# An Anterior-Posterior Gradient of Refractive Index in the Ameba and Its Significance in Ameboid Movement*

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## ABSTRACT

Sustained locomotion in *Amoeba proteus* and *Amoeba dubia* results in the establishment of a measureable gradient of refractive index along the anterior-posterior axis of the cell, provided thickness of the specimens is kept constant by even compression under a selected coverglass supported by quartz beams of uniform diameter.

The tail region of the ameba develops a higher refractive index, indicative of from 6 to 40 per cent more organic matter (expressed as protein) there than present in the front. This gradient fades on cessation of movement. The average protein concentrations in the crystal-free tails and fronts of 15 *A. proteus* were 3.9 and 3.4 per cent, respectively. In individual experiments, the tail-front difference ranged from one to eight times the accuracy of the method.

Since the gradient of refractive index was shown not to result from extraction of water from the tail by the contractile vacuole, it was interpreted as displacement of water toward the anterior part of the cell during movement. It is suggested that contraction of the ectoplasm drives forward a "tide" of syneretic fluid, the anterior border of which is visible as the hyaline cap, which contains less than 1 per cent protein. The movement of the granular endoplasm into the hyaline cap would then complete the cycle by imbibition of the fluid tide. The theoretical positions of Pantin and of Dellinger have been combined in the proposal that ameba cytoplasm consists of a network of a contractile phase which is able to expel (by syneresis) a highly mobile fluid phase. Some other possible interpretations are discussed.

Although there is an imposing number of theories of ameboid movement, no one of these can be regarded as entirely satisfactory. Nevertheless, most recent authors have favored some form of contractility (2, 8–13, 16, 17, 20). Pantin (20) and later authors considered the "ectoplasmic tube" as the only contractile part of the cell, forcing the passive endoplasm forward. Pantin further inferred a forward movement of water from the observed similarity in form of whole marine amebae in hypertonic medium to the tail of normal cells, and the similar resemblance of amebae in hypotonic medium to the anterior region of normal cells, and believed that ectoplasmic contraction resulting from syneresis was the basis of movement.

Recent developments in refractometry with the interference microscope (3–5) have made measurements of water content in various regions of the cell possible. A phase change measured with the interference microscope can be used to find refractive index if the thickness of the specimen and the refractive index of the medium are known. We have introduced a simple and precise technique for regulating the distance between coverglass and slide by interposing quartz (fused silica) beams drawn to various diameters. When the thickness of an ameba is controlled under these conditions, differences in phase change between various parts of the cell can be used to compute corresponding differences in refractive index, which, in turn, are related to the relative concentrations of organic matter (expressed as per cent protein) and water. Since measurements can be made in almost any region of the cell, it is possible by interference microscopy to follow local changes

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in water content which result from ameboid movement.

### Material and Methods

Most of the observations and measurements to be reported were made on rapidly growing specimens of *Amoeba proteus*, raised either on *Tetrahymena* (23) or in rice-wheat cultures containing *Chilomonas* and *Colpidium*. *A. dubia* were grown by the latter method exclusively. To avoid the high refractive index of crystalline inclusions in the cell, it was necessary to select, under oblique incident illumination, those organisms which scattered the least light. It was possible to find in nearly all cultures of rapidly growing organisms some which were sufficiently clear to obtain a reproducible extinction setting in the cytoplasm using the mercury green line (5461 A) with the American-Optical-Baker interference microscope (Figs. 1 and 2).

Several experiments were carried out on organisms which had been almost entirely cleared of cytoplasmic inclusions by centrifugation followed by excision of the centrifugal end of the stratified cell. Through perserverance, many of the organisms were rendered so transparent by this process that they could be scarcely seen through the dissecting microscope. Those cells which still contained a nucleus recovered rapidly after the operation and showed no abnormalities over the observation period of several hours.

The thickness of the cells was kept constant during phase change measurements by the pressure of a coverglass (selected for optical flatness) resting on two quartz (fused silica) beams approximately the length of the coverglass and with diameters ranging from 29 to 50 microns, but constant in one preparation to about 5 per cent. Since the cells were about one-fiftieth the length of the beams, variation in chamber thickness due to taper of the beams was negligible over the length of a single organism. The width of the organism was always several times the diameter of the beam. It was possible to test, after each series of phase change measurements, whether the specimen filled the depth of the chamber by pressing lightly on the coverglass with a needle; uniformly compressed specimens flattened further under this treatment. If slides and coverglasses were not flat, this could introduce an error which might be cumulative, provided the organisms continued to move in the same direction. However, they typically altered their direction several times over the period of about 15 minutes required to obtain a sufficient number of measurements. During this time, the changes in direction of migration of the cell, and of orientation of the slide due to rotation of the stage served to randomize these variations.

The procedure of measurement was as follows: a rapidly streaming, monopodial specimen was chosen which had relatively few crystals. The field of the microscope (either the 10X or 40X system of the A-O:Baker shearing interference microscope) was set to extinction with the first-order fringe using mercury green-line illumination (5461 A). The analyzer was then rotated until the desired portion of the object was brought to extinction. Pairs of readings (background and object) were taken as near together in the middle of the field as practicable. When the background extinction setting drifted (due to variation in coverglass thickness, etc.) enough to impair the sensitivity of the system, the first-order fringe was again centered in the back focal plane of the objective. Determination of the extinction point in the cytoplasm was not so precise as in the background because of the presence of cytoplasmic inclusions. For this reason, it was particularly important to avoid measurements on slowly growing or starved cells, since these contained a large number of inclusions, especially crystals, which made extinction readings unreliable. No measurements were made in parts of the cell where curvature of the surface would influence thickness.

### RESULTS

#### A. Refractive Index of the Cytoplasm:

When amebae were transferred to a slide and flattened with a coverglass supported by quartz beams, movement ceased for a period ranging from several seconds to a minute or more. During this time, it could be seen that the optical path difference between cytoplasm and the medium was constant throughout the cell. Within minutes after the cell had begun to move, an optical path difference between the front and rear of the ameba could be observed and measured. (See Figs. 1 and 2). Measurements on 15 specimens of *Amoeba proteus* and 3 of *Amoeba dubia* revealed phase changes from one-fifth to over one-half a wave length (5461 A) for specimen thicknesses between 32 and 50 microns. In thicker chambers the amebae were insufficiently compressed. Table I shows in detail the summary of results, in which the mean refractive index measured in the tail region of *A. proteus* was found to be 1.3401, and in the front 1.3391, representing a mean "protein concentration" of 3.95 per cent and 3.39 per cent protein, respectively, for the tail and front regions of these cells. This difference was an average of 5 (range: 1 to 8) times the accuracy of the method (about 0.1 per cent protein). Expressed as per cent difference in protein concentration between the rear and front of the cell

$$\left( \frac{\text{per cent protein in rear-per cent protein in front}}{\text{per cent protein in front}} \right)$$

the range was 6 to 40 per cent, with a mean of about 17 per cent in *A. proteus*, 32 per cent in *A. dubia*.

TABLE I

*Summary of Data Showing the Anterior-Posterior Gradient of Refractive Index in Amoeba; Expressed Also as Protein Concentration*

| Species and condition | Specimen thickness | Mean refractive index | | | Mean protein concentration (± 95 per cent confidence limits) | | | Per cent difference tail-front |
|---|---|---|---|---|---|---|---|---|
| | | Tail | Middle | Front | Tail | Middle | Front | Front |
| | μ | | | | | | | |
| A. proteus | 44 | 1.3406 | 1.3302 | 1.3394 | 4.22 ± .18 | 4.00 ± .14 | 3.67 ± .13 | 18.2 |
| (uncentrifuged) | 44 | 1.3406 | 1.3404 | 1.3396 | 4.22 ± .28 | 4.09 ± .19 | 3.68 ± .21 | 14.6 |
| | 46 | 1.3399 | 1.3399 | 1.3395 | 3.83 ± .14 | 3.86 ± .24 | 3.62 ± .18 | 6.0 |
| | 33.5 | 1.3398 | ——— | 1.3390 | 3.80 ± .28 | ——— | 3.31 ± .38 | 15.7 |
| | 32.5 | 1.3413 | ——— | 1.3410 | 4.61 ± .22 | ——— | 3.88 ± .33 | 18.8 |
| | 50 | 1.3395 | 1.3390 | 1.3386 | 3.61 ± .26 | 3.32 ± .22 | 3.12 ± .23 | 15.7 |
| | 50 | 1.3389 | 1.3386 | 1.3382 | 3.28 ± .10 | 3.12 ± .29 | 2.88 ± .21 | 14.0 |
| | 46 | 1.3387 | 1.3384 | 1.3381 | 3.16 ± .14 | 3.02 ± .29 | 2.88 ± .11 | 9.7 |
| | 38 | 1.3418 | 1.3406 | 1.3399 | 4.88 ± .48 | 4.21 ± .28 | 3.86 ± .19 | 26.4 |
| | 38 | 1.3417 | 1.3404 | 1.3393 | 4.83 ± .3 | 4.13 ± .22 | 3.50 ± .30 | 38.0 |
| | 40.5 | 1.3388 | ——— | 1.3380 | 3.22 ± .33 | ——— | 2.80 ± .14 | 15.0 |
| (centrifuged) | 43.5 | 1.3372 | 1.3369 | 1.3368 | 2.32 ± .08 | 2.16 ± .09 | 1.93 ± .05 | 20.2 |
| | 35 | 1.3408 | 1.3404 | 1.3349 | 4.32 ± .06 | 4.08 ± .13 | 3.54 ± .40 | 22.0 |
| | 49 | 1.3373 | 1.3372 | 1.3369 | 2.38 ± .4 | 2.32 ± .14 | 2.19 ± .41 | 8.6 |
| | 49 | 1.3377 | 1.3374 | 1.3366 | 2.60 ± .31 | 2.44 ± .39 | 2.02 ± .28 | 28.0 |
| A. dubia | 40.5 | 1.3394 | 1.3386 | 1.3376 | 3.55 ± .22 | 3.12 ± .26 | 2.54 ± .12 | 40.0 |
| (uncentrifuged) | 40.5 | 1.3403 | ——— | 1.3390 | 4.05 ± .18 | ——— | 3.34 ± .11 | 21.0 |
| ↓ | 40.5 | 1.3386 | 1.3376 | 1.3371 | 3.11 ± .15 | 2.55 ± .07 | 2.28 ± .18 | 36.4 |

Since uniformity of specimen thickness would would be an important factor in evaluating the observed difference in phase change in terms of differences in protein concentration, an experiment was designed to reverse the sign of any systematic errors due to specimen thickness differences. A solution of 8 per cent polyglucose[1] (refractive index 1.3434) was pipetted into a chamber containing an actively moving specimen which was phase retarding and had shown the usual anterior-posterior phase change gradient. Before the osmotic effects of the polyglucose solution became pronounced, there was a period of a few minutes in which measurements could be made. The specimen was now phase-advancing, and the optical path difference was now greater in the front than in the rear, indicating that the rear end of the cell (1.3419) was closer to the refractive index of the polyglucose than the front end (1.3411), confirming the higher refractive index in the tail found

earlier. These refractive indices are too high; due partly to dilution of the polyglucose solution by the water previously in the chamber, and partly to osmotic effects on the ameba. The reversal of optical path difference in a medium of high refractive index provided confirmation for evidence from rapid compression of the specimen to indicate that the thickness of the main body of the ameba in the areas used for measurement was essentially constant.

## B. The Contractile Vacuole:

Since fresh water amebae pump a considerable quantity of water to their environment by their posteriorly located contractile vauole, it is conceivable that extraction of water from this region of the cell might be due to the action of the vacuole, rather than some phase of cytoplasmic streaming and ameboid movement. Normal cells when not moving, and enucleated cells (which rarely engage in sustained movement) were observed for changes in refractive index in the neighborhood of an eccentrically situated contractile vacuole. Since no refractive index changes were observed, it is reasonable to conclude that the pumping of the contractile vacuole is not a factor in the observed gradient of refractive index in the moving cell.

[1] Kindly supplied by the Grasselli Division of Du Pont de Nemours and Co., Wilmington, Delaware. The specific refractive increment of this material was about 0.0013 in the condition supplied. Since no attempt was made to dry the material before the measurement, the true value is probably closer to that recently reported for glycogen (6).

## C. Injury Vacuoles and the Hyaline Cap:

The contracting ectoplasm hypothesis suggests that the hyaline cap fluid might be pressed from the interstices of the ectoplasmic gel by syneresis. If this idea is correct, ameba cytoplasm must consist of at least two phases, one the contractile material, and the other the syneretic fluid. The instantaneous formation of large injury vacuoles, such as occurs when an ameba is too rapidly compressed, demonstrates that the cytoplasm can be separated into two distinct phases. The cycle of hyaline cap formation is interpretable as a cyclic separation and recombination of phases. It was of interest to determine the refractive indices of the hyaline cap material and vacuolar fluid in order to learn whether these substances might be indentical.

One organism which was too rapidly compressed to a thickness of 40.5 microns provided a very favorable case in which to measure the refractive index of two large, flattened, injury vacuoles 87 and 42 microns in horizontal diameter respectively. Obviously, both of these vacuoles were flattened considerably in the vertical direction by pressure from over- and underlying ectoplasm; their vertical thickness could not be measured microscopically. Fortunately, a contractile vacuole (easily recognizable by its shell of mitochondria and by its low phase retardation) 117 microns in diameter lay nearby, presumably flattened to about the same degree. Assuming that the phase retardation presented by the contractile vacuole was caused only by over- and underlying ectoplasm (which would be nearly the same for the injury vacuoles), it was possible to subtract this phase retardation (10°) from that presented by the injury vacuoles (39 and 36 degrees respectively) and arrive at an average protein concentration of 1.15 per cent, or between a third and a fourth the concentration of whole ground cytoplasm plus unresolved granules.

The refractive index of the hyaline cap is difficult to measure because it has a curved surface. Immersion refractometry could not be used to match its refractive index, because protein solutions alter movement and cause pinocytosis in amebae, and other solutes exert an osmotic effect. However, observations on the formation of injury vacuoles from the granular cytoplasm which were squeezed into the hyaline cap region provided a means of comparison to show that the hyaline cap material had a considerably lower refractive index than the vacuolar fluid. Because of the geometric difficulties

involved, no attempt was made to arrive at an accurate figure for the hyaline cap material; however, it is probably less than 1 per cent protein.

## D. Observations on the Nucleus:

The resting nuclei of both *A. proteus* and *A. dubia* are highly heterogeneous objects, as can be seen in recently published electron micrographs (7, 21). Nevertheless, if one observes the nucleus at magnifications and numerical apertures at at which the solid bodies of the nucleus are not resolved, the phase changes recorded will give an approximate determination of average organic matter concentration. Unfortunately, another factor enters in the case of *A. proteus*, for its nucleus undergoes continual changes in shape. A series of 20 measurements of nuclear dimensions in this species revealed the following variations: long axis, 32 to 54 (mean 45) microns; width, 30 to 41 (mean 35) microns; and thickness 12 to 27 (mean 19.5) microns. In addition, continued observations on the nuclei of several cells showed that some of this variation was due to deformation of the nucleus, caused partly by collisions with more solid parts of the cells. The most reliable measurements were on very thin preparations, in which it is reasonable to assume that the nucleus was flattened to somewhere near the minimum (12 micron) thickness. If this assumption is correct, we have sufficient information to obtain a value for refractive index of the nucleus by substituting in the equation given by Barer (3, 5):

$$\phi_N = n_N t_N + n_C (t_C - t_N) - n_M t_M$$

in which $\phi$ is the phase change through the nucleus in microns,

   $n$ is refractive index,
   $t$ is thickness,
   and the subscripts $C$, $N$, $M$ refer to cytoplasm, nucleus, and medium.

$$0.334 = 12 n_N + (1.3395) (21.4) - (1.333) (33.4)$$

$n_N = 1.3491$ (equivalent to about 8.9 per cent protein).

The accuracy of this measurement suffers from the fact that simultaneous measurement of phase change and thickness are not possible with present methods. Since the dimensions of the nucleus continually change, it is necessary either to measure maximum and minimum thickness and express protein concentration as a range (from 6.3 per cent to 10.6 per cent), or pick a favorably thin prepara-

tion in which it can be assumed that the nucleus is flattened to the thinnest dimensions seen when the nucleus is on edge. The nucleus of *A. dubia* undergoes less marked changes in dimensions, and it was possible to determine nuclear refractive index at about 1.349 or equivalent to about 9 per cent protein.

## DISCUSSION

### A. Sources of Error:

In consecutive series of measurements of phase change between the anterior and the posterior regions of the cytoplasm and the nearest points in their respective environments, some variation was encountered. The accuracy to which the analyzer of the interference microscope could be set without a half-shade device was about 1 to 2 degrees, indicating that the accuracy of the method was about 0.1 per cent protein. The extinction point of the background could be set more accurately than that of any region of the cell; still, the reproducibility of rapid, consecutive settings was good. Over a period of time, variations in measurements of phase change in moving specimens increased, due to some extent to differences in thickness of the coverglass and to the fact that probably neither the coverglass nor the slide was optically flat. As indicated earlier, these errors were probably well randomized by changes in direction of movement and orientation of the preparation. The greatest variation was produced by movements of the amebae; cessation of movement, for example, brought about either a decrease in, or abolishment of the phase change difference established between the anterior and posterior ends over a period of sustained locomotion.

It is a matter of fundamental importance whether thickness was successfully controlled in these experiments. In uncompressed cells, thickness plays the greatest role in determining optical path, and indeed the ectoplasmic ridges of *A. proteus* appear quite prominent. If amebae are incompletely compressed because the supporting beams are too thick, discontinuities of thickness are reasonably obvious. With beams of the proper size range (30 to 50 microns), pressing on the coverglass with a needle caused further flattening (seen as broadening) of the part of the organism which filled the depth of the chamber. Additional evidence that a difference in thickness, if it occurred, did not account for the optical path difference came from measurements of phase change

between front and rear of a streaming organism in 8 per cent polyglucose. If the anterior end, which had shown a lower refractive index in water, had been thinner than the tail, it would have presented a refractive index greater than the tail when measured in polyglucose.

### B. Magnitude and Significance of the Anterior-Posterior Gradient in Refractive Index:

Taking the variation of measured phase changes into account, and assuming the scatter to be caused by a number of random variations discussed above, the protein concentration of the tail was found to be significantly higher than that in the front of the cell, to a confidence of 95 per cent in most experiments. The average difference of about 15 per cent more protein in the posterior end could mean any of a number of different things. Removal of water from the tail by the contractile vacuole is one possible interpretation which can be ruled out by the lack of a refractive index gradient in non-motile, normal, and enucleated cells with eccentric and actively pumping contractile vacuoles. Calculations from the data of Adolph (1) showed that the rate of water removal by the contractile vacuole was much too slow to account for the unequal distribution of water in the ameba. A gradient of cytoplasmic inclusions from posterior to anterior might be another source of the gradient in refractive index. This might be true for cells containing a large number of crystals, but in the present study the refractive index gradient remained even when the crystals were mostly removed from the cell. As stated earlier, the organisms used were selected as the most transparent in our cultures. A more likely explanation for the magnitude of the refractive index differences observed is a real difference in protein concentration in the ground cytoplasm associated with ameboid movement itself.

The observation of a gradient of large cytoplasmic inclusions (mostly crystals) in some cells suggested that a possible corresponding gradient of smaller inclusions, such as mitochondria or microsomes, might have influenced our results. It does not seem probable that an inconspicuous inequality in distribution of inclusions could account for more than a fraction of the observed difference in refractive index. *A. dubia* often collect inclusions near the tail region, then release them almost all at once into the endoplasmic stream. In one case, this was followed under the interference microscope; the refractive index gradient remained even when the inclusions had moved, showing that light scattering by

a moderate number of inclusions did not seriously influence the measurements of refractive index. Measurements on other organisms cleared of most of their inclusions by centrifugation showed the same kind of gradient in refractive index.

### C. Some Theoretical Considerations:

Although ectoplasmic contractility is a plausible theory of ameboid movement, previous evidence in support of it has so far come either from the observed shortening of the ectoplasmic tube (12, 13, 20), or from syneresis (such as occurs on contraction in actomyosin gels (26) or fibers (14)) either inferred as part of ectoplasmic contraction from observational evidence (20) or induced experimentally (12). When adequate methods become available, the ectoplasmic contraction theory should be tested at the macromolecular level, for there have been suggestions that changes of shape in protein molecules could perform work in biological systems (e.g. 18, 19). Recently an attempt has been made by Goldacre and Lorch (13) to test a theory of ameboid movement based on the folding of protein molecules in the tail, and unfolding in the front, of the ameba. On the basis of the fact that some proteins (e.g. ovalbumin) adsorb more dye when "unfolded" (denatured) than when "folded" (native), it was predicted that amebae placed in dye solutions should bind the dye on unfolded protein molecules in the ectoplasm, and that this dye should be "released" in the tail when these same protein molecules folded. Unfortunately the fact that dye does accumulate in the tail of A. discoides (13) and A. proteus (22) does not prove the correctness of the model, since this phenomenon is equally well explained by the facts that (1) the ectoplasm itself (particularly its outer region) eventually accumulates in the tail before joining the endoplasmic stream, just as the dye does, and (2) that the rate of penetration of the dye would be higher in the posterior region of the cell due to the greater surface exposed there. What remains to be shown is that the dye is bound in the ectoplasm by proteins which fold, for there are presumably many other cellular materials which might bind dye. The observations of dye accumulation under hydrostatic pressure by Prescott (22) are likewise subject to alternative interpretations; amebae in dye solutions become spherical at both 350 and 450 atmospheres of hydrostatic pressure, indicating at both pressures a solution to the state characteristic of the endoplasm (15). The failure of the cell to accumulate dye at the higher pressure could as well be due to decreased permeability as to additional folding of ectoplasmic protein molecules.

The ectoplasmic contraction theory and the Goldacre model appear to provide the most satisfactory theoretical interpretation of ameboid movement, although, as pointed out above, the evidence in support is rather slim and often subject to alternative interpretations. For example, there is no doubt that the ectoplasm shortens, but it should be kept in mind that this shortening, although compatible with the theory of ectoplasmic contraction, could also occur if the ectoplasm possessed only "elastic tension" (16, 17) and the endoplasm were "pumped out" by some mechanism operating from the front of the cell (cf. 10, p. 450).

Also compatible with the contracting ectoplasm theory is the movement of water during locomotion toward the front of the cell, which was inferred by Pantin (20), and has now been confirmed by interference microscope measurements in the present communication. Here also, it is necessary to point out that although syneresis is an attractive explanation for the movement of water, other interpretations must be considered as well. For example, according to Rashevsky (24), diffusion forces resulting from higher permeability to water at the anterior end of the cell might be adequate to explain cytoplasmic streaming associated with ameboid movement. This explanation is not satisfactory, because (1) the fluid of the hyaline cap can be seen to arise from behind, indicating it does not form from an entrance of water from the outside; (2) the concentration gradient results from movement, and fades when movement stops; and (3) sudden reversals of flow and counter-streaming (8, 10, 25) are not explained.

If the syneresis interpretation is correct, and the water which appears in the hyaline cap originates from contractions of the ectoplasm, it is reasonable to conceive of the hyaline cap as the forward border of an extensive "fluid tide" which sweeps through the endoplasm of the cell. The plasmagel sheet of the ameba has been described as a kind of "filter" through which the hyaline fluid material is "pressed" (11, 12). It follows that if water is lost by syneresis in the ectoplasm, a corresponding imbibition must occur in the endoplasm. Microscopic observation of the manner in which the haline fluid material sweeps past the endoplasm periodically during the cycles of hyaline cap formation (12, 16) suggests that the "watery" phase of the cytoplasm is highly mobile. The ease with which slight mechanical injury can cause a separation of phase in the cytoplasm may attest to this mobility. The fact that the fluid of the injury vacuoles has a higher protein concentration than

the hyaline cap material could indicate either that injury had added proteins to the "tide" of hyaline material or that the cytoplasm consists of more than two separable phases.

The principal weakness of the contracting ectoplasm theory (like the diffusion theory) is also its failure to explain satisfactorily the rapid reversals of streaming and counterstreaming within the same pseudopod (8, 10, 25). If the endoplasm were completely passive, it is difficult to conceive how it could be impelled in opposite directions by contractions of the same ectoplasmic tube. On the other hand, it is perfectly possible that the endoplasm could possess some degree of structure sufficient to allow it to contract or extend in much the same manner as this material seems to do as soon as it is incorporated into the ectoplasmic region. Evidence for this possibility was published in a note by one of us (2) a few years ago, in which it was shown that prolonged and often violent streaming activity takes place in cytoplasm from broken cells contained in quartz capillaries sealed under oil. Under these conditions, release of the endoplasm from the confines of the ectoplasmic tube rendered possible streaming movements almost certainly based on contraction and extension of endoplasmic material. However, there is little information on what changes had occurred in this naked cytoplasm that might alter the situation with respect to what occurs normally inside the cell.

In view of the highly tentative nature of the theories of ameboid movement and of the complexities of the observational evidence on which they must be based, we are inclined to prefer a combination of the theoretical positions taken by Pantin (20) and others, who have emphasized the contractile role of the ectoplasm, and of Dellinger (9), who conceived of the endoplasm and ectoplasm as one contractile network, the endoplasmic stream shaped and controlled by the ectoplasmic tube (cf. also 25). Contractions and extensions of a "contractile network" such as Dellinger proposed would require a two-phase system, since the contractile phase could not contract unless it could squeeze out the more dilute phase. It appears that such a syneresis does occur normally when the ectoplasm contracts, and it is tempting to suggest that a similar phenomenon may occur in the endoplasm when contiguous steams of endoplasm move in opposite directions.

Another aspect of ameboid motion which has been unexplained is the alternating advance in the anterior region, of the hyaline cap fluid and the granular endoplasm. While the formation of the hyaline cap could, according to the present view, be part of a fluid tide moving through the granular endoplasm even when the granules of the latter are stationary (10), movement of the granules themselves would represent the extension of the "contractile network" with simultaneous imbibition of the hyaline cap fluid. The fact that movement can occur without formation of a hyaline cap does not speak against this mechanism, for the two processes could occur simultaneously as well as alternately.

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EXPLANATION OF PLATE 249

FIG. 1. A specimen of *Amoeba proteus*, uncentrifuged and flattened to a thickness of 33.5 microns; photographed with the analyzer of the interference microscope at extinction position for the anterior part of the specimen. The pointers show the regions in the posterior (black pointer) and anterior (white pointer) where measurements were made. The hindmost part of the tail is not considered since it may vary in thickness. The role of light scattering from inclusions in various parts of the cell is discussed in the text. × 200.

FIG. 2. The anterior third of an uncentrifuged specimen of *Amoeba dubia*, showing the relatively homogeneous extinction point in the ground cytoplasm despite the presence of a moderate number of cytoplasmic inclusions. Photographed with the 40X shearing system; exposure 1 second. × 900.
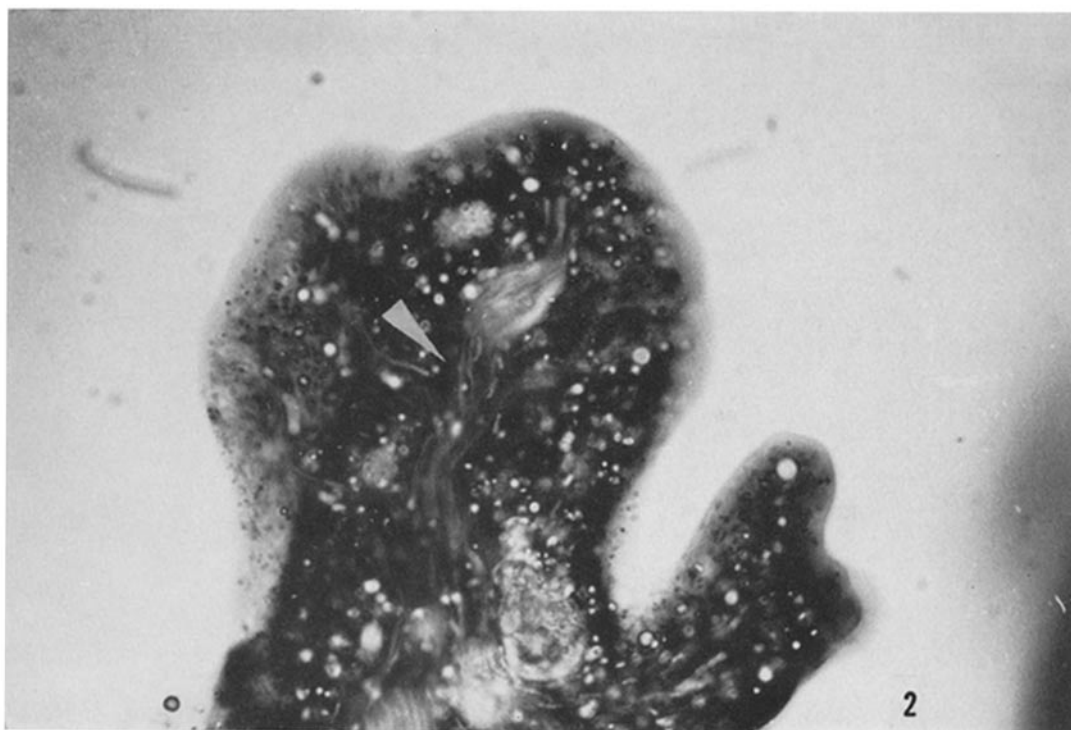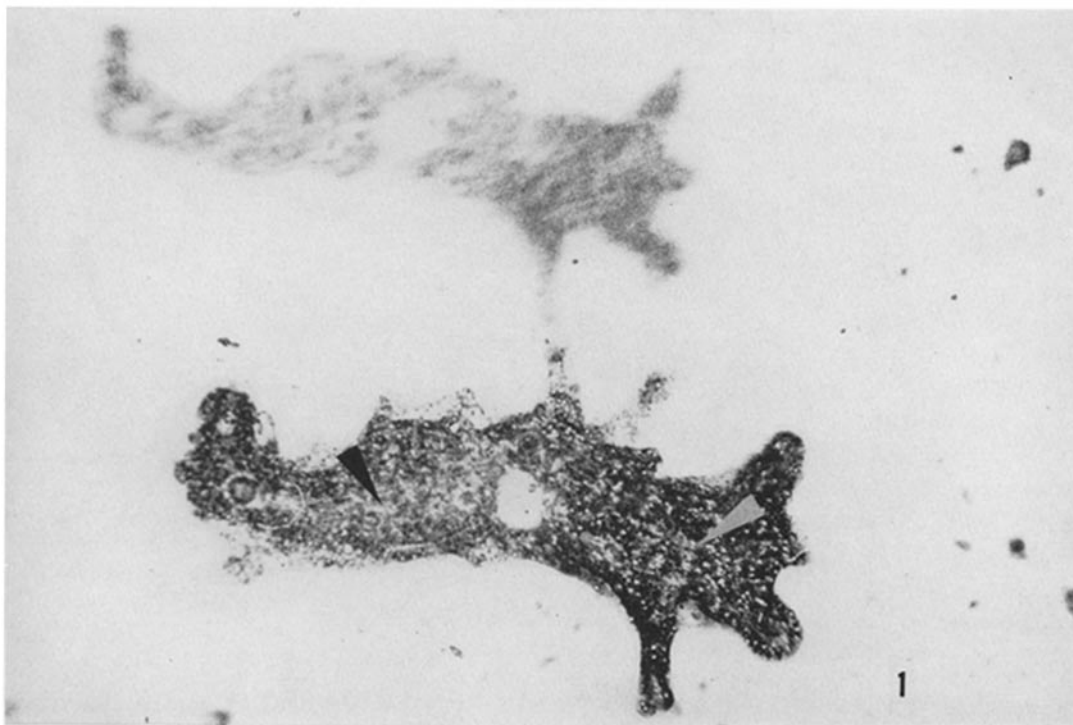
(Allen and Roslansky: Refractive index gradient in ameba)