STUDIES ON THE INTRACELLULAR AND EXTRACELLULAR POTENTIALS OF THE LYMPHATIC HEART IN *RANA NIGROMACULATA.*

MITSURU OKI

From the Department of Physiology
(Prof. G. KAWABATA)
Yamaguchi Medical School. Ube, Japan
(Received March 9, 1961)

Since an introduction of microelectrode technique by Ling & Gerard in 1949 (1), a vast number of successful intracellular potential recordings has been reported upon the electro-physiologically excitable tissue/cells, such as nerve, skeletal and cardiac muscle. Using the smooth muscle as an experimental material, however, a little results have been reported hitherto. This is because that the smooth muscle cells are so small that the insertion or fixation of microelectrode-tip into the cell is very difficult.

An anatomy of the frog's lymph-heart is described in elsewhere (2). Although its muscle-cells have the cross-striations, as can be seen in the following figure, the phylogenetic differentiation of the cell may not be so specific as cardiac one. Then, it is desirable to observe the electro-physiological behaviour of the lymph-heart under the plausible presumption that the cells might have physiological function intermediate between cardiac and smooth muscle-ones. That is, the muscle cells of lymph-heart might have a different character from that of the well-known cardiac ones, and the experimental data obtained from the present preparation would be very useful to elucidate the physiological function of the smooth muscle.

MATERIALS AND METHODS

Experiments were carried out with an uni-lateral posterior lymph-heart of the frog (*Rana nigromaculata*) in summer. The beats could be seen from outside with naked eye. The overlying skin and subcutaneous connective tissue were separated under the dissecting microscope, and the lymph-sac (*Cavum perilymphocardicum*) was exposed. A small amount of lymph was dropped out by this procedure without influencing the experimental results. To avoid dryness of the tissue it was sometimes covered with liquid paraffine.

The animal in a glass vessel was placed on the silver plate (5×5 cm^2), the surface of which was previously converted to silver chloride. This plate was an indifferent electrode. Adequate amount of Ringer's solution was poured into the vessel to acquire electrically good contanct. For an investigation of the intracellular action potentials of muscle cells, *in situ*, the glass-capillary electrode of Ling

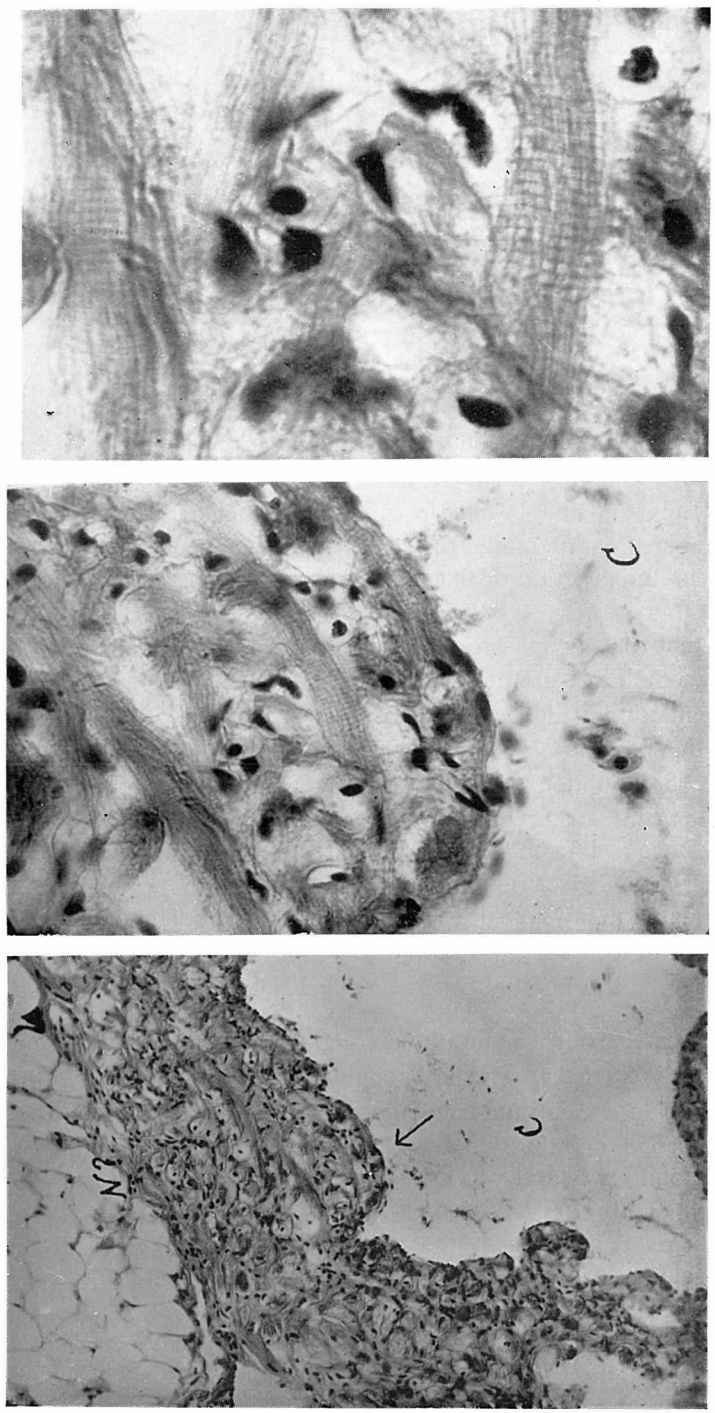

Fig. 1 Optical microscopy of frog's lymph-heart.
From left to right, magnification is larger and may be computed from the size of erythrocyte in the picture. The magnified region was indicated by the arrow in the left one. Mark C indicates "Cavum lymphocardicum", and N? may be (could not be assured as) a nervous element.
Note that the muscle cells have the transverse striations and are not organized regularly. The connective tissue cells can be seen abundantly in the middle layer, and the longer axis of the muscle cell is not directed uniformly.

& Gerard type (1) was used as an exploring one. Having the tip diameter of less than one micron, it was filled with 3 molar KCI solution by boiling under negative pressure, and was flexibly mounted on the preparation with micromanipulator after the method of Woodbury et al(3). In the case of extracellular lead, so-called "wick-electrode" was used as an exploring electrode: i.e the tip of tapered glass tude was propped by the conic Ringer-soaked cotton wool, one side of which was in contact with leading Ag-AgCl wire in the glass tude and the other sharper end was placed on the surface of the preparation. A constant volume of Ringer's solution existed at the tip of the tube, as this is the capillary in physical term.

The differerential direct-coupled amplifier and double beam cathodrayoscilloscope or ink-writing electromagnetic one were employed for recording.

RESULTS AND DISCUSSION

1. The histology and intracellular potentials.

That the muscle cells of lymph-heart have cross striations is described in the literature (2, 4), and the optical microscopy of the preperation was attempted to verify them. Their photographic pictures are shown in **figure 1.** The above mentioned cross striations are well illustrated. It must be noted, in addition, that the fusiform muscle cells are not organized regularly or compactly and that the connective tissue cells can be seen abundantly in the middle parenchymatous layer. Seeing the figure, one might well suppose that the long lasting lead-off of the intracellular potentials from the beating lymph-heart is not so easy as that from the skeletal muscle or the blood-heart.

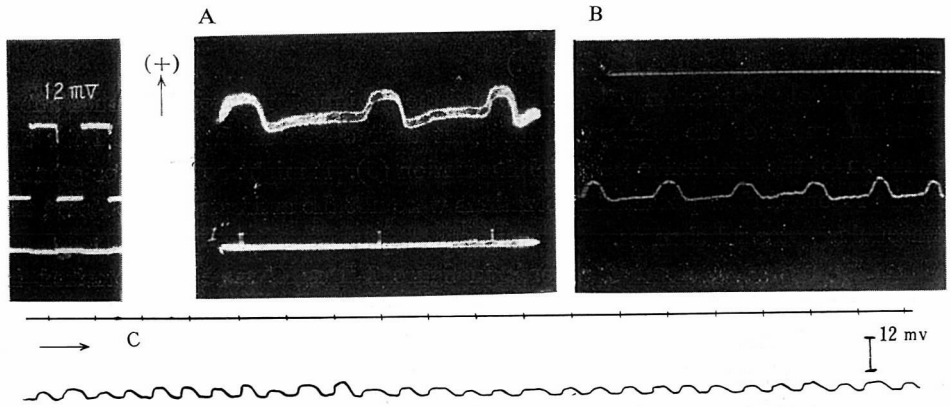

Fig. 2 Electrograms of frog's posterior lymph-heart, *in situ*, obtained with the so-called flexibly mounted microelectrode technique.
Upper figures (A & B) was recorded with double beam cathodray-oscilloscope, and lower ones (C) with ink-writing electromagnetic one. Amplification and sweep velocity were indicated in the figures. Time marks are 1 second apart.
Positivity of the exploring electrode was indicated by the upward deflection in the figure. The recording level before the insertion of microelectrode was not on the another beam (time base); the value of resting potential can not be measured from the figures.

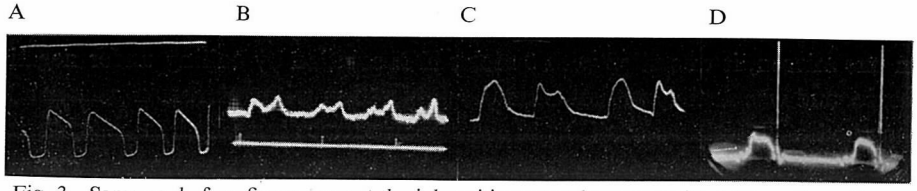

Fig. 3 Same as before figure, except the ink-writing records were omitted. (Sometimes these electrograms were obtained instead of those in figure 2. See in the text for details.)

The recorded electrograms using the microelectrode are shown in **figs. 2 and 3.** Contrary to the earliest expectation that the intracellularly recorded electrograms of lymphocardiac muscle might have similar configuration as that of cardiac one, although sometimes it was the case as shown in **fig. 3-A**, almost all records were those of **figure 2-B** or **C. Figure 3-B** or **C**, in which the wave of potential fluctuation had two or more peaks, was also obtained sometimes, but **fig. 3-D** was very rare. These differences in configuration may be explained as follows.

In the first place, it is possible that the slow potential fluctuation in the figures might be the result of mechanical or physical artifacts. Although the rate of fluctation was temporarily coincident with the beat of lymph-heart observed by the naked-eye, the corresponding changes of the contact-area of the electrode could not be considered in the experiments. As for the displacement of the electrode-tip, it was very plausible that the tip was broken or pulled out off the muscle cell by the mechanical movement. In these cases, however, the base-line for the recorded curves had to be displaced or the direction of the beam-deflection be inverted. The injury of the cell membrane would be shown by the gradual dissipation of the established potential or by the not-establishment. (Here, it was out of the question whether the establishment was full or not. See below.)

The physical polarization of electrode and the tip junction potential would affect the measurement of the resting potential, but their effects might be small for the evaluation of the qualitative potential fluctuation (5). It might be taken for granted that the recorded slow potential fluctuations were not the artifacts, but reflected the intracellular potential changes.

Secondly, the most frequent records in figure 2-B or C were characterized in their slow depolarization and equally slow repolarization. The configuration of the curve was designated as "dome-typed" by Ochiai et al (6). Also their small amplitudes were noted, and if there were not short-circuit owing to the injury, it might be reasonably postulated that the potential fluctuation would not be a spike-discharge such as could be seen in cardiac or skeletal muscle-cells, but a local potential resulted from the activity of adjacent muscle cells or of nervous elements (7). If this were a case, however, there might also have to exist another potential fluctuation corresponding the spike-discharge. The first potential fluctuation in figs. 3-B, C, or D might be a distorted spike-potential and second one a local potential. But, as the

amplitudes were small in B and C, and as D was an exceptional case, the problem could not be settled confidently. (To the authors' feeling, the first potential fluctuation may be not a spike-discharge but a local potential.)

Lastly, it is worthy to discuss that each curve had not the same amplitude and that the repolarization did not occur promptly after the depolarization. The latter might be the same as the "plateau" in the intracellular action potential of cardiac cells (8). The next figure may be consulted here.

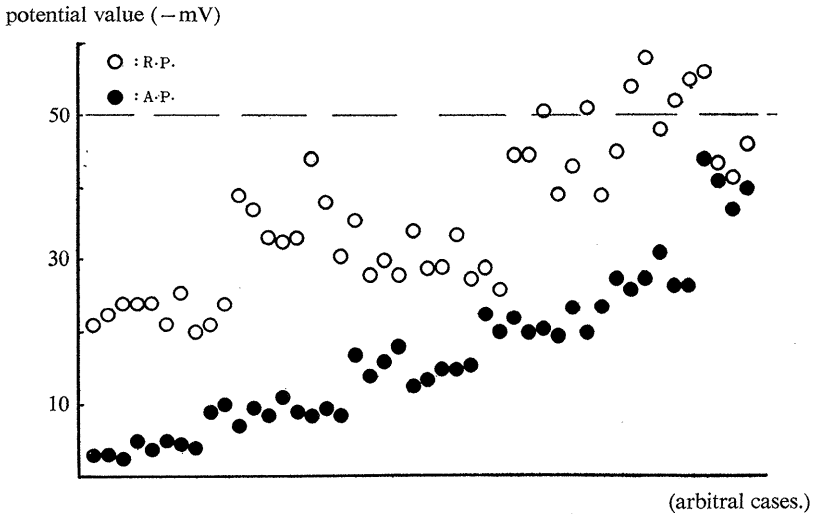

Fig. 4 A prospect of the measured potentials.
Abscisse is not a scale, showing the experimental cases arbitrarily. Each open-circle shows the deviation of the recording spot/point before and after the insertion of the microelectrode; solid circle, corresponding the above open-one, was the mean amplitude of those potential-variations, when almost the same configurations were recorded for over the 5 minutes.
Those values on the ordinate were tentatively assumed as resting potentials (R. P.) and as action potentials (A. P.) respectively.

Figure 4 is a prospect of the experimental results for potential measurement. The deviation of the recording spot before and after the insertion of the microelectrode was calibrated, and tentatively assumed it to be the resting potential. At the same time, after the tip was rightly inserted into the cell the amplitude of each potential fluctuation would be the value of action potential. A part of the experiment is written on the abscisse, case-by-case. Seeing the figure, the author felt that each value would not be consistent but variable. Some of the low potential values are, presumably, resulted from the cell injury mentioned before and from the physical tip junction potentials dealted with in the other paper (5). On the other hand, as the recordings were carried out regularly and carefully for over 5 minutes, the remaining examples might be true without those artifacts. Then, it is possible to offer the

posturation that the muscle cells of lymph-heart might have a functionally different polarized state. In other words, if the resting potential were small, i.e. the cells were in the depolarized state, small amplitude might be recorded for the intracellular action potential.

Refering to the above posturation, the following experiment of Dr. M. Kuno, will be noted: that is, when the muscle cells of lymph-heart are artificially brought to the depolarized state by the catho-electrotonus, the intracellularly recorded potential fluctuation becomes smaller. Inversely, the amplitude is larger in the case of hyperpolarization.

The graded response of the preparation, which has been frequently discussed by many authors (8.9), may be well explained from the above posturation. Also, the so-called "double-spike" (6) meaning the twice potential fluctuation in the intracellular records corresponding one mechanical beat, and which is also seen in this experiment in figure 3-C or D, may be a fluctuation of the depolarized state. It is preferable not to use the term (spike).

It has been clarified that the muscle cells of the lymph-heart are innervated by the spinal center via IX-th to XI-th spinal nerves (10, 11). How is the mechanism of intracellular potential fluctuation after the arrival of nerve-impulses? Moreover, our knowledge is scanty concerning the relationship between the established potential and the contractile mechanism of the muscle. More experiments and thinkings may be necessary to extend the posturation farther. Considering the fact of neurosecretion, however, the author realized that the slow potential fluctuation recorded in this experiment and the graded polarized state above imagined might have some relationship to the metabolic state of the cell.

2. The surface electrogram and its comparison with intracellular potential.

It is a well-known fact that the surface electrogram in this experiment is, strictly speaking, not a monopolar but a bipolar one. The recorded potential fluctuation reflects the vectorial summation of the action potentials of many contractile units (the unit, here, means not always a single muscle cell), and the possibility to involve the mechanical artifacts such as the dislocation and the change of contact area of the electrode-tip, is greater than that in the experiment with microelectrode. The analysis of the configuration of recorded waves with the surface electrode is very complicated. Moreover, in the case where intracellularly recorded waves had not the same and only one configuration (see above), the waves might have more than two configurations even if many conractile units neighbouring the electrode-tip would have temporarily similar activity. In spite of these difficulty, the surface lead is indispensable for the studies of biological action of some pharmacological agents.

The surface electrogram and the intracellular potentials were observed simultaneously, and the factors determining the configuration were studied. **Figure 5** is one of these experiments showing that the biphasic or monophasic waves could be

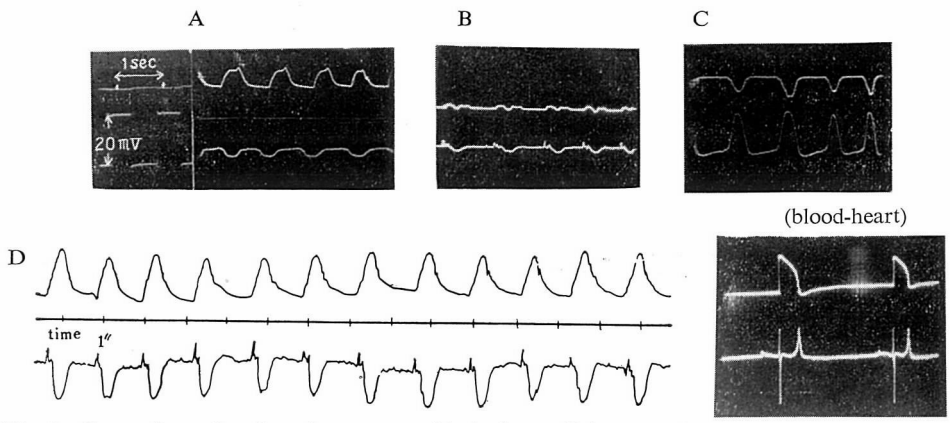

Fig. 5 Comparison of surface electrogram with the intracellular potential.
Upper beams were recorded with the microelectrode and lower ones with the wick-electrode. Time mark and amplification were written on each figure. The distance of two electrode was 1-3mm. apart. Similar record using a blood-heart as an experimental material was inserted at lower-right, for a reference.
Note the time relationship and configuration of each wave.

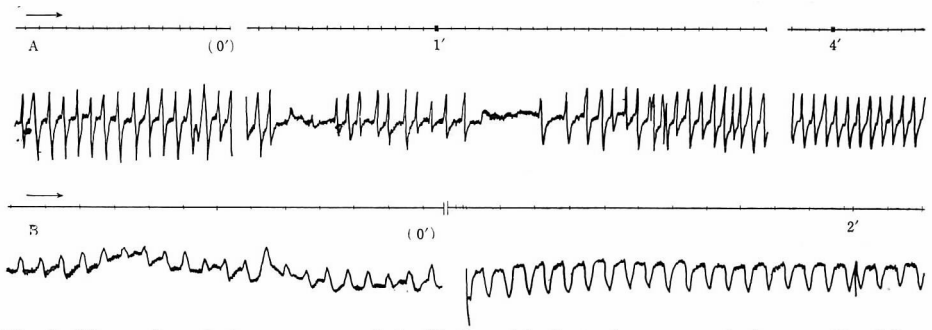

Fig. 6 The surface electrograms recorded with the wick-electrode as an exploring one. Read from left to right.
Upper series (A) indicate a change of electrogram after the administration of acetylcholine; and lower ones (B), of d-tubocurarine. The dosage of the drug was 0.1 and 1.0 mg. per 100g body-weight of the animal respectively. The control curves were shown at the left side, and the elapsed time after the administration was written on the figures. Time marks are 1 second apart; and the negativity of the exploring electrode was directed downwards.
Note the changes of configuration in A, and the displacement of base-line and the polarity in B. For details, see in the text.

recorded in the former when there were "dome-typed" monophasic potentials in the latter. The similar record with the cardiac muscle is inserted in the lower-right of the figure, for a reference. In these records, as described in the previous report (12), negatively directed waves in the surface electrogram corresponded to the depolarization of the muscle cell (i.e. positive deflection of the monophasic wave in the intracellular recording.): and positive ones in the former did to the re-

polarization in the latter. Also it has been discussed that the configuration of the wave in the extracellular records can be explained as a differential of that in the intracellular ones. These relatively simple explanation for the correspondence between the two records, however, might be applicable only when the experimental conditions were under such that many units neighbouring the electrode-tip had the synchronous activity and that the contact-area of the electrode would be very small. Indeed, another configuration was frequently observed (fig. 6-B), where the positive deflection was not remarkable and the main deflection was negatively directed, and the waves seemed to be monophasic in the figure.

In other words, if the waves in the surface electrogram were negatively directed and seemingly monophasic, the depolarization of the muscle cells might be, but the repolarization might not be studied by analysing the waves. For instance, slow and linear repolarization in the intracellular potential might not be recorded extracellularly, because the latter might be considered to be a differential of the former. Many other explanations may be possible for discussing the difference of the configuration in the two records. It is plausible, however, that the above mentioned one is mostly worthy to note.

The changes of the configuration in surface electrogram after the administration of acetylcholine or d-tubocurarine are shown in figure 6. The frequency and the amplitude of potential fluctuation were discussed in another report (13). The displacement of the tentatively posturated base-line and the direction of the waves must be noted in figure 6-B; where, if it were assumed that each potential fluctuation might have the same configuration, the polarity of each wave might be inverted owing to the effect of the pharmaca. In the case of intracellular lead with microelectrode, it is reasonable that the displacement of electrode-tip from the inside of the cell to the outside would cause the invertion of polarity. Where the lead is extracellular, on the other hand, another factor must be the cause.

It is regretable that the employed amplifier was C-R one, and the displacement of base-line was not recorded clearly. To authors' subjective feelings, however, there was indeed the dispacement accompanying the invertion of polarity above mentioned.

Generallys speaking, it is possible to regard a resting potential as the standard base-line in such an experiment as researching action potential of skeletal muscle or of nerve-fibre: in which the polarized state is a stable one and the resting-potential value is constant so long as it does not set into activity. With those preparations such as cardiac or lymphocardiac muscle, in which the intracellular potential shows the constant fluctuation, on the other hand, it is questionable to regard the base-line analogously. Especially, if the polarized state of the lymphocardiac muscle were not the only stable state, and if the depolarized one had also a similar nature, as imagined in the above section, it may be fruitless to posturate the standard base-line for the elucidation of intracellularly or extracellularly recorded potential fluc-

tuation. The working hypothesis is presented as follows. The lymphocardiac muscle is in the polarized state at the left-half of figure 6-B and the main potential fluctuations in the surface electrogram are negatively directed. After the administration of d-tubocurarine, the polarized state of the preparation is changed to the depolarized one resulting the invertion of polarity of the main wave.

SUMMARY

1. Monopolar electrograms of posterior lymphatic heart of the frog, *in situ*, were observed with the so-called flexibly mounted (3), intracellular microelectrode. The pencil-typed "wick-electrode" made of glass tube and cotton wool was also used as an exploring one.

2. The "overshoot" of action potential could not be observed. The duration and the amplitude had no constant value. It could not be assured, however, that the intracellular recordings were always successful.

3. A consistent value for the resting potential of the muscle cell was not obtained. It ranged from 20 millivolts to 58, and it is assumed that these varaitions were due not to the failure of the experiments, but to the variable polarized state of the preparation.

4. Monophasic and biphasic potential fluctuations could be seen in the extracellularly recorded electrogram. Comparing them with the simultaneously recorded intracellular potential, it was concluded that the depolarization of the cells could be studied by researching the negatively directed main deflection in the former. It was difficult to analyse the repolarization.

5. An assumption that the depolarized state might also be a stable one in the lymphatic heart seemed to be plausible; whereas, the polarized state was stationary and depolarization was transitory in the skeletal muscle or in the nerve-fibre. The finding was based on the following experimental results.

①. The intracellular potential fluctuations had not a consistent configuration, e. g. the duration of action potential or that of posturated "plateau" was not constant. ②. The adequately measured resting potential had a very low value. ③. The polarity of main deflection in the surface electrogram was inverted accompanying the displacement of base-line, after the administration of d-tubocurarine.

Grateful acknowledgements are due to prof. G. Kawabata for his supervision of this study.

REFERENCES

1) LING, G. & GERARD, R. W.: The normal membrane potential of frog sartorius fibres. *J. Cell. Comp. Physiol.* **34**: 382–393, 1949.
2) ECKER GAUPP.: *Anatomie des Frosches.* 3te Auf.: Braunschweig, 1896.

3) WOODBURY, J. W. & BRADY, A. J.: Intracellular recording from moving tissues with a flexibly mounted ultramicro-electrode. *Science*, **123**: 100p, 1956.
4) KAMIJIO, K.: Pharmacological studies of lymphatic heart. *Nihon Yakurigaku Zassi*, **47** & **46**: 1950 & '5i. (in Japanese)
5) OKI, M.: Studies on the tip junction potentials of the microelectrode and re-evaluation of intracellular potential of *Nitella flexilis*. *Yamaguchi Igaku*, **9**: 1341–1344, 1960. (in Japanese)
6) OCHIAI, M.: Comparative studies on the transmembrane potentials of the cardiac muscle fibres of lower animals. *Igaku Kenkyn* (at Kyushu University), **29**: 4589–4602, 1959. (in Japanese)
7) OBARA, S.: On the nerve muscle system of amphibian lymph-heart in relation to the skeletal muscles. *Acta Medica et Biologica* (at Niigata University), **7**: 133–147, 1959.
8) GOTO, M. & TAMAI, T.: *Modern aspect of the electrophysiology of involuntary muscles*. Kinpodo publishing Co. Kyoto: 1960. (in Japanese)
9) OGATA, T.: The lymphatic heart. *Igaku Souhou*, **1**: 319–386, 1947. (in Japanese)
10) MORITA, I.: Beiträge zur Physiologie des Lymphherzers. *Nagasaki Igakuzassi*. **15** & **16**: 895p & 371p, 1937.
11) OKADA, H.: The regulatory mechanism of the vagus nerves upon the lymphatic hearts' movement. *Nihon Seirigaku Zassi* **15**: 324–333, 1953. (in Japanese)
12) KAWABATA, G. et al.: Some considerations on repolarization process of ventricular muscle, represented by the T-was in the ECG. *Bull. Yamaguchi Med. School.*, **7**: 117–125, 1960.
13) OKI, M.: Effects of some autonomic nervous poisons on the electrogram of lymph-heart., *Yamaguchi Igaku*, **10**: 1961 (in press, in Japanese).