THE CONSISTENCY OF AMEBA CYTOPLASM AND ITS BEARING ON THE MECHANISM OF AMEBOID MOVEMENT

II. The Effects of Centrifugal Acceleration Observed in the Centrifuge Microscope

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ABSTRACT

Three species of common, free-living amebae, *Amoeba proteus*, *Amoeba dubia*, and *Chaos chaos* were directly observed and photographed while exposed to a range of centrifugal accelerations in two types of centrifuge microscopes. Cytoplasmic inclusions in all three species are displaced discontinuously (at a variable velocity) in apparently all parts of the cell, suggesting non-Newtonian behavior and/or heterogeneous consistency. The ectoplasm of all species shows the highest yield point of any region in the cell; the posterior ectoplasm is less rigid than that in the anterior part of the cell. The axial part of the endoplasm shows evidence of structure (a sharp viscosity transition if not a true yield point) by its: (a) resistance to the displacement of particles carried in that region of the cell, (b) hindrance to the passage through the cell of inclusions displaced from other regions, and its (c) support without visible back-slip of inclusion being resuspended in the axial endoplasm in a centripetal direction at accelerations as high as 170 g. At this acceleration, each crystal "weighs" the equivalent reduced weight of seven times its volume in gold at 1 g. The only regions of the normal, moving cell which show clear evidence of low apparent viscosity are the "shear zone" (see Fig. 8) and the "recruitment zone." Possible reasons for low apparent viscosity in these regions are discussed. A new scheme of ameba "structure" is presented on the basis of the combined results of velocity profile analysis and the present centrifugation study.

INTRODUCTION

In the first paper of the present series (2), it was pointed out that an accurate and detailed knowledge of the consistency[1] of the cytoplasm in various regions of the ameba would be of basic importance for a decision among theories of ameboid movement. In that paper, velocity profiles of the endo-

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[1] "Consistency" is defined as ". . . that property of a material by which it resists permanent change of shape, and is defined by the complete force-flow rela- tion" (reference 21, page 24). This term is used in preference to "viscosity" which implies that the force-flow relation is linear.

plasmic stream were presented which may be interpreted as strong evidence of non-Newtonian consistency in the endoplasm. This view is in conflict with the usual "sol-gel" concept of ameba structure, the evidence concerning which has recently been reviewed in detail (1). The present paper contains observations which verify the conclusions drawn from the previous study (2) by demonstrating the existence of structure in the axial endoplasm. It will further be shown that the entire posterior part of an ameba (endoplasm and ectoplasm) displays less structure than the anterior portion.

The first study of the ameba's cytoplasmic consistency by centrifugation was carried out by Heilbrunn (14–17) under the tacit assumption that the cytoplasm was a structureless Newtonian sol. He accelerated *Amoeba dubia* with a hand centrifuge in order to determine the displacement velocity of crystalline inclusions at a known acceleration. Since the velocity could not be measured directly, amebae were centrifuged for varying lengths of time at roughly constant acceleration (128 *g*) and subsequently transferred to a slide for observation. The total displacement divided by the time required for sedimentation was substituted into the Stokes law equation on the further tacit assumption that crystals were displaced at constant velocity. By making a number of additional assumptions which have until recently seemed reasonable, Heilbrunn arrived at a viscosity value for the entire cytoplasm of *A. dubia* which was about twice that of water, or roughly the value expected for a dilute protein sol. It now appears that several of the assumptions which led to this conclusion were unjustified or incorrect (see Discussion).

A few years later, Harvey and Marsland (13) recorded the following direct observations with the centrifuge microscope on amebae moving along attached to a glass surface while exposed to centrifugal acceleration:

"The heavy crystals of *Aoemba dubia* always fall in 'jerks' even when moving through a visibly clear field. They move and stop and move and stop as if they met invisible obstructions. The same is true of *A. proteus*. This behavior is marked and apparent in all parts of the animal and suggests that the discontinuous movement must be due to a structure in the cytoplasm rather than to adherence of the crystals as they move along a sticky external surface."

These observations of Harvey and Marsland could be regarded as strong evidence for the presence of structure throughout much of the ameba's cytoplasm: a view diametrically opposed to that arrived at by Heilbrunn on the basis of his centrifugation study on *A. dubia*. A subsequent study by Heilbrunn *et al.* (17) on *A. proteus* revealed the presence in this cell of a gelled "cortex" corresponding roughly to the "plasmagel" of Mast (18) (the ectoplasm). Still, however, the endoplasm was considered to be a "sol." The unresolved question of the consistency of ameba cytoplasm has now been settled at least in part by centrifuge microscope experiments at low accelerations at which it can be determined whether regions previously believed to be fluid in fact exhibit a sudden transition in viscosity comparable to the yield point of a gel.

MATERIAL AND METHODS

Three species of amebae were used in the present investigation. All were raised both in the customary manner on wheat infusions in either Prescott and James medium (20) (for *Amoeba proteus* and *Chaos chaos*) or a distilled water (for *Amoeba dubia* (6)), and by the improved mass-culture method recommended by Griffin (7, 8). Healthy, feeding cells were isolated in culture medium and deprived of food for 24 to 36 hours before centrifugation experiments in order to deplete them of large food vacuoles.

All experiments were carried out at room temperature (22–25°C.) in wedge-shaped centrifuge-microscope cuvettes of the type described by Harvey (11). These cuvettes were large enough to maintain 20 to 50 small amebae for periods of several days without ill effects. The tops of the cuvettes were sealed with cellophane tape to prevent excessive evaporation. At the accelerations used, there was no need for a high-density "cushion," as most cells were not detached from the glass surface.

Two different types of centrifuge microscope (12) were employed. The first was a commercial model formerly manufactured by the Bausch & Lomb Optical Company of Rochester, New York, and equipped with high and low power objectives. This instrument had a radius of 8.2 cm. (center of rotor to bottom of cuvette) and a strong motor, so that accelerations of a few thousand times gravity could be developed within a few seconds. Ordinarily the centrifuge microscope was operated from a calibrated voltage supply and allowed to come gradually to a predetermined acceleration over a period of a minute or more. Photographs were taken at intervals with a Leica camera and micro-ibso attachment.

The second instrument, a rapidly starting centri-

The Size Distribution in Microns of Crystalline Inclusions from Representative Samples of Ameba Cultures

| Species | Bipyramids | | | | Plates | | | | |
| | Length | | Width | | Length | | Width | | Thickness |
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	
Amoeba dubia	8.5	3.6–12.4	5.0	1.6–8.3	13.5	8–16	13.5	8–16	0.4
Amoeba proteus	2.7	1.1– 4.1	1.9	1–2.8					
Chaos chaos	2.4	1.1– 4.4	1.2		5		5		

fuge microscope, was especially constructed in the Department of Biology machine shop by Mr. Russell Mycock. It consisted of a brass rotor containing the required prisms and objectives, driven through a magnetic clutch by a Sorvall model SS-1 centrifuge which served as a heavy fly-wheel. The entire device was mounted in a massive welded steel frame with suitable armor protection.[2] After the fly-wheel had reached a preselected angular velocity, the magnetic clutch was engaged. The latter could be adjusted so that the centrifuge microscope rotor achieved a known terminal angular velocity within as short a time as 0.002 seconds or as much as a second by regulating the current applied to the magnets.

RESULTS

1. *The Discontinuous Displacement of Cytoplasmic Inclusions; an Observation Common to All Three Species*

Most of the cytoplasmic inclusions of all three species were not moved by centrifugation until a critical acceleration was reached; they then became displaced, not at a constant velocity, but discontinuously as Harvey and Marsland (13) described (see also page 386). This observation by itself rules out the possibility that the cytoplasm might be homogeneous and of Newtonian consistency, but does not permit a decision as to whether the cytoplasm is non-Newtonian, of heterogeneous consistency, or both of these.

Observations reported in the following sections provide some general information on variations in consistency in different regions of the ameba. In view of the discontinuous displacement of inclusions described above, no attempt was made to record individual particle displacement velocities. There is good cause to believe that the Stokes law equation is not valid for the measurement of

[2] The late Professor E. Newton Harvey very kindly contributed many centrifuge microscope components and offered several helpful suggestions.

cytoplasmic viscosity in the case of the ameba (see Discussion).

2. *Effects of Centrifugal Acceleration on Amoeba dubia*

Typical specimens of *A. dubia* are usually polypodial and contain one or more nuclei and numerous cytoplasmic inclusions, the most striking of which are crystals, numbering from several dozen to a few hundred. It is primarily these crystals which are displaced by centrifugation, although mitochondria and other components can be sedimented at higher accelerations than are considered here. The majority of the crystals are bipyramidal in form (both pointed and truncated), and the remainder are thin, flat plates. Direct measurements of crystal sizes are presented in Table I. The crystals normally lie free in the cytoplasm without any membrane surrounding them. If, however, an ameba is injured, excessively stimulated, strongly illuminated, or fixed, some of the crystals will almost invariably be found to be enclosed in a vacuole membrane which is readily visible with bright-field or phase-contrast microscopy.

In nearly all wild cultures a few amebae can be found which move sluggishly and contain larger and more numerous crystals than the amebae usually found in rapidly dividing cultures. These sluggish amebae apparently are products (at least in part) of poor culture conditions, for they are more prevalent in old, declining cultures. These cells stratify more readily than the more active ones and recover much more slowly after centrifugation.

The gradual application of acceleration to normal specimens of *A. dubia* with the Bausch & Lomb centrifuge microscope caused the first visible signs of crystal displacement at between 40 and 60 *g* (Fig. 1). At accelerations between 50 and 100 *g* the largest and heaviest inclusions suddenly disengaged themselves and moved discontinuously to a final position within several microns of the plasmalemma at the centrifugal end of the cell. On the way, they often collected temporarily in

pockets; then the pockets of crystals became displaced in the same manner. Some cells which exhibited normal movement showed no marked accumulation of crystals in any part of the cytoplasm even at 100 g. These became stratified, however, after gradual acceleration had achieved a force of 200 g.

A sudden burst of constant acceleration in the rapidly starting centrifuge microscope produced less sedimentation of inclusions than would have been expected on the basis of the effects of gradually applied acceleration. Acceleration of 70 g for 2 minutes caused only about half of the inclusions of most cells to accumulate at the centrifugal end. Application of 90 g for over a minute in several cells caused less sedimentation than 2 minutes at 70 g. More surprising still, a 10-second exposure to 220 g did not visibly alter the distribution of inclusions in many highly motile cells (Fig. 2). Exposure to this acceleration for 20 to 30 seconds, however, did sediment over half of the cells' crystals. The accumulation of these crystals in "pockets" in the cytoplasm was especially pronounced. A full minute was required at 220 g before all such pockets had been cleared from the cytoplasm.

The failure of A. dubia to stratify readily when exposed to suddenly applied acceleration of 220 g is partly explainable in terms of different experimental conditions from those reported in the past (see Discussion). The fact that the cytoplasm displayed greater resistance to displacement from suddenly—than from gradually—applied accel-

eration could be due either to some stiffening action resulting from the sudden shock to the animal, or to some liquefying action of prolonged exposure to increasing acceleration. Present evidence does not permit a decision, because some movement continues under the conditions of both experiments.

The small size and large number of pseudopodia render A. dubia less than ideal for centrifugation studies. One fact appears to be clear, however: amebae of this species contain no large volume of fluid sol. Whether limited regions of sol exist could not be determined on the basis of these experiments. It is certain also that most regions of the cytoplasm contain sufficient structure to impede the displacement of crystals and other inclusions by the forces involved in the present study.

3. Effects of Centrifugal Acceleration on Amoeba proteus

A. proteus is a larger cell with fewer pseudopodia than A. dubia, consequently, its rate of locomotion is greater. The cytoplasm contains smaller, but more numerous crystals (see Table I). A proteus also contains a large number of heavy spherical bodies or refractile bodies (3, 4), the nature of which is unknown, plus the usual mitochondria, oil droplets, etc. The crystals were observed to be free in the cytoplasm, as in A. dubia, prior to centrifugation, afterward, some were contained within vacuole membranes.

Figure 1

Photographs taken through the Bausch & Lomb centrifuge microscope of Amoeba dubia under increasing centrifugal acceleration. Each frame is 520 μ in width; the black triangles are position reference marks to show locomotion.

Frame 1: Specimen before centrifugation; acceleration 1 g.

Frame 2: Same ameba 1 minute after centrifuge started; acceleration 72 g. A few large crystals had sedimented.

Frame 3: 1½ minutes; 100 g. Note increasing slow sedimentation. Each crystal broke away and fell discontinuously.

Frame 4: 2 minutes; 124 g. Many crystals still suspended and moving normally in the endoplasmic stream.

Frame 5: 2½ minutes; 148 g. Still some crystals not sedimented; most of these now in the ectoplasm.

Frame 6: 3 minutes; 164 g. Some particles still streaming in the endoplasm.

Frame 7: 4½ minutes; 200 g. Stratification nearly complete, but the cell is moving centripetally with respect to the position marker.

Frame 8: 6 minutes; 220 g. Ameba completely stratified, but the clear endoplasm continues to stream, pseudopods extend and eventually carry the ameba centripetally out of the field of view.
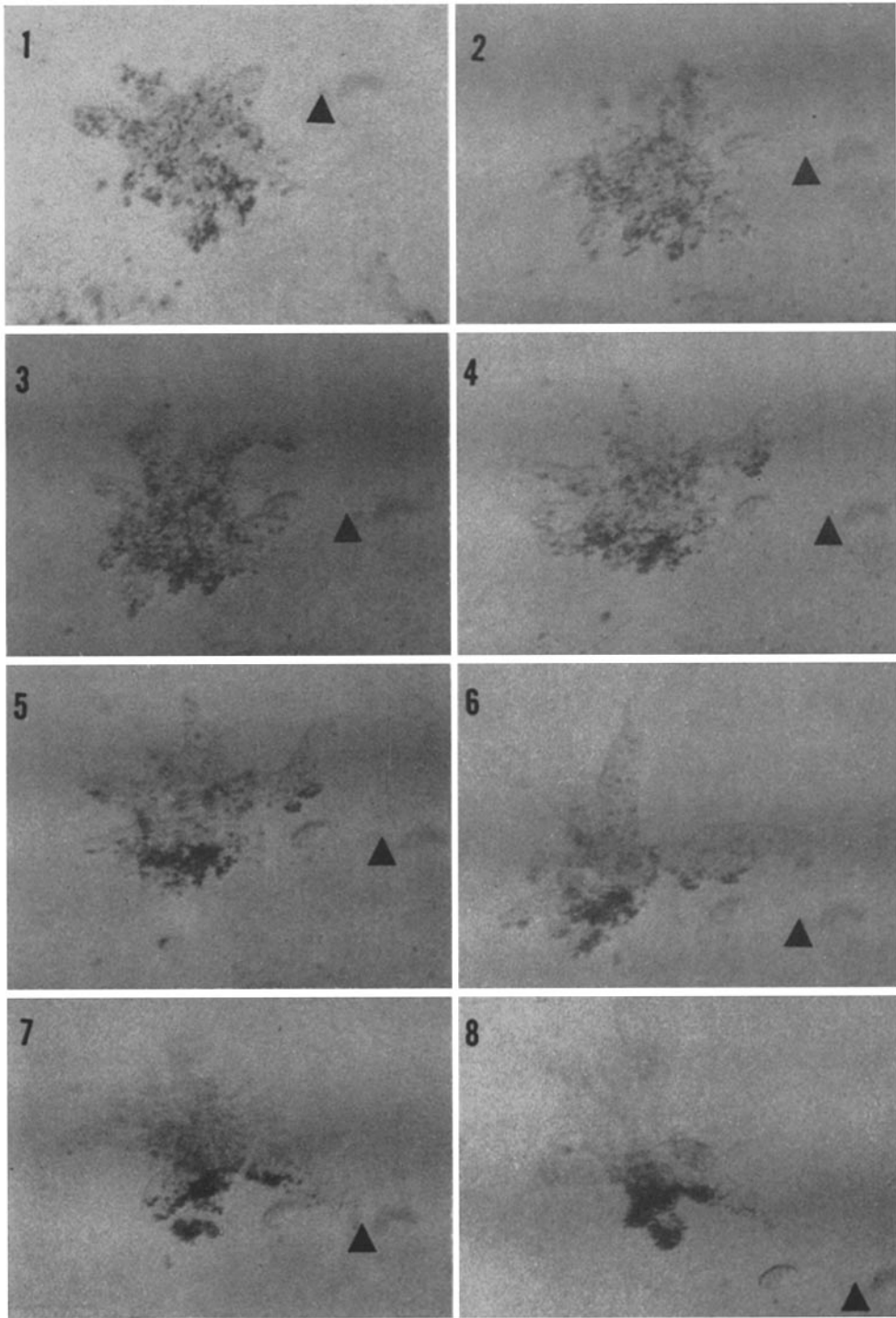
Acceleration, when gradually applied, caused the first detectable displacement of inclusions at 40 to 60 g. Exposure for 20 to 30 seconds to this range of accelerations caused a progressive accumulation of sedimented inclusions at the inner centrifugal border of the tail ectoplasm (See Frames 3 to 6 of Fig. 3, and Fig. 4). At higher accelerations, some or all inclusions traversed this border, particularly in the tail region, and came to rest near the plasmalemma (Fig. 3, Frames 1 to 10).

The most obvious accumulation of inclusions was always found in the tail region. This is explained in part by the fact that inclusions are first displaced in this region; that is, the tail appears to have a somewhat lower apparent viscosity than the anterior part of the cell. The greater displacement of particles in the tail perhaps exaggerates the consistency differences between posterior and anterior regions because the endoplasm which flows forward has already been depleted of its largest and heaviest particles, and thus appears deceptively unaffected by centrifugation.

Amebae oriented perpendicularly or obliquely to the axis of acceleration often showed some accumulation of inclusions on the centripetal side of the axial portion of the endoplasm as well as the centrifugal border of the ectoplasm when the acceleration was between 100 and 150 g (Fig. 5). This endoplasmic accumulation of crystals and other inclusions is shown in Frames 3 and 4 of Fig. 4 and in Frames 4, 5, and 7 of Fig. 3. It was quite easy to see that these inclusions actually were in the endoplasm, and not in a pocket of ectoplasm, for they moved with the endoplasmic stream.

In centrifugally directed specimens, there was surprisingly little accumulation of inclusions in the anterior region at accelerations that caused sedi-

mentation in the tails of laterally directed specimens. Those inclusions which became displaced almost invariably did so in the region close to the ectoplasmic walls, which we shall refer to below as the shear zone (Fig. 8 and Discussion). Occasionally a particularly large and/or heavy inclusion was observed to drop through the shear zone and fountain zone and pass into the hyaline cap. At much higher accelerations (300 to 500 g) the hyaline cap cometimes became filled with inclusions; this served to drag the entire pseudopod centrifugally.

One series of observations and photographs (Fig. 4) serves to illustrate the fact that the endoplasm is not structureless, but contains an axial region with some kind of tenuous structure. A centrifugally moving specimen turned a right angle; accumulations of crystals and other inclusions were forced through and along the border of the tail ectoplasm toward the bend in the pseudopod. At 63 g few if any crystals passed into, through, or around the endoplasm at the bend (see arrow, Frame 1 of Fig. 4). As the acceleration reached 73 g 30 seconds later, the resistance of the endoplasm gave away and inclusions previously unable to pass fell through or around the endoplasmic stream toward the centrifugal border of the ectoplasm.

Several observations on centripetally moving specimens added further evidence of structure within the endoplasm. Some specimens were found to be capable of resuspending most or all of their inclusions throughout the axial endoplasm against accelerations as high as 170 g (see Fig. 3 and Fig. 5). This demonstrates not only structure in the endoplasm, but also considerable power output on the part of these cells. To cite an example, a specimen was observed to move 80 microns centripetally in 30 seconds without chang-

FIGURE 2

Photographs of *Amoeba dubia* taken 2 seconds before (Frames 1 and 3) and 4 seconds after (Frames 2 and 4) a 10-second exposure to a centrifugal acceleration of 220 g. Each frame is 250 μ wide. The organism on top responded by general pseudopodial retraction but showed little sedimentation. Longer exposure to the same acceleration caused crystals to accumulate in "pockets," which later broke away and fell discontinuously through the cytoplasm. Stratification was incomplete even after 1 minute at this acceleration. The cell on the bottom (Frames 3 and 4) shows the beginning of "pocket" formation and a few sedimented inclusions in the centrifugally directed pseudopodia.

ing its over-all dimensions. Assuming a cell volume of 2.5×10^{-6} cc. (7, 8) and a density of 1.02 (perhaps a conservative estimate), the force exerted by the ameba against the glass wall of the cuvette at 100 g was 4.9×10^{-3} dynes. The work performed was 3.9×10^{-5} ergs in 30 seconds, representing a power output of 1.3×10^{-13} watts.

4. Effects of Centrifugal Acceleration on Chaos chaos

The giant ameba, *Chaos chaos*, is polynucleate and about 10 to 15 times as large as *A. proteus* (See references 7, 8 for the range of cell volumes of the species used). Despite the differences in size and in number of nuclei, the over-all body form and the
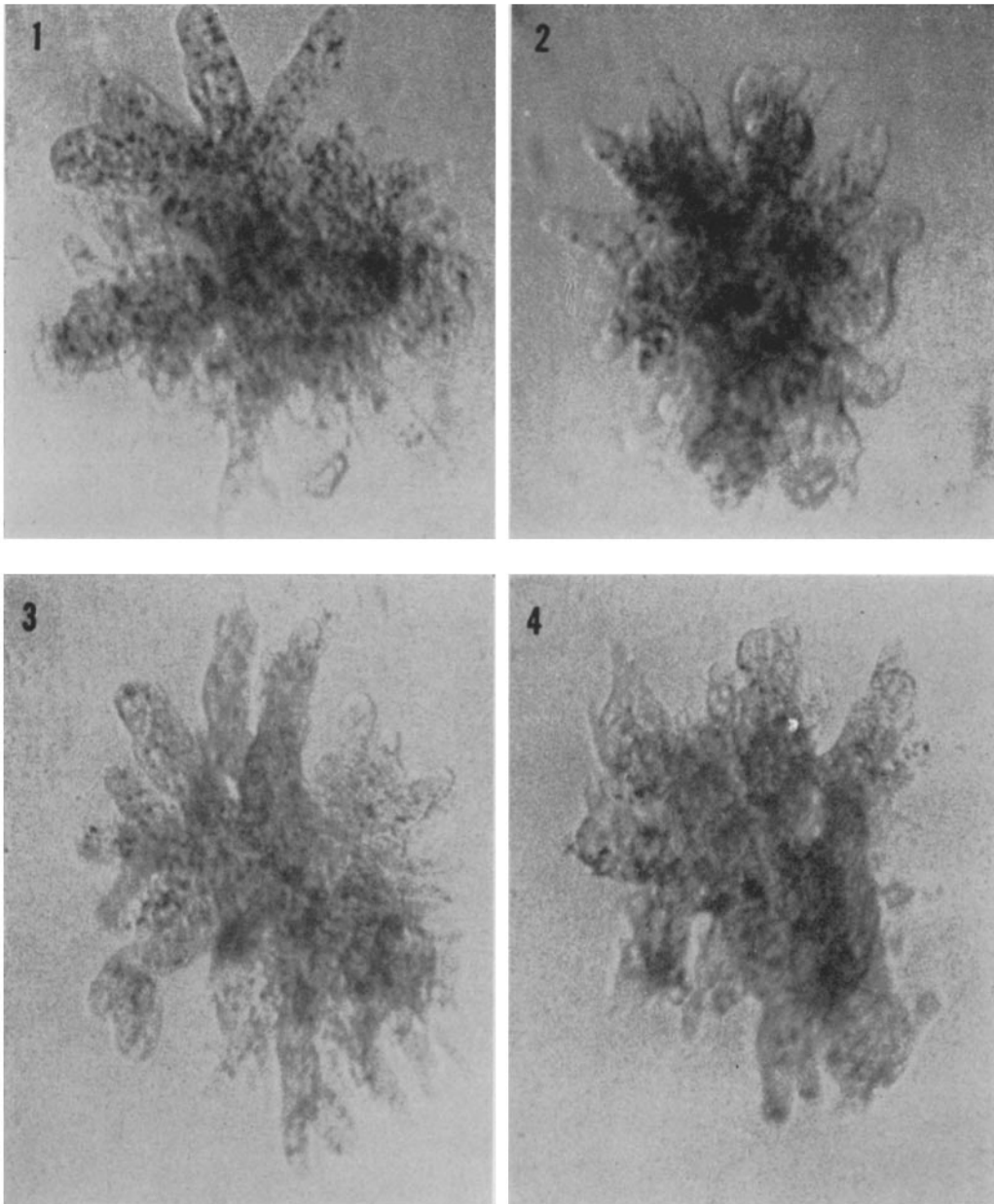
size, kind, and concentration of inclusions is so remarkably similar in these other two species that a close taxonomic relationship has been suggested (5).

The bipyramidal crystals of *C. chaos* are more spindle-shaped than those of the other two species used. A representative sample of frozen-dried cells showed an average size a little smaller than the reported average crystal dimensions of the Carlsberg strain (3, 4, see also Table I). *Chaos chaos* also contains many "heavy spherical bodies" which have an average diameter of about 4.0 microns (3). The crystals, heavy spherical bodies, and nuclei (kidney- or lentil-shaped bodies about $27 \times 32 \times 9$ to 15 microns (3)) are the principal components which move during centrifugation.

During the gradual application of centrifugal acceleration no displacement of inclusions was detected until the acceleration reached about 100 g. Above this acceleration an increasing number of inclusions broke away and suddenly moved through the cytoplasm. Discontinuous displacement was perhaps best observed in this species because of the distance each particle had to fall. The number of inclusions displaced decreased again as the acceleration reached 400 g. Shortly after centrifugal displacement ceased, streaming stopped in most cells. A few specimens, however, continued to show active cytoplasmic streaming of a rotatory nature at accelerations close to $1,000$ g.

Because of the large size of *Chaos chaos*, some unusual hydraulic effects caused by the weight of the endoplasm were encountered which were not seen in the smaller species. When the acceleration applied to a centripetally moving specimen was increased rapidly, the direction of streaming often suddenly changed, with the result that the tail became filled with endoplasm so rapidly that the ectoplasmic tube ruptured. The cell as a whole

did not rupture, but the sudden rushing of the endoplasm into gaps in the broken endoplasmic wall were clearly observed. The impression was gained that cells which had undergone this kind of disruption stratified somewhat more easily than the cells which had been moving along an axis perpendicular to the axis of acceleration.

The application of sudden acceleration with the rapidly starting centrifuge microscope to specimens moving perpendicularly to the field of acceleration caused much more marked accumulation of inclusions in the posterior than in the anterior region of the cell (see Fig. 6). This effect was especially marked at an acceleration of 225 g. In cases in which cells were subjected to sudden accelerations of from 225 to 360 g, the fall of inclusions in the posterior half of the cell either stopped or obscured the normal cytoplasmic streaming in this region, while the anterior portion of the cell, even though exposed to the same acceleration, showed little or no effect of centrifugation. In this species, there is perhaps less tendency for inclusions to halt at the inner border of the ectoplasm than in *A. proteus*; inclusions often penetrate as far as the hyaline ectoplasm, especially in the posterior region.

A few specimens were observed which had short but broad cylindrical, centrifugally directed pseudopodia when an acceleration of 225 g was suddenly applied. In these cases, a shower of inclusions fell through the shear zone, but little if any additional displacement occurred in the axial region of the endoplasmic stream (Fig. 6). It should be noted that this is what would be expected from a fluid in plug flow (2) and the exact opposite of expectation if the endoplasm were a sol in laminar flow.

It is perhaps worth recording two new observa-

FIGURE 3 *in three parts*

A series of ten 35 mm. photographs of *Amoeba proteus* taken through the revolving centrifuge microscope at 30-second intervals while the centrifugal acceleration gradually increased to 167 g. The width of each frame corresponds to 1120 microns. The black triangle in each frame marks a constant position in the cuvette and serves as a reference for the measurement of locomotion.

Frame 1: Taken $\frac{1}{2}$ minute after the centrifuge was started; acceleration 12 g. No sign of stratification.

Frame 2: 1 minute; acceleration 24 g. No stratification of inclusions.

Frame 3: $1\frac{1}{2}$ minutes; acceleration 37 g. First signs of the displacement of a few inclusions out of the tail endoplasm.
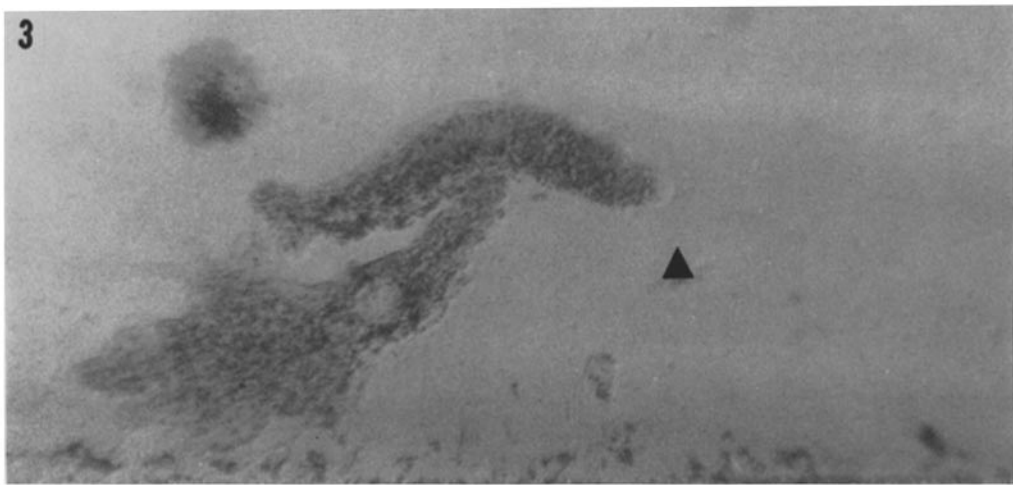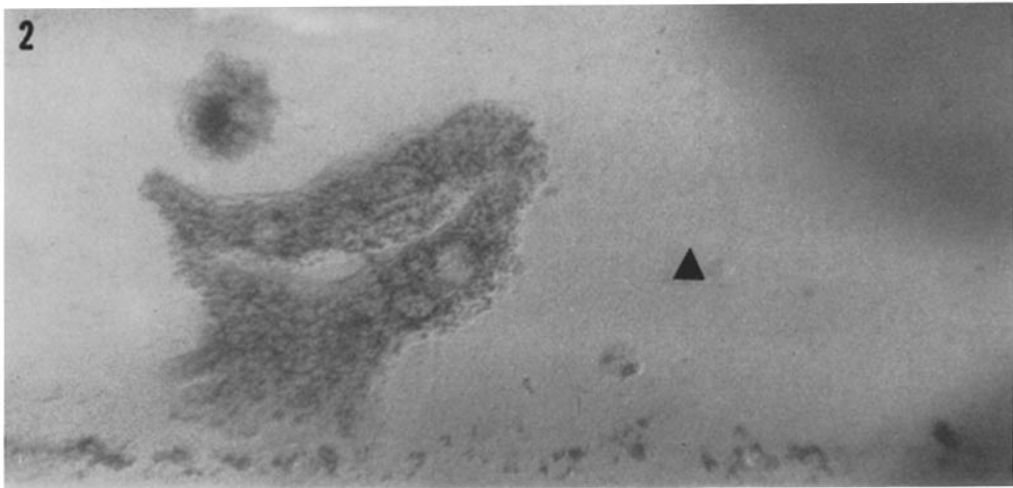
tions on the recovery of *C. chaos* following centrifugation. First, the newly resuspended inclusions in the axial endoplasm of a long pseudopod provide an excellent demonstration of plug flow; they are displaced very little relative to one another in the axial region (*cf.* reference 2). Second, recovering amebae sometimes exhibit interesting direction shifts in the tail endoplasm. Sometimes adjacent streams pass one another in opposite directions in the same cylindrical section of ectoplasmic tube. This kind of fluid behavior is, of course, inexplicable in terms of pressure-induced flow.

5. *Physical Properties and Hydrodynamic Behavior*
of the Crystalline Inclusions

Centrifugation can yield reliable information on cytoplasmic consistency only if the physical properties and hydrodynamic behavior of the displaced objects, in this case the crystalline inclusions, are known with certainty. Griffin (7, 9) has recently determined that the plate-like crystals of *A. dubia* are carbonyl diurea (triuret). This identification was based on elemental analysis and identical infra-red spectra for the crystals and synthetic carbonyl diurea. The bipyramidal crystals were also identified as carbonyl diurea after "recrystallization" from water (7, 9), but their infra-red spectrum is changed (9, 10), and they recrystallize as plates and not as bipyramids (9). The question does not yet appear to be settled whether the bipyramids are carbonyl diurea in another crystalline arrangement, or a related compound which breaks down in water to form carbonyl diurea. At any rate, Grunbaum *et al.* (10) have determined the density of ameba crystals as 1.74, while the density of synthetic

carbonyl diurea crystals is 1.745 ± 0.005. These data prove that the crystals have reduced weight some eight to nine times that assumed in previous centrifugation studies (15).

A few simple hydrodynamic experiments were carried out on isolated *A. proteus* crystals (density 1.74) contained in sealed glass capillaries filled with carbon tetrachloride (density 1.59; viscosity 8.8×10^{-3} poise at 24°C.). Since the crystals are not spheres as required for Stokes's law, their rate of fall would be expected to deviate somewhat from that of spheres. Nevertheless, inserting the range of free fall velocities (1.14 to 5.16 μ/sec.) into the Stokes's law equation, it was calculated that the crystals fell as rapidly as spheres with a radius of 1.8 to 3.8 microns. Crystals from the cytoplasm of *A. dubia* were made to fall through water by crushing an ameba on the vertical stage of a horizontal microscope in the presence of a trace of versene. The observed rate of fall was that expected of spheres with a radius of 2.5 microns and less. The fact that the calculated radius agrees reasonably well with the size measurements and density values serves as an internal check on the validity of these data and serves to fix a range of numerical values for the shear stresses exerted by the crystals on the cytoplasm of the ameba at various accelerations (see page 395).

DISCUSSION

What information about cytoplasmic consistency can be extracted from centrifugation data? Most discussions of this subject have been concerned with a special case: the free fall of spheres in a Newtonian sol. In this case, the information obtainable is the viscosity coefficient, a value which is constant for a Newtonian fluid. In this

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FIGURE 3 *continued*

Frame 4: 2 minutes after the centrifuge was started; acceleration 44 *g*. The specimen moving toward the left shows stratification in the tail region. The middle specimen moving to the right shows crystals caught in the centrifugal half of the endoplasm being carried along with the endoplasmic stream. Note the penetration of tail ectoplasm by inclusions. The right, centripetally moving specimen is suspending its crystals centripetally against 44 *g* and will shortly move out of the field of observation.

Frame 5: 2½ minutes; 60 *g*. Left specimen only slightly more stratified in the tail region. Endoplasm of right ameba still contains many inclusions.

Frame 6: 3 minutes; 72 *g*. Centripetally moving specimen has resuspended all but a few inclusions trapped in its tail. Endoplasm of other amebae partly cleared of inclusions.
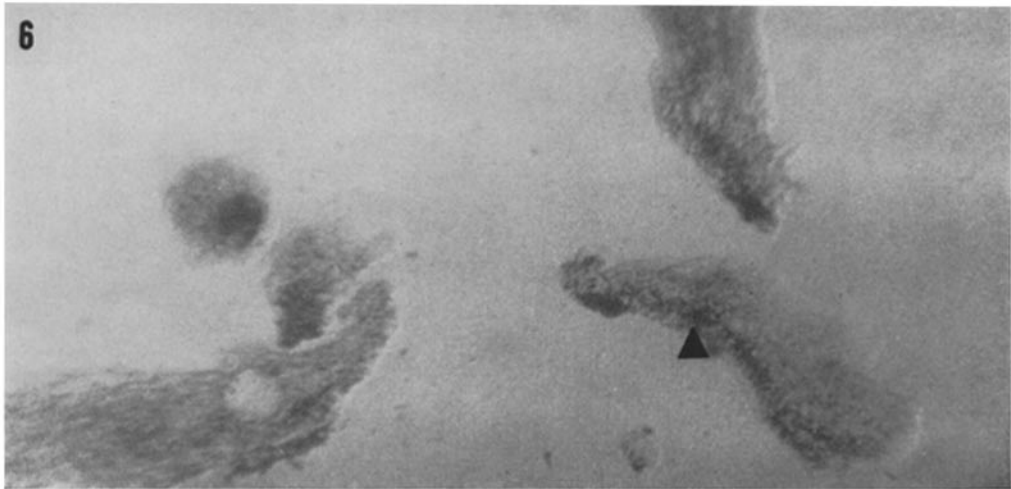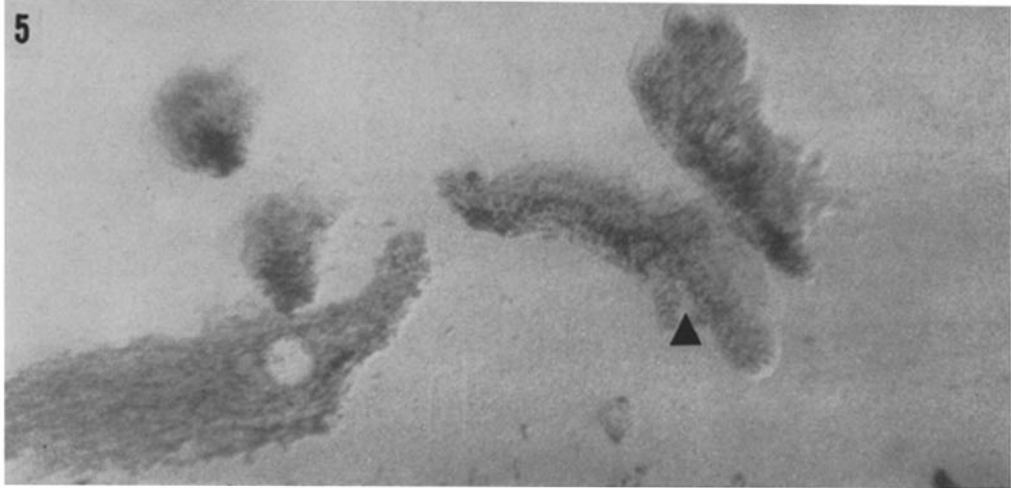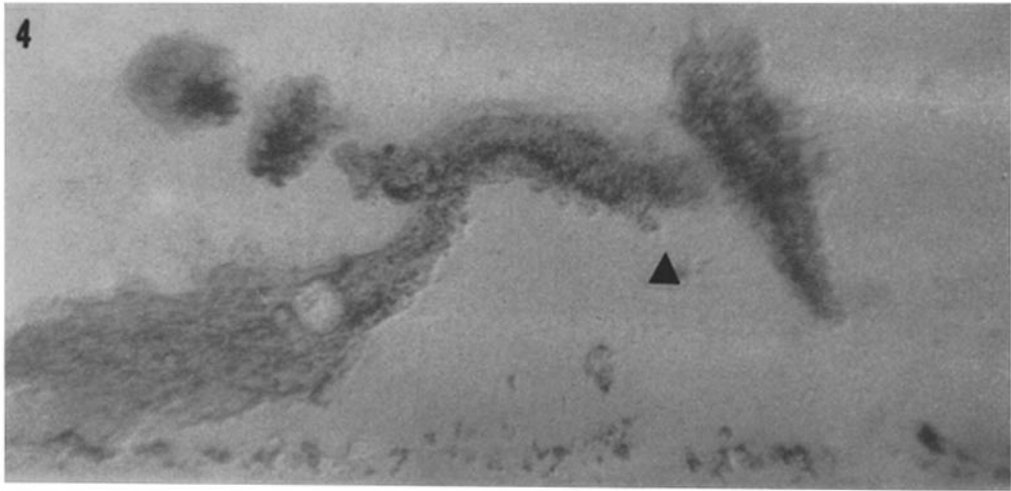
special case, the Stokes's law equation can be used to compute the viscosity of fluid if its density, and the radius, density, and velocity of a falling sphere are known. The recent review of Heilbrunn (16) gives Stokes's law and the various corrections which have been used for measurements by the centrifuge method of "protoplasmic viscosity" in living cells.

In the case of non-Newtonian fluids, Stokes's law might be expected either to apply as an approximation or to be totally inapplicable, depending on how nearly linear was the force-flow relation of the fluid. In the extreme case of a stiff gel, a heavy sphere would not fall until its applied shear stress under acceleration exceeded the yield point of the gel. Whether such a heavy sphere would then fall at a constant or variable velocity would depend on the degree of homogeneity of the gel structure, and on the extent to which the shear stress exceeded the yield point of the gel. The decision to use Stokes's law or one of the formulas for computing viscosity from thermal (Brownian) displacement of particles is not valid unless it is known in advance that the force-flow relation of the fluid in question is approximately linear. Fig. 7 illustrates the difficulties involved in measuring apparent viscosity in a non-Newtonian fluid. Curves A and B represent idealized force-flow relations for a Newtonian fluid and a gel respectively; the curves are purposely drawn so that their slopes are parallel except for a section of B at low shear stresses. It is obvious in this case that fluids A and B could be distinguished by falling sphere methods only if low shear stresses were applied, so that the force applied to a particle in fluid B would be insufficient to move it.

Previous reports of low protoplasmic viscosity

in the ameba (14–17) have been the result of some unjustified or incorrect assumptions and some unfortunate choices of experimental conditions. The use of Stokes's law without prior evidence of a Newtonian force-flow relation was unfortunate in one of these cases (15), for it set up a circular argument in which the "data" appeared to support the original unjustified assumption. In that study, it was further assumed that the crystalline inclusions had a density of about 1.10 instead of the correct value of 1.74. Thus the applied shear stress was grossly underestimated, for at the seemingly modest acceleration of 128 g, each crystal "weighed" four and one-half times the reduced weight of an equal volume of gold.

It is perhaps surprising that similar accelerations did not cause such rapid or complete sedimentation of crystals and other inclusions in the present study as Heilbrunn reported in $A.$ $dubia$ (14, 15). This is partly due to the fact that our specimens were attached and moving, although in nearly all cases the movement was not sufficient to explain lack of sedimentation by the continual resuspension of particles. Heilbrunn recognized in his study that the cells which assumed a spherical shape when moved to the centrifuge tubes became stratified most readily. Furthermore, he selected for study principally those cells that gave the lowest viscosity values (reference 14, page 61). In our experience, these are the cells which contain the largest and most numerous crystals and are the most feeble in their locomotion. Nearly all wild cultures contain some of these cells; the poorer the culture conditions, the more prevalent they are. In his original publication, Heilbrunn pointed out

FIGURE 3 *continued*

Frame 7: 3½ minutes after the centrifuge was started; 84 g. Centripetally moving specimen showed some accumulation of crystals in both shear zones. The crystals in the tail were probably the largest ones; the others moved normally except for an occasional one which broke away and fell toward the tail. The endoplasms of the other two specimens were virtually cleared of crystals.

Frame 8: 4 minutes; 96 g. The two specimens observed so far now moving out of the field of best focus. A new specimen fell to the wedge-shaped end of the cuvette and began immediately to send out pseudopodia.

Frame 9: 4½ minutes; 108 g. Specimen moving centripetally and resuspending its inclusions.

Frame 10: 5 minutes; 120 g. Further resuspension of inclusions. This cell continued to move out of the field even when the acceleration reached 167 g.
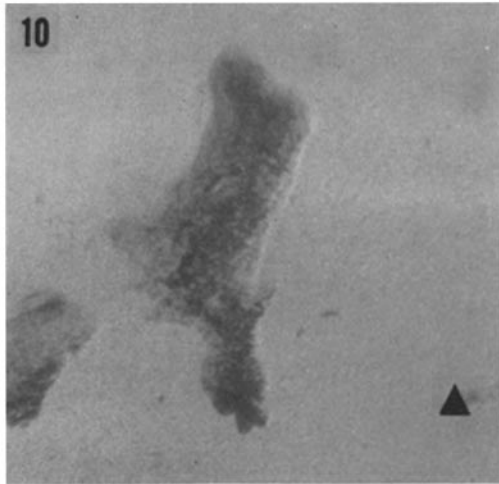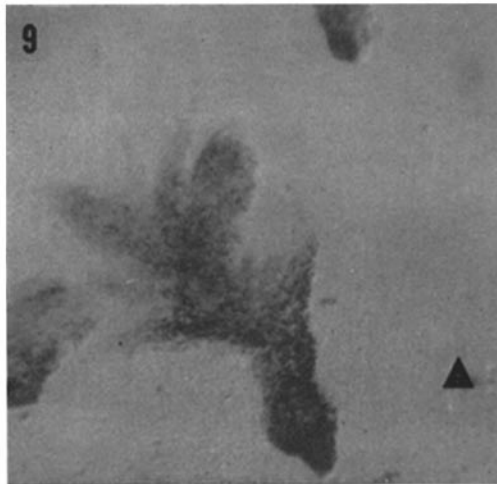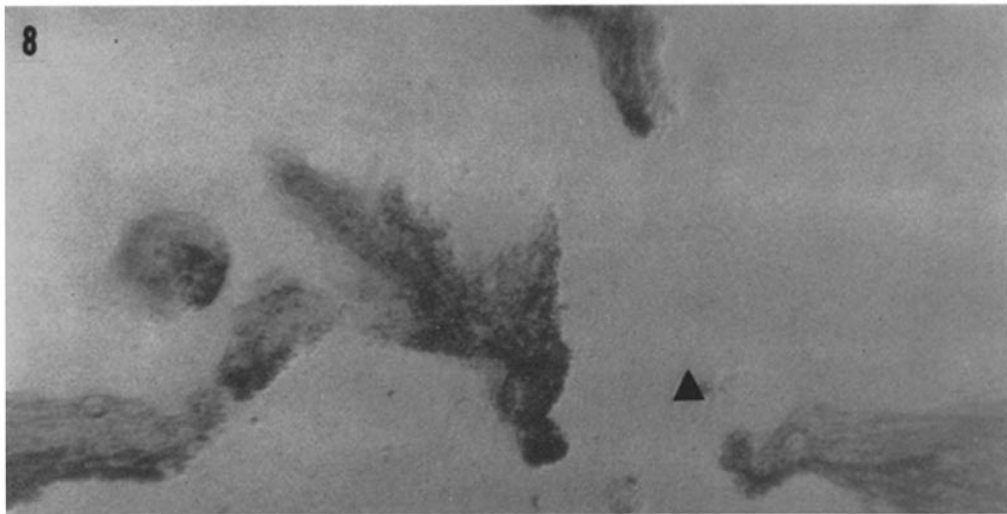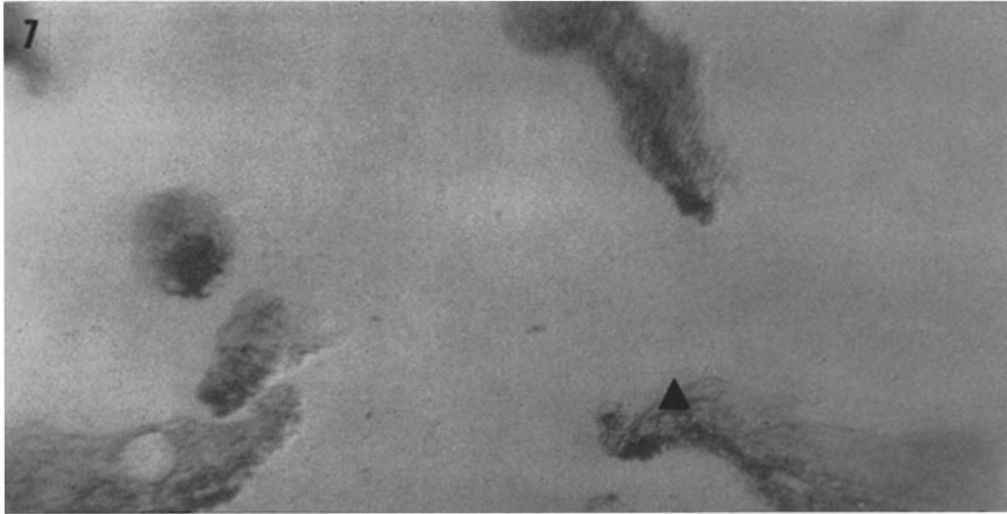
that his viscosity data applied only to rounded cells, and that actively moving specimens responded differently to centrifugation. These facts have been overlooked in more recent discussions (16).

The Brownian motion study of Pekarek (19) was carried out under somewhat less than ideal physiological conditions. Cells selected for low motility were vitally stained with neutral red and compressed in order to measure thermal displacements of inclusions without interference from streaming movements. It is unlikely that measurements made under these circumstances would yield a true picture of the normal cytoplasmic consistency during locomotion. It should also be pointed out that Newtonian consistency must be assumed in order to estimate viscosity from thermal displacements of particles.

The results of the present study show clearly that the "sol-gel" concept of ameba structure and consistency is too simple. There is no doubt that the ectoplasm has been correctly characterized as a gel, although it is quite possible that its actual molecular structure is more orderly than usually envisioned by the term "gel." The consistency of the ectoplasm is probably not homogeneous; the posterior ectoplasm shows a definitely lower resistance to the displacement of inclusions compared to that in the anterior part of the cell.

The endoplasm shows definite evidence of structure by (a) the sudden breaking away, at a critical acceleration, of particles in the axial part of the stream, and (b) the ability of the axial endoplasm to impede the displacement of particles which fall into it. The only regions of the ameba which at present show evidence of low apparent viscosity are (a) the peripheral endoplasm or shear zone; so called because of the high velocity gradients developed there during streaming, and (b) the posterior endoplasm or recruitment zone; this term was coined because the only process of which we can be certain in this region is the recruitment of endoplasm from the walls of the ectoplasm in the posterior third of the cell. In the shear zone, the *apparent* viscosity (computed from velocity profiles (2)) may dip to as low as about $\frac{1}{2}$ poise as a result either of high shear stress at the walls of the ectoplasmic tube, or of local chemical factors. In the recruitment zone, the velocity gradients are not so high, and it is more probable that local chemical factors play a role in determining a less rigid consistency in the entire posterior region of the cell.

Fig. 8 is a new scheme for ameba structure based on the best evidence from centrifuge microscope observations and velocity profile analysis. One interesting feature of the new scheme is the apparent structural continuity between the
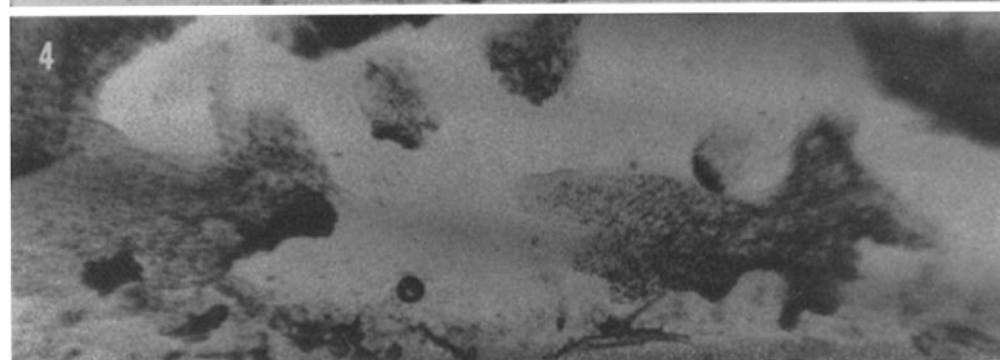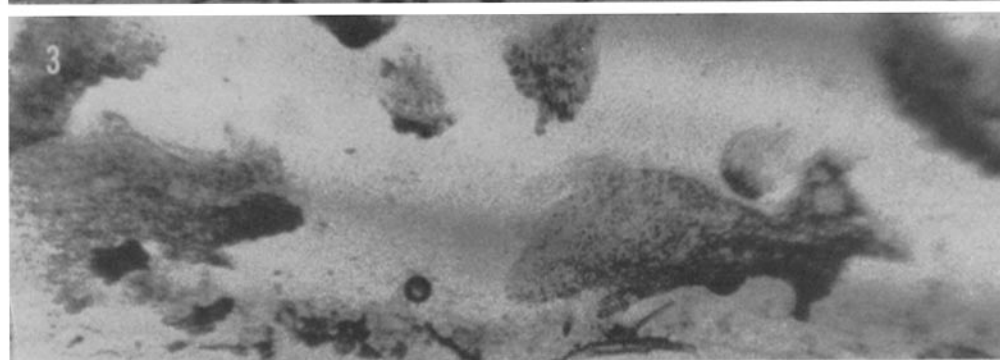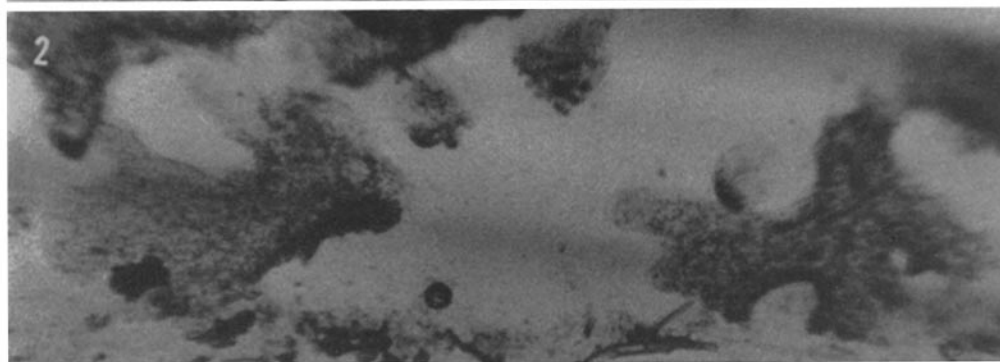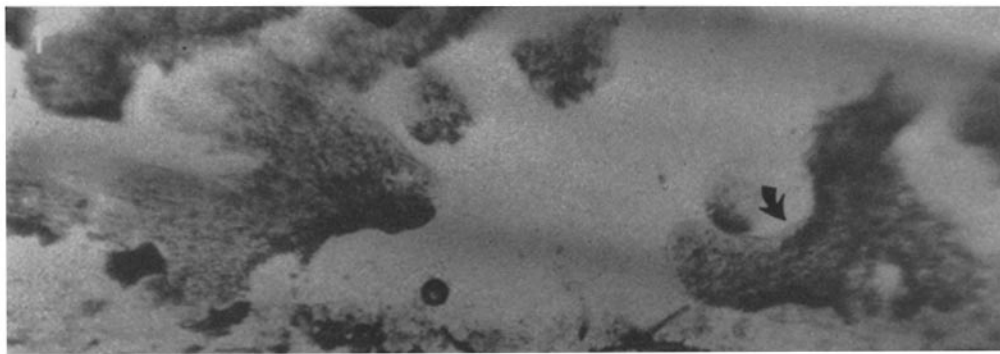
FIGURE 4

A portion of a sequence of photographs of *Amoeba proteus* taken under conditions quite similar to those in Fig. 3 and at the same magnification.

Frame 1: 3 minutes after the centrifuge was started; 63 g. In the right cell, a group of inclusions (mostly crystals) was sliding centrifugally through the shear zone in the vertically oriented portion of the cell. Where the pseudopod bent at a right angle (see arrow) these inclusions eventually passed through or around the endoplasmic stream. In so doing, they behaved as though resisted by structure within the axial portion of the endoplasmic stream. At 63 g there was little movement of inclusions through the endoplasm at the bent region.

Frame 2: 3½ minutes; 73 g. Many of the accumulated inclusions had by this time passed through the endoplasm either singly or in groups of a few. Some were caught in the endoplasm and transported perpendicularly with respect to the axis of acceleration without an apparent centrifugal velocity component.

Frame 3: 4 minutes; 84 g. Nearly all of the inclusions passed through the stream from the original accumulation. Note the layer of inclusions being carried forward in the endoplasm while the centrifugal shear zone has been cleared.

Frame 4: 4½ minutes; 93 g. Note the resuspension of particles during the formation of the most centripetal pseudopod. Even up to 170 g, it failed to sediment many of the heavy inclusions. That these were dense compared to the ground cytoplasm was proved by the application of higher acceleration at which they broke away and fell through the cytoplasm.
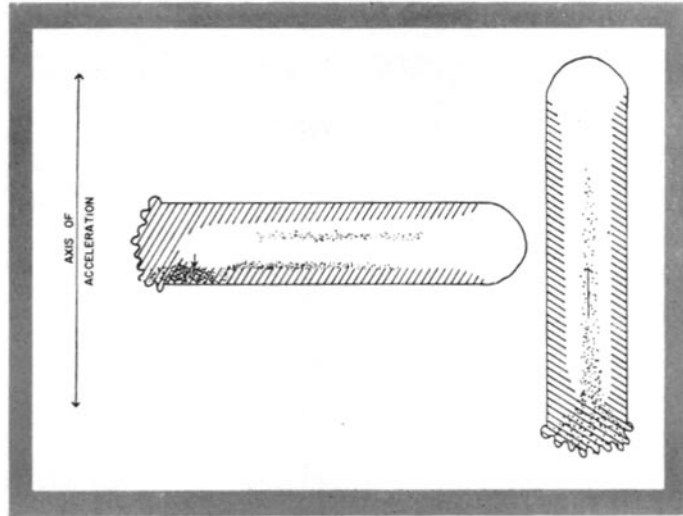
FIGURE 5

Schematic diagram of *A. proteus* under centrifugal acceleration to illustrate the resistance of the axial endoplasm to displacement of inclusions. See text for details.

axial endoplasm and the ectoplasmic tube through what we have termed the "fountain zone." Since the axial endoplasm already shows evidence of tenuous gel structure (or at least a sharp viscosity transition) its conversion to ectoplasm may be more than the simple gelation which has been assumed in the past to occur there. We are now exploring the possibility that what occurs in the fountain zone may be a contraction pulling the axial endoplasm forward toward the ever advancing ridge of the ectoplasmic tube. The evidence in support of this idea will be presented elsewhere (1).

So far, we have discussed only the qualitative information which can be drawn from the present study. Even though quantitative *viscometry* appears to be ruled out by the obvious signs of non-
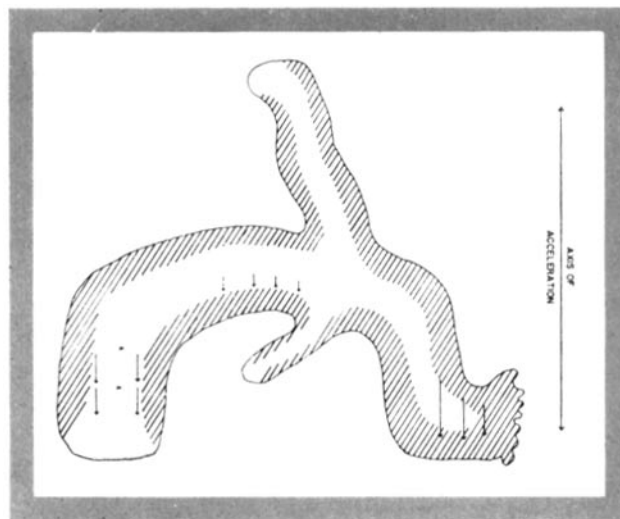

FIGURE 6

A diagram to show the regions of moving *Chaos chaos* in which inclusions are readily displaced during sudden application of centrifugal acceleration (225 *g*).

Newtonian behavior, it is possible to estimate the yield points of some regions of the cytoplasm from the physical properties of the displaced inclusions and observations of the accelerations at which these inclusions become displaced out of, or accumulate in, various regions. The shear stress, τ, exerted by a sphere of radius r and density ρp on a homogeneous Newtonian fluid of density ρ_m is given by

$$\tau = \frac{r(\rho_p - \rho_m)cg}{3}$$

in which c is centrifugal acceleration in gravities and g is 980 cm./sec.[2] In a gel, the force exerted by the sphere would be distributed more nearly over the cross-section area of the sphere rather than the whole area of the sphere, and would therefore be four times greater. The shear stress applied to a gel by a heavy crystal embedded in it would also vary with the orientation of the crystal. It was determined empirically with plasticene models of crystals that their mass is most accurately represented for shear stress estimations by assuming a radius of 0.31 times the length of the crystal. Fig. 9 is a graph representing the range of maximum shear stresses applied by bipyramidal crystals (treated as spheres) of the three ameba species at
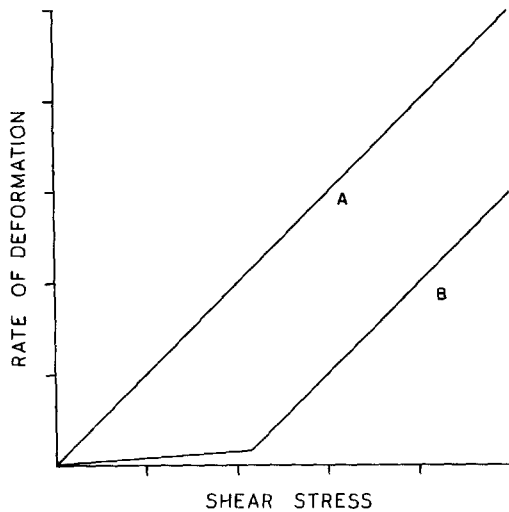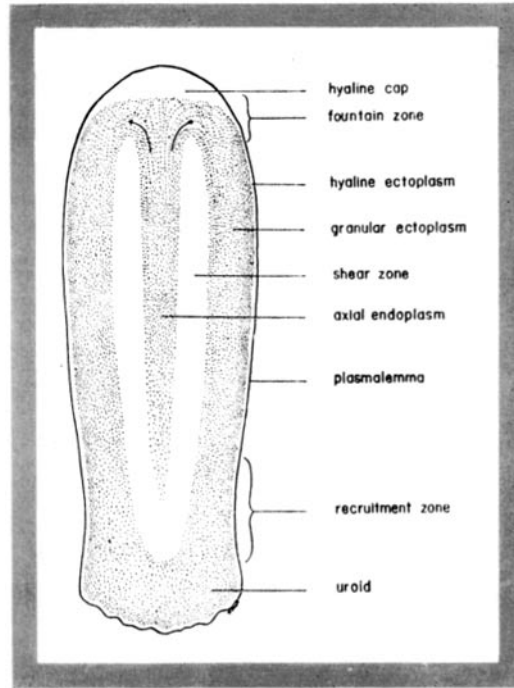

FIGURE 8

A new scheme for ameba structure based on rheological evidence discussed in the text. A new terminology is proposed for regions of the cell which are differentiated with respect to consistency.

accelerations up to 400 g. The crystal measurements on which these calculations were based are presented in Table I of the Results. Fig. 9 shows why under increasing acceleration different crystals break away suddenly at different critical accelerations. The fact that *C. chaos* inclusions begin to be displaced at accelerations of about 100 g and no further displacement occurs after about 400 g is reached suggests that the yield points of various parts of the cytoplasm lie in the range of 12 to 48 dynes/cm.[2] The minimum yield point for the endoplasm of *C. chaos* computed from velocity profile analysis was about 5 dynes/cm.[2]; this value was probably too low by a factor of about 2 because the calculation was based on the minimum motive force measured as pressure gradient. The resistance of *A. dubia* cytoplasm to sudden accelerations of 220 g suggests that much of the cytoplasm of this species must be able to resist, at least temporarily, shear stresses in the range of 20 to 70 dynes/cm.[2]

These yield point figures should serve only as a

FIGURE 7

Schematic force-flow relations for A, a Newtonian sol and B, a pseudoplastic liquid. At high shear stress the *apparent viscosities* are identical although the *consistencies* are markedly different.

rough estimate, for the experimental evidence is still very crude compared to that which can be routinely obtained by rheologists about large bodies of inanimate fluids. In living systems there is of course the added difficulty that the experimental treatment may have altered the consistency to be measured.[3]

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[3] It is not the author's intention to discuss again (*cf.* reference 2) the implications of structure in the endoplasm or possible mechanisms of ameboid movement. The reader is referred instead to a forthcoming article on "A new theory of ameboid movement" to appear in *Experimental Cell Research* (1961).

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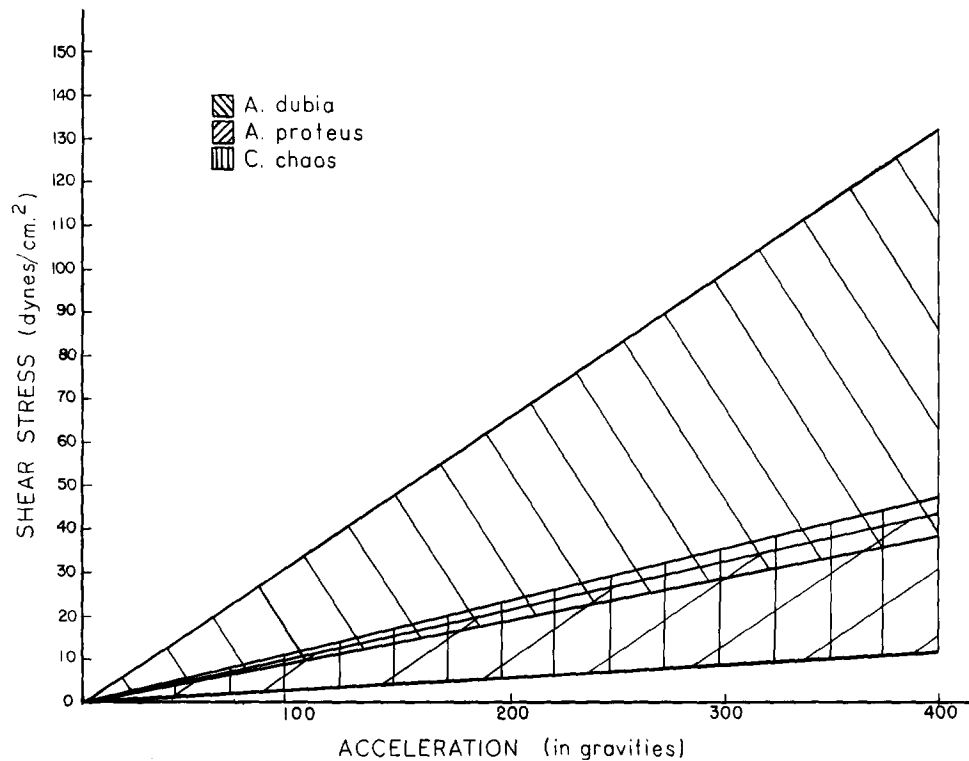15. HEILBRUNN, L. V., *Protoplasma*, 1929, **8**, 65.

FIGURE 9

The approximate range of stresses calculated to be exerted by crystals on the cytoplasm of three species of amebae under various accelerations. The crystals were treated as spheres embedded in a gel. In a homogeneous fluid the shear stress values on the ordinate would be one-fourth as large. See text for details.

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