portant quantitatively than the main motive force responsible for the thermal cycles which we have found.

While the possibility, admitted by Kamiya (*17*), that the motive force might be due to interactions between the smaller streams and their channel walls cannot be rigorously excluded on the basis of all the information now available, there is increasing evidence to suggest that the mechanochemical event responsible for streaming is a contraction of the channel wall material. For example: (i) the occurrence of pulsations, which may be interpreted as contractions; (ii) the ability of hanging plasmodial strands to show both torsional motions and isotonic contractions (*23*); (iii) microscopically observable changes in channel diameter during the streaming cycle; (iv) the demonstration of a protein system capable of both mechanochemical and enzymatic reactions similar to those of muscular "contractile proteins"; and finally (v), the recent demonstration of a dynamically organized fibrillar system the form and birefringence of which fluctuates during the streaming cycle (*24*).

We would prefer not to generalize from our present evidence concerning the mechanism of slime mold streaming to problems in the mechanism of ameboid movement, reticulopodial streaming in foraminifera, and rotational streaming in Characeae, where recent studies suggest diversity of mechanism at the cellular level (*24*). This does not exclude the possibility that the molecular basis of cytoplasmic streaming phenomena might show the unity of mechanism which many have expected to find at the cellular level (*25*).

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Virus-Like Particles in Blood of Two Acute Leukemia Patients

Abstract. *A modified negative staining technique suited to demonstrating fragile virus particles in the electron microscope revealed particles having a surrounding membranous sac and an internal filamentous component of a consistent diameter of 75 Å in the blood of two patients with acute leukemia. No particles of this type were seen in the blood of four other patients with acute leukemia or in the blood of six normal individuals.*

Since Dmochowski and Grey (*1*) in 1957 observed virus-like particles in thin sections of AK mice with spontaneous leukemia, and in C3H mice injected with Gross leukemia virus at birth, the thin-section technique has been widely used in attempts to demonstrate similar virus-like particles in the leukemic cells and tissues of man. Dmochowski (*2, 3*) has recently reviewed his and others' findings in this field. As Bryan (*4*) concludes, virus-like particles have been reported in only a small fraction of leukemic patients examined.

The negative staining technique (*5*) for the study of virus particles with the electron microscope permits the fine structure of virus particles to be discerned in great detail, making it easier to decide whether any given particle resembles a known virus particle, and also permits virus particles to be classified on a morphological basis (*6, 7*). By a modification of the technique recently evolved in our laboratory (*8, 9*) virus particles can be negatively stained in what are essentially untreated cell preparations. This method does not require purification procedures which might destroy a fragile virus particle. Hence even the fine structure of rabies virus particles (which are notoriously fragile and unstable) has been observed in cell preparations (*10*). For this reason this technique has been used to examine the leukocytes in the blood of patients with acute leukemia.

Blood from six patients with acute blast-cell leukemia and blood from six healthy subjects have now been studied. Hematological and other clinical findings indicated that three of the leukemias were lymphoblastic and the other three myeloblastic. In two of the myeloblastic cases, which clinically were similar, virus-like particles were consistently found in leukocyte preparations, and the particles in each instance were of the same morphological type.

The first patient was a 43-year old female with symptoms of 2 months' duration. On admission to the hospital her leukocyte count was 140,000/mm^3 with 88 percent primitive cells, her hemoglobin was 6.8 g per 100 ml of blood, and her platelet count 25,000/mm^3. The sternal marrow showed 95 percent primitive cells. Two specimens of peripheral blood were obtained before, and one 8 days after, the start of 6-mercaptopurine therapy. The sec-

ond patient was a 53-year old male with symptoms of 2 weeks' duration. On admission his leukocyte count was 185,000/mm³ with 80 percent primitive cells; his hemoglobin was 11.2 g per 100 ml of blood, and his platelet count 46,000/mm³. Blood-cell preparations for study with the electron microscope were obtained on the 1st and 2nd days after hospital admission. Therapy was instituted with 6-mercaptopurine and predisone but the patient died on the 4th day after admission. With Wright's stain the primitive cells seen in both cases were similar, they were 15 to 20 μ in diameter with irregular folded nuclei that contained fine chromatin. Nucleoli were seen in a few nuclei. The cytoplasm was moderately pale blue and in some instances contained fine azurophilic granules. The findings in both cases were consistent with a diagnosis of acute myeloblastic or monomyeloblastic leukemia.

For each examination 10 ml of blood was drawn and the tripotassium salt of ethylenediamine tetraacetic acid (EDTA) (2 mg/ml of blood) was used as anticoagulant. The blood was centrifuged at 2000 rev/min for 10 minutes. The buffy coat (the layer where the leukocytes are aggregated) was then removed and prepared for electron microscopy (*9*) by freezing the material in a mould and then cutting the frozen material at a setting of 4 μ on an ordinary microtome contained in a cryostat maintained at −20°C. The cell fragments so obtained were then negatively stained by mixing them with a 3-percent solution of potassium phosphotungstate adjusted to *p*H 6; they were then placed on carbon-Formvar coated grids and examined immediately in the electron microscope.

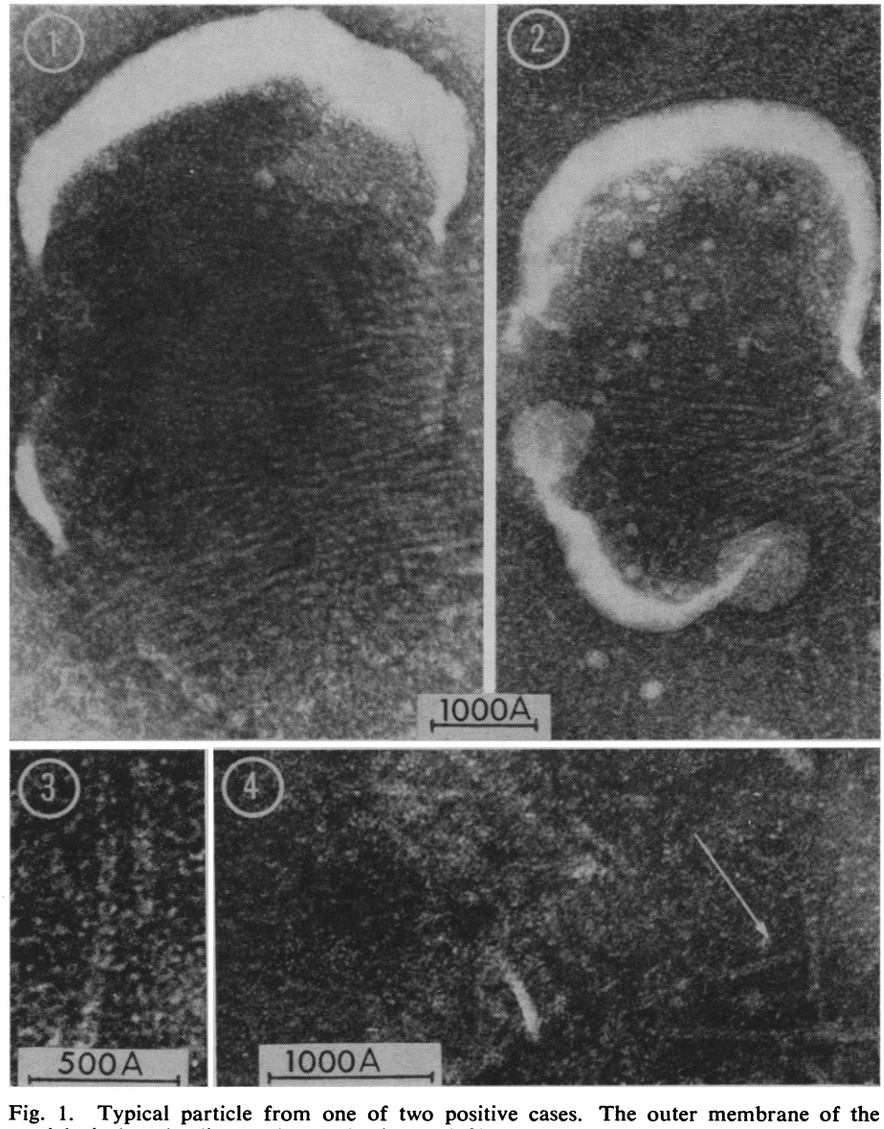

Fig. 1. Typical particle from one of two positive cases. The outer membrane of the particle is largely disrupted and the internal filamentous component can be seen. The filaments have a diameter of 75 Å and are more or less parallel. Fig. 2. A particle from the second positive case. Here the particle is more compact and the outer membrane is almost complete. The internal component is apparent and here also, a parallel arrangement can be observed. Fig. 3. A high-power micrograph of a small fragment of a filament from the internal component showing the "figure eight" appearance that suggests the possibility of a double helix. Fig. 4. Detail of a micrograph from the edge of another particle. The arrow indicates portions of the internal component that have fragmented.

In all three specimens obtained from the female patient, and in both specimens obtained from the male patient, particles of the same type were observed (Figs. 1 and 2). Each particle had a more or less disrupted surrounding membrane and an internal filamentous component. Although the disrupted surrounding membrane permitted the internal filamentous component of the particles to be seen advantageously it made the overall diameters of particles difficult to establish because the disrupted particles were always expanded to some extent. Measurements ranged from 1000 to 5000 Å. The better preserved particles had a diameter close to 1000 Å. The internal filaments were often in more or less parallel array (Figs. 1 and 2). Individual filaments had a constant diameter of 75 Å. Within the filaments, a finer component of 32 Å in diameter could be discerned. In particularly clear areas in the micrographs there was a figure-eight appearance (Fig. 3), which suggested the possibility that the internal filaments might be in the form of a double helix. The internal filaments closely resembled the internal filaments described for influenza virus particles (*11*) except that they were narrower; those of influenza virus particles have a diameter of 90 Å. The fragile nature of the filaments in the particles we observed was indicated by the fact that many short lengths, broken off from those in the main body of the particle (Fig. 4), could be seen.

In that the particles observed were 1000 Å or more in diameter with a surrounding membrane and an internal filamentous component, they possess features that are now known, from negative staining, to be the characteristics of several known animal virus particles (*12*). More particularly such particles resemble most closely those that would be classified in the compound helical group in a classification

of virus particles based on morphology such as that recently devised (*7*).

The preparations of the blood of normal people and of four of the leukemia patients revealed many particles in the same size range as virus particles. These might be mistaken for virus particles unless criteria, dependent on the observance of one of the types of morphology (*7*) now known to be associated with negatively stained virus particles, are considered. In no negatively stained preparations from the blood of these people were particles seen that exhibited any of the known types of virus-particle morphology.

It might be questioned whether the particles we observed could be the same as those occasionally seen by Dmochowski and others (*3*) in thin sections of leukemic cells and tissues of man. That both are covered with a membrane and in the same size range is in support of this possibility. Moreover, the particles we observed may be of the same general type as those described by de Harven and Friend (*13*), who, by means of a negative staining technique, found rare particles, approximately 1000 Å in diameter, resembling myxoviruses, in the blood of mice with Friend leukemia.

Since passenger viruses can be present in malignant tissue and since the method used in this study discloses fragile virus particles that might not be seen by other methods it would be unwise to attempt to draw any conclusions about whether the particles described here are directly related to the disease in which they were observed in two instances. However, even if the described particles are not directly related to the disease in which they were found, their presence in this disease poses a sufficiently interesting problem to alert those studying clinical leukemia (*14*).

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Lens Development: Fiber Elongation and Lens Orientation

Abstract. *When the lens of the 5-day chick embryo is surgically reversed so that its epithelium faces the neural retina the elongation of those lens cells which have already differentiated is arrested and the epithelial cells differentiate into a new set of lens fibers. This internal reorganization, together with a reversal in polarity at the lens equator, results in a complete reversal of the polarity of the entire lens.*

This study demonstrates that the differentiation of lens epithelial cells into lens fibers is not simply a function of their age but depends importantly on their location within the eye. The lens of the embryonic eye of the domestic fowl begins as a placode of palisaded cells between the 10 and 20 somite stages. It is induced in head ectoderm by the tip of the optic vesicle (prospective neural retina) (*1, 2*). The placode invaginates during the 21- to 25-somite stage and separates from the surface by the 35- or 36-somite stage to form the hollow lens vesicle (see *2–4*).

Simultaneously the cells of the vesicle lying toward the vitreous body elongate, approach the anterior epithelium, and obliterate the lens cavity (*4, 5*). The primitive lens thus formed has a simple cuboidal epithelium toward the cornea, and an ellipsoidal fascicle of lens fibers located behind the epithelium and continuous with it at the equator. As early as 1907 Le-Cron (*6*) recognized that the presence of the optic cup is necessary for the maintenance of a lens at this stage. The mitosis which contributes to the growth of the lens is restricted to the epithelium. At the equator the differentiation of the epithelial cells is signaled by their elongation to form meridionally oriented lens fibers which are added to the body of the lens in concentric layers.

The factors which control the elongation of lens fibers during normal development, as well as the factors which confine the zone of incipient differentiation to the equator, have not yet been identified. However, during regeneration of the lens from the dorsal iris in adult amphibians (*7*) the lens fibers develop in that part of the lens which faces the neural retina, even when the iris has been surgically reversed, back to front, before the onset of lens regeneration. The observation that the lens was oriented appropriately to the eye rather than to the iris was interpreted to indicate the presence of a factor from the posterior half of the eye which promotes elongation of the posterior cells of the lens.

We have dealt with a similar phenomenon during normal lens development in the chick embryo. The specific questions we asked were: If they are appropriately stimulated can all of the cells of the lens epithelium, and not just those near the lens equator, form lens fibers? Does the position of lens cells relative to the neural retina determine whether or not they will form lens fibers? Once lens fiber elongation has been initiated is it autonomous? Can the polarity of the equatorial zone and of the lens as a whole be reversed?

To answer these questions we studied histogenetic changes in the embryonic chick lens after it had been surgically reversed so that the lens epithelium faced the vitreous body. The embryo was exposed for surgery by cutting a window through the shell and shell membrane, making an incision through the chorion at the edge of the allantois, and opening the amnion over the right eye. The lens enclosed within its capsule was removed intact from the right eye at 5 days of incubation through an incision at the dorsal corneal limbus in 22 embryos. The same lens, or a comparable one from a 5-day donor, was reinserted into the eye after being reversed.

Separate experiments demonstrate that after this procedure the eye wall can heal in as little as 4 hours. While the incisions did not heal in a few specimens this did not affect the sequence of changes in the lens (see Fig. 1*A*). At 2-day intervals after the