SYNERESIS IN AMEBOID MOVEMENT: ITS LOCALIZATION BY INTERFERENCE MICROSCOPY AND ITS SIGNIFICANCE

ROBERT D. ALLEN and RONALD R. COWDEN, with the technical assistance of PRUDENCE JONES HALL. From the Department of Biology, Princeton University, Princeton, New Jersey, and the Division of Cell Biology, Institute for Muscle Disease, Inc., New York

The periodic appearance of a hyaline cap at the front of advancing pseudopodia is an almost universal accompaniment of movement in ameboid cells. The fluid of the hyaline cap cannot be a simple filtrate of the ground substance pressed through the "plasmagel sheet" as Mast (11, 12) believed, because the dry mass of this fluid is less than half that of the ground substance of the rest of the cell (5). Neither can this fluid be derived from the environment, for the turnover rate of water is orders of magnitude too slow (10). The low dry mass of the hyaline cap fluid, therefore, indicates that it is produced by syneresis accompanying the contraction of a gelated region somewhere within the cell.

The site of the contraction which serves as the motive force for ameboid movement has never been clearly established. The ectoplasmic contraction theory (or tail contraction theory) (7, 8, 11, 14) would lead one to predict that the origin of the hyaline cap fluid would be the contracting tail ectoplasm (7). This possibility seemed to receive strong support from the gradient of optical path difference in flattened amebae demonstrated by Allen and Roslansky (5). These data, in fact, suggested that contraction of the tail ectoplasm released a "tide" of syneretic fluid which was pressed forward, its anterior margin visible as the hyaline cap. However, Allen and Roslansky pointed out several aspects of ameboid movement which were not in good agreement with the theory.

Recently, one of us (2, 3) has proposed a new theory which is compatible with the various behavioral aspects of ameboid movement and with the limited amount of experimental data available. The new theory postulates a contraction *at the front* of each advancing pseudopod as the motive force for movement. This front contraction theory rests on the same two basic assumptions as the tail contraction theory: first, that the cytoplasm of ameboid cells can exist in at least two states, contracted and extended, and, second, that the driving contraction is propagated along the cytoplasm, as it is in muscle. According to the new theory, the contraction occurs in the endoplasm as the latter splits and becomes everted to form the ectoplasmic tube. The region just posterior to the hyaline cap where this is postulated to occur has been called the "fountain zone" (2, 3).

The front contraction theory (or fountain zone contraction theory) demands that the endoplasm: (*a*) possess "structure capable of developing and transmitting tension," (*b*) be capable of conducting a wave of contraction, (*c*) actually shorten and thicken in the fountain zone in the process of forming new ectoplasmic tube, and (*d*) give rise to the hyaline cap fluid by syneresis localized in the fountain zone.

The first three demands of the front contraction theory have been demonstrated to be fulfilled. Rheological experiments have proved that the endoplasm is not a sol, but rather contains pseudoplastic and/or elastic components in its structure (1, 2, 6). Cytoplasm dissociated from *Chaos chaos* has been shown to stream outside the intact cell by means of a propagated contraction (4). The endoplasm does, in fact, shorten and thicken in passing through the fountain zone (2, 3). Unfortunately, most of the facts which these investigations have uncovered cannot be used as decisive evidence in choosing between the front and tail contraction theories, for these facts are compatible with both theories. In only two cases so far has it been possible to test the predictions of one theory in such a way as to yield evidence which excludes the other. In the first case, Allen, Cooledge, and Hall (4) have recently shown that the streaming of dissociated cytoplasm, unexplainable previously by the tail contraction theory, could be accounted for in all aspects by the front contraction theory.

The second series of decisive experiments are those to be reported here. They concern the fourth prediction of the front contraction theory, namely, that the site of the syneresis giving rise to the hyaline cap must be the fountain zone. The prediction is testable by interference microscopy, for

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the cytoplasm of the ameba undergoes a cyclic movement: through the endoplasm, into the ectoplasm, and back again into the endoplasm. The granular cytoplasm, in order to give rise to the hyaline cap, must, according to the theory, squeeze out the low refractive index hyaline fluid from the fountain zone. Therefore, the ectoplasm, from which the hyaline fluid has been removed, should have a higher refractive index than the endoplasm.

The only information until now available on the site of syneresis in the ameba has been the demonstration of a gradient in optical path difference in cells flattened to roughly one-half to two-thirds their normal thickness by a coverglass maintained parallel to the supporting slide by quartz rods of measured, uniform thickness as spacers (5). The observed gradient confirmed the expectation according to the tail contraction theory.

An alternative explanation of the observed gradient was suggested by the new theory: this gradient could have arisen from a combination of two thickness gradients of opposite sign in the endoplasm and ectoplasm along with a *difference* in refractive index between the two layers. The data of Mast and Prosser (13) show, and simple inspection confirms, that the ectoplasm of ameboid cells is much thicker toward the posterior end of the cell. In fact, the tail region is all ectoplasm.

The present experiments were designed to test whether there is such a difference in refractive index between the two principal layers of advancing pseudopodia of large ameboid cells. The species observed were *Chaos chaos*, *Amoeba proteus*, *Amoeba dubia*, and the testacean *Difflugia pyriformis*. Advantage was taken of the fact that with a fringe-in-field interference system, such as that provided by the Leitz interference microscope, it is possible to distinguish between a homogeneous cylinder (*e.g.*, a glass rod in oil) and a hollow cylinder (*e.g.*, a glass capillary in, and filled with, oil). The optical path difference varies across a homogeneous cylinder according to the relation:

$$\Delta = 2(n_o - n_s)\sqrt{(2rd - d^2)}, \tag{1}$$

where Δ is the optical path difference (cm), r is the radius of the cylinder (cm), d the distance (cm) from the edge at which Δ is measured, n_o the refractive index of the cylinder, and n_s the refractive index of the medium.

Accordingly, the fringe displacement, S, across a homogeneous cylinder follows the following relation:

$$S = \frac{2D}{\lambda}(n_0 - n_m)\sqrt{2rd - d^2}, \tag{2}$$

where λ is the wavelength of the source. In the case of a capillary, fringe displacement as far as the lumen follows equation (2). If the contents of the capillary do not exactly match the refractive index of the capillary wall, the slope changes. The following relation describes the optical path difference across a capillary:

$$\begin{aligned}\Delta = {} & 2(n_c - n_s)\sqrt{(d - r + r_1)(r_1 + r - d)} + \\ & 2(n_o - n_s)\left[\sqrt{d(2r - d)} - \right. \\ & \left.\sqrt{(d - r + r_1)(r_1 + r - d)}\right],\end{aligned} \tag{3}$$

where r_1 is the radius of the lumen, and n_c is the refractive index of the contents of the lumen; consequently, fringe displacement across a capillary follows the relation:

$$\begin{aligned}S = {} & \frac{2D}{\lambda}(n_c - n_s)\sqrt{(d - r + r_1)(r_1 + r - d)} \\ & + \frac{2D}{\lambda}(n_0 - n_s)\left[\sqrt{d(2r - d)}\right. \\ & \left. - \sqrt{(d - r + r_1)(r_1 + r - d)}\right].\end{aligned} \tag{4}$$

The experimental method was to find and photograph pseudopodia which most closely resembled cylinders in shape. Some of the smaller, newly formed anterior pseudopodia of *C. chaos* are frequently large enough to show clearly the

FIGURE 1

A newly formed pseudopod of *Chaos chaos* photographed with Leitz interference microscope on Plus-X 35 mm film, using a 1 sec. exposure and the × 40 objective. The general shape of the displaced fringes indicates that the pseudopod is nearly circular in cross-section, and that there is a break in fringe 3 as it passes through the endoplasm. Hyaline cap cytoplasm is located distal to the last distinct fringe. × 660.

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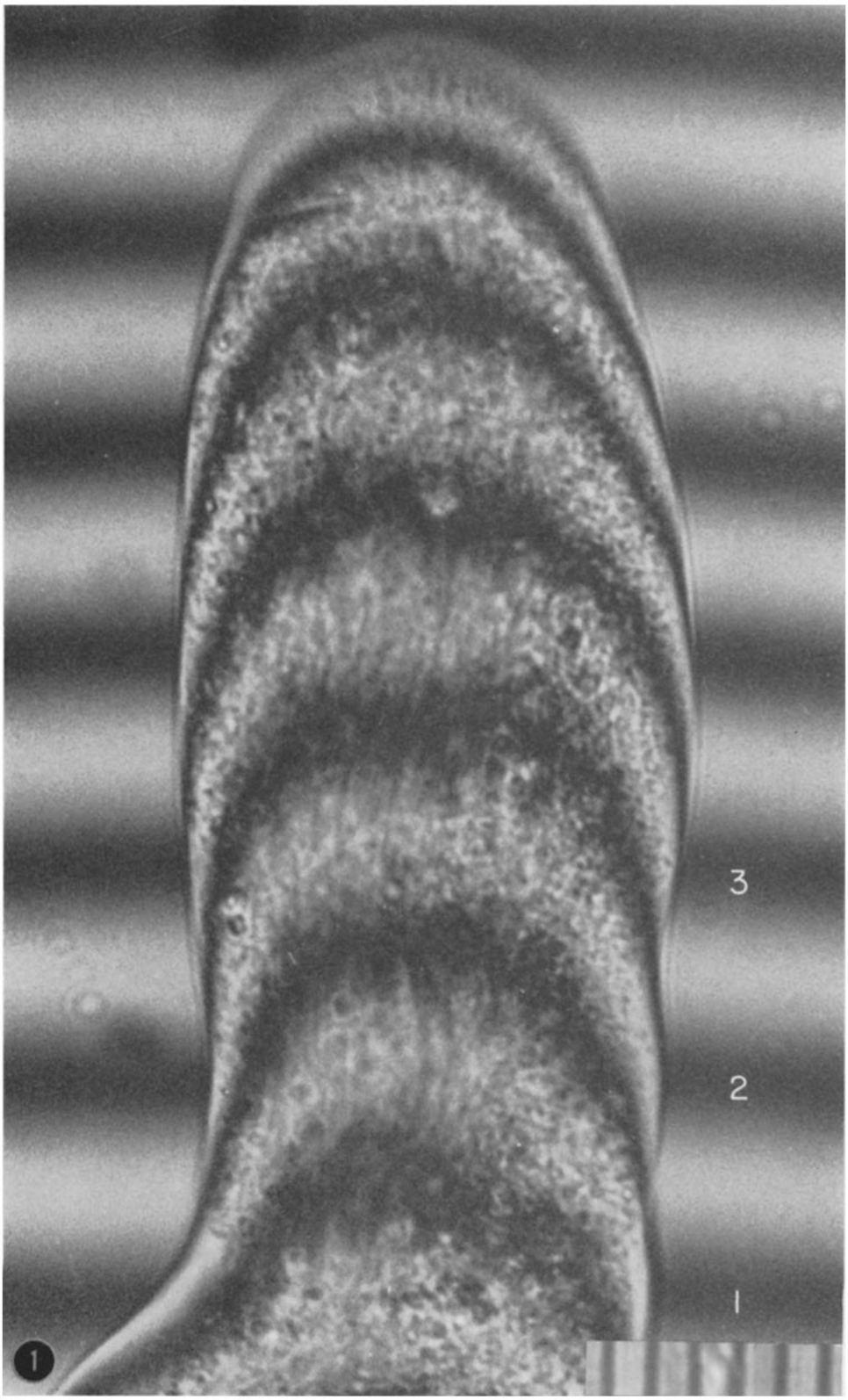

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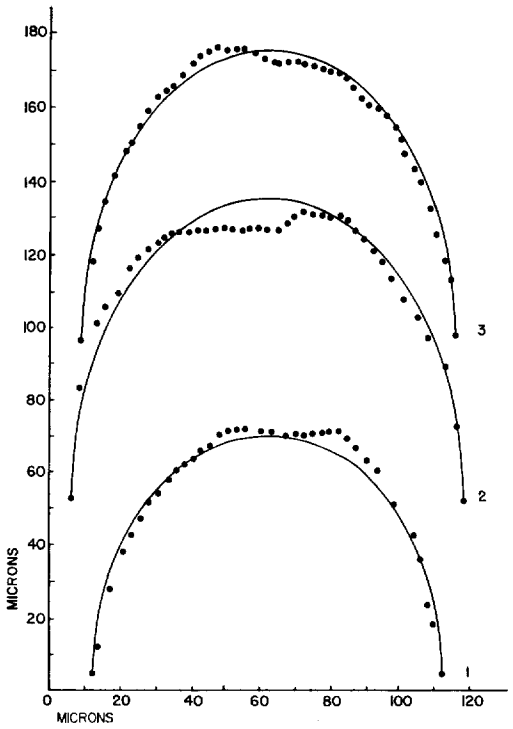

FIGURE 2

Fringe displacement curves (dotted lines) measured from Fig. 1 and plotted as a graph to show deviation from the solid line which is the calculated fringe displacement curve across a homogeneous cylindrical body. The data points were corrected for small changes in pseudopodial diameter.

expected discontinuity in the fringe displacement curve (see Figs. 1 and 2). This discontinuity also appears in broader pseudopodia of both *C. chaos* and *A. proteus*. These larger pseudopodia tend to be somewhat elliptical in cross-section, so that the pattern of fringe displacement is more difficult to interpret. In addition, pseudopodia of both these species contain other structural features, such as dorsal ectoplasmic ridges and ventral attachment zones. Both these features are recognizable and occur only some distance posterior to the regions photographed in this study.

The expected dip in the fringe displacement curve at the boundary of the endoplasm was seen in all the species examined, although in the smaller forms it was not possible to exclude deviations from circular cross-sectional outline as a contributory cause of the fringe displacement curves. Because of their size, specimens of *Chaos chaos* showed the clearest examples of discontinuous fringe displacement patterns.

What is especially noteworthy in Fig. 1 is the fact that the "dip" in the fringe displacement curve follows the wandering of the endoplasmic stream as it advances obliquely across the pseudopod. The dip is most clearly observed in the second fringe, but can also be seen in the other two fringes by comparing the slopes on the two sides. Note that the curve for fringe 2 through the ectoplasm closely follows the curve expected of a cylinder, showing that it does approximate a cylinder in shape near its anterior end. Farther back, of course, it becomes irregular, owing to the causes mentioned above. In Fig. 2 the data points measured from fringe displacement are superimposed on a "best fit" theoretical curve for a homogeneous rod with a refractive index of 1.3424, corresponding to a dry mass concentration equivalent to a 5.22 per cent protein solution.

The fringe anterior to no. 3 did not deviate significantly from the theoretical curve for a rod, but the exposure period was too long and the motion too rapid to permit firm conclusions from this fact. It is worth pointing out, however, that this result would be expected according to the front contraction theory, for in the anterior portion of the fountain zone, syneresis should be nearly complete.

By inserting the calculated value for ectoplasmic refractive index and the measured values for pseudopodial and endoplasmic radii, and measured optical path difference, into equation (4), the approximate refractive index of the endoplasm was computed as 1.3405, corresponding to a dry mass equivalent to 4.16 per cent protein.

This difference of approximately 1 per cent protein concentration between the endoplasm and ectoplasm at the front of an ameba pseudopod is typical of values obtained from several pseudopodia. For the endoplasm to change its dry mass concentration from 4.2 per cent to 5.2 per cent within a second or two, as it passes through the fountain zone to form the ectoplasmic tube, represents the loss, through syneresis, of 20 per cent of the endoplasmic water content during its contraction. It therefore appears justifiable to conclude that the front contraction theory, as far as it goes, is correct.

This conclusion is not weakened by the fact that ameboid movement *can* occur without hyaline cap formation, as Mast (12) observed. Syneresis

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is commonly observed to accompany isodiametric contraction in structurally isotropic gels. In structurally anisotropic gels, whether syneresis occurred or not would depend on whether the increased molecular cross-bonding allowed a compensatory thickening. We still know too little about the molecular organization of ameba endoplasm to state whether the absence of hyaline cap formation indicates a lack of syneresis or the disappearance of the hyaline fluid into some other part of the cell. The latter possibility is suggested by the observation that as the granular cytoplasm invades the hyaline cap, the plasmalemma appears to be forcibly elevated from the newly formed ectoplasmic tube by fluid driven posteriorly from the hyaline cap. The plasmalemma slides over the ectoplasmic tube (9), providing a channel through which the hyaline cap fluid may (in fact must) circulate toward the tail, where it somehow finds its way through the weakened ectoplasmic tube (1) to rejoin the endoplasmic stream. That the hyaline cap fluid does rejoin the endoplasmic stream in the tail is shown by the fact that the same difference in refractive index between the endoplasm and ectoplasm is seen not only near the front of new pseudopodia, but much farther posterior in long cylindrical specimens.

A simple calculation showed that the observed refractive index difference could, combined with endoplasmic and ectoplasmic thickness gradients of opposite sign, account for not more than a 5 per cent difference in optical path between the front and rear of an ameba. This is clearly insufficient to explain the 15 per cent gradient reported by Allen and Roslansky (5). When their data were examined, it was found that the steepness of the gradient in compressed cells was clearly related to the extent of compression, a fact previously interpreted to mean incomplete compression in some cells. Now, however, it seems much more likely that because of the gradient of thickness already present, and because of the relatively softer consistency of the endoplasm, the effect of greater compression was to flatten the endoplasmic stream to a greater extent in the tail than in the front. This being the case, the thickness gradient in the endoplasm would be steeper than normal. It is also possible that the optical path difference gradient may be in part due to the presence of the "hyaline ectoplasm," which we believe is the channel for the tailward transport of the hyaline cap fluid. This layer is much thicker in the anterior region than in the tail; rapid compression causes a broad hyaline layer to accumulate on the sides of the anterior portions of cells.

The loss of water by the endoplasm in the formation of the ectoplasmic tube by "gelation" and the corresponding appearance of the low refractive index hyaline cap seems to establish beyond doubt that the shortening and thickening of cytoplasm in the fountain zone is a contraction which provides the motive force for ameboid movement. It seems likely that this hyaline cap fluid is recirculated toward the tail in a channel beneath the plasmalemma.

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REFERENCES

1. ALLEN, R. D., *J. Biophysic. and Biochem. Cytol.*, 1960, **8**, 379.
2. ALLEN, R. D., *in* The Cell, (J. Brachet and A. E. Mirsky, editors), New York, Academic Press, 1961, 135.
3. ALLEN, R. D., *Exp. Cell Research*, 1961, Suppl. **8**, 17.
4. ALLEN, R. D., COOLEDGE, J. W., and HALL, P. J., *Nature*, 1960, **187**, 896.
5. ALLEN, R. D., and ROSLANSKY, J. D., *J. Biophysic. and Biochem. Cytol.*, 1958, **4**, 517.
6. ALLEN, R. D., and ROSLANSKY, J. D., *J. Biophysic. and Biochem. Cytol.*, 1959, **6**, 437.
7. GOLDACRE, R. J., *Internat. Rev. Cytol.*, 1952, **1**, 135.
8. GOLDACRE, R. J., and LORCH, I. J., *Nature*, 1950, **166**, 497.
9. GRIFFIN, J. L., and ALLEN, R. D., *Exp. Cell Research*, 1960, **20**, 619.
10. LØVTRUP, S., and PIGON, A., *Compt.-rend. trev. Lab. Carlsberg, Sér. chim.*, 1951, **28**, 1.
11. MAST, S. O., *J. Morphol. and Physiol.*, 1926, **41**, 347.
12. MAST, S. O., *Protoplasma*, 1931, **14**, 321.
13. MAST, S. O., and PROSSER, C. L., *J. Cellular and Comp. Physiol.*, 1932, **1**, 333.
14. PANTIN, C. F. A., *J. Marine Biol. Assn. United Kingdom*, 1923, **13**, 24.

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