Experimental Studies on the Protoplasmic Streaming in the Myxomycete Plasmodium I. Some observation on the motive force of protoplasmic streaming\textsuperscript{1,2}

Jirô Ohta

Botanical Institute, Faculty of Science, University of Tokyo\textsuperscript{3}

Received June 3, 1952

Protoplasmic streaming in the plasmodium shows characteristic reversal in the direction of flow and its velocity changes according to the rhythmic pattern. Therefore, in order to obtain a quantitative evaluation of this activity, we must device a suitable technique. No methods of evaluating this activity has been reported until Kamiya developed an excellent one (1940, 1942, 1943), by means of which it is now possible to measure the absolute value of the motive force responsible for the flow. It seems probable that this technique is useful for the study of the fundamental characteristic of the motive force, by applying it to various treatments of the slime molds.

In the present report, the author studied the relation between the weight of plasmodium and the magnitude of the motive force by using Kamiya's method, and on the basis of this preliminary experiments the author also investigated the effect of chloroform vapour upon the motive force. As material, the plasmodium, \textit{Physarum polycephalum}, was used.

In the following, the motive force referred to will be abbreviated as m.f..

\textbf{Method of measurement of the motive force}

The method of measurement of the motive force responsible for flow is the same as Kamiya's (1942, 1943). The whole set-up is shown diagramatically in Fig. 1. In the following, the author will only explain its outline.

A plasmodium consisting of two protoplasmic globules connected by a strand of plasmodium in the shape of a dumb-bell is placed in a double chamber. The two masses of protoplasm are in different compartments, but are connected by a single strand which passes through

\begin{footnotesize}
\textsuperscript{1} Contributions from the Divisions of Genetics and of Cytology, Botanical, Institute, Faculty of Science, University of Tokyo, No. 343.

\textsuperscript{2} Some parts of the present work were reported briefly at the annual meeting of the Japanese Botanical Society in 1949 at Tokyo, an abstract of which was published in Japanese.

\textsuperscript{3} This work was supported partly by a grant from the Science Research Fund, Ministry of Education.

\textsuperscript{3} Present address: Laboratory of Botany, Department of Science, Ochanomizu University, P. O. Koishikawa, Tokyo
\end{footnotesize}
the agar-wall. The structure of the chamber is such that each compartment may be kept air tight without interfering with the protoplasmic flow in the connecting strand. One of these two compartments is kept at atmospheric pressure, whereas pressure in the other compartment is under control. When there is no pressure difference between them, protoplasmic streaming goes on normally, showing regular reversal in the direction of flow. When, however, the air pressure of one compartment is modified, the velocity of protoplasmic flow in the connecting strand is accelerated or retarded. By controlling the air pressure, it is possible to oppose the m.f. in such a way as to hold the protoplasm at a standstill. This counter-pressure (*balance pressure*) is

![Diagram showing the arrangement of the whole system consisting of the double chamber having two compartments, A and B, rubber aspirator, As, controlled by screw, S, manometer, M, three-way stop cock, T, and stop cock, SC. (The direction of cocks indicates the position when in operation.) W is a drop of water. Agar-wall is shaded. Protoplasm in A and B is designated a and b, respectively, a measure of the absolute value of the m.f. responsible for the streaming of protoplasm.](image)

In order to determine the changes of the m.f. in relation to time, instantaneous value of the counter-pressure was read at a certain intervals. By plotting a series of these succeeding values as ordinate against time as abscissas, undulating curves, which Kamiya calls "dynamoplasmogram", were obtained. The graphs thus obtained gives a complete pattern of the rhythm in the protoplasmic activity. All the characteristic of rhythm such as wave form, frequency, polarity and amplitude are portrayed by graphical representation.

**Part I. The relation between the weight of plasmodium and the magnitude of the motive force**

*Experiments* In this section, the magnitude of the m.f. is expressed
as a combined estimate of the frequency and the amplitude of the dynamoplasmogram.

1) Two plasma masses and the connecting strand were weighed by torsion balance (accuracy within 0.1 mg.), then measurement of the m.f. was made. The resultant rhythms of the plasmodia of various weights were compared with one another. These results are shown in Table 1. There are no direct relationship between the magnitude of the m.f. and the weight of plasmodium within certain limits (0.5 ~ 50 mg.).

<table>
<thead>
<tr>
<th>Exp. No.</th>
<th>The weight of plasmodium (mg.)</th>
<th>The magnitude of the m.f.</th>
<th>Exp. temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Connecting strand</td>
<td>Protoplasm in A.</td>
<td>Protoplasm in B.</td>
</tr>
<tr>
<td>1</td>
<td>0.8</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>0.7</td>
<td>0.6</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>0.7</td>
<td>5.8</td>
<td>6.4</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>11.2</td>
<td>15.6</td>
</tr>
<tr>
<td>5</td>
<td>1.0</td>
<td>28.8</td>
<td>25.3</td>
</tr>
<tr>
<td>6</td>
<td>0.8</td>
<td>41.3</td>
<td>42.7</td>
</tr>
<tr>
<td>7</td>
<td>1.3</td>
<td>50.1</td>
<td>48.8</td>
</tr>
</tbody>
</table>

In this report, the word "Amplitude" and "Frequency" has the following meanings.

Amplitude: the ordinate difference between the maximum of a wave and the point with the same time coordinate on a line connecting its immediately preceding and following minima.

Frequency: the difference between the time coordinates for successive maxima or minima. Even though exactly the same wave form is not reported, this interval is nearly constant as long as the cyclic oscillation of the wave is vigorous.
2) After the same measurements as in the case of (1) were made, about half of the plasmodium in one or both compartments were cut off as in Fig. 2A. Then the same procedures were repeated again. An instance of this, is shown in Fig. 2A.

When we compare curves obtained before and after the treatments, we find considerable changes in the wave forms, but the magnitude of the m.f. decreases or increases according to circumstances.

3) The other plasmodia were placed close to the dumbbell-shaped plasmodium in one or two compartments of the double chamber (as shown in Fig. 2B). In some cases, these two adjacent plasmodia fused together, during the measurement of the m.f. generated in the dumbbell-shaped plasmodium. Soon after their fusion, wave form of dynamoplasmogram considerably changed, and the protoplasm showed the tendency to reverse the direction of displacement, as indicated by the shifting of the wave downwards (see, Fig. 2B).

In these experiments, the weight of plasmodium undoubtedly increased after the fusion, however, the magnitude of the m.f. did not always increase.

4) When only one blob of the plasmodium was added to the strand, or even when nothing was put to it, the m.f. was also possible to measure. In each case, however, we have had many difficult technical problems.

When the two plasma-blobs were cut off, namely, when the strand remained alone, the protoplasmic flow in the strand stopped for some time. Then feeble movements having short zigzag locus, were originated at the various parts in the strand. With the lapse of time, these movements were gradually organized in a normal reversible flow. Therefore, it may be an important problem for the study of the mechanism of flow, to investigate the mechanism of this organization.

Conclusion All above experiments indicate that there is no direct relation between the magnitude of the m.f. and the weight of plasmodium within certain limits. This result forms the foundation of the experiments concerning to the effects of various agents upon the m.f.

Part II. The effects of chloroform-vapour upon the protoplasmic streaming

On the basis of the result of the previous part, the author studied the effects of chloroform-vapour upon the m.f. by using 30~50 mg. plasmodium in each compartment.

Experiment

1) When the vapour was applied to both sides of the compartments:
   a) In the case of low concentrations\(^5\). (Chloroform-saturated air

\(^5\) Plasmodium shows various response to the same concentration of the vapour,
was diluted four times at 25°C.

When the plasmodium was placed in this vapour, the magnitude of the m.f. increased considerably. In this state, the plasmodium maintained active streaming and the instantaneous velocity of the flow was raised at certain times.

After the vapour in the chamber was replaced by fresh air, the magnitude of the m.f. decreased, as shown in Fig. 3.

b) In the case of high concentration (Chloroform-saturated air was diluted three times at 25°C.).

After this concentration of the vapour was applied to the double chamber, dynamoplasmogram came to lose its rhythmic pattern (see Fig. 4). If the removal of the vapour was made in a comparatively short length of time, the curve got a rhythm again. However, there are differences of the wave-form between curves before and after the treatment. This fact was not observed in the case of low concentration. It may be probable that irreversible changes in the mechanism of flowing, occurred in this high concentration of the vapour.

The microscopic observation in this case showed faint streaming, but prolonged exposure to the vapour brought about the cessation of the flow.

according to circumstances. So the author could not determine the definite relation between the concentration of the vapour and the action of it on plasmodium.
In higher concentrations, immediate stoppage of the flow and irreversible coagulation of the protoplasm were observed.

2) When the vapour was applied to only one side of compartment:
   a) In the case of low concentration.
   When only one half of the plasmodium in the double chamber was treated with this vapour, protoplasm shows a tendency to move into
the non-treatment compartment, as indicated by the shifting of the wave upwards (see Fig. 5). However, this tendency gradually disappeared, though the condition in the chamber was kept the same.

b) In the case of high concentration.

Soon after this vapour administered to one compartment of the chamber, dynamoplasmogram shifted to one side of the abscissa and almost complete loss of rhythmicity was observed as shown in Fig. 6.

![Fig. 6.](image)

When fresh air was applied in a short time, gradual recovery of rhythmicity was observed.

When a piece of filter paper, which was moistened by chloroform-saturated water, was placed in one compartment of the double chamber, the plasmodium also showed the tendency to flow towards the other compartment.

Discussion It is a well-known fact that dilute concentrations of fat-solvents cause an increase in the rate of streaming in various plant cells, (Ewart, 1903; Seifriz, 1943; and others,) and it has been considered that this effect is presumably due to the action of these substances on protoplasmic viscosity. However, as has been mentioned in previous section, the magnitude of the m.f. was increased considerably by the application of low concentration of chloroform vapour. Thus, the action of fat-solvents in dilute concentration on protoplasmic streaming must be considered not only on the basis of the changes of the viscosity, but also of the m.f.
For a long time, many authors tried to explain the mechanism of protoplasmic streaming by the surface-tension changes of the protoplasm—such authors as Berthold, Verworn, Bitschili and others. At the present days, various arguments have been advanced against this theory. If this explanation was true, protoplasm would have to flow towards the fat-solvents. However, when one half of the plasmodium in the double chamber was treated by chloroform vapour, the plasmodium shows negative taxis to that vapour. Therefore, surface-tension theory does not seem to be adequate for the explanation of the mechanism to flow in the plasmodium.

After the chloroform vapour of low concentration was applied to one compartment of the double chamber, the plasmodium shows the tendency to flow into non-treatment compartment. However, this tendency did not last long even if the plasmodium was left in this state. These phenomena seem to indicate that the plasmodium develops a "tolerance" for the chloroform. Seifriz (1941) has already pointed out similar responses of the plasmodium to various agents, and the author also found them in the case of hydrogen cyanide vapour.6

In conclusion, the author wishes to express his gratitude to Prof. B. Wada, University of Tokyo, for his constant encouragement and criticism, and to Prof. N. Kamiya, University of Osaka, at whose valuable suggestions and under whose guidance and continuous help, this work was carried out.

Summary

By the application of Kamiya's dynamoplasmometry, the author found the following results concerning the motive force of protoplasmic streaming in the plasmodium, Physarum polycephalum.

1. There was no direct relation between the magnitude of the motive force and the weight of the plasmodium within certain limits.

2. After the plasmodium in one or both sides of the compartments was cut off, or after two plasmodia in the same compartment were fused together, the magnitude and the wave-form of the dynamoplasmogram changed considerably. In these cases, the above conclusion was also supported.

3. When the chloroform vapour was applied to both sides of the compartments, the magnitude of the motive force increased in low concentrations and decreased in high concentrations. In the latter case, changes in the wave-form of the dynamoplasmogram were observed.

4. When these vapours were applied to only one compartment,

6 The effects of this and several other agents upon the protoplasmic streaming in the plasmodium will be reported elsewhere.
protoplasm showed a tendency to move into the non-treatment compartment, as indicated by the shifting of the wave upwards. In this treatment, the plasmodium develops a “tolerance” for the vapour in the case of low concentration.

Reference


