

CONTRACTION OF ISOLATED CONTRACTILE VACUOLES FROM *AMOEBA PROTEUS*

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The contractile vacuole of freshwater protozoa is involved in regulation of cell volume and of intracellular solutes (6, 13, 22). Despite the long-standing interest in this organelle and knowledge of its functional significance, little is known of the mechanisms of the primary actions of the contractile vacuole, namely, the formation and expulsion of the vacuolar fluid.

This report is concerned with the origin of the force for systole, the expulsion of the vacuolar fluid. The force could arise from tension generated in the wall of the vacuole, or from one of several processes in the adjacent cytoplasm, or by a combination of forces. Kitching in 1956 emphasized the lack of pertinent information, but concluded from indirect evidence that the walls of contractile vacuoles develop tension (12). On the other hand, Wigg et al. (29) suggested that the contractile vacuole (or water-expulsion vesicle) of *Amoeba proteus* is not contractile, and that the vacuolar fluid is expelled by force generated in the adjacent endoplasm. This conclusion was based on cinemicrography of the vacuolar cycle. The movement of the cytoplasm toward the vacuole during systole and the collapse of the vacuole were taken as evidence for generation of force by the endoplasm, and not by the wall of the vacuole. Similar observations were made on *Paramecium* (18) and *Tetrahymena* (17).

We found that the application of adenosine triphosphate (ATP) to contractile vacuoles isolated from *Amoeba proteus* caused the vacuoles to contract. The contraction had specific requirements for ATP and Mg. This observation suggests that the force for systole is generated by the vacuole itself. A preliminary report of these results has been published (21).

MATERIALS AND METHODS

Amoeba proteus were cultured at 18°C in the dark in a dilute, mixed salt solution (20), and were fed washed *Tetrahymena pyriformis*.

Amoeba proteus normally has one contractile vacuole per cell. The contractile vacuole was identified and

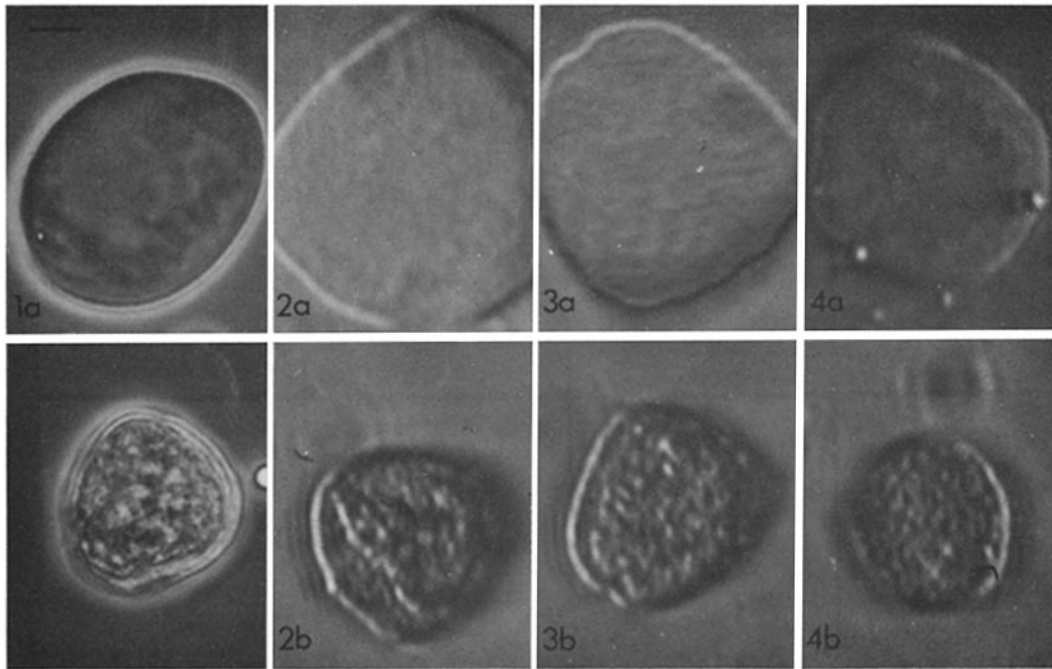
distinguished from food vacuoles by its gradually increasing volume (diastole) and by its posterior position in the cell. The vacuole was isolated from the cell with a glass micropipette (outside tip diameter about 30 μ) held by hand on the cell just adjacent to the contractile vacuole. A slight, abrupt suction exerted by mouth ruptured the cell plasmalemma and drew the contractile vacuole and a small volume of cytoplasm, free of other vacuoles, into the pipette. The vacuole was placed in a drop of culture medium or other solution in a depression slide. The test solutions applied to the vacuole with micropipettes usually contained ATP or another nucleotide (Sigma Chemical Co., St. Louis, Mo.), one or more salts, and were adjusted to pH 7.0. The isolated vacuole was photographed on Polaroid film, before and after treatment, through a microscope equipped with phase-contrast optics.

OBSERVATIONS

The isolated contractile vacuoles were spherical or slightly oval in shape. The diameters were between 20 and 40 μ , and were the same before and for several hours after isolation (cf. 22). The walls of the isolated vacuoles appeared to be without substructure and free from adhering cytoplasm. After about an hour, the vacuoles became granular in appearance, but they remained intact for several hours. They tended to stick to the glass slides, allowing the application of test solutions without losing the vacuole from the visual field.

The application of a solution containing ATP and MgCl₂ (pH 7.0) to an isolated vacuole caused a contraction, characterized by a folding or wrinkling of the wall of the vacuole and a reduction of the volume of the vacuole. Figs. 1-4 show four isolated vacuoles before and immediately after treatment with ATP and Mg. The contractions were always complete in 0.5 sec. In attempts on several hundred vacuoles, failures to contract were rare.

The isolated vacuoles were tested for contractions in either the culture medium, or in solutions of various K salts at 30 mM, near the cytoplasmic K concentration (Prusch and Dunham, unpub-



FIGURES 1-4 Phase-contrast micrographs showing contractions of four contractile vacuoles isolated from *Amoeba proteus*. The bar in Fig. 1 *a* indicates 5μ . The figures designated (*a*) show the isolated vacuoles before treatment. Figures (*b*) show the same vacuoles after application of a solution containing 10 mM ATP, 5 mM CaCl_2 , and 5 mM MgCl_2 . $\times 1400$.

lished results). The contractions of the vacuoles were the same in all of the solutions.

The lowest concentration of ATP causing contraction was between 1.5 and 2.5 mM. ATP at 10 mM with 5 mM MgCl_2 was sufficient to give the maximal response. Application of ATP alone caused no response. CaCl_2 at 5 mM did not substitute for MgCl_2 . In the presence of 5 mM CaCl_2 , the contractions caused by ATP and Mg were irreversible, whereas, in the absence of added Ca, the vacuoles relaxed to near their original configuration within 1 min.

ATP is required specifically for the contraction. Inosine triphosphate, guanosine triphosphate, adenosine diphosphate, and adenosine monophosphate, all at 10 mM, applied along with 5 mM CaCl_2 and 5 mM MgCl_2 , all failed to elicit any signs of contraction in the isolated vacuoles. These observations also show that the contraction is not due to a change in the ionic strength of the medium.

The contractions are also not a passive osmotic phenomenon. The application of 100 mM sucrose

or 30 mM Na_2SO_4 caused no noticeable contraction, nor any reduction in volume of the vacuoles. This result also suggests a low permeability to water, which would be of obvious functional significance for a structure which in vivo expels a fluid hyposmotic to the cytoplasm (22, 24).

DISCUSSION

The contraction of the isolated contractile vacuole suggests that the force for systole in *Amoeba proteus* is generated at least in part by the wall of the vacuole. The contractile property of the isolated vacuole does not constitute proof for this mechanism of systole in the intact cell, but it does make this possibility much more likely.

The isolated vacuole contracted by ATP does not resemble exactly the fully contracted vacuole in vivo. However, it is comparable in appearance to one of the intermediate stages of systole shown by Wigg et al. (29). Occasionally the isolated vacuoles contracted by ATP were flattened in the plane of the light path, and more nearly resembled the vacuole in vivo just before the end

of systole. It is possible that the contracted vacuoles in Figs. 1-4, while circular in outline, are flattened in the plane of the photographs.

The observations of Wigg et al. (29) on the contractile vacuole in vivo in *Amoeba proteus* are consistent with the authors' conclusion that the force for systole is generated by the adjacent cytoplasm. However, the observations do not exclude the possibility that the force arises in the wall of the vacuole. The movement of cytoplasmic particles toward the vacuole during systole was interpreted to indicate that the vacuole is collapsing due to a force from the cytoplasm, but this movement could as well be due to contraction of the vacuole and hydraulic coupling to the adjacent cytoplasm. The collapsing of the vacuole can no longer be cited as evidence of a passive vacuole since the contraction in vitro could equally well be described as collapse.

The requirements for ATP and Mg for contraction of the isolated vacuole provide little basis for speculation about the control of the triggering of expulsion. Addition of Ca was not necessary for contraction in vitro, but this in no way rules out a requirement for a low Ca concentration, which was undoubtedly present in all of the solutions. The relaxation of the vacuole after contraction in media to which Ca was not added is most likely not the same process as the filling of the vacuole in vivo. The relaxation is faster than diastole in vivo. Furthermore, it remains to be demonstrated that the conditions necessary for diastole exist for the isolated vacuole. Thus the ability of the isolated vacuole to relax raises questions about the control of diastole in vivo.

In 1920, Chambers reported the isolation of contractile vacuoles from *Amoeba proteus* (5). Chambers observed apparently spontaneous contractions of the isolated vacuoles, a phenomenon never observed in the present study. Although the account was extremely brief, the description of the contractions was otherwise similar to that given here. Contractile vacuoles have also been isolated from other species of amoeba (10, 22), but no observations were made of their contractile properties.

The contraction of the isolated vacuole requires the presence of contractile elements in or on the wall of the vacuole. There were early reports, from electron microscopic studies, of fibrils associated with the contractile vacuole of *Amoeba*

proteus (1, 8, 14), but the fibrils appear to be fixation artifacts. In other studies no fibrils were seen around the contractile vacuole of *Amoeba proteus* (15) or *Chaos chaos* (19), but these studies probably suffered from inadequate fixation or imbedding techniques (3). With new techniques, fibrils have been seen in the cytoplasm of *Amoeba proteus* (3, 30) and *Chaos chaos* (16), but no special attention was paid to the contractile vacuole. The smaller soil amoebae offer less difficulties with fixation. Microtubules or filaments have been reported bordering the contractile vacuoles of two species of soil amoeba (2, 9), but were not observed in three other species (4, 26, 28). An early demonstration of birefringence of the wall of the contractile vacuole of *Amoeba verrucosa* is perhaps relevant (23). Fibrils are associated with the contractile vacuoles of several ciliates (7, 25), but there is no reason that contractile vacuoles of ciliates and amoebae, let alone all amoebae, should share a common mechanism for systole. Further studies are necessary for clear evidence on the structural basis for contraction of the contractile vacuole in amoebae.

It might be possible to speculate about the nature of the contractile proteins in the wall of the vacuole by comparison with the properties, particularly with regard to the role of divalent cations, of actomyosin and its components (cf. reference 11). Both myosin-like and actomyosin-like proteins have been identified in homogenates of *Amoeba proteus* (27). However, the evidence on the roles of Mg and Ca in the contraction of the isolated vacuole is not sufficiently clear to warrant any conclusions.

This investigation was supported by United States Public Health Service Grant NB 08089.

Received for publication 20 February 1970, and in revised form 27 March 1970.

REFERENCES

1. BAIRATI, A., and F. E. LEHMANN. 1956. *Protoplasma*. 45:525.
2. BHOWMICK, D. K. 1967. *Exp. Cell Res.* 45:570.
3. BHOWMICK, D. K., and K. E. WOHLFARTH-BOTTERMANN. 1965. *Exp. Cell Res.* 40:252.
4. BOWERS, B., and E. D. KORN. 1968. *J. Cell Biol.* 39:95.
5. CHAMBERS, R. 1920. *Anat. Rec.* 18:225.
6. DUNHAM, P. B. 1970. In *Biology of Tetrahymena*. A. M. Elliot, editor. Appleton-Century-Crofts Inc., New York. In press.

7. ELLIOTT, A. M., and I. J. BAK. 1964. *J. Protozool.* **11**:250.
8. GREIDER, M. B., W. J. KOSTIR, and W. J. FRAJOLA. 1958. *J. Protozool.* **5**:139.
9. HOLBERTON, D. 1969. *Nature (London)*. **223**:680.
10. HOPKINS, D. L. 1946. *Biol. Bull. (Woods Hole)*. **90**:158.
11. JAHN, T. L., and E. C. BOVEE. 1969. *Physiol. Rev.* **49**:793.
12. KITCHING, J. A. 1956. *Protoplasmatologia*. **IIID 3a**:8.
13. KITCHING, J. A. 1967. In *Research in Protozoology*. T.-T. Chen, editor. Pergamon Press Inc., New York. **1**:307.
14. LEHMANN, F. E. 1959. *Ergeb. Biol.* **21**:88.
15. MERGER, E. H. 1959. *Proc. Roy. Soc. Ser. B.* **150**. 216.
16. NACHMIAS, V. T. 1968. *J. Cell Biol.* **38**:40.
17. ORGAN, A. E., E. C. BOVEE, and T. L. JAHN. 1968. *J. Protozool.* **15** (Suppl.):23.
18. ORGAN, A. E., E. C. BOVEE, T. L. JAHN, D. WIGG, and J. R. FONSECA. 1968. *J. Cell Biol.* **37**:139.
19. PAPPAS, G. D., and P. W. BRANDT. 1958. *J. Biophys. Biochem. Cytol.* **4**:485.
20. PRESCOTT, D. M., and T. W. JAMES. 1955. *Exp. Cell Res.* **8**:256.
21. PRUSCH, R. D., and P. B. DUNHAM. 1967. *J. Gen. Physiol.* **50**:1083.
22. RIDDICK, D. H. 1968. *Amer. J. Physiol.* **215**:736.
23. SCHMIDT, W. J. 1939. *Protoplasma*. **33**:44.
24. SCHMIDT-NIELSEN, B., and C. R. SCHRAUGER. 1963. *Science (Washington)*. **139**:606.
25. SCHNEIDER, L. 1960. *J. Protozool.* **7**:75.
26. SCHUSTER, F. 1963. *J. Protozool.* **10**:297.
27. SIMARD-DUQUESNE, N., and P. COUILLARD. 1962. *Exp. Cell Res.* **28**:92.
28. VICKERMAN, K. 1962. *Exp. Cell Res.* **26**:497.
29. WIGG, D., E. C. BOVEE, and T. L. JAHN. 1967. *J. Protozool.* **14**:104.
30. WOLPERT, L., C. M. THOMPSON, and C. H. O'NEILL. 1964. In *Primitive Motile Systems*. R. D. Allen and N. Kamiya, editors. Academic Press Inc., New York. 143.