RHYTHMICITY IN THE PROTOPLASMIC STREAMING OF A SLIME MOLD, PHYSARUM POLYCEPHALUM*

II. THEORETICAL TREATMENT OF THE ELECTRIC POTENTIAL RHYTHM

BY UICHIRO KISHIMOTO

(From the Department of Biology, Osaka University, Osaka, Japan)

(Received for publication, July 15, 1957)

ABSTRACT

The electric potential difference (1 to 15 mv.) between two loci of the slime mold connected with a strand of protoplasm changes rhythmically with the same period (60 to 180 seconds) as that of back and forth protoplasmic streaming along the strand. When atmospheric pressure at a part of the plasmodium is increased (about 10 cm. H₂O), the electric potential at this part becomes positive (0 to 20 mv.) to another part with a time constant of 2 to 15 minutes. If the atmospheric pressure at a part of the plasmodium is changed (about 10 cm. H₂O) periodically, the electric potential rhythm also changes with the same period as that of the applied pressure change, and the amplitude of the former grows to a new level (i.e., forced oscillation). The electric potential rhythm, in this case, is generally delayed about 90° in phase angle from the external pressure change, and the amplitude of the former grows to a new level (i.e., forced oscillation). The electric potential rhythm which coincided with that of the pressure change is maintained for a while after stopping the application of the pressure change, if the period is not much different from the native flow rhythm. Such a pressure effect is brought about by the forced transport of protoplasm and is reversible as a rule. In the statistical analysis made by Kishimoto (1958) and in the rheological treatment made in the report, the rhythmic deformation of the contractile protein networks is supposed to be the cause of the protoplasmic flow along the strand and of the electric potential rhythm. The role of such submicroscopic networks in the protoplasm in various kinds of protoplasmic movement is emphasized.

The cells, in which we can study the protoplasmic movement, all show sol-like inner protoplasm (plasmasol) and a gel-like outer protoplasmic layer (plasmagel). Microscopic granules are in lively movement (i.e., Brownian movement or flow) in the endoplasm where the viscosity is considered to be comparatively low, while in the outer protoplasmic layer they are motionless. According to Goldacre (1952) and Goldacre and Lorch (1950), the protein molecules are in a folded state in the endoplasm, and in an unfolded state in the ectoplasm. However, a “folding and unfolding” mechanism alone is per-

* Supported by a Grant for Fundamental Scientific Research from the Ministry of Education.
haps not enough to bring about an organized protoplasmic movement. Each contractile protein in the protoplasm may perhaps maintain an organized framework which would assist some aspects of protoplasmic flow (Seifriz, 1942, 1943, 1953; Frey-Wyssling, 1949, 1953, 1955). Therefore, we can assume that there are "couplings" among contractile proteins, though perhaps not so strong as those in muscle cells. Accordingly, protoplasmic movement such as streaming is an object of rheology.

A B
Pressure Control Systems

\[ \text{Pressure Control Systems} \]

\[ \text{Direct Current Amplifier} \]

\[ \text{Automatic Recorder} \]

FIG. 1. A vessel for measuring the electric potential rhythms of a slime mold, \textit{Physarum polycephalum}, is shown with pressure control systems and with a recording system.

In the preceding paper the author showed that there is a close correlation between the electric potential rhythm and the protoplasmic streaming of a slime mold, \textit{Physarum polycephalum} (Kishimoto, 1958). In this present paper, some facts about the electric potential rhythm of the slime mold and theoretical treatment of them will be reported.

\textit{Material and Apparatus}

The plasmodium of \textit{Physarum polycephalum} was used throughout the experiment. The method of recording the electric potential rhythm is the same as described in the previous report (Kishimoto, 1958). In order to control the rate of the protoplasmic flow along the connecting strand the atmospheric pressure at \(B\) compartment was changed artificially. This was carried out by pressing a rubber aspirator by hand or by moving a syringe coupled to a phonomotor through a reduction gear as shown in Fig. 1.
RESULTS

We define the sign of the potential difference in such a way that the curve comes downwards when the potential at B is higher than that at A and comes upwards in the reverse case. The direction of the protoplasmic streaming is shown with upward arrows at the time when its direction changes from $A \rightarrow B$ to $B \rightarrow A$ and with downward arrows at the time when it changes from $B \rightarrow A$ to $A \rightarrow B$.

Pressure Effect.—When the atmospheric pressure is increased at a part of a plasmodium, the electric potential at this part becomes, with no exception, positive to other parts. In other words, if the atmospheric pressure at B is increased in a single step about 10 cm. H$_2$O against that of A with a pressure control device (Fig. 1), the electric potential at B becomes positive, i.e. 0 to 20 mv., to A (Fig. 2). A similar result can be obtained even if we study the effect by filling each compartment with 0.001 M KCl solution (Fig. 2 b). However, if the connecting strand is very thin or if the protoplasmic streaming through the strand is very weak or suspended abnormally, the pressure effect is not remarkable, although the electric potential rhythm can be observed as usual. Therefore, we can suppose that the effect is brought about by the forced protoplasmic flow through the connecting strand due to the uneven application of external pressure. This phenomenon is generally reversible.

Although the back and forth protoplasmic streaming is continuous during this process, a certain net amount of the protoplasm is certainly transported to A judging by the fact that the protoplasmic flow from B to A is much stronger and is of much longer duration than that in the opposite direction at the early stage of this process, although at the steady state these are nearly equal. As shown in Fig. 2, the neutral line of the potential rhythm goes upwards or downwards with a time constant of 2 to 15 minutes. The potential rhythm, however, keeps on going as before. Therefore we can assume that the slow potential drift is a measure of a slow unidirectional flow of protoplasm that occurs with the same time course. The following equation will be adopted for the movement of the protoplasm along the strand

$$J \ddot{x} + B \dot{x} + C x = F$$

$$F = 0 \quad \text{for} \quad t = 0$$

$$= k \Delta P \quad \text{for} \quad t > 0$$

$x$ is the displacement of the protoplasm along the strand, $\dot{x}$ is its velocity, and $\ddot{x}$ is its acceleration. $J$, $B$, and $C$ are assumed as constants which are related to the physical properties of the protoplasm. In the strict sense, however, these are not constants, but will change more or less with time, because the plasmodium changes its form to some extent during experiment, which is also true for the connecting strands. Therefore, we must be content here with studying the qualitative aspects of flowing through the strand. The first term, $J \ddot{x}$, of the
Fig. 2. Examples of "pressure effect." If external pressure at compartment $B$ is increased (decreased) about 10 cm. $H_2O$ against $A$ (Fig. 1), the electric potential at $B$ becomes positive (negative) to $A$. This process is reversible. In (a) the plasmodium is attached to open air (at 27° C.), while in (b) it is immersed in 0.001 M KCl solution ($pH = 6.0$, at 24.5° C.).
left side of this equation corresponds to the force of inertia of the flowing protoplasm, \( B \xi \) to the frictional force, and \( C \xi \) to the elastic force. \( F \) stands for the force due to the imposed pressure difference which forces the protoplasm to flow in a direction, \( B \rightarrow A \). The solutions of equation (1) are as follows:

\[
\dot{\xi} = K \cdot \Delta P e^{-\left(\frac{B}{2J}\right)t} \cdot \sin \left( \sqrt{\frac{C}{J}} - \left(\frac{B}{2J}\right)^2 \cdot t \right)
\]

\[
\xi = \frac{K \cdot \Delta P}{(C/J)} \left\{ \sin \varphi - e^{-(B/2J)t} \cdot \sin \left( \sqrt{\frac{C}{J}} - \left(\frac{B}{2J}\right)^2 \cdot t + \varphi \right) \right\}
\]

\[
\tan \varphi = \sqrt{\frac{C}{J} - \left(\frac{B}{2J}\right)^2} / \left(\frac{B}{2J}\right)
\]

Fig. 3. Displacement (\( \xi \)) and velocity (\( \dot{\xi} \)) of the flowing protoplasm are plotted in arbitrary units as ordinate against time as abscissa using equations (2) and (3).

\( K \) is a constant and \( \varphi \) is the phase lag of the displacement of the protoplasm with respect to the applied pressure change.

These relationships are shown graphically in Fig. 3. If the atmospheric pressure at \( B \) is increased, i.e., \( \Delta P \), a certain amount of protoplasm is forced to flow towards \( A \). This forced transport of the protoplasm, however, never does continue so far that the whole of the protoplasm at \( B \) disappears, but it stops after a certain amount of the protoplasm has been transported. The rhythmic protoplasmic streaming can be observed again at this steady state.

At the steady state the inner pressure at \( A \), (i.e., \( p_A \)), will be equal to

\[
p_A = p_o + T_A
\]

while the inner pressure at \( B \), (i.e., \( p_B \)), is equal to

\[
p_B = p_o + \Delta P + T_B
\]
Fig. 4. Examples of "periodic pressure effect." (a) the period of the applied external pressure is different from that of native rhythm.
\[ \Delta P = \rho \sin\left(\frac{2\pi}{\lambda}\right)t \]
\[ \rho = 10.5 \text{ cm. H}_2\text{O} \]

Time Marks, 5 min.

Time Marks, 1 min.

age is a little larger than (at 21.0°C.), (b) just the same as (at 24.5°C.), (c) much shorter than (at...
in which \( p_0 \) is the original atmospheric pressure and \( T_A \) and \( T_B \) are the pressures due to the tension of the outer protoplasmic layer at \( A \) and at \( B \) respectively. At the steady state these two should be equal.

\[
p_0 + T_A = p_0 + \Delta P + T_B
\]

or

\[
T_A = T_B + \Delta P
\]

Therefore, the tension of the outer gel layer of the plasmodium at \( A \) will be higher than that of \( B \) at the steady state. Or it can also be supposed that the protoplasm at \( A \) becomes more densely packed on the average than it is at \( B \). Anyway, the electric potential difference at the part where the atmospheric pressure is higher is always positive to the other part. This result may be explained by saying that the electric potential is decreased at \( A \) where the protoplasm is more densely packed than at \( B \).

What will happen if the atmospheric pressure at \( B \) is changed periodically? Three representative examples are shown in Fig. 4: (a) the period of the applied external pressure is a little longer than, (b) just the same as, and (c) much shorter than the period of the native electric potential rhythm. The amount of applied external pressure (i.e. amplitude) is about 10 cm. H_2O in (a) and (c). In (b) the balancing pressure which is necessary to stop the flow (generally less than 20 cm. H_2O) is applied.

The electric potential shows a response which is a forced oscillation. The protoplasmic streaming along the strand is forced to flow at the same pace as the external pressure change and becomes more vigorous than before ((a) and (c)). One thing which should be noted here is that the pattern of the electric potential change can be modified by the uneven application of the external pressure. However, the pressure effect, whether the pressure is applied in a single step or periodically, is supposed to correspond, in general, to a change in the static membrane potential (i.e. \( \epsilon_A \) and \( \epsilon_B \) in equations (1) and (2) of the preceding report (Kishimoto, 1958)) and is not supposed to be a result of alterations in the rhythmic mechanism of the protoplasmic streaming. Periodic change in density from compression by protoplasm at \( A \) or at \( B \) is supposed to be the cause of such electric potential changes. Again, the electric potential is always negative where the protoplasm is thought to be more densely packed owing to compression by transported protoplasm.

Another notable result is that the electric potential change goes, generally, behind the applied pressure change about 90° in the phase angle. Such a phenomenon could be observed in every sample, notwithstanding the former phase relation which is not always retardation, but more probably a phase advance, as will be discussed later. Therefore, Kamiya and Abe's conclusion (1950) that the electric potential change occurs about 90° later than the flow
rhythm may not be true on undisturbed samples, as their experiment was done by applying a balancing pressure.

Plotting the period and the amplitudes in the process of the periodic pressure effect against successive waves in numerical order, we can get Fig. 5. The period of the electric potential rhythm is defined as the time interval between adjacent troughs and the amplitude as the vertical distance from the top of a peak to the line connecting adjacent troughs. The amplitude is increased during application of the pressure as the result of forced oscillation. It is worth while to notice that the period, having become equal to that of the applied pressure change at B, is maintained for a while after stopping rhythmic pressure applications. Perhaps, the forced transport of the protoplasm has had some effect on the rhythm of contraction and expansion of the contractile protein networks in this case. In other words, the rhythm of the latter might have been entrained by that of the former. Such a phenomenon is more evident, if the period of the applied pressure change does not differ too much from that of the native rhythm.

Phase Difference between the Electric Potential Rhythm and the Protoplasmic Streaming.—As for the existence of a close correlation between protoplasmic streaming and electric potential change, Watanabe, Kodachi, and Kinoshita (1937) reported in some detail on a slime mold, Didymium nigripes. Using the microelectrode technique, they found that the electric potential at the posterior end (tail) of the plasmodium was negative to the anterior end (head). From the fact that granules in the protoplasm are generally negatively charged, they claimed that the protoplasm was forced to flow towards its head, where the electric potential is positive with respect to its tail (i.e., an electrophoresis theory of protoplasmic streaming). According to them, therefore, the electric potential rhythm creates the rhythmic streaming.

On the other hand, Kamiya (1942, 1950, 1953) devised a double chamber method, by which he measured the balancing pressure necessary to stop the flow and concluded that he could measure the motive force of the protoplasmic flow with it. Measuring the electric potential change simultaneously with the motive force of flow (i.e., by applying balancing pressure), he claimed that the electric potential change lags behind the rhythmic flow. Because of this and because the externally applied electric potential (0.01 to 4.0 volts) of a shorter duration did not give any noticeable effect on the wave pattern of the motive force of flow, he denied the electrophoresis theory of protoplasmic flow. However, as mentioned in the preceding paragraph the applied balancing pressure retards the electric potential rhythm about 90° in phase angle from the former (Fig. 4 b), and his evidence is therefore anything but convincing, at least, for the phase relation.

Ordinary phase relations between electric potential rhythm and flow rhythm are shown in Fig. 6. The most frequent is the case in which the flow changes its
FIG. 5. Changes in period and in amplitude in Figs. 4 a and 4 c are plotted against successive waves. Amplitude becomes greater during the application of periodic external pressure change. Protoplasmic flow in this process evidently becomes stronger than before (i.e., forced oscillation). Period coincides with that of the applied external pressure change. If the period of external pressure change is not so much different from that of native rhythm as in Fig. 4 a, the modified period remains for a while even after removing the pressure change.
direction from \(A \rightarrow B\) to \(B \rightarrow A\) at each peak (Fig. 6 a). In some cases, however, other phase relations were also observed (Fig. 6 b and 6 c). Such a phase relation is not always constant during the time course of the rhythmic protoplasmic streaming, but often changes temporarily.

The velocity distribution of the protoplasmic flow along the strand was observed to take the form of non-Newtonian passive flow (Kamiya, 1950). Accordingly, we can assume that the motive force of the protoplasmic flow along the strand locates mainly at each plasmodium and not at the connecting strand. The strand, however, cannot be regarded as a mere resistance to the protoplasmic flow, but it should be regarded as an impedance element, because the flow takes place through it, changing its direction alternately. As protoplasm is well known to have a viscoelastic property, the following equation for the protoplasmic movement through the strand will be applied

\[
\dddot{\xi} + \dddot{\xi} + \dot{\xi} = M \sin \omega_0 t
\]

\(\dddot{\xi}, \dddot{\xi}, \text{and } \ddot{\xi}\) are displacement, velocity, and acceleration of the protoplasm in the strand. Three terms on the left side of this equation have meanings similar to those of equation (1). \(M \sin \omega_0 t\) is the motive force of the protoplasmic flow which is assumed to be located at each plasmodium blob. The solution of this equation at steady state is as follows:

\[
\ddot{\xi} = \frac{M \sin \omega_0 t}{B + j \left( J \omega_0 - \frac{C}{\omega_0} \right)}
\]

\(j\) stands for the square root of \(-1\). Equation (8) shows that there is a certain amount of phase difference between the velocity of flowing protoplasm and the rhythmic change in motive force of protoplasmic flow.

The analysis made on the pressure effect has shown that the average time course of the response can be expressed by \(e^{-(\omega/J)t}\) and its damping overshoot is expressed by \(\sin \sqrt{(C/J) - (B/2J)^2} \cdot t\) (equations (2) and (3)). Adopting 120 seconds for the period of the protoplasmic flow, \(J \omega_0 - C/\omega_0\) is generally a positive quantity and much greater than \(B\). Then, according to equation (8), the change in velocity of the protoplasmic flow along the connecting strand should be delayed about 90° in phase angle from the changes of the motive force which is located mainly at each plasmodium blob.

\(1\) As the time constant of the electric potential drift due to the pressure effect is generally about 10 minutes (Fig. 2 a), \((B/2J) = (1/60 \cdot 10) = 1/600\). On the other hand, as the period of damping overshoot is generally greater than 30 minutes (Fig. 2 a), \((C/J) \leq (B/2J)^3 + (2\pi/60 \cdot 30)^3 = (1/600)^3 + (2\pi/1800)^3 \approx 5/(600)^3\). Then, \(J \omega_0 - C/\omega_0 = (J/\omega_0)(\omega_0^2 - (C/J)) = (J/\omega_0)((2\pi/120)^3 - 5/(600)^3) \approx (J/\omega_0)(2\pi/120)^3 = J \omega_0 > 0\). As \(B/2J = 1/600, B/J \omega_0 = (2/600)(120/2\pi) = 3\pi/5 < 1\). Therefore, we can suppose: \(J \omega_0 - C/\omega_0 \gg B > 0\).
Fig. 6. Phase relations between electric potential rhythm and flow rhythm. Generally, flow changes its direction from $A \rightarrow B$ to $B \rightarrow A$ at the peak of the electric potential rhythm (a). In some cases, however, such a phase relation does not hold, but changes in more or less labile manner ((b) and (c)). At 25.5°C.
From the viewpoint that the protoplasmic flow is brought about by the contraction of an organized framework of contractile proteins in the protoplasm, the motive force is the result of such a mechanochemical process. In accompaniment with such a mechanochemical process, ions will be released or rebound, which will give rise to changes in membrane potential almost at the same time. Then, it may be quite reasonable to suppose that the electric potential change precedes the protoplasmic flow along the connecting strand. Therefore, if we assume that the electric potential decreases when the protoplasmic networks are in a contracted state, the flow along the connecting strand occurs towards the electrically positive side about 90° later than the electric potential change, as shown in Fig. 6 a. Similar observations were made on another slime mold, Didymium nigripes, by Watanabe, Kodachi, and Kinoshita (1937).

As stated earlier, variations in phase relation between the electric potential rhythm and the flow rhythm are often observed (Fig. 6 b and 6 c). This fact makes it difficult to suppose that the protoplasm is forced to flow towards A simply because the plasmagel at B contracts like a pressed rubber ball. The motive force of the protoplasmic flow along the connecting strand should be understood in the following way. Protoplasmic streamings along many ramifying canals in the plasmodium blob pour into the connecting strand, resulting in a vigorous flow through it. Such a summated force of flowing at the end of the strand is supposed to correspond to the motive force of the protoplasmic flow along the strand. As the electric potential change is a vector sum of all elementary rhythms in the plasmodium (Kishimoto, 1958), while the protoplasmic flow along the connecting strand does not always depend on all the elementary rhythms, such variations in phase angle as shown in Figs. 6 b and 6 c are also expected.

Building Up a Rhythm of a Definite Periodicity.—At the steady state, when the protoplasmic streaming along the strand is vigorous, the electric potential rhythm has a dominant period (Kishimoto, 1958). At the early stage, however, after placing a plasmodium in the vessel, the potential rhythm is weak and shows many irregularities (Fig. 7). At this stage the protoplasmic flow along the strand has not yet begun and the protoplasmic streamings along the canals in the plasmodium blob are in a state of disorder. Protoplasmic connections between the two plasmodia and the connecting strand are not yet numerous enough. As the rhythmic flow begins to take place along the strand the electric potential rhythm also grows gradually in amplitude and regularity in period is increased (Fig. 7).

In the beginning elementary rhythms of flowing in different parts of a plasmodium are supposed to occur with their own period, amplitude, and phase. If, however, some of the periods happen to coincide with the characteristic period $T_c$ of the flow rhythm of the whole plasmodium, they will grow greater and greater. Neighboring elementary rhythm will be forced to change by this
strong oscillation. With such a process an organized protoplasmic flow of nearly constant periodicity is supposed to be built up.

Forced oscillation of this type can be expressed with equation (9)

\[ J_1 \dot{\xi}_i + B_1 \xi_i + C_1 \dot{\xi}_i = M_i \sin \omega_i t \quad (9) \]

\( \xi_i \) is the displacement of protoplasm due to the elementary flow rhythm at a canal, not at a connecting strand in this case, of the plasmodium, \( \dot{\xi}_i \) is its velocity, and \( \ddot{\xi}_i \) is its acceleration. Such an elementary rhythm is forced to change by the strong force of the flow rhythm, the period of which is equal to the characteristic period (\( T_0 \)). \( \omega_0 \) is the characteristic angular velocity (\( \omega_0 = 2\pi/T_0 \)). \( J_1, B_1, \) and \( C_1 \) have meanings similar to those of \( J, B, \) and \( C \) of equation (7), but are generally different from the latter in magnitude, as in this case the protoplasm in a canal of the plasmodium blob, not in the connecting strand, is the object of study. Actually, the protoplasmic flow through a canal in the plasmodium is comparatively slow and is surrounded by a thick gel layer, while it is remarkably vigorous in the connecting strand and is surrounded by a comparatively thin gel layer. The solution of equation (9) is given as follows:

\[ \xi_i = A_i e^{-\lambda t} \sin (\omega_i t + \alpha_i) + B_i \sin (\omega_i t - \epsilon_i) \quad (10) \]

\[ \tan \epsilon_i = \omega_i (B_i / J_i) / \left| (C_i / J_i) - \omega_i^2 \right| \]

\[ \omega_i = \sqrt{(C_i / J_i) - (B_i / J_i)^2} \]

An elementary flow rhythm will soon die out with the time constant \( \lambda \), if its period is different from \( T_0 \). Each elementary flow rhythm will be forced to
move with the same period (i.e., $T_0$) after a time. It goes, generally, behind the compulsory force (i.e., phase retardation, $\phi$). A similar mechanism is supposed to occur also in the submicroscopic rhythms of deformation of contractile protein networks, the frequency of which may be much greater than that of the actual flow rhythm as will be discussed later. In this way the rhythm of a definite periodicity is supposed to grow up to a certain level. Accordingly, it is possible to suppose that elementary flow rhythms, if not the same in amplitude or phase among themselves, are distributed throughout the plasmodium and contribute, as a whole, to an organized streaming system. If we assume that the left side of equation (9) corresponds to the movement of protoplasm along the connecting strand and the right side to the force given to the plasmodium at $B$ by the periodic external pressure change, the experiments shown in Fig. 4 (i.e., periodic pressure effect) may be explained in the same way as stated above.

**DISCUSSION**

It has been shown above that several facts about the protoplasmic streaming of *Physarum polycephalum* can be explained with a linear theory. The linear theory, however, fails to answer the question of the stability of the rhythm in the strict sense. The way in which non-linearity is introduced into the equation will be the problem to be studied in the future. As this is generally very difficult, it might be better, at present, to show a qualitative explanation. A trial, however, was made by Minorsky (1948) in the study on the stability of mechanical oscillation, introducing an equation which involves non-linear terms due to a feedback mechanism. He obtained a solution of the equation in a form, $\xi = a_0 \sin (\omega_0 t + \varphi_0)$, at the steady state where average balance per cycle between the energies absorbed and the energy dissipated is reached. The amplitude, $a_0$, the angular velocity, $\omega_0$, and the phase angle, $\varphi_0$, at the steady state, however, are not constant as they are in the linear theory, but these quantities fluctuate around constant values. Such a treatment is very important because it seems to explain why the period of the electric potential rhythm shows a Gaussian distribution, and why the amplitude and the phase angle are not constant (Kishimoto, 1958).

Recently, the author found a close correlation between the protoplasmic streaming along the strand and the electric impedance changes of each plasmodium blob (Kishimoto and Fukui, 1954). Although the electric potential rhythm was not measured, simultaneously, it is certain that the impedance and the potential difference change almost at the same time judging from their phase relations to the rhythm of protoplasmic streaming. Such an impedance change or electric potential change is supposed to be the result of mechano-chemical processes in the plasmodium. From this and from the analysis made in this report, we can suppose, with good reason, that the electric potential
change and the protoplasmic streaming are two different results of the same mechanism; i.e., the rhythmic deformation of contractile protein networks in the plasmodium. Although the electric potential change is thought to occur without retardation, mechanical changes such as flow along the strand will be delayed to some extent.

With regard to the existence of the networks of contractile proteins in the plasmodium, there is no decisive experiment at present to identify it. However, actomyosin-like contractile proteins were found by several authors (Loewy, 1949, 1950, 1952; Ts'o et al., 1956 a, 1956 b, 1957; Nakajima, 1956). The metabolic pattern of Physarum is very similar to that of muscle cells (Ohta, 1954). Electron microscope studies on the protoplasm of Physarum show the existence of network-like structure (Sponsler and Bath, 1953; Kishimoto and Terada, 1956). However, such submicroscopic networks of the contractile proteins may be labile compared with those of muscle cells. But they may be strong enough to maintain a structural continuity, contributing to the establishment of a coordinated streaming such as flow.

Within the outer rim of the plasmodium, we can see that there is a sheet of protoplasm and this sheet as it approaches the center part of the mass becomes a reticulum consisting of many ramifying canals. Protoplastic streaming through each of these canals seems to occur at the same pace, at least when the plasmodium is not so large. According to Seifriz' time lapse motion picture, the plasmodium of Physarum pulsates like a beating heart. Whether the pulsation is the cause of or the result of the protoplasmic flow, there should be rather strong coupling among parts of the protoplasm in order to maintain such an organized movement. The waves of pulsation propagate in one direction and then back through the plasmodium. One part of the force of flow summed up in this way is given to the strand connecting two plasmodia, resulting in a strong forced streaming of protoplasm through it.

Ameboid movement may be brought about by a similar mechanism. Another type of protoplasmic movement such as cyclosis of Nitella or Elodea may also be under the control of the rhythmic contraction of contractile protein networks in the flowing protoplasm, although in this case the protoplasmic flow is a one way traffic along the cell wall. One possible explanation of the mechanism of such a coordinated movement of protoplasm is to assume that there is a suitable phase lag for each successive rhythmic contraction in the direction of flow. For such a situation Seifriz proposed the existence of many rhythms of different periodicities (1942). However, this is not always necessary, and seems to be less probable, if we consider the rather strong couplings among contractile proteins in the plasmodium (Kishimoto, 1958). An assumption of suitable phase relations for each rhythmic contraction along the canal in the direction of flow is enough to explain the establishment of the coordinated flow. Some of the metabolic energy is consumed in maintaining the mechanism of contraction and expansion of contractile protein networks.
With regard to the location of such a coordinated mechanism of contraction, many authors suppose it to be at the gel layer. If so, it is very difficult to explain another type of protoplasmic flow such as cyclosis. Actually in the *Nitella* cell, protoplasm flows along the cell wall. The thickness of the plasmagel layer of this cell, if it really exists, is less than 0.5 μ. The tonoplast is also very thin and cannot be supposed to be highly elastic. In order to compel the protoplasm which is about 10 μ thick to flow, these two membrane should deform remarkably. However, this cannot be observed. Therefore, we must discard this trial to look for the seat of motive force of protoplasmic flow in the plasmagel layer, at least in the case of cyclosis of *Nitella* cell. Even in slime mold and in ameba, there is evidence against the plasmagel contraction theory for the protoplasmic flow. Contraction should shorten and therefore smooth out the surface of plasmagel at the posterior end as it is emptied of protoplasm. However, photographs of slime mold (Seifriz, 1942) and of ameba (Abe, 1957) showed that the periphery of the plasmagel at the posterior end is wrinkled, whereas the periphery of the expanded protoplasmic mass at the anterior region is smooth. Wrinkling of the periphery with a decrease in volume due to loss of protoplasm indicates that there has been no change in the total surface area and therefore, no essential role for contraction of plasmagel in the protoplasmic flow. The so called sol endoplasm is rather viscous and elastic compared with a liquid and shows a structural continuity (Seifriz, 1942). Therefore, it is more reasonable to suppose that the plasmagel and plasmasol both have the ability to contract and that the plasmasol is flowing as a thixotropic sol, while the plasmagel remains as a gel because of its denser packing. Microscopic observations on the plasmodium generally show that the thickness of the outer gel layer in the strand changes, more or less, with time and that the inner region has an appearance of being melted away. These two states of protoplasm are easily interchangeable and there seems to be no structural difference except a difference between a denser packing or a looser packing.

Many questions, however, remain to be answered. Namely, how is the elementary flow along the canal brought about by the rhythmic deformation of contractile protein networks in the protoplasm? Why is the protoplasmic flow of the slime mold of the back and forth type, while it is cyclosis (i.e., one-way traffic type) in other plant cells such as the *Nitella* or *Elodea* cell? What is the factor which determines the period of the back and forth protoplasmic flow?

In order to study the protoplasmic movements on unified ground, the author made the following speculation. Ts'o *et al.* (1957) reported that the myxomyosin, i.e. contractile protein of *Physarum polycephalum*, is an ellipsoid (70 × 4500 A). According to Kishimoto and Terada's electron microscopic picture (1956) of the plasmodium, a large number of globular proteins (500 to 700 A in diameter) form a three dimensional network-like structure or a somewhat regular aggregation. This difference may be due to difference in
the method of preparation, i.e., purified protein was used in the experiments of Ts'o et al., while Kishimoto and Terada used crude protein solution. In any case, if we assume that these proteins displace about 300 Å in each oscillation (i.e., amplitude of submicroscopic oscillation), and the velocity of the displacement is 50 to 100 μ per second (about same as that of protoplasmic flow along the canal), and that the oscillation is a simple harmonic curve (i.e., \( \xi = a_r \cdot \sin 2\pi \left( \frac{t}{T} - \frac{x}{\lambda} \right) \), \( x \) is distance along the direction of flowing, \( \lambda \) is wave length), the frequency of such an oscillation can be calculated as 260 to 530 cycles per second. There is no decisive evidence, at present, to identify the existence of such a rhythm. However, the impedance measurement made by Iwamura (1952) on this organism with varying A.C. frequency showed that there was a reactance maximum at about 400 cycles per second, which seems to show the existence of some process that absorbs energy maximally per cycle at such a frequency. This process may perhaps correspond to the submicroscopic deformation of contractile protein networks.

As the velocity of propagation of such a wave (i.e., phase velocity, and not the actual velocity of displacement of the contractile protein networks) may be very great, a “standing wave” will appear along the canal. Therefore, node and loop (i.e., regions of contraction and of expansion of submicroscopic networks) come successively along the canal. If both ends of the canal are fixed ends for the wave, node and loop will not move along the canal. Then, no actual net flow will occur. However, when one end, \( A \), is a fixed end, while another end, \( B \), is a free end, node and loop will move towards \( B \). In this case, energy flow is greater in a direction \((A \rightarrow B)\) than in the reverse direction; that is, protoplasmic flow towards \( B \) will be realized. Such a flow of protoplasm along a canal may bring about an accumulation of protoplasm at the anterior end of the canal, where the protoplasm will be compressed by the flowing protoplasm. If the protoplasm is packed closely at the anterior end in this way, this end will change into a fixed end for the wave. On the other hand, a reverse change (i.e., from fixed end into free end) is supposed to occur at the posterior end, where the degree of compression might have been decreased considerably due to the protoplasmic flow towards the anterior end. Then, reversal of flow direction will occur. If the phase reversals at each end of the flowing bring about an actual back and forth protoplasmic flow in this way, each time interval at which the end of flowing is changed from free to fixed end for the wave will determine the period of the flow rhythm along the canal. Then, the period of protoplasmic flow should depend closely on the physical or mechanical properties of the flowing protoplasm itself and of the plasmagel.

If it so happens that one end, \( A \), of the plasmodium does not change easily
into a fixed end because of some change in its physical properties, the time for net flowing towards A will be longer than the reverse flowing. In such a situation the plasmodium moves towards A as a whole; i.e., taxis. On the other hand, if one end, A, is kept as a fixed end (i.e., gelated state due to application of some agents) for a long time, the plasmodium will show a tendency to escape from A. In the case of cyclosis (i.e., the Nitella or Elodea cell) where no such phase reversals occur in any part of the protoplasm, the protoplasm continues to flow in only one direction. In order to explain a unidirectional flow strictly, it is very necessary to suppose the non-linearity of the submicroscopic oscillation (i.e., greater displacement in one direction and smaller displacement in the reverse direction, or quick contraction and slow expansion). Such a non-linearity comes not only from the difference in boundary conditions between the posterior end and the anterior end, but also from the fact that protoplasm moves in one direction as the result of submicroscopic oscillation. Anyway, if we can suppose that protoplasmic flow is necessary not only for the locomotion of the organism, but also for its growth and development, the existence of submicroscopic oscillation (about 400 cycles per second in frequency) may be very important for the probability of enzymatic reactions (i.e., metabolism, protein synthesis, etc.). There is, generally, a very close parallelism between the activity of growth or development and of protoplasmic flow.

The velocity of protoplasmic flow is 50 to 100 μ per second along ramifying canals, while it is as great as 1000 μ per second along the connecting strand. The period, however, is about 100 seconds and it does not vary too much among many flow rhythms. Then, the amplitude of flow rhythm is calculated to be 0.7 to 1.4 mm. along the canal and 1.4 cm. along the strand. Actually, protoplasm does not always move from one end to the other end of a canal or strand in a half-period, but reverses its direction at the half way. Such a situation can be understood easily, if we observe the protoplasmic flow along a long strand which can reach 15 cm. or more in its development. The velocity and the period of protoplasmic flow along such a long strand do not differ so much from those of the usual connecting strand. Therefore, it is impossible to suppose that the protoplasm can travel such a long distance; i.e., 15 cm. along the strand in a half-period. Generally, the protoplasm can move, as a structural unit, only a certain distance along the canal or along the strand in a half-period. This may correspond to the wave length or to the distance between two nodes of submicroscopic oscillation. Pulsation of the plasmodium in a time lapse motion picture may perhaps correspond to the rhythmic displacement of node and/or loop of the submicroscopic oscillation. The period of the former is equal to that of the actual rhythm of protoplasmic flow.

As assumed earlier the electric potential rhythm is supposed to be the
result of the deformation of submicroscopic networks of contractile protein. On the average this will be lower when protoplasm is in a state of contraction and higher at the time of expansion. Therefore, it will be negative at a “tail” and positive at a “head” of flowing. If the protoplasm is compressed by flowing at the head, this part will be changed to a state of condensation or new tail and the electric potential will become negative to the former tail. The electric potential change, however, is not localized at head or at tail, but should be observed at any place along the strand as would be expected from the mechanism of protoplasmic flow discussed above. Actually, Tauc (1954) observed that the electric potential of any one part of the isolated strand placed on agar changed periodically against agar. Such a rhythm of electric potential occurs in every subdivided canal or strand, contributing to the resultant electric potential rhythm as shown in the previous report (Kishimoto, 1958).

As stated above, there should be couplings among each contractile protein in the protoplasm in order to maintain an organized protoplasmic movement such as flow. Fig. 8 a and 8 b are the schemes of two representative types of protoplasmic flow: the one is for ameboid movement where the flowing protoplasm is surrounded by a thick layer of plasmagel (e.g., slime mold or ameba) and the other is for cyclosis where the flowing protoplasm is in touch with the thin plasmagel layer only at one side (e.g., the Nitella or Elodea cell). Networks in these figures should be understood as three dimensional ones. The actual structural organization of protoplasm may be more complex. However, as any organized protoplasmic movement can never be realized without regularity, exaggerations of this were made in these figures. Many protoplasmic inclusions (i.e., nuclei, mitochondria, microsomes, etc.) have been omitted to avoid complexity. At the layer of plasmagel the contractile proteins (expressed with black circles) are in denser packing and couplings among them are comparatively strong as expressed with the full lines. On the other hand, such intermolecular couplings are loose in the flowing plasmasol and these are expressed with the dotted lines. At the boundary region (i.e., transition zone), the sol ⇄ gel transition (thixotropy) is supposed to take place easily due to the shearing force between the plasmagel and the flowing plasmasol. If the strength of couplings among such contractile proteins changes periodically, intermolecular distances also will be changed periodically, resulting in a submicroscopic rhythm of contraction or expansion of the contractile protein networks. If such a submicroscopic wave of contraction moves in one direction as discussed above, the protoplasmic flow in this direction will be realized. There seems to be no essential difference between these two types of protoplasmic flow. The plasmasol itself has the ability to flow actively along the layer of plasmagel.

The author wishes to emphasize the importance of the role of submicro-
Fig. 8. Schemes of protoplasmic streaming: (a) of ameboid movement (i.e., slime mold, ameba), and (b) of cyclosis (i.e., *Nitella*, *Elodea*). •, contractile protein; ---, intermolecular couplings are strong; ----, intermolecular couplings are weak or in some places these are torn off (i.e., in transition zone). If waves of contraction and expansion of such submicroscopic networks move entirely along in one direction, protoplasmic streaming in this direction will be realized. See text.
scopic networks constructed by the contractile proteins in the endoplasm in various kinds of protoplasmic movements.

The author wishes to acknowledge his indebtedness to Professor N. Kamiya for his criticism throughout this investigation, to Professor K. Fushimi (Department of Physics) for his theoretical suggestion, and to Dr. S. Harada (Department of Mathematics) for his mathematical advice and discussion.

BIBLIOGRAPHY

Abe, T., Kagaku (printed in Japanese), 1957, 27, 357.
Iwamura, T., Cytologia, 1952, 17, 322.
Kamiya, N., Cytologia, 1950, 15, 183.
Kamiya, N., and Abe, S., J. Colloid Sc., 1950, 5, 149.