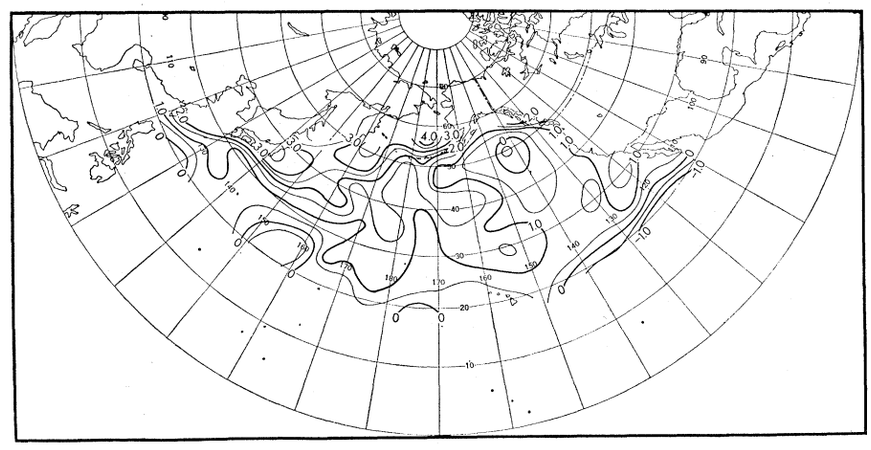

Fig. 3. Average departures of sea-surface temperature for the ten winters, 1960–70, from long-term (about 40-year) means ending about 1945. Temperatures were extracted from monthly means published by the Bureau of Commercial Fisheries (*7*) and from values furnished by the Japanese Meteorological Agency. Isopleths are constructed at 0.5°F (approximately 0.3°C) intervals.

sure level and by isopleths of departure from normal (here, normal is defined as an average for the winters of 1947–63) for the ten winters (Fig. 2).

Since the prevailing winds flow along the contours with low heights to the left, this chart shows that the long wave pattern affecting much of the Northern Hemisphere, and especially North America, was appreciably amplified above the normal. With a stronger ridge (northward bulge in the flow) over western North America and a stronger trough (southward bulge) to the east, the more frequent deployment of Arctic air masses into the eastern half of the nation is assured. This is obvious from the isopleths of 700-mb height anomaly and is verifiable from an objective system (*2*) for specifying mean temperature anomalies at the earth's surface from 700-mb height anomalies. In view of this relationship, it seems unlikely that increased air pollution, variation in volcanic activity, or human intervention was the cause of the decadal temperature fluctuation over the United States. If these factors do not cause the U.S. temperature fluctuation, then it is possible that fluctuations elsewhere are caused by direct regional interactions and their further consequences (*3*).

At the same time that the eastern United States was abnormally *cold*, the sea surface over much of the North Pacific was abnormally *warm*. Figure 3 shows the average departures of sea-surface temperatures in the winters of the 1960's from normals based on a period of more than 40 years prior to 1945 (*4*). Whatever the cause of the oceanic warming, it is likely to have produced an aberration in the wintertime atmospheric circulation over the North Pacific—most probably by abnormal excitation of cyclones, as I have described elsewhere (*3*). Once the cyclonic activity increased and the vorticity (or curl of the winds) was transported aloft, the resulting standing (or forced) long-wave central Pacific trough created downstream perturbations in the manner shown in Fig. 2. These perturbations are the well-known long or Rossby waves, and their observed positions agree with theoretical (*5*) or empirically derived (*6*) teleconnections.

The *fall* sea-surface temperature anomalies of the last decade (not shown) averaged up to 1°F (approximately 0.5°C) warmer than those of the following winters over much of the central North Pacific. Thus, the winter storms had an unusually warm initial reservoir on which to feed, and some of the anomalous heat was extracted through increased latent and sensible heat losses associated with stronger winds.

There seems to be no strong reason why repetitive conditions such as those described cannot lead to climatic fluctuations of a much longer time scale than a decade. It may be shortsighted to invoke extraterrestrial or man-made activity to explain these fluctuations.

JEROME NAMIAS

Extended Forecast Division, Weather Bureau, ESSA, Washington, D.C. 20233, and *Scripps Institution of Oceanography, La Jolla, California 92037*

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20 August 1970

Actomyosin from Physarum polycephalum: Electron Microscopy of Myosin-Enriched Preparations

Abstract. *Negatively stained slime mold actomyosin examined by electron microscopy consists mainly of actin-like filaments with occasional angular projections. If some of the actin is removed, the myosin-enriched actomyosin appears as continuous arrowhead structures similar to those of vertebrate striated muscle actomyosin. Together with other evidence, the findings suggest that cytoplasmic streaming in* Physarum *may involve a contractile process operating at a relatively low myosin-actin ratio.*

Actomyosin preparations purified from *Physarum polycephalum* by a slight modification of the method of Hatano and Tazawa (*1*) have been shown (*2*), when examined in the electron microscope after negative staining, to consist of beaded filaments of various lengths up to about 1.2 μm long with projections at intervals. Sometimes the projections are clearly at an angle with respect to the filaments, and occasionally thin (about

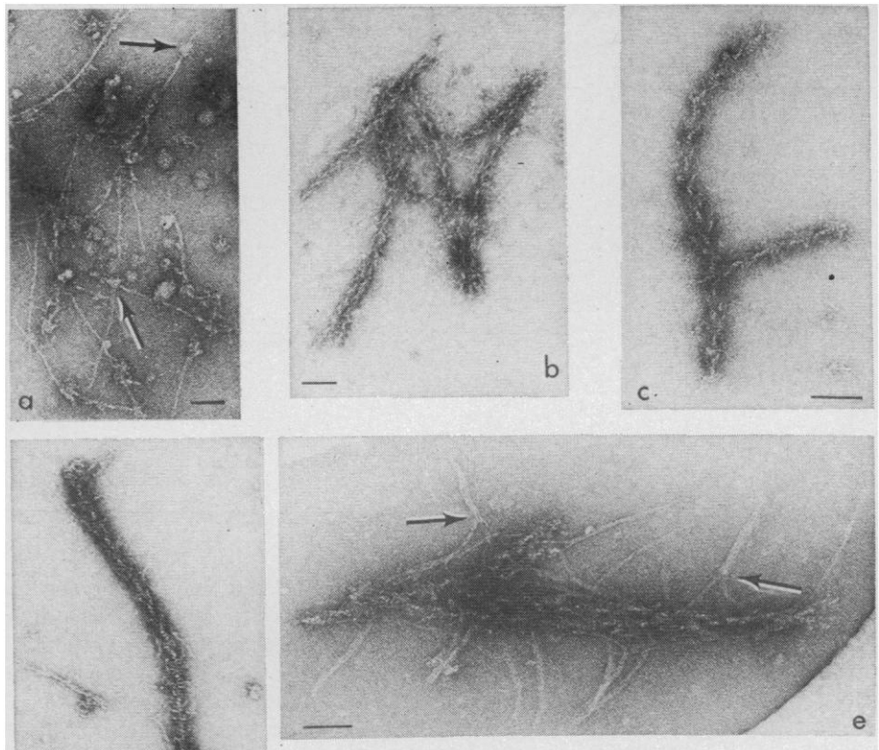

Fig. 1. (a) Partially purified *Physarum* actomyosin in 0.5*M* KCl. Long filaments with occasional angular projections (arrows) are seen together with globular impurities. Bar, 0.1 μm. (b) Myosin-enriched *Physarum* actomyosin from a low ionic strength dialysis. Bar, 0.1 μm (c) Myosin-enriched *Physarum* actomyosin from a dialysis against 0.6*M* buffered KCl. Bar, 500 Å. (d) Natural actomyosin from rabbit striated muscles in buffered 0.6*M* KCl. Bar, 500 Å. (e) Myosin-enriched *Physarum* actomyosin from a dialysis against 0.06*M* KCl, 0.01*M* tris (*p*H 7). Arrows point to places where extensions from arrowhead regions apparently join to form thicker extensions. Bar, 500 Å. All figures are of negatively stained preparations using 1 percent uranyl acetate (see text).

20 Å) extensions of the projections are seen. The projections are removed by treatment with adenosine triphosphate (ATP) and magnesium salts leaving unbranched beaded filaments. Independent evidence has been obtained for the existence of a myosin in *Physarum* (*1, 3*). It was suggested that the projections on the filaments represented *Physarum myosin* (*2*). Their scarcity is in keeping with estimates for the myosin-actin ratio in *Physarum* of 1:1 or 1:2 (*1, 3*) rather than 2:1 or 3:1 as for natural actomyosin from vertebrate striated muscle (*4*), which has a greater number of angulated projections (*5*). The interpretation is also consistent with data showing *Physarum* myosin to be an elongated molecule with a molecular weight and *S* value close to those for vertebrate striated muscle myosin (*1, 3, 6*).

If correct, this interpretation predicts that an experimental increase in the myosin-actin ratio in *Physarum* actomyosin should increase the number of such projections. The resultant complex would be expected to resemble the actomyosin of vertebrate striated muscle. The following experiment shows that *Physarum* actomyosin can be so enriched, and that the resultant preparations do closely resemble muscle actomyosin.

The procedure used is essentially that used to separate myosin and actin in muscle actomyosin (*7*). Partially purified *Physarum* actomyosin preparations in 0.5*M* KCl (Fig. 1a) were made 5 m*M* in ATP and magnesium chloride to dissociate the actomyosin complex. The preparations were then centrifuged at 100,000*g* for 1½ to 3 hours. The small, pale yellow pellets were discarded. The water-clear supernatants were dialyzed against either 0.05*M* KCl in 0.01*M* tris(hydroxymethyl)aminomethane or imidazole buffer (*p*H 7), or 0.5*M* KCl in 0.1*M* imidazole buffer. "Natural" actomyosin from rabbit skeletal muscle was prepared by the method of Szent-Györgyi (*8*). Electron microscopy was performed with a Hitachi HU-11A microscope equipped with a 50-μm thin foil objective aperture. Substrates were holey carbon or carbon-celloidin filmed grids. The negative stain procedure has been described (*2*). Magnifications were calibrated with a standard replica grating (E. F. Fullam, Inc.). Adenosine triphosphatase assays were done on the initial preparations according to Adelman and Taylor (*3*) at *p*H 8 in the presence of 10 m*M* $CaCl_2$. Estimates of protein concentration were by the Lowry method (*9*) with bovine serum albumin as a standard. Optical densities were measured in a Gilford modified Beckman spectrophotometer. Relative viscosities were measured with Cannon semimicro viscometers with outflow times of 30 to 40 seconds.

When supernatants from ultracentrifuged *Physarum* actomyosin were dialyzed against low ionic strength mediums, flocculent turbidity developed in all cases. Electron microscopy of these supernatants showed clumps of filaments which appeared as arrowhead structures (Fig. 1, b and e). When the supernatants were dialyzed against high ionic strength mediums, no turbidity developed. Separated filaments were present (Fig. 1c), appearing much like natural actomyosin from muscle (compare Fig. 1, c and d). In some areas in both types of *Physarum* preparations, the arrowheads bore long extensions of the order of 20 Å in diameter and up to about 1400 Å in length. At low ionic strength some of these appeared to join to form thicker extensions (Fig. 1e, arrows).

The actomyosin preparations that were centrifuged had adenosine triphosphatase activities (expressed as the number of micromoles of P_i produced per milligram per minute) from 0.17 to 0.37. The rate was proportional to the amount of actomyosin added. Viscosity drops for the preparations used here were 0.1 to 0.15 relative units when 1 m*M* ATP or 2 m*M* sodium pyrophosphate plus 1 m*M* magnesium salts were added. When ATP was added the viscosity usually rose to a level higher than the initial value; when pyrophosphate was used the viscosity did not recover during the observation period (1 hour). Optical densities were measured from 230 to 300 nm on preparations before centrifugation and after dialysis at high ionic strength. When pyrophosphate (10 m*M*) and magnesium chloride (5 m*M*) were used to dissociate the actomyosin, a

small increase in the optical density ratio at 280/260 was found—from 1.12 in the original actomyosin to 1.23 in the supernatant. However, when ATP was used to dissociate the actomyosin, strong absorption persisted at 260 nm even after dialysis, and the 280/260 ratio was 0.35 and 0.57 in two experiments. Calculations showed that the ATP concentration should have been too low for significant absorption. Residual binding of ATP or ADP by the proteins is a likely explanation for this. A value of 1.66 has been reported for the 280/260 ratio for *Physarum* myosin free of actin (*3*).

From all the results taken together, we conclude that ultracentrifugation in the presence of ATP or pyrophosphate sedimented much of the actin fraction (as well as some impurities visible in Fig. 1a) while both myosin and short F-actin fragments remained in the supernatant. Removal of the ATP by dialysis allowed the actomyosin complex to reform, now with a higher ratio of myosin to actin. Our results are in agreement with recent findings by Hatano and Ohnuma (*3*). They also found free myosin in the supernatant fraction after comparable treatments, and it is possible that this accounts for some residual binding of nucleotide in our experiments.

The demonstration that *Physarum* actomyosin enriched in myosin displays a polarity similar to that of muscle actomyosin confirms the previous report of polarity for *Physarum* filaments reacted with HMM S-1, the subunit from rabbit muscle myosin which retains both actin-binding ability and the enzymatic activity of myosin, from rabbit striated muscle (*2*). This similarity between the two actomyosins suggests that part of the process of cytoplasmic streaming depends on events fundamentally similar to those occurring in muscle contractions. But the myosin component from *Physarum* remains soluble at low ionic strength (*1*, *3*), forming only small aggregates. The present findings show that *Physarum* myosin can aggregate in a tail-to-tail fashion at least when attached to actin. However, the aggregations seen clearly so far seem to be in a parallel direction. It will be interesting to see if antiparallel tail-to-tail aggregations can occur with *Physarum* myosin in the absence of actin (*10*).

V. T. NACHMIAS
W. C. INGRAM
Department of Biology, Haverford College, Haverford, Pennsylvania 19041

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11. We thank Dr. S. Matacić for natural actomyosin from rabbit striated muscle, Mrs. S. Shane for *Physarum* cultures, Miss B. Herzog for assistance with photography, and Drs. A. Loewy and D. Kessler for discussions. Supported by the Sloan Foundation and in part by NIH grants A108732 and HE04385.

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Prolactin: Evidence That It Is Separate from Growth Hormone in Human Blood

Abstract. *A highly sensitive bioassay has been developed for prolactin, which uses a mammalian end organ and which is capable of measuring the hormone in unextracted human plasma. High prolactin activity, largely neutralizable with antiserum to human growth hormone, is stimulated along with high immunoassayable growth hormone by insulin-induced hypoglycemia. High prolactin activity, not neutralizable with antiserum to growth hormone, exists with low concentrations of plasma growth hormone in postpartum patients and patients with galactorrhea.*

Since the initial demonstrations that purified growth hormone extracted from human pituitaries possessed lactogenic activity (*1*), efforts have been made to isolate a human prolactin separate from growth hormone. Although fractions that have variable ratios of prolactin to growth hormone have been prepared (*2*), the two biological activities have never been completely separated, and doubt has been expressed that human beings possess a separate prolactin comparable to that of lower animals (*3*). Nevertheless, several lines of evidence—including the absence of acromegalic changes in most patients with galactorrhea (*4*), the presence of postpartum lactation in patients with isolated growth hormone deficiency (*5*), histological studies of human pituitaries from postpartum subjects (*6*), and histological and bioassay studies of a pituitary tumor from a patient with galactorrhea, who showed low growth hormone in extracts of the tumor and high prolactin that was not neutralizable with antiserum to growth hormone (*7*)—suggest that there may be a distinct human prolactin. The inability of the pigeon crop-sac assay to measure the hormone in blood without elaborate extraction procedures has delayed resolution of this problem. We report here findings based on a more sensitive bioassay for prolactin in human blood which indicate (i) that prolactin exists in man and can circulate independently of growth hormone and (ii) that human growth hormone as it circulates in blood is intrinsically lactogenic.

The assay we have devised (*8*) depends on the ability of prolactin to cause differentiation and milk secretion of mouse breast tissue in organ culture (*9*). Fragments of tissue from Swiss albino mice, pregnant for 8 days and bred in our laboratory, are incubated 4 days in medium 199 containing insulin (10 μg/ml) and hydrocortisone (20 μg/ml) in an atmosphere of 95 percent O_2 and 5 percent CO_2. After incubation, tissues are fixed and stained with hematoxylin, phloxine, and saffron. Ovine prolactin (NIH-S8, 28 unit/mg) added to the medium at concentrations ranging from 1 to 50 ng/ml produces clearly visible and distinct secretory changes that are graded on a scale of 0 to 4+ (Fig. 1). When pooled plasma without detectable prolactin activity, from normal males, was added to the medium in 30 percent concentrations, it was found not to interfere with the response.

By partially or completely replacing this plasma with a sample to be tested, it has been possible to measure the prolactin concentration in circu-