HIGH HYDROSTATIC PRESSURE EFFECTS ON *AMOEBA PROTEUS*

Changes in Shape, Volume, and Surface Area

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Upon application of hydrostatic pressure to *Amoeba proteus*, all movement stops and the organism assumes a spherical shape (1). The attainment of a completely spherical shape is a gradual process requiring from 5 to 20 minutes, depending upon the pressure and temperature level. This "sphering" under pressure has been attributed to the reversible disruption of the plasmagel integrity and consequent loss of structural opposition to the tensional forces at the surface of the ameba. However, one of the initial events is the collapse of the contractile vacuole, and this has posed some questions as to the nature of the "sphering" phenomenon.

The disruption of osmoregulation under pressure could result in swelling, with the final diameter of the sphere being determined by the eventual equilibration of the osmotic forces involved and the elastic tension of the membrane. Of further consideration is the fact that the sphere represents a minimal surface area for a given volume. Should there be no change (or even a slight decrease) in the volume of the organism during "sphering," a decrease in surface area must occur. Similarly, on release of pressure, as pseudopodial flow returns, an increase in surface area must occur. However, should there be some increase in volume due to osmosis, no change in surface area need occur since the sphere surface might then represent the minimal surface for an increased volume.

While theoretical considerations of relative vapor pressures under conditions of high hydrostatic pressure would seem to eliminate osmosis as an experimental factor, it was thought necessary to perform experiments concerned with actual volume changes in *A. proteus* during the period of pressure application.

MATERIAL AND METHODS

A. proteus were cultured in Chalkley's medium and fed washed *Tetrahymena pyriformis* in accordance with the method of Griffin (2). In order to facilitate measurement of relative changes in volume, the amebas were entrapped in fine glass capillary tubes. The diameter of the capillary had to be large enough, however, to permit adequate free membrane surface for possible osmotic transfer of solvent.

The pressure methods and equipment were similar to those previously described (1). A diagrammatic representation of the experimental apparatus is shown in Fig. 1. The specimen chamber is made by mounting two coverslips on either end of a glass ring rimmed with a moderately thick layer of stopcock grease.

Several amebas suffered membrane damage while being sucked into the capillary tube. Upon observation in the pressure chamber, the membrane had disappeared and a flow of naked cytoplasm could be detected for a period of 1 or 2 minutes, sufficient time for the performance of additional studies on the naked cytoplasm.

RESULTS

Volume Experiments

The results are recorded in a series of photographs taken at specific times prior to, during, and following the application and release of pressure at 25°C (Fig. 2, *a* to *f*). Fig. 2 *a* represents an ameba as first viewed in the pressure chamber.

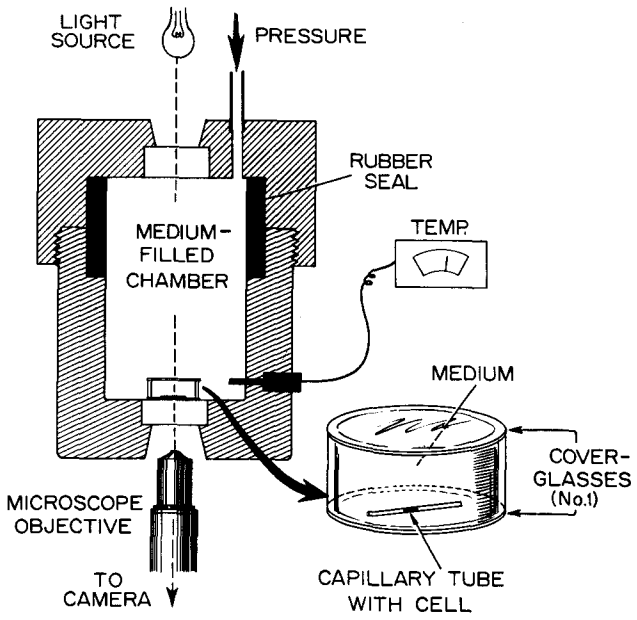

FIGURE 1 A diagrammatic representation of the pressure apparatus.

After 2 minutes at atmospheric pressure, the remaining pseudopodium had retracted and 8000 psi of pressure was applied (Fig. 2 *b*). Some 15 seconds later (Fig. 2 *c*) the ameba presented uniformly rounded ends and the contractile vacuole had collapsed. The pressure was maintained for 20 minutes (Fig. 2 *d*) and a careful overlay comparison made from enlargements of Figs. 2 *c* and 2 *d* showed a slight decrease in the critical dimension from end to end with no change in shape. This slight decrease in volume actually occurred within 2 minutes of pressure application and may have been anticipated on the basis of Bridgman's data (3). The pressure was released and the expected contraction of cytoplasmic granular material occurred (2 *e*). After 3 to 5 minutes normal pseudopodial flow commenced (Fig. 2 *f*).

Naked Cytoplasm Experiments

Cytoplasmic flow was maintained for no longer than 2 minutes following the disruption of the membrane. No adjustments of medium were made to increase this time period. When flow was evident in the absence of a membrane, a pressure of 8000 psi was immediately applied. The pressure caused an immediate cessation of flow followed in a few seconds by a small but sharp movement of granules from the periphery toward the center of the cytoplasmic mass. The appearance of the cytoplasm under pressure is shown diagrammatically in Fig. 3 *a*. The tailing of granular material at each end of the specimen represents peripheral cytoplasmic material which has fallen to the bottom of the capillary tube. When the pressure was abruptly released within 2 minutes of application, an intense contraction of the cytoplasmic material occurred (Fig. 3 *b*). After this phase, flow never resumed. Instead, there occurred a gradual disintegration of the compact mass.

The time factor was of utmost importance in these experiments. No contractile response was evident on release of pressure if pressure was applied after cytoplasmic flow had ceased. Also no such response occurred if the pressure was maintained longer than 2 minutes.

DISCUSSION

As a result of these experiments it can be said that osmotic forces seem to play no role in the "sphering" process caused by pressure application. If there is any volume change in the organism during the pressure period, it is most likely a small negative one. Therefore the change from a random shaped, multipseudopodial organism to a sphere necessitates a reduction in surface area, while the reverse situation necessitates an increase.

If the membrane of the pressure-induced sphere were folded on an ultramicroscopic level and this

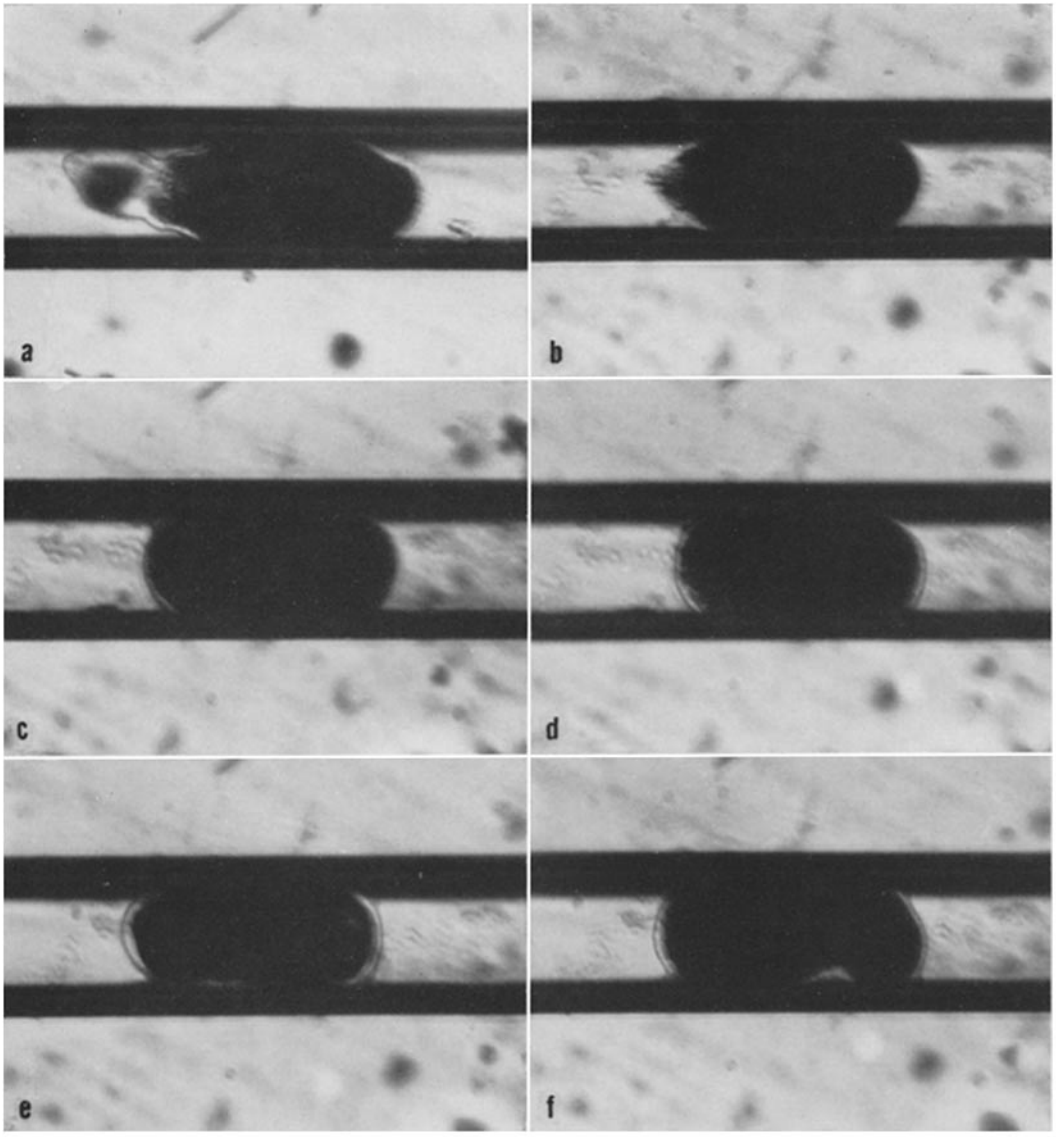

FIGURE 2 The reaction of *A. proteus* to high hydrostatic pressure. The ameba as seen (*a*) at atmospheric pressure, (*b*) upon application of 8,000 psi, (*c*) 15 seconds later, (*d*) 20 minutes later, (*e*) upon release of pressure, and (*f*) upon resumption of flow. See text for description.

was not apparent at ordinary magnifications, one could postulate a folding and unfolding to account for the apparent changes in surface area. However, electron micrographs reveal the membrane of the organism in the spherical state to be remarkably smooth in contour (4).

There remain at least two further possibilities to account for surface area changes: (*a*) a plastic stretch with only slight development of elastic tension, or (*b*) the formation of new and resorption of excess plasma membrane.

The first possibility might involve macromolecular realignments of the membrane components. As of now, there is little definitive evidence to

support or refute such an hypothesis. High resolution electron microscopy involving a comparison of the membrane structure of the pressurized spherical organism with that of the postpressure or normal *A. proteus* may help in resolving this problem .

The second possibility has been indicated by Goldacre (5). He has suggested that the plasma membrane is newly formed each time the ameba passes through its own length. However, Wolpert and O'Neill (6) on the basis of antibody labeling of the surface of the ameba, have expressed doubts that membrane turnover is rapid enough for such extensive new formation. Recently Jeon and Bell (7) have questioned Wolpert and O'Neill's assumptions in calculating that a high turnover rate is necessary for formation of new membrane at the tips of advancing pseudopodia. They indicate that the turnover need not be very rapid during normal pseudopodial flow. If the assumption is made that new membrane is formed, the present data indicate that, in the change from a spherical ameba to a normally shaped ameba, this new membrane formation need not be extensive but must be quite rapid.

The ability of the cytoplasm to form new internal membranous components is well established and the formation of such components as a result of high pressure treatment has been shown (4, 8). Whether this intracellular membrane can be converted to plasma membrane is still open to conjecture.

In the experiments on naked cytoplasm, the cessation of flow, the slight immediate contraction upon application of pressure, and the marked postpressure contraction duplicate the reactions of the intact organism. Theoretically, the negative volume condition imposed by pressure would initially assist in the active contraction of the gel network just prior to the disruption of polymeric linkages. This type of contraction upon application of pressure has been noted before (9) but is more distinctly seen in naked cytoplasm.

Except for the slight initial contraction, there is no tendency for the granules of the naked cytoplasm toward closer aggregation under pressure. Therefore, the role of the membrane in the sphering of the organism seems to be as earlier postulated; *i.e.*, the tensional forces at the surface overcome the lowered structural strength of the plasmagel layer.

While a marked decrease in relative plasma gel strength at 8000 psi and 25°C has been previously shown (1), the results of the experiments on naked cytoplasm reveal that at this temperature and pressure the gel structure is not totally disrupted. Although cytoplasmic flow stops immediately and some peripheral granules seem to fall free, the bulk of the cytoplasmic granular material remains in three-dimensional conformity. The relatively short period of exposure to pressure (2 minutes) in these experiments as compared to the 20 minutes exposure in the previous experiments could quite likely explain the retention of some gel structure. In the present experiments, the time of exposure to pressure is severely limited by the inability of the medium to maintain a functional cytoplasm for any reasonable duration. It would be interest-

FIGURE 3 A diagrammatic representation of the effect of release of pressure on naked cytoplasm. *a*, The cytoplasm under pressure. *b*, The contractile response following pressure release.

ing to see whether an extended period of exposure to pressure would allow the cytoplasm to retain its contractile potential and ability to resume flow. Such experiments utilizing the medium and techniques of Allen (10) on *Chaos carolinensis* cytoplasm are presently contemplated.

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