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Studies on Protoplasmic Streaming in Myxomycete Plasmodium

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Plasmodia of Myxomycetes, commonly known as slime moulds, have various favourable characteristics for conducting studies on protoplasmic streaming. In a series of experiments, the author[22),23),24)25)] investigated the physiological properties of protoplasmic flow by using the plasmodium of a Myxomycete, *Physarum polycephalum*, as material. The purpose of the present paper is to develop theoretical considerations on the experimental data obtained by our group of investigators with special reference to the mechanism of protoplasmic streaming. Some unpublished data are also described.

To explain the mechanism of protoplasmic flow, it is necessary to elucidate the following points:
(1) Source of energy for streaming, (2) mechanism, by which chemical energy is converted into mechanical work (mechano-chemical system), (3) intraplasmic localization of the mechano-chemical system in protoplasm.

At the present time, however, our knowledge is too meager to explain the last problem. Therefore, the first two problems are mainly discussed in the following.

I. Energy supply for protoplasmic streaming

1. Metabolism in plasmodium

To discuss the energy supply for protoplasmic streaming, it is important to elucidate the metabolic characteristics of the plasmodium. The respiration of *Physarum polycephalum* has been studied in considerable detail by Allen and Price[1)], and further information of the metabolism, including fermentation, has been reported by the author[24)].

By using Warburg manometer, the author has obtained the following results:

(1) Qo_2 value on a wet weight basis was found to be 1.08 (M/45 phosphate buffer, pH 6.0), or 7.3 on a dry weight basis, 140 on a nitrogen basis.

(2) R.Q. value was found to be 0.83 on an average.

(3) O_2-uptake was strongly inhibited by various metabolic poisons such as cyanide, NaN_3 and monoiodoacetate. CO inhibited the respira-

tion considerably but this inhibition was reversed to some extent on illumination. Considering the effects of these inhibitors, it is inferred that cytochrome oxidase is involved, as terminal oxidase in the respiration of the plasmodium. Further support in this respect was afforded by the spectroscopic confirmation of cytochromes *a*, *b* and *c* in the plasmodium.

(4) Under an anaerobic condition, the plasmodium showed an endogenous fermentation which was accompanied by acid formation. No sign of aerobic fermentation was observed.

Recently the author* has investigated the enzymatic activities in the homogenates of the plasmodium by using Thunberg tubes and Warburg manometer. In this work, the presence of various enzymes such as succinic dehydrogenase, malic dehydrogenase, pyruvic oxidase and lactic dehydrogenase was discovered in the plasmodium. Therefore, it seems likely that the Tricarboxylic acid cycle exists in the plasmodium. Production of lactic acid under anaerobic condition was also confirmed.

All the above results incidate a close similarity of the metabolic system of the plasmodium to that of animal tissues, especially muscle. Therefore, the main path of the metabolism of this organism is inferred to be as follows:

Glycogen is decomposed to pyruvic acid through a series of glycolytic enzymes. In anaerobiosis, the pyruvic acid is reduced to lactic acid by lactic dehydrogenase. In the presence of oxygen, however, pyruvic acid goes into the Tricarboxylic acid cycle and is oxidatively decomposed to carbon dioxide with the aid of cytochrome system, as terminal oxidase. In addition to these mechanism for carbohydrate metabolism, a system for fat metabolism must also be present, because the appearance and disapperance of fat droplets are often found to take place in the plasmodium. The fact that the R.Q. value here is as low as 0.83 also speaks for the supposedly important role of fat in the metabolism of this organism.

2. Effects of various metabolic poisons and reduced oxygen tension on the motive force of protoplasmic streaming

To express the protoplasmic flow in the plasmodium on a quantitative basis, the author used the technique originally developed by Kamiya[6),7),8)] (double-chamber method). According to this method, the oscillatory changes in the motive force responsible for the protoplasmic flow can be continuously measured by following changes in magnitude of counterbalancing hydrostatic pressure which is required to stop the flow. In order to determine the time course of these changes in the motive force, values for the counterpressure were read at intervals. By plotting these

* Unpublished data.

succesive values (ordinates) against time (abscissae), a series of undulating curves (dynamoplasmogram, after Kamiya), is obtained reflecting the patterns of the rhythms in the protoplasmic activity.*

By using the double-chamber method, the author[23] has investigated the effects of some respiratory poisons and reduced oxygen tension on the motive force of protoplasmic streaming.

a. Low oxygen tension

The effect of oxygen tension on the protoplasmic streaming in the plasmodium of *Physarum polycephalum* has been studied by Kitching and Pirenne[13], Allen and Price[1], Loewy[16], Ohta[23], Kamiya[8], and Kamiya et al[9),10]. Using the double-chamber method, the present author[23] and Kamiya et al.[9),10] have recorded dynamoplasmogram of the plasmodium under reduced oxygen tension.

It was observed that anaerobiosis had no (or a slightly enhancing) effect on the motive force. On replacing nitrogen in the double-chamber with fresh air, the motive force showed temporary drop to return to the original level after some time (Fig. 1). When the plasmodium was

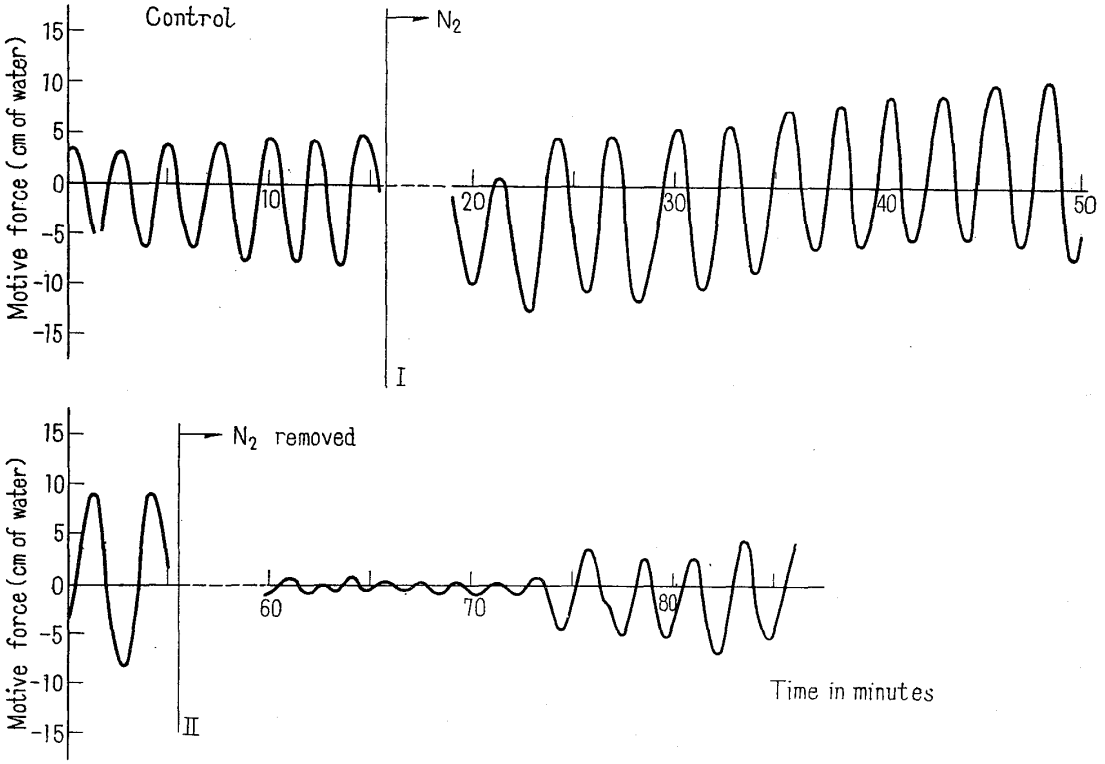

Fig. 1. Dynamoplasmogram under anaerobic state. During the period lying between the two vertical lines, I and II, the whole plasmodium was treated with nitrogen.

left under anaerobic condition, it continued its protoplasmic flow as long as 10 hours, without any sign of injury. When the nitrogen gas or air in the double-chamber was replaced with a mixture of 95 per cent N_2:

* Details concerning this problem have been reported in earlier papers.

5 per cent O_2, no significant effect on the motive force was observed.

The dispensability of oxygen for protoplasmic flow has also been confirmed by Kamiya et al. These investigators, however, observed a marked increase in the motive force in anaerobiosis, which was not found in the present study. The discrepancy between these observations remains to be investigated.

When the one side of the double-chamber was filled with N_2 instead of air, protoplasm showed a tendency to flow into the latter chamber. A positive taxis toward aerobic atmosphere was thus confirmed.

On the other hand, Seifriz and Urbach[29] have pointed out the interesting fact that the spreading of the plasmodium over the surface of a solid medium is inhibited under the anaerobic condition.—A finding which has also been reconfirmed in the present study. The bearing of this observation on our experimental results concerning the motive force of flow, is also open to further elucidation.

b. Carbon monoxide (CO)

According to Allen and Price[1], the protoplasmic streaming in plasmodium retains its normal features in an atmosphere of 95 per cent CO:

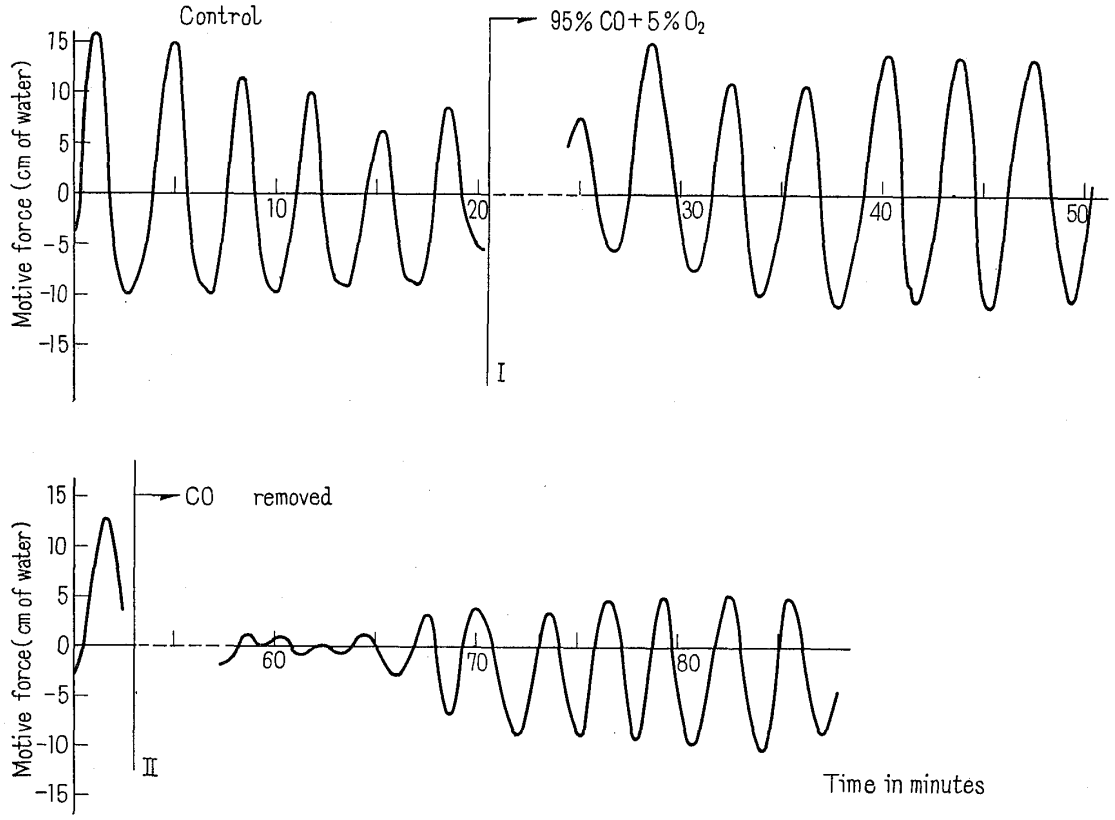

Fig. 2. Dynamoplasmogram under the effect of CO. During the period lying between the two vertical lines, I and II, the plasmodium was placed in an atmosphere of 95 per cent CO:5 per cent O_2

5 per cent O_2. The author[23] reexamined this point by means of the double-chamber method.

When the air in the double-chamber was replaced with this gas mixture, the motive force of the flow slightly increased. In this gas mixture, the plasmodium maintained active streaming even after 10 hours. After CO was removed, the motive force decreased for some time prior to its recovery, to the normal level, as in the case of nitrogen (Fig. 2).

When the atmosphere of one half of the double-chamber was replaced by 95 per cent CO: 5 per cent O_2, a negative taxis was observed, the plasmodium flowing towards the air-filled chamber.

The spreading of the plasmodium was also inhibited in CO.

c. Cyanide

Seifriz and Urbach[29] reported that 10^{-3} M KCN had no detectable effect on the protoplasmic streaming in the plasmodium, while the expansion of the surface area was completely stopped at this concentration of the reagent. Allen and Price[1] also found that protoplasmic flow was not stopped within 6–8 hours at cyanide concentrations up to 1×10^{-3} M.

The present author[23] has investigated the effects of HCN on the motive force of flow. When HCN was added to the plasmodium at concentrations ranging from 0.36 mg./*l.* to 3.60 mg./*l.*, the motive force increased slightly. When the plasmodium was treated with higher concentrations of HCN (18.0 mg./*l.*), the motive force increased considerably for a short time before it gradually decreased, eventually approaching nil. By applying an alternating pressure difference between the two compartments of the double chamber, it was possible, in earlier stages of the HCN-treatment, to move the plasmodium to and fro in the desired direction, thus confirming that no coagulation of the protoplasm had occurred at this stage. On prolonged treatment of the plasmodium with this poison, there finally occurred death and coagulation of the protoplasm. Judging from the dynamoplasmograms, it seems that the plasmodium gradually increases its tolerance towards HCN when the organism is repeatedly treated with this gas.

On introducing the HCN-gas to one side of the double-chamber, the protoplasm was found to migrate rapidly toward the other chamber, indicating a negative taxis toward this poison. This tendency, however, was found to disappear before long, although it is inconceivable that the HCN had disappeared in so short a period of incubation.

Kamiya et al.[9),10] have also reported the effects of KCN on the motive force of flow. They found that the motive force had a tendency to increase its amplitude under the influence of 10^{-3} M KCN (pH 7.0) and that, soon after the replacement of the cyanide solution with fresh medium, the motive force showed a slight decrease before it gradually

regained its normal magnitude. Recently, they[11] performed simultaneous measurements of the motive force and oxygen-uptake with the same plasmodium, using a specially deviced apparatus. In this case, KCN was found to reduce the oxygen-uptake and to increase the motive force.

d. Monoiodoacetic acid (IAA)

Kamiya et al.[10] found that $1-2\times10^{-3}$ M IAA considerably inhibited the generation of the motive force.

e. Sodium fluoride (NaF)

According to Kamiya et al.,[10] 5×10^{-3} M NaF conspicuously suppressed the production of the motive force responsible for protoplasmic flow.

f. 2, 4-Dinitrophenol (2,4-DNP)

Allen and Price[1] found that the protoplasmic flow was brought to a complete standstill within a few minutes after exposure to 2×10^{-4} M 2,4-DNP and that a less rapid inhibition was also observed at 1.25×10^{-4} M 2,4-DNP.

Kamiya et al.[10] reported the effect of 2,4-DNP on the generation of the motive force of protoplasmic flow. Their results are as follows. When 5×10^{-4} M 2,4-DNP was admitted to the plasmodium, during the first 10 minutes the motive force showed a temporary increase to an extent even greater than in the control and then it diminished to disappear almost completely. The motive force under anaerobic condition was also destroyed by 2,4-DNP in the same concentration. It was therefore concluded that the inhibition of the motive force by 2,4-DNP was not due to the uncoupling of oxidative phosphorylation caused by this reagent.

3. Relationship between motive force of protoplasmic streaming and the metabolism in the plasmodium

The results described above show that the motive force of protoplasmic flow is not affected when the plasmodium is placed under low oxygen tension or when it is treated with respiratory inhibitors such as CO and cyanide. Fermentation poisons such as monoiodoacetic acid and sodium fluoride, on the other hand, significantly depress the motive force. These facts suggest that the energy available for the protoplasmic flow in the plasmodium is supplied by fermentation. The increase in the motive force caused by anaerobiosis, or treatments with CO or cyanide, may be due to the increase in fermentation capacity under these conditions. As a matter of fact, the author[24] has observed the Pasteur effect in this organism. This opinion has also been presented by Kamiya et al[10].

II. Mechano-chemical system in the plasmodium

1. Adenosinetriphosphate (ATP) as an energy source

As is well known, ATP occupies a unique status in cell metabolism, being the main supplier of energy for various biological activities. For example, muscular movement is primarily dependent upon ATP as energy source. Accordingly, it will be natural to assume that ATP also constitutes the immediate energy source for protoplasmic flow in the plasmodium. As a matter of fact, Takeuchi and Hatano[31] have recently discovered the presence of ATP in the plasmodium of *Physarum polycephalum* in a concentration of $0.5\text{-}1.0\times10^{-3}$ M/*l*. It was also demonstrated by them that the ATP obtained from the slime mould is able to evoke contraction of glycerinated rabbit muscle, as muscle-ATP does.

The effect of ATP on the motive force of protoplasmic flow has been investigated by Kamiya et al.[10] According to them, a pronounced augmentation in the motive force occurs when ATP is added to the plasmodium. They also studied the effects of muscle- and yeast-adenylic acids (AMP) on the protoplasmic flow. In these cases, however, they could not observe any effect comparable to that produced by ATP. Therefore, they assumed that the augmentation of the motive force brought about by ATP was caused by the energy-rich phosphate bond of ATP.

Kuroda[14] has reported another interesting experiment. She observed the behaviour of minute protoplasmic blobs obtained by cutting the plasmodium of *Physarum polycephalum*. The protoplasmic blobs which had a diameter of about 30μ showed apparently disordered, complicated movements. On application of 5×10^{-4} M ATP solution (containing 0.1 M KCl and 0.001 M $MgCl_2$), the motility of the blobs increased considerably. This fact also suggests that ATP plays an important role in the protoplasmic activity of the plasmodium.

As has been mentioned in the previous section, it seems likely that the ATP formed through the fermentative process occurring in the protoplasm is solely responsible for producing the motive force of protoplasmic flow, whereas the ATP formed in the respiratory process cannot be utilized for this purpose. An explanation of this has been presented by Kamiya et al[10]. They consider that ATP synthesized in mitochondria cannot readily reach the mechano-chemical system which is supposed to be located in the hyaloplasm, while the ATP produced through fermentation which takes place in the "soluble part of the cytoplasm" (i.e., hyaloplasm), is readily utilized *in situ*.

2. Contractile protein of plasmodium

As mentioned above, the immediate energy source for protoplasmic streaming in the plasmodium seems to be produced through fermentation in the protoplasm. The next important point that arises concerns the

system which is responsible for the conversion of the chemical energy of ATP into mechanical work.

Loewy[17] was the first who actually discovered an ATP-sensitive protein in Myxomycete plasmodium. He extracted the plasmodium of *Physarum polycephalum* with 1.2 M KCl (pH 8.2) at 0°C and obtained a protein fraction, of which the viscosity was abruptly lowered on addition of ATP. The ATP-induced lowering of viscosity was found to be followed by a slow rise, which was interpreted to be due to the formation of AMP as a result of dephosphorylation of the added ATP. This viscosity response was found to be specific to muscle-ATP and some of the nucleotides related to it (e.g., muscle-inosinic acid). Thus yeast AMP was entirely without effect. The ATP-sensitive protein, named myxomyosin by Ts'o et al[32),33),34], has been purified by these authors by salt fractionation, and its physicochemical properties, including electrophoretic and ultracentrifugal data, have been reported, as well as its ATPase activity.

Details concerning this protein have also been reported by Nakajima[18),19),20]. He extracted the plasmodium with Weber-Edsall solution (0.6 M KCl, 0.01 M Na_2CO_3, 0.04 M $NaHCO_3$) and purified it by the dilution-method. He has found that the properties of the purified sample thus obtained are markedly similar to those of muscle-actomyosin with respect to its response to ATP. In disaccord with the results obtained by Loewy[17], AMP caused no change in viscosity of Nakajima's sample. The effects of Ca^{++} and Mg^{++} were found to be essentially the same as those observed on the case of muscle-actomyosin. Super-precipitation has also been observed with a 0.1 M KCl solution of the plasmodium contractile protein. The ATPase activity and the effects of Mg^{++} and Ca^{++} on this reaction have also been investigated in detail. The conclusion reached through these minute examinations is that the contractile protein in question is very closely related to actomyosin, especially that of smooth muscle.

An experiment was also carried out by the present author with glycerol-treated preparation of the plasmodium. In this experiment, a protoplasmic strand was treated with a 50 per cent glycerol-water solution, containing 0.001 M $MgCl_2$, 0.16 M KCl and 0.05 M phosphate buffer (pH 6.8), at 0°C for 7–10 days. Before the measurements, it was washed in 20 per cent glycerol solution for 2 hours and then it was again washed in 0.16 M KCl for ca. 20 minutes at room temperature. The strand was hung vertically in an aqueous solution (containing 0.001 M $MgCl_2$, 0.16 M KCl and 0.001 M phosphate buffer) with a small load on its free end. On replacing the medium with the same solution but containing 0.2 per cent ATP, a slight, although evident contraction occurred amounting to about 4 per cent of the original length. The presence of Ca^{++} and Mg^{++}

was found to intensify the extent of contraction, in a concentration range of 0.001–0.002 M. Without the addition of these ions, the results were rather unreproducible, the amount of contraction thus obtained fluctuating between 0 to 1.5 per cent. Pyrophosphate and AMP caused no detectable effects on glycerol-treated strand. We could not find any functional differences in this respect between muscle- and yeast-ATP.*

As has been reported recently[25], ethylenediaminetetraacetic acid (EDTA) inhibits the generation of the motive force responsible for protoplasmic streaming in the plasmodium and this inhibition is reversed by the addition of Mg^{++} (Fig. 3). This fact is interpreted to indicate

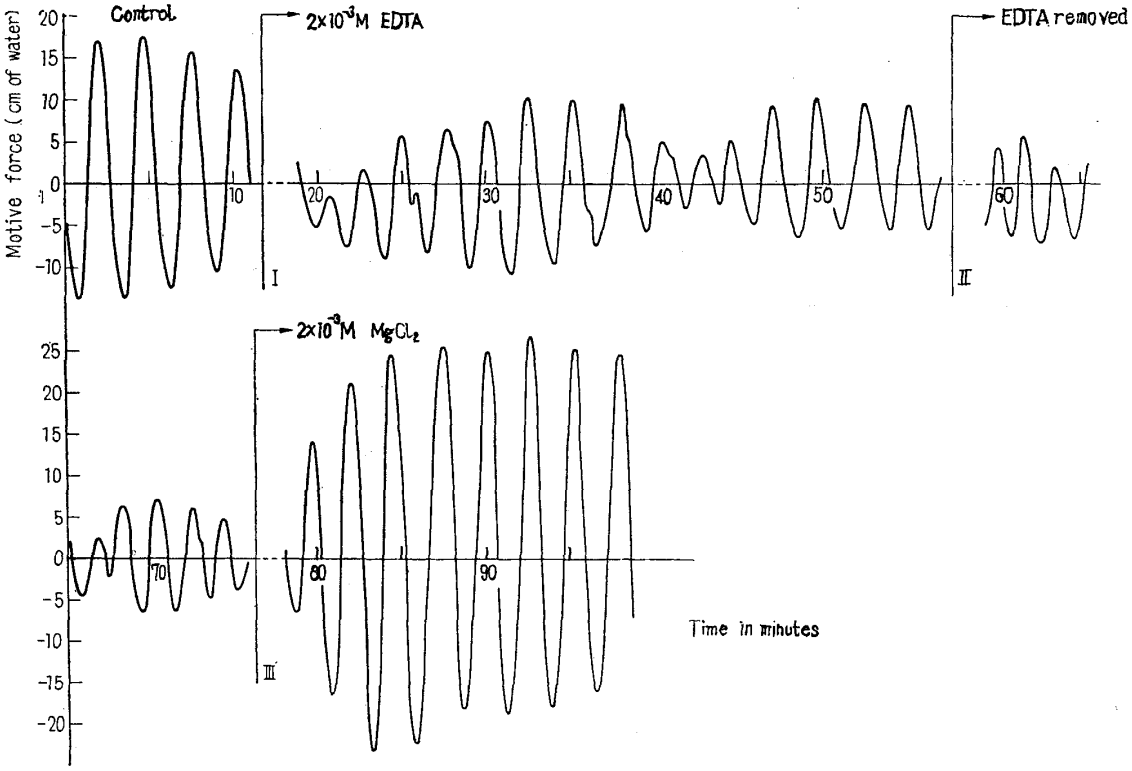

Fig. 3. Dynamoplasmogram under the effect of 2×10^{-3} M EDTA. The whole plasmodium was treated with EDTA during the period lying between the two vertical lines, I and II. At the time marked with the vertical line III, 2×10^{-3} M $MgCl_2$ was admitted to both compartments of the double-chamber.

that the removal of Mg^{++} caused by the addition of EDTA suppresses the reaction of the contractile protein with ATP.

On the contrary, the addition of amino acids, such as histidine, cysteine and glycine was found to increase the motive force of flow (Fig. 4). This increase of the motive force seems to be due to the metal-chelating action of amino acids. In fact, the histidine solution in which the plasmodium had been suspended for 30 minutes, always gave a positive dithizone reaction, indicating the combination of the added

* Further details of this work will be published elsewhere.

amino acid with some metal (e.g., zinc). The same colour reaction was also observed in the case of cysteine and glycine. Similar results have been reported concerning various protoplasmic activities (e.g., motility of starfish spermatozoa) and explained as the removal of inhibition due to zinc. The question remains open to future investigation of the same mechanism applies to the present case.

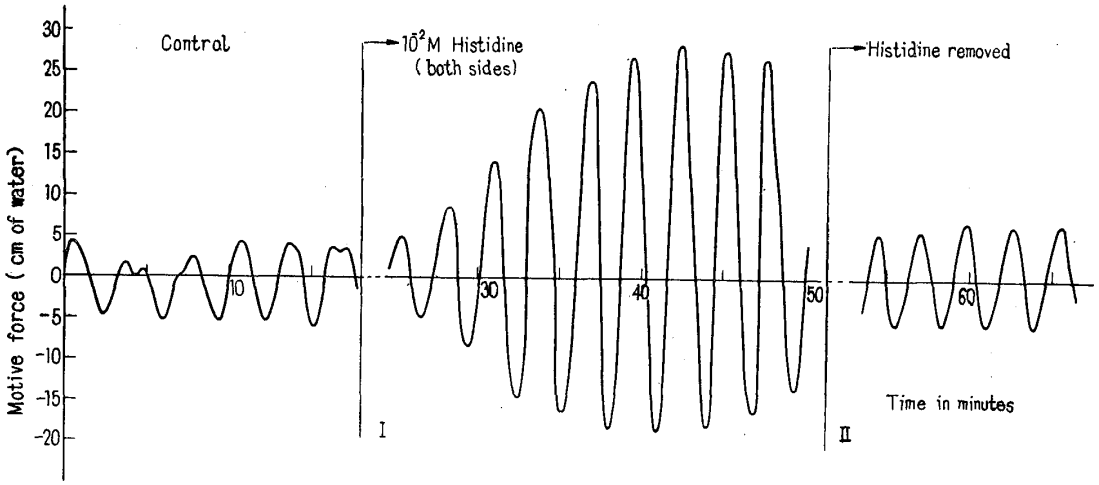

Fig. 4. Dynamoplasmogram under the effect of 10^{-2} M histidine. The whole plasmodium was treated with histidine during the period lying between the two vertical lines, I and II.

III. Discussion

In conclusion, it will be deduced from the above-compiled body of evidence that a contractile protein similar to muscle actomyosin is operative in the protoplasmic flow of *Physarum polycephalum*, converting the chemical energy of ATP into mechanical work. A similar assumption has also been presented by Kamiya et al[10].

The next problem that follows is about the intraplasmic organization of this contractile system in the plasmodium. In this respect, however, no sufficient data have so far been obtained, but some speculations are proposed in relation to the theory of protoplasmic streaming[2),3),15),30)].

A point which remains open for further investigation, perphaps from another angle of approach, concerns the problem of the periodicity which is always observed in the protoplasmic streaming of Myxomycete plasmodium. From the metabolic point of view, with which the present study is primarily concerned, a search must be carried out to discover the nature of the metabolic process which directly governs the switching of the protoplasmic streaming in one direction to another or the direction of twisting of a living protoplasmic strand as originally observed by Kamiya and Seifriz[12].

The effect of anesthesia on the motive force of protoplasmic flow

has been investigated by the present author[22] as well as by Kamiya[8]. Chloroform at high concentrations was found to supress the motive force of flow. As still higher concentrations, the periodicity also disappeared, resulting in an irregular, and faint oscillatory movement of the protoplasm. This effect of chloroform, however, was found to be reversible, being removed by fresh air. According to Kamiya, both periodic activity and the magnitude of the motive force disappeared completely on application of 1 per cent ether solution. The effects of eserin and caffein on the motive force of protoplasmic streaming have also been studied by the present author.* When the plasmodium was treated with these reagents, changes in the pattern of the dynamoplasmogram were observed in some cases without, however, any detectable effect in other cases. Judging from these findings it seems likely that a certain conductive system is involved in the protoplasmic flow.

The beating of cilia and flagella, the streaming of protoplasm, the movements of amoeboid cells, the contraction of muscle, are all manifestations of the ability of living matter to transform chemical energy into mechanical work. Since early days, various investigators have suggested the resemblance among these movements mainly from the view point of cellular contractility[2),5),21),26),27),29)]. The fact discussed in this paper give further evidence to support this assumption.

IV. Acknowledgement

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V. Summary

1. The motive force of flow in the plasmodium of a Myxomycete, *Physarum polycephalum*, was investigated, using Kamiya's double-chamber method. The results thus obtained, together with data published by the present author, as well as by other investigators, are discussed with special reference to the mechanism underlying the protoplasmic streaming of the plasmodium.

2. The metabolism of the plasmodium under the influence of metabolic poisons such as carbon monoxide, cyanide, monoiodoacetic acid, sodium fluoride and 2, 4-dinitrophenol, and reduced oxygen tension was investigated. The results thus obtained are compared with observations concerning the effects of these factors on the motive force of proto-

* Unpublished data.

plasmic flow.

3. Judging from these experimental results, it is inferred that the ATP formed in the fermentative process in the plasmodium makes the immediate source of energy for the production of the motive force of flow.

4. It is concluded that an actomyosin-like protein participates as the mechanism converting the chemical energy of ATP into mechanical work. The simultaneous participation of a conductive system in the process is also suggested.

VI. References

1) Allen P. J. and W. H. Price 1950 Amer. Journ. Bot. **37** 393-402.
2) de Bruyn P. P. H. 1947 Quart Rev. Biol. **22** 1-24.
3) Frey-Wyssling B. 1948 Proc. 6th Intern. Congress of Exp. Cytology.
4) ——— 1955 Die submikroskopische Struktur des Cytoplasmas, Protoplasmatologia, Band H, A 2.
5) Gray J. 1931 A Text-Book of Experimental Cytology, Cambridge.
6) Kamiya N. 1940 Science **92** 462-463.
7) Kamiya N. In "The Structure of Protoplasm" (ed. by W. Seifriz) Monogr. Amer. Soc. Plant Physiol. (Ames-Iowa), 199-244.
8) ——— 1953 Ann. Rep. Sci. Works, Osaka Univ. **1** 53-83.
9) ———, Nakajima H. and S. Abe 1954 Seitai no Kagaku (Medical Science) **5** 148-157 (in Japanese).
10) ———, ———, ———, 1957 Protoplasma **48** 94-112.
11) ———, S. Abe and Nakajima H. 1957 Proc. Japan Acad. **33** 407-409.
12) ——— and W. Seifriz 1954 Exp. Cell Res. **6** 1-16.
13) Kitching J. A. and M. H. Pirenne 1940 Journ. cell. comp. Physiol. **16** 131-133.
14) Kuroda K. 1958 C. r. s. biol., Paris **152** 392-394.
15) Loewy A. G. 1949 Proc. Amer. Philosophycal Soc. **93** 4.
16) ——— 1950 Journ. cell. comp. Physiol. **35** 151-153.
17) ——— 1952 Journ. cell. com. Physiol. **40** 127-156.
18) Nakajima H. 1955 XX Ann. Meeting Bot. Soc. Japan.
19) ——— 1956 Seitai no Kagaku (Medical Science) **7** 49-52 (in Japanese).
20) ——— 1957 XXII Ann. Meeting Bot. Soc. Japan.
21) Ohta J. 1950 Seibutsu Kagaku (Biological Science) **2** 10-24 (in Japanese).
22) ——— 1952 Cytologia **17** 210-218.
23) ——— 1952 Cytologia **17** 300-310.
24) ——— 1954 Journ. Biochem. (Japan) **39** 489-497.
25) ——— 1958 Cytologia **23** 232-238.
26) Pantin C. F. A. 1930 Proc. Royal. Soc. London B **105** 538.
27) ——— 1930 Proc. Royal Soc. London B **105** 555.
28) ——— 1930 Proc. Royal Soc. London B **105** 565.
29) Seifriz W. and F. Urbach 1944 Growth **8** 221-233.
30) Stewart R. A. and B. J. Stewart 1959 Exp. Cell Res. **17** 44-58.
31) Takeuchi I. and S. Hatano 1955 XX Ann. Meeting Bot. Soc. Japan.
32) Ts'O P. O. P., Bonner J., Eggman L. and J. Vinograd 1956 Journ. Gen. Physiol. **39** 325-347.
33) ———, Eggman L. and J. Vinograd 1956 Journ. Gen. Physiol. **39** 801-812.
34) ———, ———, ——— 1957 Arch. Biochem. Biophys. **66** 64-70.

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