lbs./inch^2. The hydrostatic pressure was developed by means of an Aminco pressure pump at the rate of 5,000 lbs./inch^2/stroke. The pressure can be released almost instantaneously by means of a needle valve. The microscope and pressure chamber, as well as all glassware and testing solutions, were equilibrated at 20°C. The amoebae were placed into the pressure chamber immediately after immersion into the pinocytosis-inducing solution. Observations of the cells began 2 to 3 minutes later and continued throughout the experimental procedures.

In early experiments, one observer scanned 10 to 30 cells to ascertain the presence of pinocytosis channels. When the channels disappeared from the pressurized cells, he and either one or two independent observers examined these cells again to see whether or not channels were present. Since channels reappeared at some pressures, it was necessary to perform more critical experiments in which two to five cells were kept under continuous observation by two alternating observers. Since time did not permit either direct channel counting or photographs at numerous focal planes, the data were recorded in terms of the presence or absence of channels in each cell with an additional notation of "many" or "few" channels.

RESULTS

In general, active protoplasmic streaming diminished and the amoebae retracted their long pseudopodia after being placed in one of the pinocytosis-inducing solutions. The retracted amoebae produced short, relatively agranular pseudopodia. Subsequently, it was possible to observe discrete channels 1 to 2 μ in diameter in some of the pseudopodia. Induction of the pinocytotic channels usually occurred in 3 to 5 minutes. Channels persisted for approximately 30 minutes after induction.

PRESSURE EFFECTS: At 20°C, pinocytosis was blocked by a pressure of 3,000 lbs./inch^2. Comparable results were obtained when the different inducing agents were employed. At 3,000 lbs./inch^2 the first noticeable effect of the pressure was a loss of channels, which occurred within 1 to 2 minutes following the initiation of pressure (see Table I). Occasionally, some small pseudopodia persisted for periods up to 10 minutes, but channels did not reform in these pseudopodia. In a few experiments, some small pseudopodia appeared during the pressure treatment, but these pseudopodia did not develop channels.

Within 5 to 10 seconds following the release of pressure, there was a generalized contraction of the cytoplasm in the interior of the cell. This was accompanied by a burst of active blebbing on the surface of the amoebae. Channels started to form during the next 30 seconds, in some cases, as early as 20 seconds after the release of pressure. Within 1 to 2 minutes, cytoplasmic activity returned to a level comparable to that seen in the non-pressurized pinocytosing cells. The same effects occurred when the pressure was applied and released 5 or 6 times with the same group of cells. Each time the pressure was applied the pinocytosis was blocked, and upon return to atmospheric pressure the pinocytosis channels returned.

At 2,000 lbs./inch^2 a different pattern of effects was observed. Following the application of pressure, most of the channels disappeared within 1 minute. However, the small pseudopodia persisted. Protoplasmic activity was not markedly affected and new small pseudopodia were formed. Occasionally, channels appeared in these newly formed pseudopodia (see Table I). The formation of new pseudopodia and channels have been observed as long as 30 minutes after the initiation of pressure.

At 1,000 lbs./inch^2, the channels and pseudopodia were not modified to any appreciable extent.

TABLE I

The Effects of Hydrostatic Pressure on Pinocytosis in Amoeba proteus

The results of experiment No. 62-06-08 in which the cells were subjected to various pressures following immersion into 1.0 per cent albumin. At each pressure level a new group of amoebae was induced and pressurized, except at the 2,000 lb./inch^2 level where the same group of cells was pressurized twice. The recorded data are from one representative optical field.

Pressure (lbs./inch^2)	No. of cells with channels before pressure	No. of cells with channels after pressure	Observations
1,000	2	2	Some of the original channels disappeared; new channels reappeared.
2,000	2	2	Most of the channels disappeared in 0.5 minutes; some new channels formed within 2 minutes.
2,000	2	1	All but 1 channel disappeared within 0.4 minutes; the remaining channel disappeared in 2.5 minutes. New channels reappeared in one cell in 6 minutes; no channels were evident in the other cell.
3,000	4	0	All channels disappeared within 1 minute; no channels reappeared.
4,000	3	0	All channels disappeared within 1.3 minutes; no channels reappeared.

Pseudopodia and channels continued to form at this pressure.

DISCUSSION

In general, the experiments demonstrated that high pressure can reversibly block pinocytosis in *Amoeba proteus*. Furthermore, the experiments indicate that pinocytosis may involve at least two distinct physiologic processes, the formation of small pseudopodia and the formation of channels.

Amoebae become spherical when subjected to a pressure of 5,000 lbs./inch^2 at 20°C (13, 16). Recently, Landau and Thibodeau (17) reported an absence of surface openings in electron micro-

graphs of amoebae which had been fixed under pressure after 30 minutes treatment at 8,000 lbs./inch^2. These surface openings are believed to be micropinocytosis channels in the uroid of normally streaming amoebae. The present experiments demonstrated that chemically induced pinocytosis can be blocked reversibly within 1 to 2 minutes by a pressure of 3,000 lbs /inch^2. Existing channels regressed and no new channels were formed during the course of the treatment. However, channels reappeared as soon as 20 seconds after the pressure was released. In some experiments at this pressure, small pseudopodia persisted for periods up to 10 minutes. At 2,000 lbs./inch^2, although most of the channels in the small pseudopodia disappeared from view, the formation of numerous small pseudopodia and occasional channels continued. These experiments demonstrate a difference between the channel and the pseudopod in the maintenance of their surface morphology. Neither polarizing microscopy (7) nor electron microscopy (18–20) have revealed any differences. However, surface frilling and the induction of small pseudopodia can occur when pinocytosis in albumin-stimulated cells is blocked with cyanide (11).

High pressure causes solation of gel structures in a variety of cells (12, 13). Hence, the present data are compatible with the hypothesis that the plasmagel is involved in pinocytosis. However, alternative sites of pressure action may exist. The plasma membrane (unit membrane) and the extracellular coats would be subjected to the same pressure actions as the plasmagel. In the absence of detailed information concerning the effect of pressure on these structures, hypotheses concerning the possible role of surface tension in pinocytosis (9, 21) cannot be evaluated. Since channel formation requires chemical energy, presumably in the form of ATP (11), thermodynamic disturbances of respiration or of the pathways of ATP utilization could yield the same results.

Mast and Doyle (6) suggested that pinocytosis might result from changes in a very thin layer of plasmagel near the membranes of the pseudopod and channel. Unfortunately, no such gelled region has ever been demonstrated in the optically clear pseudopods. Brandt (9) observed that the cell surface was initially attached to the plasmagel at the point of channel formation. He suggested that the channel is drawn down at this point of attachment and further elongated by extension of the pseudopod. A pressure-induced weakening of the plasmagel would be expected to release such attachments. However, after pressure release some channels were formed in pseudopods which did not undergo significant changes in shape. Since the observed plasmagel does not extend into the pseudopod, reattachment at the same point does not appear possible. If Brandt's hypothesis should prove correct, a more permanent submicroscopic connection would have to exist.

Although the site of expenditure of mechanical energy during pinocytosis remains unknown, the present experiments have demonstrated that the maintenance of channels is more sensitive to high hydrostatic pressure than the maintenance of pseudopods.

SUMMARY

The effect of hydrostatic pressure on pinocytosis in amoebae was investigated. A pressure of 1,000 lbs./inch^2 had no detectable effect on pinocytosis. At 2,000 lbs./inch^2 most pinocytosis channels disappeared, but small pseudopodia and some new channels reappeared. At 3,000 lbs./inch^2 all the channels disappeared within 1 to 2 minutes and no new channels were formed. As early as 30 seconds after decompression, the channels returned. The results are discussed in terms of the effects of pressure on the sol-gel equilibrium in amoeba and the possible role of gelation reactions in pinocytosis.

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REFERENCES

1. EDWARDS, G. J., *Biol. Bull.*, 1925, **48,** 236.
2. HOLTER, H., *Internat. Rev. Cytol.*, 1959, **8,** 481.
3. RUSTAD, R. C., *Scient. Am.*, 1961, **204,** 121.

4. RUSTAD, R. C., *in* Recent Progress in Surface Science, (J. F. Danielli, K. G. A. Pankhurst, and A. C. Riddiford, editors), New York, Academic Press, Inc., 1964, **2,** 353.
5. CHAPMAN-ANDRESEN, C., *Compt. rend. Lab. Carlsberg*, 1962, **33,** 73.
6. MAST, S. O., and DOYLE, W. L., *Protoplasma*, 1934, **20,** 555.
7. RUSTAD, R. C., *Nature*, 1959, **183,** 1058.
8. SCHUMAKER, V. N., *Exp. Cell Research*, 1958, **15,** 314.
9. BRANDT, P. W., *Exp. Cell Research*, 1958, **15,** 300.
10. BHIDE, S. V., and BRACHET, J., *Exp. Cell Research*, 1960, **21,** 303.
11. DETERRA, N., and RUSTAD, R. C., *Exp. Cell Research*, 1959, **17,** 191.
12. MARSLAND, D. A., *Internat. Rev. Cytol.*, 1956, **5,** 199.
13. LANDAU, J. V., ZIMMERMAN, A. M., and MARSLAND, D. A., *J. Cell. and Comp. Physiol.*, 1954, **44,** 211.
14. PRESCOTT, D. M., and JAMES, T. W., *Exp. Cell Research*, 1955, **8,** 256.
15. MARSLAND, D. A., *J. Cell. and Comp. Physiol.*, 1950, **36,** 205.
16. MARSLAND, D. A., and BROWN, D. E. S., *J. Cell. and Comp. Physiol.*, 1936, **8,** 167.
17. LANDAU, J. V., and THIBODEAU, L., *Exp. Cell Research*, 1962, **27,** 591.
18. BRANDT, P. W., and PAPPAS, G. D., *J. Biophysic. and Biochem. Cytol.*, 1960, **8,** 675.
19. NACHMIAS, V. T., and MARSHALL, J. M., JR., *in* Proceedings of the First IUB/IUBS International Symposium, (T. W. Goodwin and O. Lindberg, editors), New York, Academic Press, Inc., 1961, **2,** 605.
20. CHAPMAN-ANDRESEN, C., and NILSSON, J. R., *Exp. Cell Research*, 1960, **19,** 631.
21. GOLDACRE, R. J., *in* Surface Phenomena in Chemistry and Biology, (J. F. Danielli, K. G. A. Pankhurst, and A. C. Riddiford, editors), New York, Pergamon Press, 1958, 278.