

# CYTOPLASMIC FILAMENTS OF *AMOEBA PROTEUS*

## I. The Role of Filaments in Consistency Changes and Movement

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### ABSTRACT

The role of filaments in consistency changes and movement in a motile cytoplasmic extract of *Amoeba proteus* was investigated by correlating light and electron microscopic observations with viscosity measurements. The extract is prepared by the method of Thompson and Wolpert (1963). At 0°C, this extract is nonmotile and similar in structure to amoeba cytoplasm, consisting of groundplasm, vesicles, mitochondria, and a few 160 Å filaments. The extract undergoes striking ATP-stimulated streaming when warmed to 22°C. Two phases of movement are distinguished. During the first phase, the apparent viscosity usually increases and numerous 50–70 Å filaments appear in samples of the extract prepared for electron microscopy, suggesting that the increase in viscosity is caused, at least in part, by the formation of these thin filaments. During this initial phase of ATP-stimulated movement, these thin filaments are not detectable by phase-contrast or polarization microscopy, but later, in the second phase of movement, 70 Å filaments aggregate to form birefringent microscopic *fibrils*. A preparation of pure groundplasm with no 160 Å filaments or membranous organelles exhibits little or no ATP-stimulated movement, but 50–70 Å filaments form and aggregate into birefringent fibrils. This observation and the structural relationship of the 70 Å and the 160 Å filaments in the motile extract suggest that both types of filaments may be required for movement. These two types of filaments, 50–70 Å and 160 Å, are also present in the cytoplasm of intact amoebas. Fixed cells could not be used to study the distribution of these filaments during natural amoeboid movement because of difficulties in preserving the normal structure of the amoeba during preparation for electron microscopy.

### INTRODUCTION

Although cytoplasmic streaming is a basic property of cells, the mechanism of the movement of subcellular particles is not clearly understood. Since cytoplasm isolated from animal and plant cells exhibits independent motility (Thompson and Wolpert, 1963; Jarosch, 1956), the motive force must be generated in the cytoplasm. The structures

thought most likely to produce movement are the two types of cytoplasmic filaments: (a) hollow filamentous microtubules, and (b) solid filaments, which occur in several sizes. Microtubules serve a cytoskeletal function in many cells and have been implicated in some types of motility, such as the movement of chromosomes in the mitotic spindle

and of pigment granules in melanophores (Porter, 1966). As in the well studied contractile system of muscle, morphological evidence suggests that solid filaments may be involved in cytoplasmic movements in many other cell types, including *Physarum* (Wohlfarth-Botterman, 1964), *Nitella* (Nagai and Rebhun, 1966), ascidian epidermal cells (Cloney, 1966), cultured fibroblasts (Buckley and Porter, 1967), *Dictyoglia* (Wohlman and Allen, 1968) and sea urchin mesenchymal cells (Tilney and Gibbins, 1969). This view has been strengthened by two lines of evidence: (a) the isolation of actin from *Physarum* (Hatano and Oosawa, 1966), *Acanthamoeba* (Weihsing and Korn, 1969), and sea urchin eggs (Hatano, Kondo, and Miki-Noumura, 1969); and (b) the demonstration that muscle heavy meromyosin forms specific, ATP-dissociated, arrowhead-shaped complexes with thin filaments in a variety of chick embryo cells (Ishikawa, Bischoff, and Holtzer, 1969) and in *Acanthamoeba* (Pollard, Shelton, Weihsing, and Korn, 1970) which are nearly identical with the complex between heavy meromyosin and actin filaments.

The closely related freshwater amebas, *Amoeba proteus* and *Chaos carolinensis*, have been used extensively in studies of ameboid movement. Allen and his coworkers (Allen, 1961) determined that the axial endoplasm of *Chaos* has a lower consistency than the ectoplasm.<sup>1</sup> The higher consistency ectoplasm had been designated the GEL and the lower consistency endoplasm the SOL. This terminology must be used cautiously, since Allen's studies have shown that the endoplasm is not a structureless SOL, but is a low consistency "gel." Although light microscope studies have not directly demonstrated gel structures in the ectoplasm, the restricted movement of ectoplasmic particles suggests that the ectoplasm is a two-phase system with the organelles suspended in a loose gel network (Allen, 1961). Movement is dependent on gel

<sup>1</sup> The terms endoplasm and ectoplasm will be used as defined by Allen (1961). The endoplasm is the low consistency granular region in the central part of the ameba. In moving monopodial specimens of *Amoeba proteus*, the endoplasm flows toward the advancing tip where it is everted in the *fountain zone* to form the higher consistency, peripheral *ectoplasm*. The ectoplasm is divided into *granular ectoplasm* and *hyaline ectoplasm*. The hyaline ectoplasm, the clear area just inside the cell membrane, is sometimes referred to as *the* ectoplasm, but this is confusing and will not be used. The endoplasm is separated from the ectoplasm by the *shear zone*.

structure (Allen, 1961), but no detailed studies on the fine structure of the high and low consistency regions of the cytoplasm have been reported.

Nachmias (1964) first reported fibrillar cytoplasmic structures in electron micrographs of *Chaos carolinensis*. Long, thin filaments, about 75 Å in diameter, and thicker filaments, 150 Å in diameter and up to 0.5 μ long, were seen in cells whose plasma membranes were torn before fixation and in intact *nonmotile* cells exposed to a pinocytosis-inducing solution.

In a related study, Simard-Duquesne and Couillard (1962) demonstrated that glycerinated specimens of *Amoeba proteus* contract in the presence of ATP and magnesium. Schäfer-Danneel (1967) examined the fine structure of such cells and showed that a network of filaments 40–100 Å in diameter associated with thicker filaments 160–220 Å in diameter becomes apparent during glycerin extraction. ATP-induced contraction of these extracted amebas produced only a slight "condensation" of the filament network. She suggested that the filaments play a role in the contraction of the glycerinated amebas and that filaments may also be important for the movement of the living ameba.

A major advance in our understanding of the mechanism of ameboid movement was the discovery by Allen, Cooledge, and Hall (1960) that particles in cytoplasm freed from *Chaos carolinensis* had independent motility similar to the movement of particles in the intact cell. This approach was carried further by Thompson and Wolpert (1963) who described a cytoplasmic fraction from homogenized *Amoeba proteus* which exhibited vigorous movement when ATP or ADP were added and it was warmed from 4° to 20°C. Electron microscope examination of aggregates formed at the end of the reaction revealed filaments which were 120 Å in diameter and 5,000 Å long. A more purified extract contained filaments about 90 Å in diameter (Wolpert, Thompson, and O'Neill, 1964). Subsequent reports (Morgan et al., 1967) have shown much thinner filaments (20 Å) in high-speed supernatant fractions of the extract. This high-speed supernatant exhibited ATP-stimulated movement only after the addition of the "vesicle fraction" which had previously been removed. Wolpert (1965) suggested that the thin filaments aggregate to form the thick filaments and that some interaction of these filaments results in contraction.

We have reexamined the motile cytoplasmic ex-

tract of *Amoeba proteus* described by Thompson and Wolpert (1963) and have correlated light and electron microscope observations with viscosity measurements to elucidate mechanisms of cytoplasmic consistency changes and movement in this amoeba. Our observations suggest that the "gelation" of amoeba cytoplasm during movement is related to the formation of labile 50–70 Å filaments from precursors in the groundplasm. We speculate that cytoplasmic movement may depend on the interaction of these thin filaments with 160 Å filaments constantly present in the groundplasm.

## MATERIALS AND METHODS

### *Amoeba Cultures*

*Amoeba proteus*, strain PROT 1, was kindly supplied by Dr. J. Griffin. The cells were grown in Prescott and Carrier medium (Prescott and Carrier, 1964) and fed *Tetrahymena pyriformis* according to the method of Griffin (1960), modified for large scale (Griffin, personal communication).

### *Preparation for Electron Microscopy*

Several thousand amoebas were pipetted into a glass vial, allowed to attach themselves to the surface and to undergo amoeboid movement. A few milliliters of fixative at 0° or 22°C were gently added while the cells were observed with a phase-contrast or dissecting microscope. Fixatives used were based on the formaldehyde-glutaraldehyde fixative of Karnovsky (1965) modified according to Ito and Karnovsky (1968) by the addition of picric acid or related trinitrocompounds (trinitroresorcinol, trinitrocresol) at the concentration of 0.01%. 2% OsO<sub>4</sub> was added in some trials instead of the trinitrocompounds. The fixatives were buffered with 0.1 M cacodylate, pH 7.2. The fixatives were designated as follows: formaldehyde-glutaraldehyde (FG), FG + picric acid (FGP), FG + trinitroresorcinol (FGR), FG + trinitrocresol (FGC), and FG + OsO<sub>4</sub> (FG-OsO<sub>4</sub>). After 10–90 min in one of the fixatives, the cells were washed several times in 0.1 M cacodylate buffer, pH 7.2, and left in this solution overnight at 4° or 22°C before further fixation in 1% OsO<sub>4</sub> buffered with 0.1 M cacodylate, pH 7.2, at 22°C for 1 hr. Some samples were treated with 0.5 or 3.0% uranyl acetate in 0.05 M Tris-maleate buffer pH 5.2 at 22°C for 1 hr after treatment with OsO<sub>4</sub>. After ethanol dehydration, the cells were passed through propylene oxide and embedded in Araldite. Light gold and silver sections were stained with saturated or 3% aqueous uranyl acetate for 15 sec to 30 min followed by lead citrate (Venable and Coggeshall, 1965) for 15 sec to 2 min.

Electron micrographs were taken on either an RCA EMU 3F or a Phillips 200 electron microscope.

### *Preparation of Cytoplasmic Extracts*

The motile fraction of amoeba cytoplasm was prepared by a modification of the method of Thompson and Wolpert (1963). Mass cultures of amoebas were washed in Chalkley's medium (Chalkley, 1930) containing 0.5 mM MgCl<sub>2</sub> and cooled to 4°C for 12–24 hr. The cooled cells were then concentrated by low-speed centrifugation, and 5 cc of the resultant slurry were centrifuged for 10 min at 18,000 rpm in a Type SW 39L Rotor (Spinco Division, Beckman Instruments, Palo Alto, California). This gave a maximum centrifugal force of 35,000 *g* at the tip of the tube and fragmented the cells into four distinct layers similar to those described by Thompson and Wolpert (1963). The top clear layer was the suspending medium. The second layer was a viscous white material consisting of membrane-bounded bags of cytoplasm. The third layer was brown and consisted of heavy fragments of the cells (food vacuoles, pieces of nuclei, mitochondria, and clusters of 150 Å filaments). The fourth layer was white and consisted of crystals. The second layer (about 1–2 cc), consisting of membrane-bounded bags of cytoplasm, was transferred to a teflon-glass homogenizer with an equal volume of glass-distilled water or Tris-maleate buffer pH 7.0 (10 or 20 mM) and homogenized with five gentle strokes. Large fragments (mainly plasma membrane) were removed by centrifugation at 1000 *g* for 5 min. The supernatant was called Extract 1. Further centrifugation of Extract 1 at 10,500 rpm in a Type 40 Rotor (Spinco) (maximum of 10,000 *g*) for 10 min removed clusters of 150 Å filaments, mitochondria, rough endoplasmic reticulum, smooth vesicles, and plasma membrane fragments as a substantial pellet. The supernatant was designated Extract 2. All steps in the preparative procedure were carried out at 0–4°C.

ATP (disodium salt—Sigma Chemical Co., St. Louis, Mo.) was freshly prepared for each experiment in glass-distilled water or 10 mM tris-maleate buffer and brought to pH 7.0 with NaOH.

Light microscope observations and ciné-photomicrographs were made with Zeiss phase-contrast and polarizing optics. Samples of extract were carefully sealed under coverslips with petroleum jelly, so that there was no movement of particles in control samples except random thermal (Brownian) motion.

Samples of the extract were prepared for electron microscopy by fixing with 3% glutaraldehyde in 0.2 M cacodylate buffer, pH 7.0, FGC, or 1% OsO<sub>4</sub> in 0.1 M cacodylate buffer, pH 7.2 for 2–15 hr at the temperature of the sample (either 0° or 22°C). The fixed extract was concentrated into a pellet by centrifugation at 140,000 *g* for 90 min. An alternate

method of fixation, which avoided concentrating the extract by centrifugation, was to solidify an entire sample of extract by adding a small amount of unbuffered 25% glutaraldehyde to a final concentration of about 3% for about 15 hr at 22°C. The fixed samples were washed with cacodylate buffer, osmicated, dehydrated, and embedded as described above.

#### *Viscosity Measurement*

An apparent viscosity of the extracts was measured reproducibly with vertically mounted 0.1 ml pipets. The pipet tips were heat constricted and were submerged in 0.2 ml of sample during viscosity measurements. The time for 0.07 cc of extract to flow from the pipet was compared with the time for an equal volume of distilled water to flow out.

#### *Dimension Measurement*

The dimensions of the various types of filaments were measured precisely with a Nikon Profile Projector (Nippon Kogaku K.K., Japan) directly from original negative electron micrographs taken at  $\times 20,580$ . The microscope was calibrated with carbon replica of a diffraction grating (E. F. Fullam, Inc., Schenectady, N. Y.).

### OBSERVATIONS

#### *The Effect of Fixation on Amoeba proteus*

Adding any of the above fixatives to cultures of actively moving amebas caused *normal* cytoplasmic streaming to cease in less than 30 sec. With FG-OsO<sub>4</sub> at 0° or 22°C or with any of the other fixatives at 0°, there was no further visible cytoplasmic movement. With the formaldehyde-glutaraldehyde-based fixatives at 22°C, the appearance of many of the amebas was distorted by violent *abnormal* contractions and changes of shape for 1–2 min following the addition of the fixative.

Substantial shrinkage of the amebas, up to one-half the original volume, occurred during fixation and embedding regardless of the technique used. High osmolarity fixatives caused more rapid shrinkage than low osmolarity fixatives. If the amebas were passed quickly through fixatives and buffer washes for avoiding shrinkage there, they would shrink during dehydration.

Although the ectoplasm was readily distinguished from endoplasm in living amebas, a clear distinction of these regions was no longer apparent in most fixed amebas.

#### *Electron Microscopy of Intact Amebas*

Both cytoplasmic matrix and membranous organelles were preserved satisfactorily by Karnovsky's formaldehyde-glutaraldehyde (FG) fixative (Flickinger, 1968), but there was further improvement in preservation when picric acid or related trinitro compounds were added to the FG fixative (Ito and Karnovsky, 1968). The addition of trinitrocresol to FG (FGC) gave a more satisfactory fixation with fewer myelin figures than FGR or FGP.

Washing fixed cells overnight in buffer solutions before treatment with OsO<sub>4</sub> removed most of the small black cytoplasmic particles which puzzled previous investigators. Flickinger (1968) obtained similar results with an overnight wash of the FG-fixed amebas in distilled water.

Fig. 1 is a low power electron micrograph of a small portion of an ameba showing the plasma membrane, a cluster of thick filaments, mitochondria, two Golgi complexes, endoplasmic reticulum, and several types of vacuoles. The cytoplasmic matrix (or groundplasm), which is preserved by the improved fixation, contains free ribosomes and glycogen particles suspended by gray background material which may appear amorphous, granular, or reticular (Fig. 2).

Two types of filaments but no microtubules were observed in the cytoplasm of randomly selected amebas, confirming the observation of Nachmias (1968) on *Chaos*. Most sections of amebas had 160 Å wide, solid filaments up to 5000 Å long (Figs. 1–3). These filaments were most often found in randomly oriented clusters near the plasma membrane or groups of mitochondria. Occasionally, they were packed in a parallel array as if they were in a stream of flow or under tension (Nachmias, 1964). In some cells most of the 160 Å filaments were found singly, distributed throughout the cytoplasm.

The second type of filament observed was about 70 Å wide and indefinite in length (Figs. 3 and 4). These thin filaments had to be searched for in the sectioned cells. They usually were found close to groups of 160 Å filaments oriented in a parallel or reticular array.

In an effort to localize these filaments in an ameba undergoing a well defined movement, single fountain-streaming amebas were fixed in FGP and embedded. Phase-contrast photomicro-

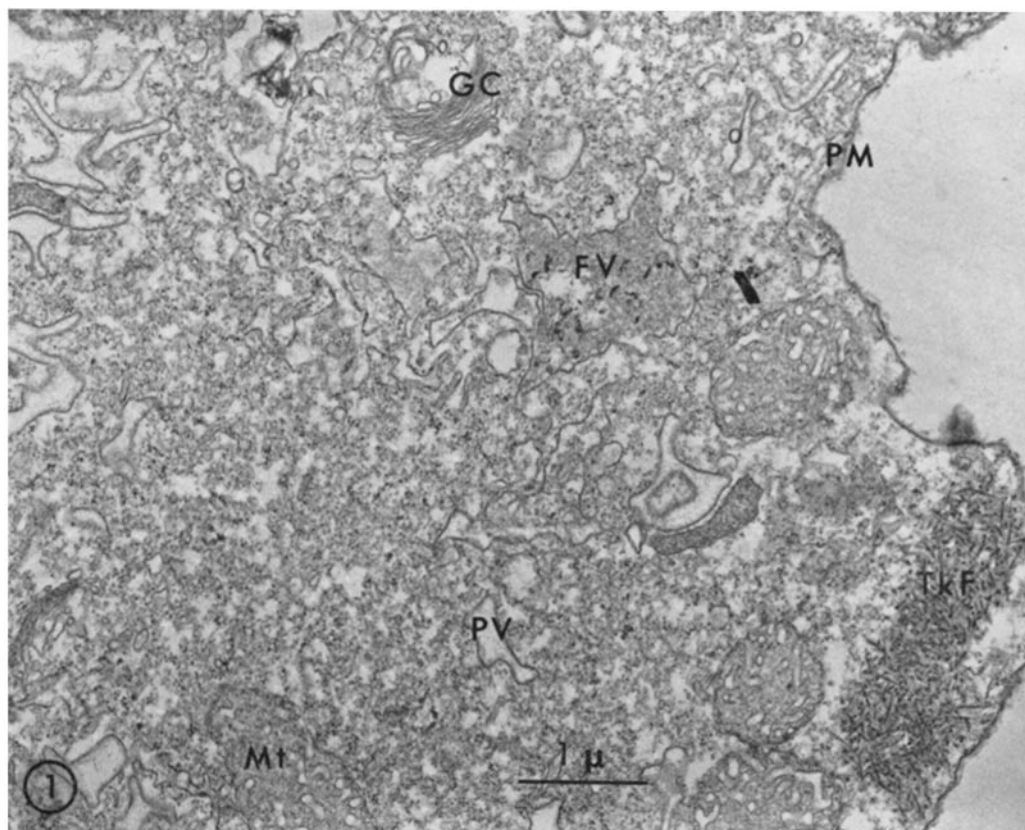


FIGURE 1 An electron micrograph of a small peripheral portion of *Amoeba proteus* showing the cytoplasmic structures: thick filaments (*TkF*), plasma membrane (*PM*), pinocytotic vesicles (*PV*), food vacuoles (*FV*), Golgi complexes (*GC*), mitochondria (*Mt*), and the cytoplasmic matrix, groundplasm. FGC fixation.  $\times 17,000$ .

graphs were taken at each step in the procedure. Shrinkage and abnormal movements during fixation usually distorted the normal morphology. One specimen which appeared to be well preserved except for shrinkage and loss of distinction of the endoplasm from the ectoplasm was serial sectioned. Scattered 160 Å filaments were found in both the endoplasm and the ectoplasm, without clear differences in any region. Few 70 Å filaments were seen.

Cooling amebas to 4°C for several hours and fixing at 4°C did not reduce the apparent number of 160 Å filaments. There were too few 70 Å filaments in fixed amebas for estimating the number of these filaments at different temperatures.

#### *Fine Structure of the Extracts*

Electron microscope examination of thin sections of Extract 1 revealed it to be a crude cytoplasmic fraction of groundplasm, mitochondria, endoplasmic reticulum, smooth membranous vesicles, glycogen particles, and small fragments of plasma membrane (Fig. 5). Occasionally, groups of 160 Å filaments and, very rarely, a few small bundles of 70 Å filaments were seen in Extract 1 kept at 0–4°C (Table I).

Centrifugation of Extract 1 for 10 min at 10,000 *g* removed most of the membranous organelles and bundles of filaments. The supernatant, Extract 2, was almost pure groundplasm (Fig. 6) with very few small vesicles and microsomes. No

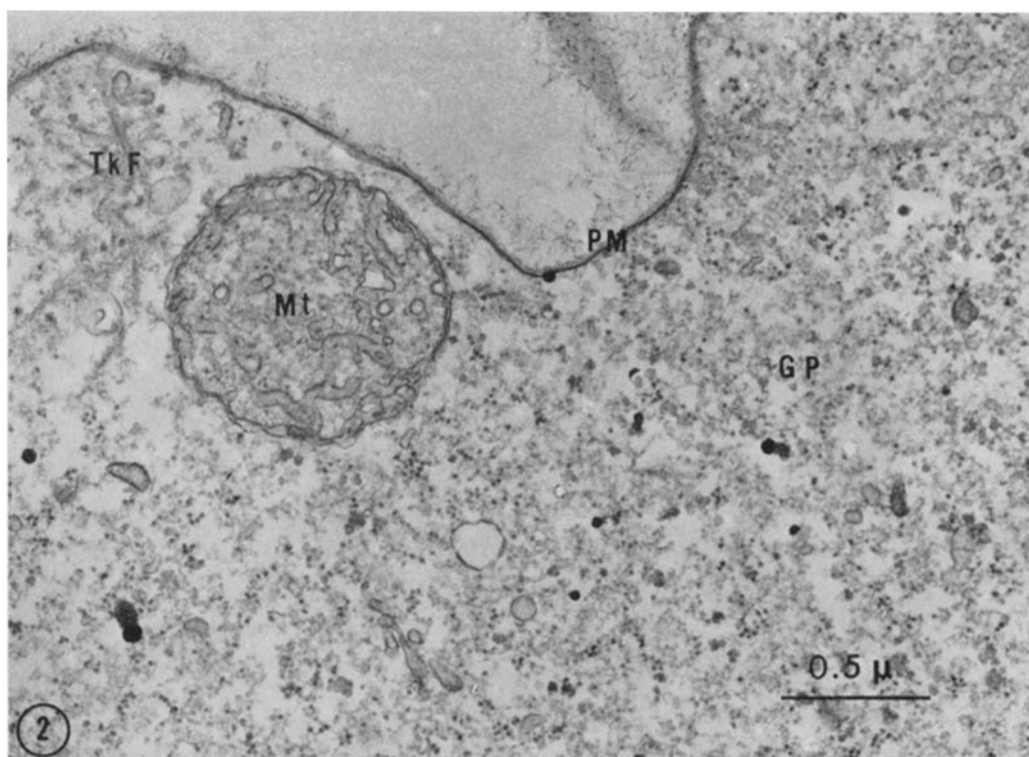


FIGURE 2 An electron micrograph showing the detailed structure of the cytoplasmic matrix or ground-plasm (GP), which consists of free ribosomes suspended in a matrix which is amorphous, granular, or reticular in different areas. A small group of thick filaments (TkF), plasma membrane, and a mitochondria are also pictured. FGP fixation.  $\times 39,000$ .

filaments were observed in Extract 2 kept at 0–4°C (Table I).

In the phase-contrast microscope, the mitochondria and vesicles in the extracts appeared as dark particles suspended in an amorphous gray background (see the background in Figs. 7 and 8).

#### *Light Microscope Observation of the Motile Extracts*

Extract 1 underwent dramatic streaming and contraction when warmed from 0° to 22°C in the presence of 2–5 mM ATP as described by Thompson and Wolpert (1963) and demonstrated in a movie at the 1968 meeting of the American Society for Cell Biology (Pollard and Ito, 1968). Movement was divided into two phases: Phase 1 was the movement observed before the formation of fibrils visible in the phase-contrast microscope, and Phase 2 was the movement seen after the formation of visible fibrils. (Note: *fibrils* were ob-

served in the light microscope and *filaments* were observed in the electron microscope).

In samples of extract sealed carefully under coverslips, there was an even distribution of particles surrounded by gray amorphous background material. In control samples, and initially in motile samples, there was only Brownian movement of the particles. In Extract 1, the crude cytoplasmic fraction, the initial phase of movement, Phase 1, began from 30 sec to 3 min after the slides were transferred from 0° to 22°C. First, there were saltatory movements of individual particles. Gradually, larger groups of particles moved in unison and appeared to be held in a semirigid structure invisible in the phase-contrast and polarizing microscopes. Large streams of particles formed and flowed in different directions. Two streams moving in opposite directions separated by an apparently gelled area were often seen within one microscope field. The effect was strikingly similar to the flow of

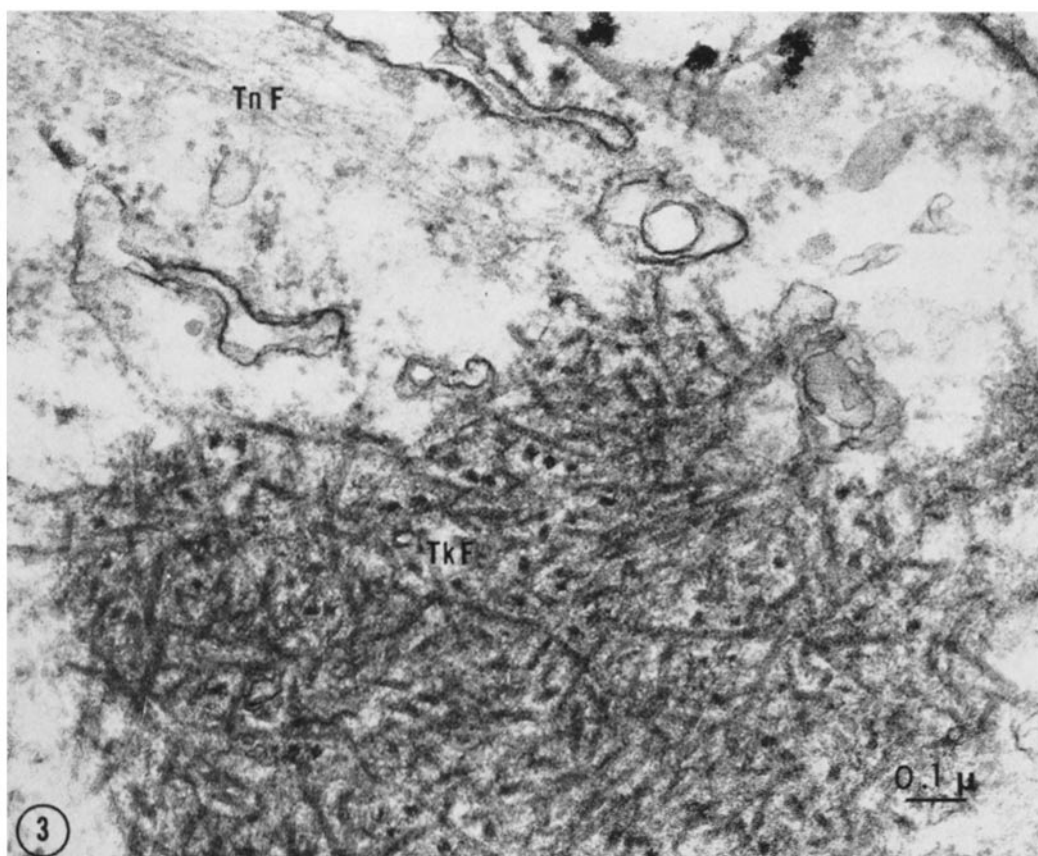


FIGURE 3 An electron micrograph showing the two types of cytoplasmic filaments: a large cluster of randomly oriented thick filaments (*TkF*) and a small parallel array of 50-70 Å thin filaments (*TnF*). FGC fixation.  $\times 81,000$ .

axial endoplasm in a shell of ectoplasmic gel in an intact ameba and to the observations of streaming in broken amebas (Allen, Cooledge, and Hall, 1960). The movement could be quite violent as areas of the extract contracted and retracted from each other; this resulted in the concentration of particles in the apparently gelled areas. This initial phase of movement lasted up to 10 min in Extract 1, before movement gradually subsided.

Control samples of extract without ATP continued to show only Brownian motion after warming to 22°C, and the particles remained evenly distributed. Consequently, it was easy to tell which samples had moved, even after the movement had ceased, by the variation in concentration of particles in the gelled areas and elsewhere. Convincing movement was observed in eight out of eleven preparations of Extract 1 prepared by the method

described above. Two of the failures occurred when dying ameba cultures were used to make the extract.

Phase 2 movement began after about 10 min at 22°C with the formation of very fine fibrils which gradually increased in thickness as they were observed in the phase-contrast microscope. The fibrils showed strong, positive birefringence. Particles were often attached to these fibrils and appeared to be pulled into clusters of other particles and fibrils as the fibril to which they were attached shortened. As the fibrils shortened, there was no detectable increase in their diameter; their proximal end appeared to vanish into the cluster of particles toward which they moved, giving one the impression that the fibril was being "pulled into" the cluster. Circular arrays of fibrils hundreds of microns in diameter sometimes formed and con-

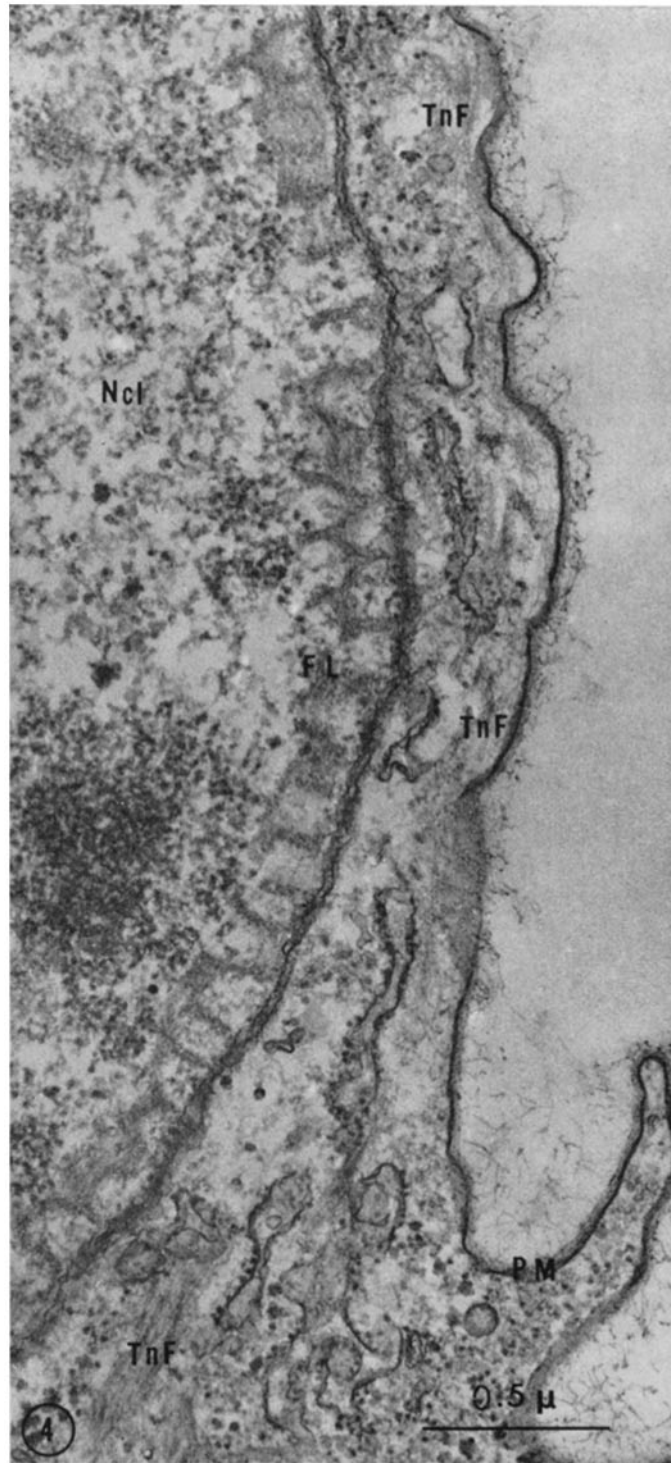
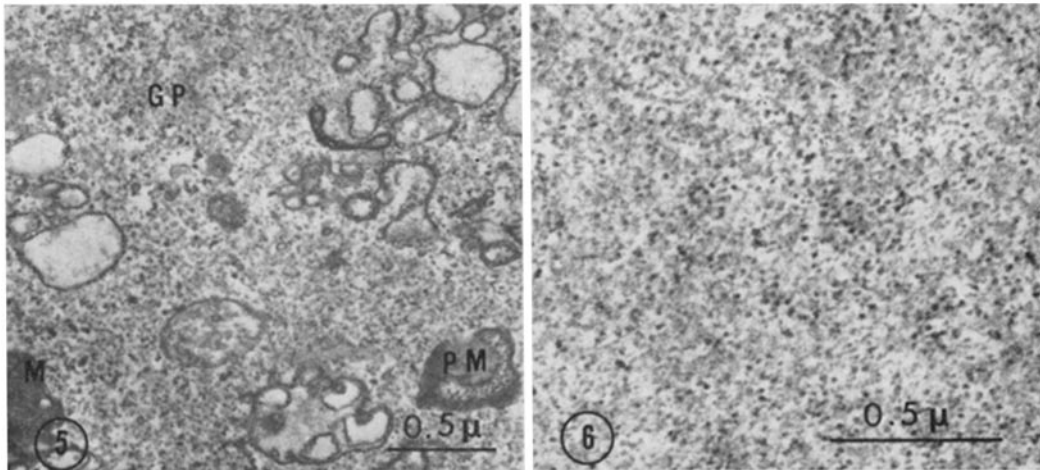


FIGURE 4 An electron micrograph of the periphery of an ameba showing an extended reticular array of 50-70 Å thin filaments (*TnF*) between the plasma membrane and the nucleus (*Ncl*). The thin filaments are organized into parallel arrays in some areas. The groundplasm is present in areas not occupied by the thin filaments. The fibrous lamina (*FL*) is prominent in the nucleus. FGP fixation.  $\times 49,000$ .





FIGURES 5 and 6 Electron micrographs of thin sections of extracts of *Amoeba proteus* at 0°C. Fixation with 3% glutaraldehyde at 0°C. Collection by centrifugation at 140,000 *g*.

FIGURE 5 Extract 1 at 0°C consists of mitochondria, vesicles, fragments of plasma membrane (PM), endoplasmic reticulum, and groundplasm (GP). Occasional 160 Å filaments and very rare 50–70 Å filaments are present in Extract 1 at 0°C—not shown.  $\times 26,000$ .

FIGURE 6 Extract 2 at 0°C is essentially pure groundplasm. Rare vesicles, but no filaments are present in samples kept at 0–4°C.  $\times 45,000$ .

tracted toward their center, sweeping all of the particles ahead of them into a large cluster visible with the naked eye. In both of these types of movement, the fibrils appeared to transmit the tension necessary to move the particles. Loose clusters of particles and fibrils contracted into tight clusters (Fig. 7). This phase of movement lasted up to 90 min and occurred in five out of eleven preparations of Extract 1.

An alternate to this second phase of movement was the formation of fibrils and spindle-shaped tactoids (Fig. 8) without visible movement or the formation of clusters of particles. The tactoids were also birefringent (Fig. 9). This was observed in four out of eleven preparations of Extract 1.

Phase 1 movement was observed in only two out of seven preparations of Extract 2, the purified cytoplasmic fraction, after warming to 22°C with ATP. When it did occur, it was transient and lasted less than 2 min. In six of seven preparations of Extract 2, extensive networks of birefringent fibrils and tactoids formed, starting about 10 min after warming with ATP. These fibrils and tactoids increased in diameter, but convincing movement was not observed. Consequently, little or no movement was observed in either phase in preparations of Extract 2.

The fibrils and tactoids were cold labile. They disappeared in samples stored overnight at 4°C and returned in 10–20 min after rewarming to 22°C. There was no movement during the reformation of the fibrils.

#### *Conditions for Movement and Fibril Formation*

Warming of Extract 1 from 4° to 22°C was required for movement. Convincing Phase 1 movement was observed on one occasion in Extract 1 *without the addition of ATP*, but ATP (2–5 mM) stimulated movement in multiple samples of eight out of eleven preparations of the extract. Extracts prepared with Tris-maleate buffer moved more consistently than those prepared with distilled water. Extract stored at 4°C lost the capacity for ATP-stimulated movement after about 1–2 hr, as described by Wolpert, Thompson, and O'Neill (1964).

For examining the calcium requirement for movement, the calcium chelating agent EGTA (1,2-bis(2-bicarboxymethylaminoethoxyethane)) at 0.33 mM was added to Extract 1 with ATP. The movement on warming to 22°C was equivalent to Phase 1 and 2 movements stimulated by ATP alone. EGTA (0.33–1.0 mM) alone did not stimulate movement.

TABLE I  
Morphological Analysis of Extracts of *Amoeba proteus*

	Extract 1	Extract 2	
Components at 0°C	Major:	Groundplasm Free ribosomes Glycogen particles Rough endoplasmic reticulum Smooth vesicles Plasma membrane vesicles Mitochondria	Groundplasm Free ribosomes Glycogen particles
	Minor:	160 A filaments 70 A filaments (rare)	Rough endoplasmic reticulum Smooth vesicles Plasma membrane vesicles
New components after warming to 22°C with ATP	50-70 A filaments (numerous)	50-70 A filaments (numerous)	
ATP-stimulated motility	Reliable	Rare and unconvincing	

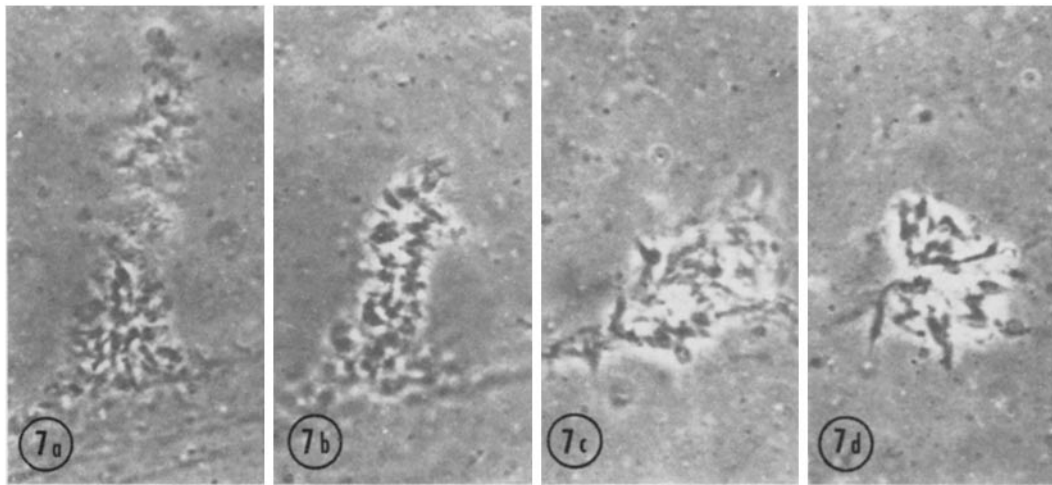


FIGURE 7 Phase-contrast photomicrographs of Extract 1 at 22°C with 4 mM of ATP. One type of ATP-stimulated movement observed in Phase 2—the condensation of a cluster of particles and fibrils—is shown (Fig. 7 a). The length of the cluster decreases about 35% in Fig. 7 b and about 60% in Fig. 7 c and d. This sequence was taken over a period of 10 min. The vesicles and mitochondria in the extract appear as small dark particles surrounded by amorphous gray material.  $\times 250$ .

Fibril formation also required warming to 22°C, but in contrast to movement, both ATP and EGTA stimulated fibril formation. EGTA promoted only a small amount of fibril formation compared with ATP.

#### Viscosity Measurements

Serial measurements of the apparent viscosity were made on samples from five preparations of Extract 1, while movement was monitored in two other samples of the same preparation sealed under

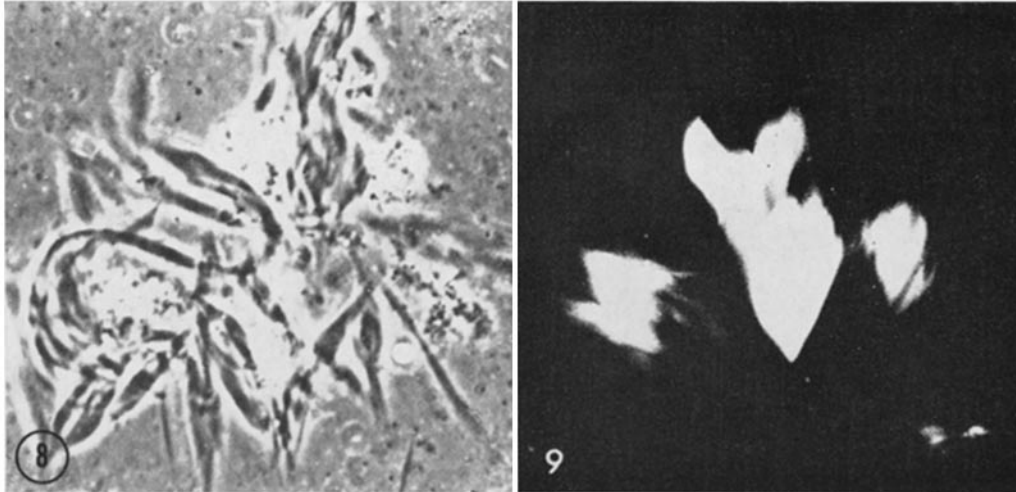


FIGURE 8 A phase-contrast photomicrograph of fibrils and tactoids formed in Extract 1 after warming to 22°C with ATP.  $\times 430$ .

FIGURE 9 A polarizing photomicrograph of birefringent tactoids formed from Extract 1 with ATP at 22°C.  $\times 930$ .

coverslips. The apparent viscosity of Extract 1 warmed to 22°C with ATP increased to a maximum, coinciding with the peak of Phase 1 movement, and then fell to control values as aggregates of the extract precipitated out of solution in Phase 2 (Fig. 10). This increase in apparent viscosity occurred only if there was active Phase 1 movement and never occurred if there was no movement, as in the controls without ATP.

The extent of the rise in viscosity during Phase 1 appeared to be determined by the length of time before the precipitation of aggregates, after which the viscosity always decreased to control values. The preparation used for Fig. 10 demonstrated the greatest increase in viscosity and no precipitate formed until after 8 min at 22°C. In two preparations, little or no rise in viscosity could be measured despite good Phase 1 movement, but visible precipitates formed in the viscometer after 2 or 3 min. In the remaining two preparations, in which intermediate viscosity increases were measured, precipitates formed after 5 or 6 min. These precipitates were not apparent in the samples sealed on microscope slides or in test tubes; so it seemed likely that the repeated flow of the extract into and out of the viscometer promoted aggregate formation. These aggregates consisted of clusters of particles, fibrils, and tactoids similar to those observed in Phase 2.

#### *Electron Microscopic Observations of the Motile Extracts*

Samples of Extract 1 fixed during active movement (Figs. 11 and 12) had extensive regions in which the membrane fragments and organelles were enmeshed in dense network of thin 50–70 Å filaments and clusters of thick 160 Å filaments (see Table I). The thick filaments were frequently found at points at which groups of thin filaments appeared to merge as in Fig. 11. This arrangement of thick filaments at the center of radiating thin filaments was also seen in moving samples of Extract 1 prepared for electron microscopy without 140,000 *g* centrifugation. This gave some assurance that this organization existed in the extract and was not an artifact of the preparation. Some of the thin filaments appeared to abut on the surface of the thick filaments, membrane fragments, or organelles (Figs. 11 and 12).

In samples of Extract 1 fixed after the formation of birefringent fibrils, 70 Å filaments were frequently arranged on long parallel arrays (Fig. 13). The parallel arrays of thin filaments would be expected to be birefringent, and the dimensions of these aggregates were the same as the dimensions of the fibrils observed in the light microscope, strongly suggesting that the birefringent fibrils were formed from 70 Å filaments.

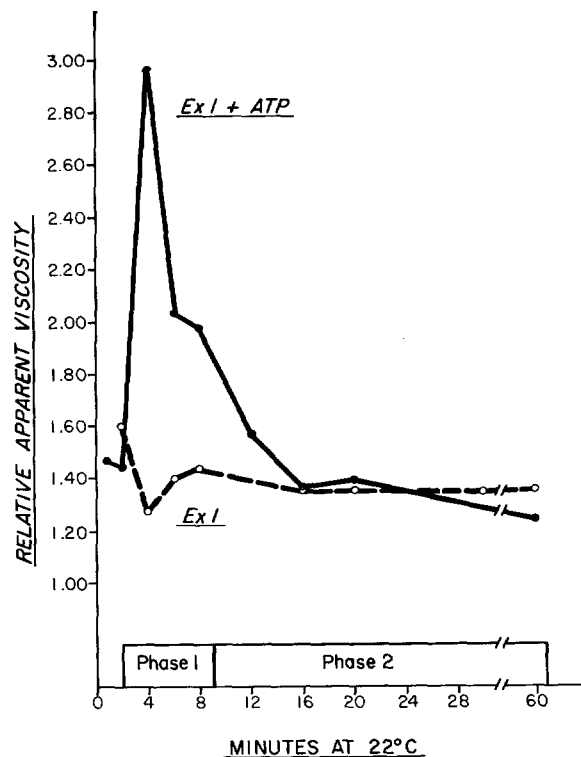


FIGURE 10 The change of relative apparent viscosity of Extract 1 after warming to  $22^{\circ}\text{C} \pm 4$  mM ATP. Relative apparent viscosity =  $\frac{t}{t_0} \eta_0$  where  $t$  = time for 0.07 ml of Extract to flow from pipet viscometer,  $t_0$  = time for 0.07 ml of water to flow from pipet viscometer,  $\eta_0$  = the apparent viscosity of water, taken to be 1.00 here. ●—●, relative apparent viscosity of Extract 1 + 4 mM of ATP. ○—○, relative apparent viscosity of Extract 1 + volume of water equal to the volume of added ATP. Two samples each of the Extract + ATP and Extract alone were observed in the phase-contrast microscope by an independent observer, while the viscosity was measured. Phase 1 movement began at 2 min after warming to  $22^{\circ}\text{C}$ . Fibrils appeared after 9 min, marking the beginning of Phase 2 movement.

Extract 1 warmed to  $22^{\circ}\text{C}$  without added ATP was found to have a few small clusters of thin filaments more frequently than similar samples kept at  $0-4^{\circ}\text{C}$ , but these filaments did not aggregate into large fibrils visible in the light microscope.

The extensive arrays of birefringent *fibrils* which form in Extract 2 after warming with ATP also comprised parallel arrays of 50–70 Å filaments (Fig. 14), just as the fibrils in Extract 1. No 160 Å thick filaments were seen in Extract 2 (see Table I).

At the end stage of ATP-induced movement of Extract 1, large pseudocrystalline aggregates of thick or thin filaments formed (Figs. 15–17). The pseudocrystals of parallel thin filaments were the birefringent tactoids seen in the light microscope (Figs. 8 and 9). The parallel, tightly packed arrays

of thin 70 Å filaments excluded all other structural elements. The aggregates of thick filaments generally excluded other structures, but there were some amorphous matrix materials or a few thin filaments between the thick filaments (Fig. 15).

In cross-section, the thick filaments seen in Extract 1 were solid with some fine lateral projections (Fig. 18). In longitudinal section, the thick filaments measured up to  $0.5 \mu$  long. The mean width of the thick filaments (such as those in Fig. 18) was 157 Å (SD = 22 Å). Some thick filaments had distinct longitudinal striations, and others appeared to end in a spray of very fine threads.

The long, thin filaments in tactoids and fibrils (Figs. 13–16) were 72 Å (SD = 10 Å) wide and appeared slightly beaded at high magnification. A cross-section of a tactoid showed that the “70

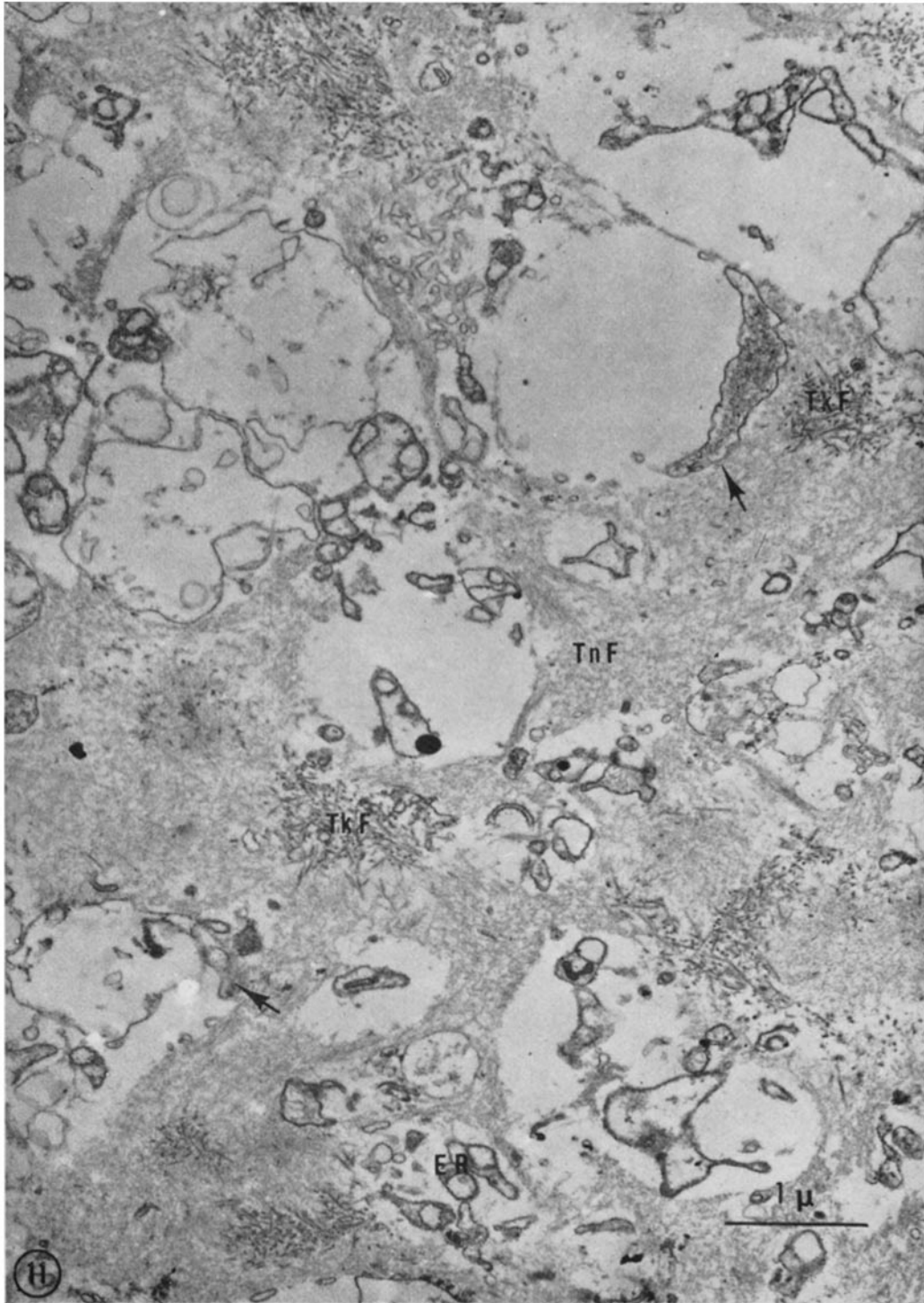


FIGURE 11 An electron micrograph of Extract 1 undergoing Phase 1 movement 8 min after warming to 22°C with ATP. An extensive filamentous network surrounds and abuts on (arrow) the membrane fragments and vesicles. Groups of 160 Å thick filaments are located at points where groups of thin filaments intersect. Fixation with 3% glutaraldehyde at 22°C. Collection at 140,000 *g*.  $\times 21,000$ .

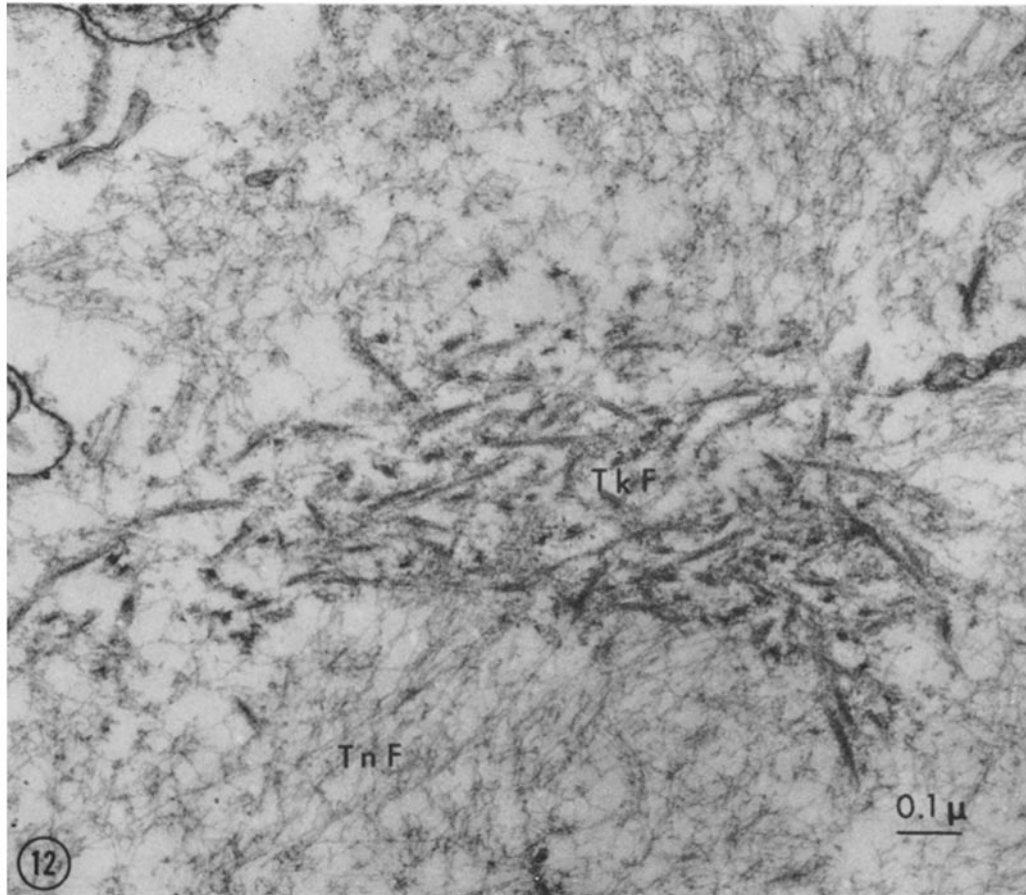


FIGURE 12 An electron micrograph of Extract 1 undergoing Phase 1 movement 8 min after warming to 22°C with ATP. Thin filaments 50–70 Å wide radiate from a cluster of 160 Å thick filaments. Preparation as in Fig. 11.  $\times 80,000$ .

Å" filaments were clearly separated from adjacent filaments in an irregular array (Fig. 17). In samples fixed during Phase 1 and early Phase 2 movement, there were 70 Å filaments identical with those seen in the fibrils and tactoids, but there were also substantial numbers of filaments which appeared thinner (Figs. 12 and 13). These thinner filaments were 54 Å ( $SD = 6$  Å) wide. These "50 Å" thin filaments were frequently seen in continuity with the 70 Å thin filaments (Fig. 13). There was no population of filaments intermediate in diameter between the 160 Å thick and 70 Å thin filaments at any stage.

#### DISCUSSION

This study of ameboid movement was undertaken to investigate the mechanisms of consistency

changes and movement in *Amoeba proteus*. The observations reported above led us to conclude, as explained in detail below, that increases in cytoplasmic consistency are caused by the formation of 50–70 Å filaments from precursors in the ground-plasm. A second type of filament, about 160 Å in diameter, was observed, and we postulate that these thicker filaments may interact with the thin filaments to cause contraction of the cytoplasm.

#### *Fixed Specimens of Amoeba proteus*

Studies of living giant amebas (Allen, 1961) have shown that the low consistency endoplasm of the ameba appears to be reversibly converted to higher consistency ectoplasm during movement. It was hoped that it would be possible to fix an ameba during movement and to examine the various

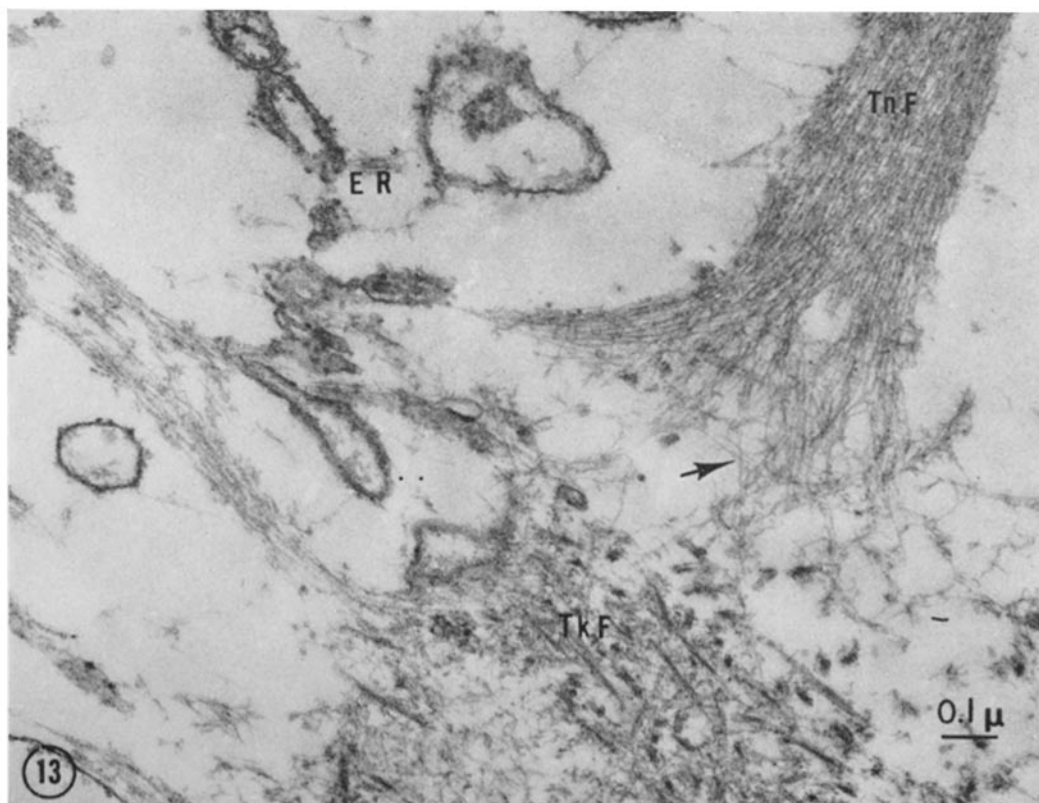


FIGURE 13 An electron micrograph of Extract 1 at 22°C for 45 min with ATP. The parallel arrays of 70 Å filaments are thought to be the birefringent fibrils observed to form during Phase 2 movement in samples warmed with ATP. The 70 Å filaments in the upper parallel array (*TnF*) appear continuous with thinner (about 50 Å) filaments (arrow) bridging them to the cluster of 160 Å filaments in the lower right (*TkF*). Preparation as in Fig. 11. *ER*, endoplasmic reticulum.  $\times 70,000$ .

regions of the cell in the electron microscope to directly observe which components of the cell are responsible for the difference in consistency between the endoplasm and the ectoplasm. Such studies were previously attempted with *Chaos carolinensis* (Komnick and Wohlfarth-Botterman, 1965) and *Saccamoeba* sp.<sup>2</sup> (Bhowmick, 1967), but the results were not convincing because of the following problems:

(a) One must observe the cell before and during preparation for electron microscopy to be certain that the cell being studied was moving normally when fixed and to precisely identify areas in electron micrographs as specific parts of the living cell. This has not previously been done.

<sup>2</sup> Originally stated to be *Trichamoeba villosa* but actually *Saccamoeba* sp., strain F-13, Griffin (J. Griffin, personal communication).

(b) Agonal contractions during fixation alter the gross morphology and presumably the fine structure of the amebas. Certain structural features that are obvious in living amebas may disappear during fixation. For example, the clear distinction between the endoplasm and the ectoplasm in living amebas is usually lost during fixation. This may explain the absence of ultrastructural distinction between the endoplasm and the ectoplasm in fixed amebas.

(c) Shrinkage of the fixed cells concentrates the cytoplasm and may also disorganize some of the cytoplasmic structures. Schäfer-Danneel (1967) considered this problem but also was unable to circumvent it.

(d) The cells should be moving—not stationary, as when exposed to pinocytosis-inducing solutions (Nachmias, 1964) or cold (Schäfer-Danneel, 1967).



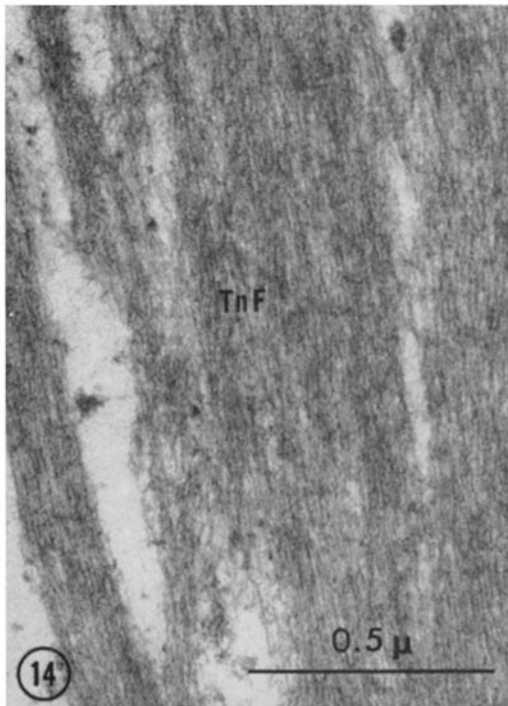


FIGURE 14 An electron micrograph of parallel arrays of 50-70 A filaments (*TnF*) in Extract 2 after warming to 22°C for 8 min with ATP. These thin filaments are thought to form the birefringent fibrils and tactoids which appear in Extract 2 warmed with ATP. Fixation with FGC at 22°C.  $\times 65,000$ .

In an effort to avoid these problems, single fountain-streaming amoebas were fixed and embedded under close microscopic observation. Careful selection of specimens with minimal abnormal movements during fixation and serial sectioning avoided the first problem, but shrinkage and imperfect fixation, as observed in the phase-contrast microscope, limited this approach.

No fine structural difference could be detected between regions of the cell known to be endoplasm and ectoplasm. This, of course, does not mean that there are no structural differences between these regions in the living amoeba. The most likely explanation for our inability to preserve this postulated differentiation is that the structures involved, presumably thin filaments (see below), are labile, and that the chemical and physical irritation of the cell by the fixative caused them to depolymerize. The loss of visual distinction of the ectoplasm from the endoplasm observed by phase-contrast micros-

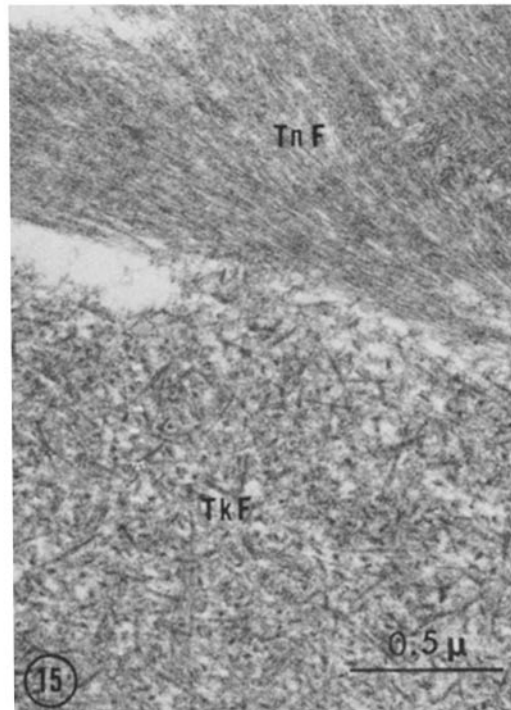


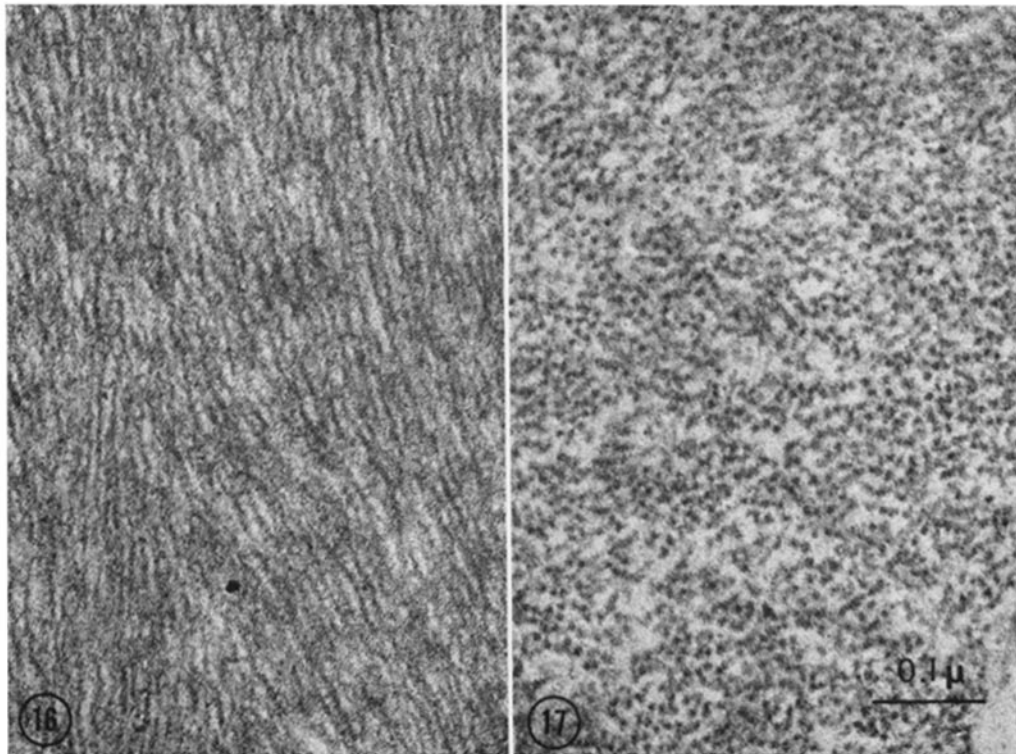
FIGURE 15 An electron micrograph of Extract 1 45 min after warming to 22°C with ATP. Pseudocrystalline arrays of thick (*TtF*) and thin (*TnF*) filaments are homogeneous except for some amorphous material between the thick filaments. The pseudocrystals of 70 A filaments are thought to be the birefringent tactoids observed in the light microscope. Preparations as in Fig. 11.  $\times 39,000$ .

copy during fixation, and the observation of Allen and Griffin (Allen, 1961) that the gel structure is labile and disrupted by agitation or other mild treatments of the cell, are in agreement with this explanation.

#### *Two Classes of Filaments in Amoeba proteus*

Previous investigators working on *Amoeba proteus* and *Chaos carolinensis* have observed cytoplasmic filaments ranging in size from 20 to 220 A in diameter (Nachmias, 1964, 1968; Wolpert, Thompson, and O'Neil, 1964; Morgan, Fyfe and Wolpert, 1967; Schäfer-Danneel, 1967). All hypothesized that the thicker filaments were aggregations of thinner filaments. The following observations are in favor of there actually being two classes of filaments in *Amoeba proteus*; 160 A diameter rods about





FIGURES 16 and 17 Extract 1 after 45 min at 22°C with ATP. Preparation as in Fig. 11.

FIGURE 16 A longitudinal section of a parallel array of thin 70 Å filaments. The filaments have no definite substructure in this preparation, but they may be slightly beaded.  $\times 145,000$ .

FIGURE 17 A cross-section of 70 Å filaments in an irregular array. Individual filaments are clearly separated from each other.  $\times 145,000$ .

0.5  $\mu$  long and 50–70 Å thin filaments of indefinite length:

(a) The exact length of the thin filaments could not be determined in sectioned material, but they appear to be quite long. The 160 Å filaments are less than 0.5  $\mu$  long. It seems unlikely that the long 70 Å filaments would break up into 0.5  $\mu$  lengths during aggregation to form the 160 Å filaments.

(b) There is no significant population of filaments of intermediate size in the cells or cytoplasmic extracts. Careful measurement of the distribution of filaments sizes supports this view. Assuming a random distribution of filament size around the mean width, 95% (mean  $\pm 2$  sd) of the filaments would fall into these ranges: 160 Å thick filaments, 113–201 Å; and 70 Å thin filaments, 52–92 Å. There is no overlap in these distributions. Overlap might be expected if the

70 Å filaments aggregated to form the 160 Å filaments.

(c) Large numbers of 50–70 Å filaments form in Extract 2 warmed to room temperature with ATP, but no 160 Å filaments are found in Extract 2 either before or after warming. Therefore, either the 160 Å filaments or factors necessary for their formation are removed by centrifugation of Extract 1 at 10,000 *g* for 10 min. The observation of scattered bundles of 160 Å filaments in the 10,000 *g* pellet favors the former explanation and makes it seem unlikely that the 70 Å filaments aggregate to form the 160 Å filaments.

(d) Pseudocrystals made up entirely of 160 Å rods or 70 Å filaments form after ATP-stimulated movement in Extract 1. Mixed or heterogeneous crystalline arrays are not observed. This may be an

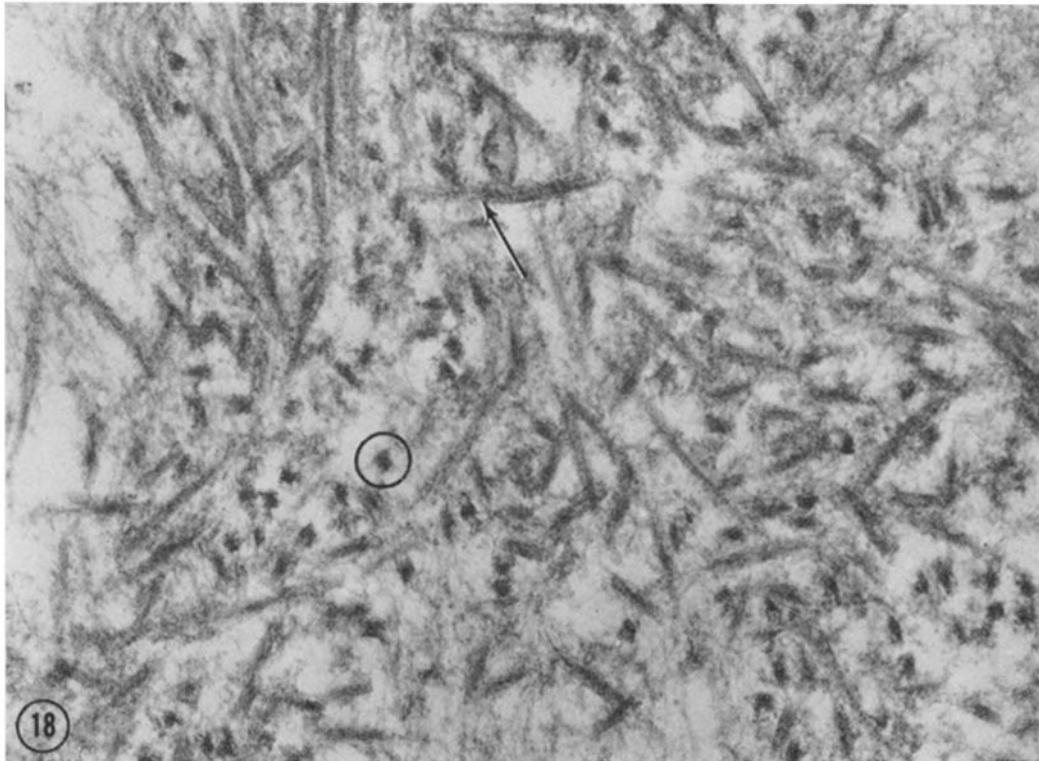


FIGURE 18 160 Å filaments in longitudinal and cross-section. Extract 1 8 min at 22°C with ATP. The thick filaments are solid in cross-section with some fine lateral projections (circle). There are distinct longitudinal striations, and some filaments end in a spray of fine filaments (arrow). Preparation as in Fig. 11.  $\times 145,000$ .

indication that the two types of filaments have different chemical compositions.

(e) The microscopic fibrils composed of 50–70 Å filaments are cold labile, while the 160 Å rods are not. The 50–70 Å filaments, if present, are found in very small numbers in extracts fixed at 0°C. On the other hand, substantial numbers of 160 Å filaments are seen in whole cells and cell fractions at 0–4°C.

(f) In the intact amoeba, the 50–70 Å filaments appear to be more labile and difficult to preserve than the 160 Å filaments. Both types of filaments are found in preparations of amoeba extracts, where, as hypothesized above, the thin filaments' state of polymerization cannot be influenced by changes in the cell membrane. Large numbers of 160 Å filaments are preserved in the cell and in cytoplasmic extracts.

Clarification or reinterpretation of earlier observations supports our hypothesis. Nachmias

(1964) described 150 and 75 Å filaments in *Chaos carolinensis* which are identical with the thick and thin filaments of *Amoeba proteus*. Wolpert, Thompson, and O'Neill (1964) demonstrated that the filaments formed from Extract 2 were thinner (80–90 vs. 120 Å) than the filaments formed from Extract 1. They did not appreciate that they may have been dealing with two classes of filaments. With improved fixation techniques, the thinner filaments appear 50–70 Å wide and are visualized in both Extract 1 and Extract 2. Their "spongy" material of Extract 1 is now resolved into networks of thin filaments. Their thicker filaments, some of which measure up to 150 Å wide in their Fig. 6, are identical with our 160 Å filaments. Of special interest is their failure to find thick filaments in Extract 2, which supports our similar observation. Morgan, Fyfe, and Wolpert (1967) described 20–30 Å filaments in negatively stained extracts of *Amoeba proteus*. These filaments appeared about

40 Å wide in sectioned material and may be the same as the thinnest filaments which we observed in sectioned material. Schäfer-Danneel (1967) also observed thick and thin filaments in glycerinated specimens of *Amoeba proteus*.

Although the definite answer to the question of how many types of filaments are found in these amoebas will have to await their isolation and chemical characterization, these observations suggest that the thick and thin filaments are two chemically distinct types.

### Thin Filaments

Long thin 50–70 Å filaments are observed in intact cells and Extracts 1 and 2. Very few thin filaments are seen in extracts at 0–4°C, so the numerous filaments seen at 22°C probably form from precursors in the groundplasm, the principal component of these extracts.

At least two factors participate in the control of thin filament formation: temperature and ATP. Warming the extracts to room temperature is an absolute requirement for filament formation. A few filaments form after warming, even in the absence of ATP or EGTA, and neither of these compounds stimulates filament formation at 4°C. In addition, fibrils of thin filaments disappear when cooled to 4°C overnight. Although it is not known whether the filaments simply disaggregate or whether they break down into subunits in the cold, these observations are consistent with filament formation being a reversible endothermic reaction. Under appropriate conditions, the polymerization of muscle actin has similar properties (Grant, 1965).

Addition of ATP greatly increases the number of filaments observed after warming the extracts to room temperature. Because some filaments form without added ATP, it is not clear whether the presence of ATP is an absolute requirement for polymerization. If it is required, endogenous ATP must be responsible for those filaments formed without its addition. Since the system probably is ATP-poor after 24 hr at 4°C, the number of filaments formed is limited.

Under suitable conditions, thin filaments aggregate laterally to form the birefringent fibrils observed in the light microscope. Morgan, Fyfe, and Wolpert (1967) reported that EGTA or EDTA will aggregate thin filaments from a high-speed supernatant of *Amoeba proteus* cytoplasm into fibrils, suggesting that the chelation of divalent

cations, especially calcium, is important in the aggregation process. We observed that fibril formation occurs in the crude cytoplasmic preparations warmed with EGTA, ATP, or EGTA and ATP. More fibrils formed in the presence of ATP, probably because more filaments formed, as discussed above. Aggregation may be a separate process involving chelation of calcium by EGTA, EDTA, or ATP.

The thin filaments vary in size between about 40 and 90 Å. Three observations suggest that 50 Å filaments aggregate to form 70 Å filaments: (a) There is considerable overlap in the distribution of the sizes of the filaments. The 95% ranges are 42–66 Å for the “50 Å” filaments and 52–92 Å for the “70 Å” filaments. (b) The 50 and 70 Å filaments are frequently observed in continuity. (c) The number of 70 Å filaments in the extracts gradually increases as the proportion of 50 Å filaments decreases with time after warming to 22°C. This concept of the relation of these two sizes of thin filaments agrees with the observations of Morgan, Fyfe, and Wolpert (1967) on negatively stained filaments from *Amoeba proteus*.

These thin filaments are similar to muscle actin in size, shape, (Hanson and Lowy, 1963), formation with the absorption of heat and proposed molecular structure, including interaction with ATP. Are they formed from an actin-like molecule? Evidence is rapidly accumulating that thin cytoplasmic filaments from nonmuscle cells such as these are formed from actin-like proteins. Hatano and Oosawa (1966) isolated actin from slime mold, and Wehling and Korn (1969) purified another from *Acanthamoeba castellanii*. Both the purified amoeba F-actin from *Acanthamoeba* and the thin filaments in glycerinated specimens of *Acanthamoeba* form “arrowhead” complexes with rabbit muscle heavy meromyosin (HMM) identical with the complexes formed from muscle actin and HMM, demonstrating that the 60 Å filaments in that amoeba are amoeba F-actin (Pollard, Shelton, Wehling, and Korn, 1970). In a subsequent report (Pollard and Worn, 1970), we will show that thin filaments in extracts of *Amoeba proteus* specifically bind muscle heavy meromyosin to form arrowhead complexes which can be dissociated by Mg-ATP, strongly suggesting that these filaments are also actin.

To summarize what is known about the thin filaments: they are actin-like 70 Å filaments which are cold labile, require both heat and ATP for

complete polymerization, and aggregate into microscopic birefringent fibrils in the presence of calcium chelators.

### *Thick Filaments*

The 160 Å wide, 0.5  $\mu$  long rodlike filaments appear to be stable components of the amoeba. They are present in cells and extracts at 0°C as well as 22°C, in motile and nonmotile amoebas and extracts, and in high and low viscosity extracts. It is possible that more of these thick filaments form in Extract 1 after warming to 22°C with ATP, but this was not certain from our observations.

Nachmias (1968) observed in negatively stained preparations that the thick filaments of *Chaos* had a filamentous substructure similar to that which we have observed in sectioned material. She, therefore, postulated that the thick filaments formed by the aggregation of 50–70 Å thin filaments. As discussed above, it seems likely that the very fine filaments which form these thick filaments may be distinct from the 50–70 Å filaments.

Just as the thin filaments are similar to muscle actin, the thick filaments bear some superficial resemblance to myosin of striated muscle: both are approximately 150 Å rods with proposed filamentous substructure and lateral projections. If these thick filaments are myosin, it is unusual. Except for striated muscle, myosin is not aggregated into stable filaments under normal intracellular conditions. The myosin isolated from slime mold (Hatano and Tazawa, 1968; Adelman et al., 1968) is soluble at intracellular ionic strengths. Smooth muscle myosin sometimes aggregates into filaments, but only under special conditions related to the state of contraction, pH, and concentration of divalent cations (Kelly and Rice, 1969; Schoenberg, 1969).

### *Mechanism of Viscosity Changes*

The technical difficulties discussed above make it impossible to directly demonstrate in an intact amoeba any structural differences between the endoplasm and ectoplasm which may account for their difference in consistency. Therefore, we have had to rely on the study of the motile extracts of amoeba cytoplasm to formulate a hypothesis for the mechanism of viscosity changes and contraction.

The *initial* phase of ATP-stimulated movement of Extract 1, Phase 1, closely resembles the cytoplasmic streaming described in intact and broken

amoebas (Allen, Cooledge, and Hall, 1960): no microscopic fibrils or strong birefringence develop, and yet the extract moves vigorously and certain areas appear to have increased in consistency. The apparent viscosity of the extract increases during this phase of movement (Fig. 10), and electron microscopy reveals that an extended network of thin 50–70 Å filaments has formed in certain regions of the extract (Fig. 11).

The formation of thin filaments from precursors in the groundplasm is probably the cause of the increase in apparent viscosity of the extract. This is supported by the correlation of viscosity increase with the appearance of large numbers of thin filaments in the extract and is analogous to the increase in viscosity associated with the polymerization of other filamentous macromolecules such as actin (Straub and Feuer, 1950).

In addition, gel formation in amoebas is known to be an endothermic reaction (Marsland, 1964), so that the observation that heat is required for the formation of the thin filaments in amoeba extracts agrees with the concept that thin filaments account for the increased consistency of "gelled" cytoplasm.

An alternate, but more complex, mechanism for consistency changes is that the actin-like thin filaments first polymerize and then interact with a myosin-like component to form an actomyosin gel. If the thick filaments are the myosin-like component, this mechanism is compatible with our observations, although it is not certain that an actomyosin gel would form in the presence of the millimolar quantities of ATP in Extract 1.

These observations suggest that the variations in the consistency of different regions of the amoeba cytoplasm may be directly related to variations in the proportion of thin filament precursors polymerized into filaments. A network of thin filaments could account for the limited movement of particles in the ectoplasm and the invisible obstructions to the fall of crystals in centrifuged amoebas (Allen, 1961). In addition, thin filaments which became oriented under tension could account for the increase in birefringence observed in specimens of *Chaos carolinensis* stretched by the application of pressure and by sporadic reversals in the direction of streaming (Allen, Francis, and Nakajima, 1965). These filaments must be present in low concentrations, and/or they may be randomly oriented, because the birefringence of amoeba cytoplasm is low, with retarda-

tions on the order of  $10^{-4}$ – $10^{-5}$  (Allen, Francis, and Nakajima, 1965).

A difficulty with this interpretation is that very few thin filaments were seen in fixed amebas, but as discussed above, the ectoplasmic gel may break down during fixation. It seems likely (Wolpert and Gingell, 1968) that changes in the cell membrane in response to external stimuli may control the state of gelation of the cytoplasm. The abnormal movements and changes in the ectoplasm of the amebas exposed to fixatives also may be mediated by the effects of the fixative on the cell membrane. This defensive mechanism is absent in the cell-free extracts and may account for the ease of fixing the thin filaments there.

### *Mechanism of Contraction*

Contraction requires the development and transmission of tension. It is clear from our observations in the light microscope and those earlier of Thompson and Wolpert (1963) that the fibrils, which form in Phase 2 movement of the cytoplasmic extract, can transmit tension. We have shown that these fibrils are birefringent and consist of parallel arrays of 70 Å filaments. Fibrils of any size, from those just resolved in the phase-contrast microscope to those more than 2  $\mu$  wide, appear to transmit tension. By analogy, one can argue that submicroscopic fibrils of thin filaments, or perhaps even single thin filaments, may also be capable of transmitting tension, such as that necessary for the saltatory movement of subcellular extracts and in the cells.

The mechanism for the development of tension is speculative, but a comparison of the motile Extract 1 with nonmotile Extract 2 gives some preliminary data on this important problem (see Table I). Nonmotile Extract 2 contains the precursors of thin filaments, which readily polymerized in the presence of ATP at 22°C; therefore, the polymerization of thin filaments is alone insufficient to cause movement. This is consistent with our light microscope observation that there is no visible change in the shape of the fibrils of thin filaments as they move toward aggregates of particles, suggesting that the fibrils are playing a passive role in the transmission of tension and are not shortening or contracting themselves. Centrifugation of motile Extract 1 at 10,000  $g$  to make nonmotile Extract 2 removes thick filaments, vesicles, endoplasmic reticulum, and mitochondria. Any or all of these organelles may

be important for movement. This differs slightly from the observations on the motility of extracts reported by Wolpert's group (Wolpert, Thompson, and O'Neill, 1964; Morgan, Fyfe, and Wolpert, 1967). They found that the 9,000  $g$  supernatant was motile, but that 35,000 and 150,000  $g$  supernatants were nonmotile unless a "vesicle fraction," the "10–35,000  $g$  pellet," was added to these high-speed supernatants. They have not reported the components of this vesicle fraction, but this fraction may be similar to the 10,000  $g$  pellet in the present study, as both have the light microscope appearance of a vesicle fraction and both are required for the movement of the supernatant.

Which of the components of the 10,000  $g$  pellet are required for movement? This has not been proven, but speculation based on the accepted model for muscle contraction (Hanson and Huxley, 1953) would suggest that the thick filaments may be required to interact with the thin filaments to develop tension. The organization of the two types of filaments in the motile extract (Fig. 9), with thin filaments radiating from clusters of thick filaments, is consistent with the idea that the two types of filaments interact to produce the movement observed in these extracts. At the end stage of the reaction, when the extract no longer moves, this organization is lost, and the filaments aggregate into large pseudocrystals of thin or thick filaments (Fig. 15). On the other hand, the vesicles removed in the 10,000  $g$  pellet may be important for the control of the development of tension as suggested by Hoffman-Berling (1964), or the mitochondria may be necessary to supply energy.

Wolpert, Thompson, and O'Neill (1964) found that Extract 1 moved only when warmed to room temperature with ATP or ADP, with ADP stimulating much less movement than ATP. They also noted that the reaction was inhibited by millimolar quantities of calcium. We noted that movement with ATP plus EGTA was equivalent to the movement stimulated by ATP alone. These observations suggest that the contractile mechanism of *Amoeba proteus* is similar to that of muscle in its requirement for ATP but dissimilar in its control mechanism, because it is not calcium activated like muscle (Weber, Herz, and Reiss, 1964).

## CONCLUSION

This study confirms earlier observations that extracts of *Amoeba proteus* move in a manner similar to the intact cell when warmed with ATP. During this movement, there is an increase in apparent viscosity associated with the formation of 50–70 Å filaments from precursors in the groundplasm. It is postulated that interaction of these labile thin

filaments with 160 Å filaments constantly present in the groundplasm causes contraction of the cytoplasm responsible for amoeboid movement.

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