Electrochemical Analysis of Intracellular Protein Colloid of an Isolated Single Muscle Fibre from the Frog

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(With 3 Text-figures)

According to Tamasige (1952), the survival of the frog muscle fibre is more prolonged when it is immersed in the hen's egg white, although the threshold value of electric excitation increases to 300−500 times the normal fibre. If, the muscle fibre is surrounded by protein colloid which is very similar to the inside one, the function of the cell membrane may disappear, since the chemical gradient between inside and outside of the membrane becomes nil. Or not? Thus if, the changes of membrane potential of muscle fibres placed in various protein colloids are determined, a physiological state in intracellular protein colloid may be explainable. On the other hand, one of the intracellular chemical properties may also be understandable by means of measurement of junction potential between sarcoplasm and electrolytes.

In order to clarify the states of intracellular protein colloid electrochemical investigations were undertaken. The experiments dealt with membrane potentials and responses to electrical stimuli in single muscle fibres immersed in various protein colloids, and with junction potentials between sarcoplasm and various electrolyte solutions.

The materials used were single muscle fibres isolated free of injury from muscle of hind legs of the frog, *Rana nigromaculata*, and the method of electrical examination was the same as that of the previous papers (Tamasige 1950, Umezawa 1957).

I. Physiological States In Intracellular Protein Colloid

A. *Membrane potential of single muscle fibres immersed in various egg white solutions* A fresh preparation of single muscle fibre was placed in various hen's egg white solutions* and the membrane potential was measured (Fig. 1). The test egg white solutions used were as follows: pure egg white (pH: 8.0), M/8 KCl-egg white (pH: 8.3), M/8 KH_2PO_4-egg white (pH: 6.3), M/8 K_2HPO_4-egg white (pH: 9.0), M/8 NaCl-egg white (pH: 8.5), M/8 NaH_2PO_4-egg white (pH: 6.7), and M/8 Na_2HPO_4-egg white (pH: 8.4).

As is shown in Fig. 1, the membrane potential changes in the following order: K_2HPO_4-egg white>KH_2PO_4-egg white>KCl-egg white, the values in average being +2 mV, −3 mV, and −9 mV, respectively. In the former two cases the potential difference across the cell membrane is hardly distinguished, although the polarity of

* The immersion of the fibre in various egg whites scarcely induce the contraction, and the striation is clearly observable under the microscope (cf. Tamasige 1952).

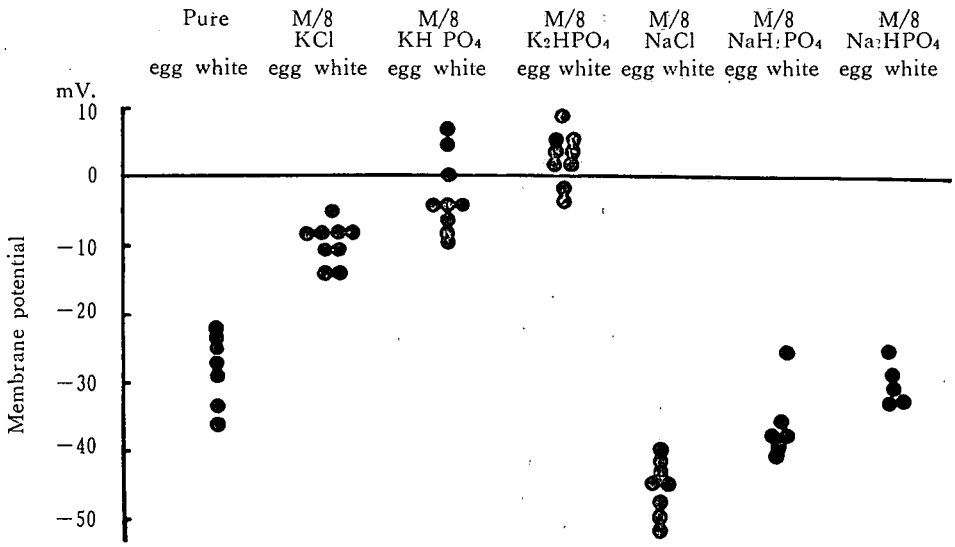

Fig 1. Membrane potentials of single muscle fibres in various egg white solutions of electrolytes measured with intracellular glass microelectrode filled with 3M KCl solution; room temp.: 28°−30°C.

potential is slightly reversed (inside positive) in the several fibres. It is suggested, therefore, that two kinds of K-phosphate-egg white are similar to the state of intracellular protein colloid. This is ascertained by the fact that the membrane potentials in pure egg white, in NaCl-egg white, and in Na-phosphate-egg white are higher than those in K-phosphate-egg white. In pure egg white the value in average being −28 mV; in Na_2HPO_4-egg white it was −30 mV; in NaH_2PO_4-egg white, −37 mV; and in NaCl-egg white, −45 mV. These results coincide with the results obtained on the chemical analysis of intracellular and extracellular electrolytic ions by Fenn (1936), and Boyle and Conway (1941). According to them, inside the muscle fibre the concentration of potassium ions is comparatively much higher than that of anions. Although the intracellular phosphate ions are most abundant among the anions, the total sum of cations exceeds the sum of all anions, and the anion deficit seems to be made up by proteins and amino acids. Also the present results seem to indicate that the Donnan's membrane equilibrium does not hold and it cannot be used to explain the ionic distribution between inside and outside the fibre, when it is surrounded by various egg whites, as was already pointed out on the two sides of the vitelline membrane by Needham (1950).

B. *Junction potential between sarcoplasm and various electrolyte solutions* In order to determine the junction potential between sarcoplasm and electrolyte solution measurements of the membrane potential of muscle fibre were made with the intracellular microelectrode at 30°C (Fig. 2). Table 1 shows the results obtained, in which the measurement is made with intracellular microectrodes filled with 3M KCl-, 3M K_2HPO_4- or 3M NaH_2PO_4 solution. The absolute value of potentials (inside negative) with 3M KCl-filled microelectrode is smaller than that with 3M K_2HPO_4-filled microelectrode, and the value is larger than that with 3M NaH_2PO_4-filled microelectrode; the differences

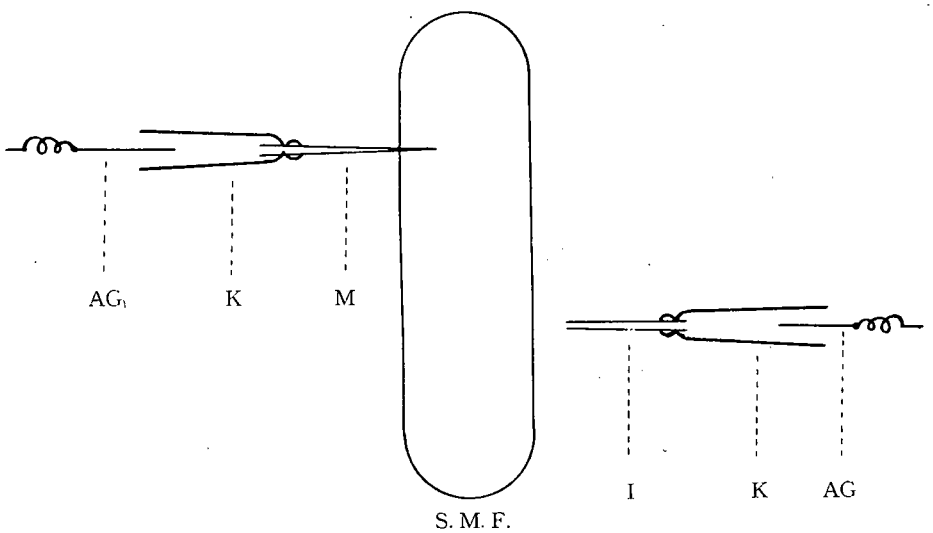

Fig. 2. Membrane potentials are measured between the inside and outside of single muscle fibre. AG: Ag-AgCl, I: indifferent electrode filled with 3M KCl solution, K: 3M KCl-agar bridge, M: intracellular microelectrode filled with various electrolyte solutions, and S. M. F.: single muscle fibre.

Table 1. Membrane potentials of single muscle fibres measured with intracellular microelectrode filled with various electrolyte solutions; room temp.: 30° C.

I. Microelectrode filled with	Membrane potentials in Ca-free Ringer's solution (−mV.)									
a) 3M KCl	74	54	82	74	54	84	88	49	79	Average
b) 3M K_2HPO_4	80	56	88	82	64	88	92	54	86	
a) − b)										+ 6 mV.
II. Microelectrode filled with	Membrane potentials in M/8 KCl solution (−mV.)									
c) 3M KCl	3	4	3	6	7	6	9	12	8	Average
d) 3M K_2HPO_4	11	7	6	14	14	11	13	14	14	
c) − d)										+ 5 mV.
III. Microelectrode filled with	Membrane potentials in Ringer's solution (−mV.)									
e) 3M KCl	116	126	116	111	103	113	108			Average
f) 3M NaH_2PO_4	110	120	106	105	92	106	104			
e) − f)										− 8 mV.
IV. Microelectrode filled with	Membrane potentials in M/8 NaCl solution (−mV.)									
g) 3M KCl	38	25	60	58	46	65	46	46		Average
h) 3M NaH_2PO_4	27	15	52	52	40	62	36	40		
g) − h)										− 8 mV.

between the values with KCl and K_2HPO_4 and between the values with KCl and NaH_2PO_4 are 6 mV and 8 mV in average, respectively. Such differences in membrane potential of the same fibre seem to result from differences in junction potential between sarcoplasm and various electrolyte solutions with which the electrode is filled. The membrane potential when 3M KCl-filled microelectrode is used (E_k) may consist of the following chain of potential differences.

$$E_k = E'_m + E'_{j(k-r)} + E'_{j(k-s)}, \quad \cdots\cdots (1)$$

where E'_m: the real value of membrane potential, $E'_{j(k-r)}$: liquid potential between 3M KCl solution and environmental solution, $E'_{j(k-s)}$: junction potential between 3M KCl solution and sarcoplasm.

In the same way, when the measurement of membrane potential is made with 3M K_2HPO_4-filled microelectrode, the potential obtained (E_{kp}) may consist of the following chain of potential differences.

$$E_{kp} = E'_m + E'_{j(k-r)} + E'_{j(kp-s)} + E'_{j(kp-k)}, \quad \cdots\cdots (2)$$

where $E'_{j(kp-s)}$: junction potential between 3M K_2HPO_4 solution and sarcoplasm, $E'_{j(kp-k)}$: liquid potential between 3M K_2HPO_4 solution and 3M KCl solution. From (1) and (2)

$$E_k - E_{kp} = E'_{j(k-s)} - E'_{j(kp-s)} - E'_{j(kp-k)}.$$

Thus, if the liquid potential between 3M K_2HPO_4 and 3M KCl, $E'_{j(kp-k)}$, is considered as zero millivolt, the difference between membrane potentials measured with KCl and K_2HPO_4 becomes to indicate the difference between junction potentials between each electrolyte solution and sarcoplasm. From the results shown in Table 1, the magnitude of junction potential between the sarcoplasm and the various electrolyte solutions (the side of electrolyte solution positive) are arranged as the following order:

$$3M\ K_2HPO_4 \underbrace{<}_{6\ mV}\ 3M\ KCl \underbrace{<}_{8\ mV}\ 3M\ NaH_2PO_4 \quad \cdots\cdots (3)$$

On the other hand, the mobility of various ions at a definite temperature is high in the order of

$$Cl > K > \tfrac{1}{2}HPO_4 > Na > H_2PO_4,$$

where the difference between Cl ions and K ions is too slight to be almost negligible. But, there are wide differences between K ions and the other ions (Landolt-Börnstein 1927, 1931, and The Chemical Society of Japan 1952). Thus, if the liquid potential between 3M K_2HPO_4 and 3M KCl, $E'_{j(kp-k)}$, is not zero millivolt, in the relation represented in (3) the difference between results with K_2HPO_4 and KCl may increase, since the sign of liquid potential mentioned above being positive at the side of 3M KCl. In the same way, the difference between results with KCl and NaH_2PO_4 may be lower than that shown in (3).

Concerning the junction potential between the electrolyte solution and sarcoplasm, the difference between results with KCl and K_2HPO_4 seems to be due to difference in concentration of ions inside the muscle fibre in which the concentration of phosphate ions is very much higher than that of Cl ions; the difference between results with KCl and NaH_2PO_4 seems to be due to difference in mobility of ions since the rate of movement of phosphate ions is lower than that of Cl ions and also due to difference between intracellular ionic concentrations (the concentration of Na ions is much lower than that of K ions). Besides, it is implied that the K- and phosphate ions are free diffusible inside the muscle fibre (cf. Umezawa 1958).

II. EFFECT OF EXTRACELLULAR PROTEIN COLLOID ON EXCITABILITY AND CONTRACTILITY

C. *Responses of single muscle fibres surrounded by egg white to electrical stimuli*

A muscle fibre was placed in various solutions of egg white (pure egg white, M/8 KCl-egg white, M/8 KH_2PO_4-egg white, and M/8 K_2HPO_4-egg white) and the electrical stimulation was made with two liquid external electrodes under the condition that the interpolar length was 5 mm, and the interpolar region of the fibre was electrically insulated in moistened air (Tamasige 1950, Umezawa 1957). In all cases the responses of fibre to electrical stimuli were a local contraction around the cathode, although the threshold values were higher than the normal value. The value in average in pure egg white was 20 V, 2 msec. or 2 V, 5 μA, 100 msec.; in KCl-egg white it was 35 V, 2 msec. or 8 V, 5 μA, 100 msec.; in KH_2PO_4-egg white, 2 V, 1 μA, 200 msec.; in K_2HPO_4-egg white, 2.5 V, 5 μA, 200 msec.; and in normal Ringer, 0.1 V, 0.5 msec.

In order to ascertain more precisely the differences of threshold the electric excitation of the same fibre surrounded by the test egg white solutions was comparatively studied. Fig. 3 shows the results obtained for several specimens. The threshold values obtained in K-phosphate-egg white are higher than these obtained in the others. It is clear,

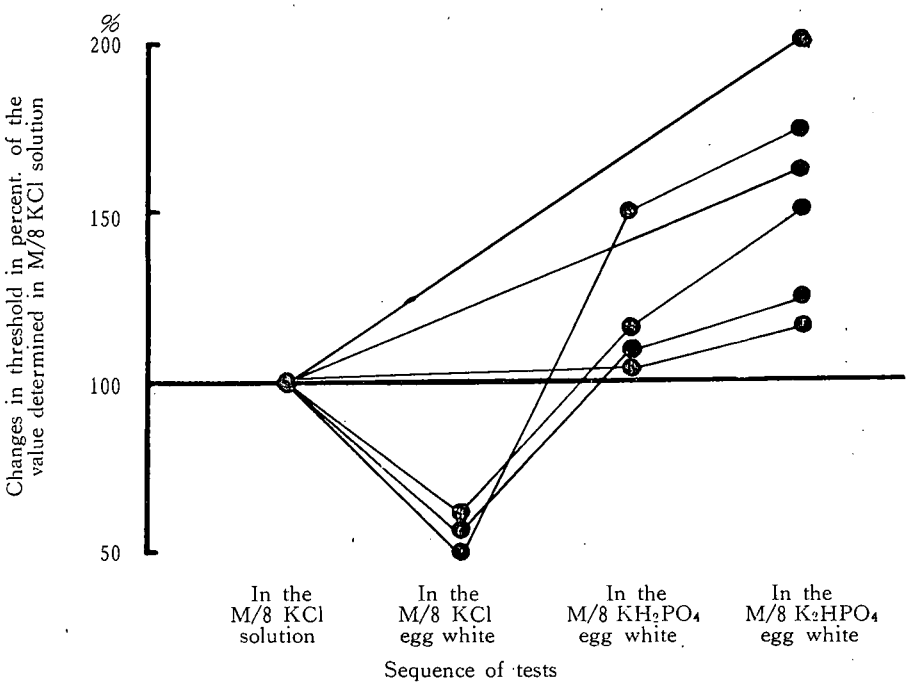

Fig. 3. Effects of various egg white solutions of electrolytes upon the excitability of muscle fibres; room temp.: 28°C.

therefore, that environmental phosphate to be contained in such a protein colloid has an important role on the decrease in excitability of the fibre. Moreover, the fact that the threshold values in KCl-egg white are lower than the values in KCl aqueous solution seems to indicate the inefficacy of potassium in the protein colloid, as was already

pointed out by Tamasige (1952).

Another series of stimulation was made on the muscle fibres treated with M/8 K_2HPO_4-egg white solution, with the one intracellular microelectrode filled with 3M K_2HPO_4 solution and another extracellular, or with the two intracellular microelectrodes. The threshold value of electric excitation in application of the one intracellular negative microelectrode was lower than that in application of the one intracellular positive microelectrode, and was less than 1 μA, 0.2 sec. In the case of application of the two intracellular microelectrodes, being kept inserted within a distance of 50 μ (cf. Hagiwara and Watanabe 1955, Umezawa 1958), the responses of the fibre to electrical stimuli took the form of a local contraction around the tip of the negative electrode. The threshold value was less than 1 μA, 0.2 sec. This indicates that the electrical stimulation is applied to the sarcoplasm without involving the membrane function (the polarizing effect on the membrane).

D. *Myofibrils surrounded by M/8 K-phosphate-egg white* A muscle fibre was placed at first in M/8 KH_2PO_4- or M/8 K_2HPO_4- egg white solution and was torn longitudinally into myofibrils by means of a fine needle. After the treatment, stimuli were applied to the myofibrils is the same manner described in (C). In the myofibrils the cross striation was clearly observed under the microscope. When the applied current was very strong, the local contraction of the myofibrils around the cathode occurred; the threshold value being 90 V, 10 μA, 0.2 sec.

After stimulation the myofibrils were transferred into M/8 KCl solution and the excitability was also examined. The recovery of excitability was fairly complete. The threshold in M/8 KCl solution was lower than that in M/8 K-phosphate-egg white solution; the value being 3V, 2 μA, 0.2 sec. Then after that, when the myofibrils were placed in M/12 $CaCl_2$ solution, the contraction gradually occurred. This seems to be due to the formation of Ca-coagulum. Therefore, it is clear that the isolated myofibrils in the protein colloid which contains phosphate ions at such a concentration has yet contractile mechanism.

From these experimental results, in short, when the muscle fibre is surrounded by a protein colloid which is similar to the inside of muscle fibre, the contractility of the fibre is very inhibited although it is never lost. Thus, if the effect of external medium which is made artificially quite similar to the state of intracellular protoplasm on the physiological phenomena of itself is investigated, both the chemical property of living protoplasm and its physiological function may be able to be understood.

Summary And Conclusion

1. The membrane potential and the responses of single muscle fibres of the frog, *Rana nigromaculata*, treated with various hen's egg white solutions were studied. Besides, the junction potential between sarcoplasm and various electrolyte solutions was measured. Further the myofibrils isolated from the muscle fibre in the egg white were investigated. From these results, the physiological state of living sarcoplasm and the effect of extracellular protein colloid on excitability and contractility in the fibre were

discussed.

2. In K_2HPO_4-egg white the membrane potential is the lowest, the value in average is +2 mV (inside positive); in KH_2PO_4-egg white it is −3 mV; in KCl-egg white, −9 mV; in pure egg white, −28 mV; in Na_2HPO_4-egg white, −30 mV; in NaH_2PO_4-egg white, −37 mV; and in NaCl-egg white, −45 mV. Therefore, the egg white contained with K-phosphate seems to be similar to the intracellular protein colloid, for the electrochemical difference between the inside and outside of the cell is most decreased.

3. The junction potential between the sarcoplasm and 3M K-phosphate is the lowest; and it is lower than the junction potential between the sarcoplasm and 3M KCl, and also is much lower than the potential between the sarcoplasm and 3M Na-phosphate. The difference between results with K-phosphate and KCl and that between results with KCl and Na-phosphate is about 6 mV and 8 mV respectively. This seems to be due to difference among concentrations of intracellular ions, e.g. K-, phosphate-, Na- and Cl-ions.

4. Mechanical responses of the fibre to electrical stimuli are a local contraction around the cathode in all test egg white solutions used in the present experiments. In the myofibrils placed in K-phosphate-egg white solution also a local contraction around the cathode occur, when a strong current is applied.

5. It is suggested that both potassium- and phosphate-ions are much contained and freely diffusible in the intracellular protein colloid whereas the contractility of the fibre surrounded by a protein colloid is very inhibited although it is never lost.

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REFERENCES

Boyle, P. J. & E. J. Conway 1941 J. Physiol., 100, 1.
Fenn, W. O. 1936 Physiol. Rev., 16, 450.
Hagiwara, S. & A. Watanabe 1955 J. Physiol., 129, 513.
Landolt, H. H., R. Börnstein, W. A. Roth & K. Scheel 1927, 1931 Physikalisch-Chemische Tabellen, I. Erg.-Bd., 619, II. Erg.-Bd., 1061.
Needham, J. 1950 Biochemistry and Morphogenesis. Cambridge Univ. Press.
Tamasige, M. 1950 Annot. Zool. Japon., 23, 125.
———— 1952 Ibid., 25, 15.
The Chemical Society of Japan 1952 Chemical Table, 2nd, 614.
Umezawa, S. 1957 J. Fac. Sci. Hokkaido Univ. Ser. VI (Zool.), 13, 479.
———— 1958 Annot. Zool. Japon., 31, 127.

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