Shuttle-Streaming: Synchronization with Heat Production in Slime Mold

Abstract. *Two small blobs and a channel excised from a slime mold plasmodium were allowed to fuse into a dumbbell-shaped mass in a thermally insulated Kamiya double chamber equipped with naked bead thermistors in contact with the blobs. Cyclic temperature differences of from 1 × 10^{-4} to 5 × 10^{-2} deg Celsius were recorded by a sensitive lock-in amplifier method with a basal noise level of less than 2 × 10^{-5} deg Celsius and a time constant of 0.5 second. The temperature differences were caused by periodic bursts of heat production synchronized perfectly with the shuttle-streaming cycle and invariably localized at the source rather than the destination of the streaming cytoplasm. The results support the theory that the motive force for cytoplasmic streaming in the slime mold is pressure, probably generated by contraction of elements in the channel walls.*

The two central problems in the analysis of the mechanism of cytoplasmic streaming phenomena at the cellular or organismal level are (i) identification of the motive force (that is, as pressure, tension, or other force), and (ii) localization of the site of its application within the cell, or in the case of the slime mold, the plasmodium.

The solutions to these problems are not so simple as some have believed. Accounts of shuttle-streaming, ameboid movement, cytokinesis, mitosis and other dynamic cellular events often include the description of "contractions." We claim that the human eye is capable only of observing deformations, and has not been demonstrated to have receptors for determining which deformations are active or passive (*1*). By way of contrast, muscular contractions can be measured isometrically as tension, or isotonically as work performed.

The present experiments, designed to localize the site of application of the motive force for shuttle-streaming in the slime mold, are based on the well-known axiom of classical muscle physiology, that during contraction significant amounts of heat are invariably released (*2*), and on the assumption that the same principle should hold true for the mechanochemical processes responsible for cytoplasmic streaming in the slime mold.

Plasmodia of *Physarum polycephalum* were cultured on filter paper saturated with tap water and sprinkled with oatmeal (*3*), and then allowed to fast for a day before two blobs (about 5 mm in diameter) and a channel (about 6 mm long, 0.2 mm in diameter) were excised and allowed to fuse into a dumbbell-shaped mass in a modified Kamiya double chamber (*4*). The chambers were made of lucite (Fig. 1) and so designed that small bead thermistors (*5*) (0.1 mm in diameter) would lie in contact with the terminal blobs. The connecting channel was embedded in vaseline and a cover glass placed over the two compartments. The double chamber was then lowered into a thermally insulated (styrofoam) box fitted with double-layer plastic windows adequate for observing the direction of streaming with a dissecting microscope.

Leads from the thermistors were passed through the insulated box to opposite sides of a Wheatstone bridge circuit containing two additional resistances, one of which was a sensitive potentiometer for balancing the bridge (Fig. 2). An oscillator in the lock-in amplifier (*6*) provided a 400 cy/sec modulation for the bridge. Any thermal imbalance between the two thermistors resulted in a signal at the single-ended output across the bridge. This signal was amplified by a vacuum-tube voltmeter (*7*) before reaching the tuned amplifier of the lock-in. The use of a lock-in amplifier allowed two major improvements over a somewhat similar thermistor thermometer described by Stow (*8*) in that the phase-sensitive detector allowed the sign of the temperature differences to be registered, and the narrow-band detection afforded by this technique resulted in a significant reduction in the basal noise level (*9*). The deflection sensitivity was determined by direct calibration in solutions of known temperatures a few degrees apart with the instrument operated at very low gains. The basal noise level was determined by allowing wads of moist cotton to lie in contact with the thermistors until thermal equilibrium had been established. At maximum gain, the rms noise was equivalent to less than 2 × 10^{-5} deg Celsius when the filtering time constant of the lock-in amplifier was matched with that of the thermistors ($\tau \approx 0.5$ second).

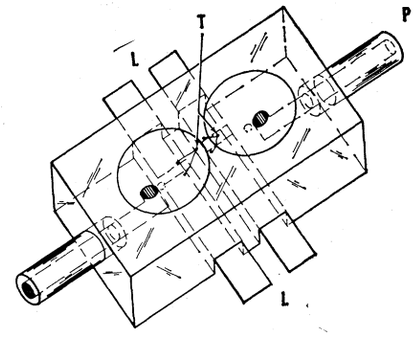

Fig. 1. A diagram of the modified Kamiya double chamber showing the position of the thermistors (*T*), pressure outlets (*P*) and terminals connected to the thermistor leads (*L*).

The measurements reported in the present study were comparable in sensitivity to the elegant thermopile-galvanometer measurements of heat production in muscle and nerve by A. V. Hill and co-workers (*10*). The present technique was chosen principally for its simplicity, convenience and economy. In addition, thermistors are available commercially in sizes considerably smaller than the plasmodial blobs.

A 30- to 50-minute interval of thermal isolation was required to ensure the onset of vigorous cytoplasmic streaming and to allow thermal perturbations in the double chamber to subside. To visualize streaming, a monochromatic light source was chosen to lie outside the normal absorption range of the plasmodial pigments (220 to 440 mμ) (*11*). The mercury green line (546 mμ) of a General Electric AH-4 lamp was filtered by 2 cm of water, an interference filter, and a variable-density polarizing filter adjusted to transmit barely enough light to render the direction of streaming visible.

The tendency of plasmodia to migrate caused some to lose contact with one or both thermistors, and therefore

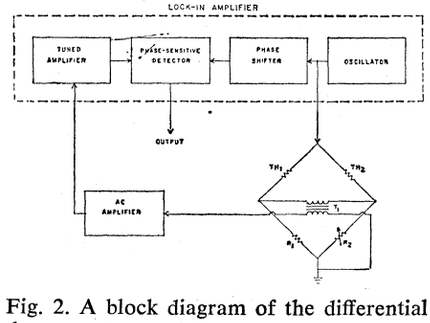

Fig. 2. A block diagram of the differential thermometer.

only about half of the preparations showed thermal cycles. In the remaining 20 successful experiments, the temperature differences recorded ranged from 1×10^{-4} and 5×10^{-2} deg. Celsius. The wide differences in magnitude probably reflected not only the movement of plasmodia out of contact with the thermistors, but a tendency of some plasmodia to secrete an insulating layer of slime.

Changes in the sign of the temperature difference were perfectly synchronized with changes in the direction of streaming. Invariably, the greater heat production was detected at the source of the streaming cytoplasm (Fig. 3). It was established that the thermal cycles were produced by the plasmodia and not by instrumental factors by the following facts. (i) In some experiments which were initiated in the dark, there were thermal cycles before the illumination was turned on; the light neither caused nor detectibly affected the thermal cycles. (ii) In control experiments with wads of moist cotton, there were no thermal cycles. (iii) The possibility that the thermal cycles were produced by the transport away from the thermistors of cytoplasm heated by the thermistors was eliminated by measuring the thermal cycles in the same plasmodium at a constant deflection sensitivity maintained by various different combinations of input voltage to the bridge and amplifier gain. No correspondence was found between the input voltage to the bridge and the amplitude of the thermal cycles found in the plasmodium.

A single blob of plasmodium measured against a wad of moist cotton showed a gradual tendency to become warmer relative to the cotton coupled with nonrhythmic bursts of heat production (Fig. 4). Two blobs of plasmodium not connected by a strand showed similar but smaller bursts of thermal activity without rhythmicity (Fig. 5). Similarly, dumbbell-shaped plasmodia which had shown thermal cycles lost their rhythmicity when the connecting channel was excised. One exception was an experiment in which there remained a passage of water in the vaseline seal between the two blobs. The observation of thermal cycles in this experiment led us to connect two plasmodial blobs with a wet cotton string, a procedure found by Kamiya and Abé (*12*) to permit the continuance of surface potential difference waves. Under these conditions, thermal cycles of the characteristic frequency were found (Fig. 6) although they were not so regular as those observed in dumbbell-shaped plasmodia (compare Figs. 3 and 6).

Opinions have differed as to whether shuttle-streaming in the slime mold should be considered to be a process

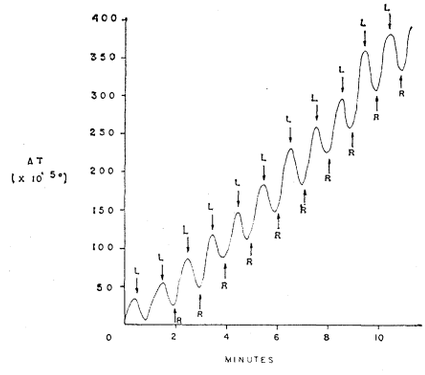

Fig. 3. A sample of a record of the temperature differences between thermistors in contact with the two plasmodial blobs (see Fig. 1).

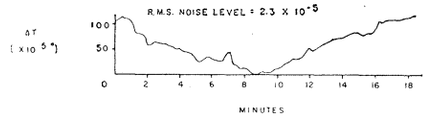

Fig. 4. A record of the temperature differences between a single plasmodial blob and a piece of moistened cotton of roughly equal size.

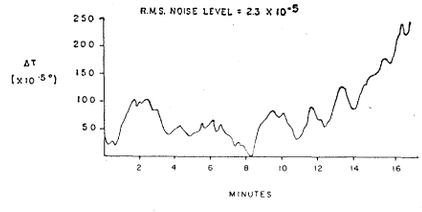

Fig. 5. A record of the temperature differences between two plasmodial blobs thermally and electrically insulated from one another.

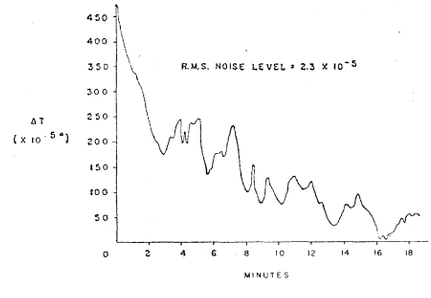

Fig. 6. A record of the temperature differences between two plasmodial blobs connected only by a wet cotton thread.

phenomenologically distinct from other examples of cytoplasmic streaming (such as ameboid movement, and cytoplasmic rotation), or to be one manifestation of a universal (but unknown) mechanism of cytoplasmic streaming. Seifriz, on observing time-lapse motion pictures of the "pulsations" in plasmodia (*13*), advanced the theory that streaming in slime molds was induced by pressure differences resulting from localized contractions. Later, he abandoned this theory on the basis that it was incompatible with the details of streaming phenomena in other organisms (*14*). Hilton (*15*) was probably the first to show that the direction of streaming could be shifted by mechanical pressure applied to the plasmodium from outside, and he concluded that streaming was caused by "expansions and contractions" of the plasmodial walls. More recently, Kamiya and Kuroda (*16*) showed that the truncated velocity profiles across a main channel were the same, whether the streaming was natural or induced by gas pressure; on this basis it was reasoned that pressure was the normal motive force, although Kamiya (*17, 18*) later admitted that (i) "suction" or another force acting from the front, or (ii) active shearing along thinner channel walls, could not be eliminated as an explanation. The latter (active shearing) was said possibly to offer a common mechanism for shuttle-streaming and rotational streaming. The truncated velocity profiles would also be compatible with a frontal contraction mechanism, such as that recently proposed for the ameba by Allen (*19*), particularly in view of some of the complexities of movement that have been pointed out by Kamiya (*17*) and by the Stewarts (*20, 21*). That is, it is conceivable that contractions at the front (destination) of cytoplasmic streams might exert tension on structural elements which have now been demonstrated to exist in the axial region of the endoplasmic stream of the slime mold (*16, 21*).

The present results appear to establish that if streaming can be attributed to a single motive force mechanism, that force must be applied where the greater part of the heat synchronized with the streaming cycle is produced; that is, at the origin of the stream. If more than one motive force is in operation, they must either operate in the same general region of the plasmodium, or the second force not applied at the source must be much less im-

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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25. Supported by research grant RG–8691 from the National Institutes of Health. We thank Noburo Kamiya and H. Nakajima for their helpful suggestions, and William Maddux for his participation in the early stages of this work.

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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-