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**Experimental Studies on the Relationship between
Electrical and Mechanical Events in the Toad Heart (II)**

ON THE RELATIONSHIP BETWEEN THE CHAIN-DOUBLETS THEORY
AND CARDIAC METABOLISM

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INTRODUCTION

As mentioned in the previous report, studies on the electrical and mechanical events of the cardiac muscle have developed not out of the medical or biological basis but out of the physical basis, such as mechanics or energetics.

As regards the muscle contraction, Kalcker¹⁾ made an exact research on the energy aspects of the metabolism of the muscular system with a mathematical treatment, and the energy expended in movement was attributed to the energy liberated in the degradation of the energy source material. According to Bethe^{2,3)}, on the other hand, the relaxation is the forerunner of the muscle contraction, and only the relaxation renders the muscle capable of new work, while in the phase of contraction there is no working capacity for excitation of the muscle. Fick, von Kries and Blix⁴⁾ demonstrated a linear relation between the contractile force of a skeletal muscle and the degree of length and of tension to which this muscle was subjected prior to stimulation. Such a relation was also first observed by Howell and Duke⁵⁾ in the cardiac muscle, in relationship to the venous influence, the systolic output and minute volume. Later Frank⁶⁾ has conclusively shown that the law of initial length was applicable in the cardiac muscle as well as in the skeletal muscle. In 1941, Maestrini⁷⁾ showed that there was a direct ratio between the myocardial fibre length and the work done in the frog heart. In the same year, Starling⁸⁾, Wiggers^{9,10)}, and Straub^{11,12)} con-

firmed the results obtained by Frank. Wiggers has eventually come to the conclusion that the law of the heart is the law of the initial length. The conception proposed by Bethe has been thus supported by the law of the initial length.

On the other hand, the energy-mechanical interpretation on the muscular machine has recently made progress. In 1868, myosin could be for the first time extracted with salt solution by Kuhne, and its physico-chemical properties was minutely studied by Edsall. Weber further succeeded to obtain a thread by jetting myosin-salt solution into water through a small hole. Since then, several studies have been focused on physico-chemical properties of myosin thread or myosin membrane, such as swelling, X-ray diffraction and elasticity. It was, however, not until 1939 that the main contractile protein was confirmed to be myosin.

As regards sources of energy, Lundsgaard demonstrated that the muscular contraction was still preserved under the complete obstruction of glycolysis and respiration. The first chemical reaction during muscular contractions was proved by Lohmann and Meyerhof to be the splitting of ATP to ADP and inorganic phosphate. The discovery by Engelhardt and Ljubimova, that myosin has enzymatic activity, acting as ATPase, suggested a close relation between the reaction of myosin with ATP and the muscular contraction. Szent-Györgyi^{13,14)} discovered that myosin threads had a contractile property when immersed in ATP solutions containing small amounts of potassium and magnesium ions. The addition

of ATP to myosin solutions resulted in an increase in viscosity and changes in its physico-chemical properties (Szent-Györgyi and Needham). From further work made by Szent-Györgyi and Straub, it appeared that myosin extracted by Kuhne was not a single substance but a compound of actin and myosin in a narrow sense, and that the action of ATPase was shown only in the form of actomyosin but not in the form of myosin in a narrow sense. Further work soon led to the discovery, by Bailly¹⁵⁻¹⁷, of a third protein, tropomyosin which was obtained in the crystalline form. Thus, in the present time it has been generally accepted that myosin, actin and tropomyosin constitute the main proteins in the muscle.

When proposed the chain-doublets theory for the first time, Prof. Maekawa¹⁸⁻²⁰ has likened electrical generation, contraction and light refraction occurred during the excitation of cardiac muscles to a piezoelectricity phenomenon. However, with a progress in physico-chemical studies on the muscular contraction, the interpretation by the chain-doublets theory was improved, and a plausible entity of the muscular contraction was attributed to the ATP-actomyosin system. And the electrical generation in excitation was supposed to be due to the intramolecular transformation of myosin. In the electrocardiographic study generally accepted up to the present, it has been supposed that the cause of electrical events consists in the cell membrane and that of mechanical events in the myocardial parenchyma. These two events, however, was unitarily attributable to the myocardial parenchyma in the theory of chain-doublets. The theoretical conclusion deduced from this theory would be then that the mechanogram of cardiac muscle is always in accordance with the integration curve of the monophasic action current under any circumstances. And when the ATP-actomyosin system is directly or indirectly affected by some treatments, changes in the contraction should be in parallel with changes in the electrical generation.

METHODS AND RESULTS

The experimental procedures have been essentially similar to those described in the previous report. The excised toad heart perfused with Straub's cannula was placed in a glass dish on which cotton moistened with Locke's solution was spread. The perfusion pressure was maintained at 4 to 6

cm H₂O during the contraction and at 3 to 5 cm H₂O during the relaxation. The total perfused fluid was 5 to 7 cc. The experimental temperature ranged from 3.0°C. to 12.0°C. A nonpolarizable electrode of silver-silver chloride type was used. For recording the monophasic action current a different electrode was covered with a 3 M-KCl solution soaked cotton gauze pad and was put on the surface of the heart. An electrical integrator was used to obtain the integration curve. The indifferent lead electrode was placed at the circumference of the glass dish. The mechanogram was recorded with a recorder which made use of the Hartley circuit.

Thus simultaneous recording on three events being made, potential changes under administration of some drugs and salt solutions were photographed with long run camera. The amount of drugs or salt solutions added to the perfusate was 0.05 cc., and the concentration was adequately adjusted, in order to minimize the effects of changes in pressure and concentration of the perfusion fluid. In Summer, the excised perfused heart was placed in an ice-box so as to keep the experimental temperature at 3.0°C. to 12.0°C. All observations were made for about 30 minutes.

(1) *Electrolytes (Sodium chloride, Potassium chloride, Calcium chloride, Magnesium chloride and Sodium fluoride)*

(a) Sodium chloride: The concentration of sodium chloride in the perfusate was altered from 110mM to 150mM by dropping 0.05 cc. of 4 M-NaCl solution. Under such circumstances the mechanogram was converted into the contraction type, and the fluid level during relaxation was raised. The integration curve showed a slight increase in amplitude without changes in the gradient. In the monophasic action current there was little change or occasionally a slight increase in the voltage. A prolongation in the Q-T time was observed. Consequently the total amount of electricity generated increased (Fig. 1). Sodium chloride concentration more than 200mM caused a remarkable decrease in the monophasic action current, while no appreciable change in the contraction was observed. This may likely be due to an artificial short circuit between tissues of cardiac muscles by high concentration of sodium chloride. When the heart ceased to beat at a late stage of perfusion, an alteration of the concentration of sodium chloride from 110mM to 150mM often caused to restore the

beating. High concentration of sodium chloride in the perfusate also caused to decrease the rate of beat.

(b) Potassium chloride: Locke's solution for the frog contains 1 mM of potassium chloride. In the present experiment the concentration of potassium chloride in the perfusate was altered, and observations were carried out at varied concentration from 1 mM to 6 mM. In the higher concentration than 4 mM of potassium chloride, the heart usually ceased to beat in a few seconds after the application. Within the range of concentration of 2 to 3 mM, the heart continued to beat over long periods. Perfusion with high concentration of potassium chloride caused to convert the mechanogram into the relaxation type, and in consequence the fluid level fell in both the phases, systolic and diastolic. The pulsation pressure decreased. The integration curve also decreased in the gradient and the amplitude. In the monophasic action current there was a reduction in the voltage. The Q-T time was shortened. The total amount of electricity generated therefore, decreased. There was also an increase in the pulse rate. Thus, the effect of high concentration of potassium chloride on these three curves was just opposite to that of high concentration

of sodium chloride (Fig. 2).

(c) Calcium chloride: Locke's solution for the frog contains 2 mM of calcium chloride. In the present study observations were made at varied concentration of calcium chloride, 2 mM to 10 mM. In about 4 mM calcium chloride the mechanogram became the contraction type, and the fluid level was raised in both the systolic and diastolic phases. In further high concentration of calcium chloride the cardiac muscle fell into the contracture state, the relaxation being incomplete. High concentration of calcium chloride caused to increase the gradient and amplitude of the integration curve. In the monophasic action current there was an increase in the voltage, but changes in the Q-T time were irregular. Generally speaking, the Q-T time seemed to be shortened by dropping calcium chloride when the heart showed a powerful beat in the winter toads, while the results were the reverse under the deteriorated condition or in the summer stage. The pulse rate was unchanged.

(d) Magnesium chloride: Locke's solution used in the present experiment did not contain magnesium chloride. But observations were performed at varied concentration (1 mM to 5 mM) of magnesium chloride added to the perfusate. No

Fig. 1 left (A): before the application of NaCl solution. right (B): 5 min. after the application of NaCl solution. from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c).

Fig. 2 left (A): before the application of KCl solution. right (B): 5 min. after application of KCl solution. from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c) respectively.

appreciable change was observed in the mechanogram, the integration curve and the monophasic action current.

(e) Sodium fluoride: A small amount (0.05 cc) of 500mM sodium fluoride was dropped into the perfusion fluid (5 cc). In first several minutes there was little change in the mechanogram, the integration curve and the monophasic action current. After a few minutes, however, the mechanogram became the relaxation type, and the fluid level fell in both the systolic and diastolic phases. The gradient of the integration curve became small and its amplitude decreased. In the monophasic action current there was a decrease in the voltage. The Q-T time was shortened. The pulse rate was little changed (Fig. 3).

(2) *Salts of heavy metals (Copper sulfate, Silver nitrate, Ferric chloride and Zinc sulfate)*

(a) Copper sulfate: A small amount (0.05 cc) of 500mM copper sulfate solution was dropped into the perfused fluid (5 cc). In a few beats after the application, the mechanogram showed the relaxation type, and the fluid level fell in the systolic and diastolic phases. This change reached a maximum in about twenty beats after the application, and gradually transferred to the contracture state. The

heart ceased to beat in the contracture state in about 10 minutes after the application of copper sulfate. The integration curve first showed a decrease in the gradient, but as the contracture state advanced both the gradient and the amplitude increased. In the monophasic action current first the voltage decreased, and the Q-T time was unchanged. But the contracture state was followed by an increase in the voltage of the monophasic action current and a decrease in the Q-T time. The pulse rate remained unchanged.

(b) Silver nitrate: A small amount (0.05 cc) of 500mM silver nitrate was dropped into the perfused fluid (5 cc). Though the perfused fluid became muddy by precipitation of silver chloride, the effect of silver nitrate was still observed. In five minutes after the application the mechanogram became the relaxation type, and the contracture state was attained in further five minutes. In the relaxation period the integration curve showed a decrease in the gradient and the amplitude. This decrease, however, was restored as the contracture state was attained. In the relaxation period there was a decrease in the voltage of the monophasic action current, and the Q-T time was little changed. As the contracture state advanced, the voltage of

Fig. 3 left (A): before the application of NaF solution. right (B): 5 min. after application of NaF solution. from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c) respectively.

Fig. 4 FeCl_3 solution was dropped at the arrow. from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c).

the monophasic action current gradually increased and the Q-T time was shortened. The pulse rate was unchanged.

(c) Ferric chloride: A small amount (0.05 cc) of 500mM ferric chloride was dropped into the perfused fluid (5 cc). The mechanogram first showed the relaxation type but immediately transferred into the contracture state. The gradient and amplitude of the integration curve decreased until the contracture state was accomplished. In the monophasic action current the voltage decreased and the Q-T time was shortened. The pulse rate was not changed (Fig. 4).

(d) Zinc sulfate: A small amount (0.05 cc) of 500mM zinc sulfate was dropped in the perfused fluid (5 cc). The mechanogram became the relaxation type, and the fluid level fell in both the systolic and diastolic phases. The effect of zinc sulfate, however, was different from that of other salts of heavy metals in that the contracture state was hardly attained. In the integration curve there was a decrease in the gradient and the amplitude. The voltage of the monophasic action current also decreased. The Q-T time was little changed.

(3) *Adrenaline and other drugs (Adrenaline, Noradrenaline, Acetylcholine, Phlorhizin, Dinitrophenol and Monoiodoacetic acid)*

(a) Adrenaline and Noradrenaline: Both these drugs caused to increase the contraction and the electrical generation even in very low concentration. With 10^{-4} to 10^{-5} per cent of these drugs the effects could be obviously observed (Fig. 5). In a few beats after the application of adrenaline or noradrenaline, and contraction became stronger, and the mechanogram showed the contraction type. In consequence, the perfused fluid level was elevated in both the systolic and diastolic phases. In about twenty beats after the application of drug the contraction height gradually increased and then continued to maintain the maximal height. In the integration curve there was an increase in the gradient and the amplitude. In the monophasic action current the enhanced voltage and the prolongation of the Q-T time were observed. The positive after-potential was conspicuously observed.

The effect of adrenaline was also observed on the excised heart which was previously administrated with several salts or drugs, such as sodium chloride,

Fig. 5 left (A): before application of adrenaline.
right (B): 5 min. after application of adrenaline.
from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c).

Fig. 6 left (A): before application of NaCl solution.
middle (B): 5 min. after application of NaCl solution.
right (C): 5 min. after addition of adrenaline to the above
from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c) respectively.

acetylcholine, phlorhizin, dinitrophenol and monoiodoacetic acid.

(b) Sodium chloride: The concentration of sodium chloride in the perfused fluid was altered from 110mM to 150mM, and consequently the contraction and the electrical generation increased. Adrenaline was dropped into the perfused fluid provided so as to reach 10^{-4} per cent of the perfused fluid. The mechanogram became the contraction type so that the perfused fluid level was raised. In the integration curve there was an increase in the gradient and the amplitude. The voltage of the monophasic action current remarkably enhanced, and the positive after-potential appeared conspicuously. There was little change in the Q-T time. From this it may likely be considered that adrenaline has an action similar to sodium chloride (Fig. 6).

(c) Potassium chloride: The concentration of potassium chloride in the perfused was previously altered from 1mM to 3mM so as to make the contraction and the electrical generation increase. Adrenaline was dropped until the concentration reached 10^{-4} per cent of the perfused fluid. The heart, however, was not affected by this addition of adrenaline. The action of adrenaline was thus apparently suppressed by potassium chloride which was previously given (Fig. 7).

(d) Acetylcholine: Perfusion with 10^{-6} – 10^{-7} per cent acetylcholine added to the original perfused fluid cause a obvious reduction in the contraction and the electrical generation. By dropping acetylcholine solution the mechanogram became the relaxation type so that the fluid level fell in both the systolic and diastolic phases. In the integration curve both the gradient and the amplitude decreased. There was a decrease in the voltage of the mono-

phasic action current. The Q-T time was unchanged. An addition of adrenaline (10^{-4} per cent) after the previous application of acetylcholine caused to restore the contraction and the electrical generation to the normal state.

(e) Phlorhizin: A small amount (0.00cc) of saturated phlorhizin solution was dropped into the perfused fluid (5cc). In about five minutes after the application the mechanogram became the relaxation type so that the fluid level fell in the systolic and the diastolic phases. There was a decrease in the gradient and the amplitude of the integration curve. The voltage of the monophasic action potential decreased but the Q-T time was unchanged. The pulse rate was little changed. An addition of adrenaline after the previous application of phlorhizin caused to abolish completely the effects of phlorhizin on the mechanogram, the integration curve and the monophasic action current.

(f) Dinitrophenol: A small amount (0.05 cc) of saturated dinitrophenol was dropped into the perfused fluid (5cc). In about five minutes after the application the mechanogram became the relaxation type, and the fluid level fell in the systolic and diastolic phases. In the integration curve there was a decrease in the gradient and the amplitude. The voltage of the monophasic action current decreased, and the Q-T time was shortened. The pulse rate was unchanged. By an addition of adrenaline (0.01 per cent, 0.05 cc) after the administration of dinitrophenol, the effect of dinitrophenol was abolished and that of adrenaline appeared.

(g) Monoiodoacetic: Monoiodoacetic acid was neutralized by sodium carbonate and used at pH 7.0. A small amount (0.05 cc) of 500mM monoiodoacetic acid was dropped into the perfused fluid (5cc). In about 30 beats after the application, the

Fig. 7 left (A): before application of KCl solution. middle (B): 5min. after application of KCl solution. right (C): 5min. after addition of adrenaline to the above from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c).

mechanogram became gradually the relaxation type, and the perfused fluid level fell in both the systolic and diastolic phases. The heart showed a weak beat for a while and was gradually transferred to the contracture state. During this period the abnormal pulsation was observed. In about twenty minutes after the application the heart fell in a complete contracture state (Fig. 8). In the integration curve there was a decrease in the gradient and the amplitude. The spike height of the monophasic action current decreased and the Q-T time was shortened. The pulse rate was unchanged. When monoiodoacetic acid was applied after the previous administration of adrenaline (10^{-4} per cent) the effect was almost the same as the case without the previous administration of adrenaline.

When adrenaline (10^{-4} per cent) was applied during the relaxation period caused by monoiodoacetic acid, the mechanogram showed a strong contraction immediately after the application of adrenaline and became gradually the relaxation type and was finally transferred to the contracture state.

In summary both adrenaline and noradrenaline had an action to increase the contraction and the electrical generation of the excised toad heart. This action was similar to that of sodium chloride. A decrease in the contraction and the electrical generation by acetylcholine, phlorhizin and dinitrophenol was completely restored by adrenaline. The inhibitory action of monoiodoacetic acid on the con-

traction and the electrical generation was released by adrenaline, while that of potassium chloride was not abolished by adrenaline.

(4) *pH*

The pH of the perfused fluid was about 7.0. The pH was altered by dropping hydrochloric acid or sodium hydroxide, and observations were made at varied values of the pH. However, within the range of pH of 6.5 to 10.0, there was almost no effect on the mechanogram, the integration curve and the monophasic action current. When the pH was more than 10.0 or less than 6.5, a diminution in the contraction and the electrical generation was observed. The mechanogram became the relaxation type so that the fluid level fell in both the systolic and diastolic phases. In the integration curve there was a decrease in the gradient and the amplitude. The spike height of the monophasic action current decreased, and the Q-T time was shortened. The pulse rate was unchanged.

(5) *ATP and ATPase*

(a) ATP: Observations were carried out at varied concentration of ATP-Na in the perfusing fluid, ranging from 0.001mM to 10mM. In 0.001 mM of ATP-Na, the mechanogram became apparently the contraction type, and the fluid level was elevated in both the systolic and diastolic phases. By dropping of ATP-Na above 0.01mM in concentration, the mechanogram became first the relaxation type, then the contraction type. In the integration curve there was an increase in the

Fig. 8 Monoiodoacetic acid was dropped at the arrow. Mechanogram.

gradient and the amplitude. The height of the monophasic action current enhanced, and a prolongation in the Q-T time was observed. There was also a reduction in the pulse rate. The endocardium was apparently injured by perfusion with ATP-Na more than 1mM in concentration. However, even under such deteriorated condition the enhanced contraction was still obtained. Usually immediately after the application of ATP, an increase in the pulse rate and the voltage was temporarily observed, and the temporal contracture state appeared. When perfusion lasted for a considerably long period, the heart ceased to beat and the auricle and ventricle remained extremely relaxed (especially in Summer). An addition of ATP solution (0.01mM) to the perfusion fluid caused to reappear the pulsation (Fig. 9). The effect of ATP on the auricle was also observed, perfusing through Straub's cannula which was inserted to the right auricle from the ventricle. The effect of 0.01mM ATP on the auricle was quite the same as that on the ventricle, though the results were more conspicuous on the latter (Fig. 10).

(b) Actomyosin: In order to obtain actomyosin solution, the high muscle of the toad was ground in a dish, being mixed with Weber's solution. After having placed this in an ice-box for twenty-four

hours, this was diluted three times with 0.6 M-KCl solution and centrifuged for thirty minutes at 4,000 revolutions a second. The supernatant fluid was sufficiently dialysed so as to remove electrolytes. To the solution obtained in this way, sodium chloride, potassium chloride, calcium chloride, sodium bicarbonate and glucose were added in the same composition as Locke's solution. The solution provided thus was henceforth called 'the skeletal muscle actomyosin solution of the toad.'

A small amount of the toad skeletal muscle actomyosin solution (0.05 cc) was dropped into the perfused fluid (5 cc). In a few minutes the mechanogram became gradually the contraction type, so that the fluid level was elevated in the systolic and diastolic phases. The mechanogram showed a steady state within about twenty minutes after the application. In the integration curve there was an increase in the gradient and the amplitude. In the monophasic action current, with the exception of period of the endocardial injury immediately after the application of the toad skeletal muscle actomyosin solution, a gradual increase in the spike height was observed. The Q-T time was shortened and the pulse rate increased.

(c) Renal ATPase: Methods to make the renal ATPase solution of the dog were essentially

Fig. 9 ATP solution was dropped at the arrow.
a: mechanogram, b: integration curve, c: monophasic curve.

Fig. 10 left (A): before application of ATP solution.
right (B): 5 min. after application of ATP solution.
from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c).

the same as in the skeletal muscle actomyosin solution of the toad. A small amount of the dog ATPase solution (0.05 cc) was dropped into the perfused fluid. The mechanogram became gradually the contraction type in a few minutes after the application, and the fluid level was elevated in both the systolic and diastolic phases. The steady of contractibility was attained in about twenty minutes after the application. Both the gradient and the height of the integration curve increased. In the monophasic action current there was an increase in the spike height, and the Q-T time was shortened. The pulse rate increased. The endocardial injury by the renal ATPase solution of the dog was more slight as compared with that by the toad skeletal muscle actomyosin solution. In general, the effect of the renal ATPase solution on the toad heart was weaker than that of the toad actomyosin solution, though there was a similarity in both the actions (Fig. 11).

(6) *Vitamins and other drugs (Vitamin B₁, Vitamin C, Ephedrine, Prostigmine, Chlorophyllin, Theophylline-ethylenediamine, Pituitrin, Nitrite and Urethane)*

(a) Vitamin B₁: Perfusion with Vitamin B₁, 0.1mg per cc. in concentration had no effect on the contraction and the electrical generation.

(b) Vitamin C: Perfusion with vitamin C, 0.5mg per cc. in concentration had no effect on the contraction and the electrical generation.

(c) Ephedrine: Perfusion with ephedrine, 0.4 mg per cc. in concentration had no effect on the contraction and the electrical generation.

(d) Prostigmine: With prostigmine, 0.0005 per cent in concentration, there was no change in the contraction and the electrical generation.

(e) Chlorophyllin: An addition of a small amount of 2.5 mM chlorophyllin solution (0.05 cc)

to the perfused fluid (5 cc) caused to render the mechanogram the contraction type in a few minutes. The perfused fluid level was elevated in both the systolic and diastolic phases. In the integration curve there was an increase in the gradient and the amplitude. The spike height of the monophasic action current increased, and the Q-T time was shortened. The pulse rate was almost unchanged.

(f) Theophylline-ethylenediamine: With this drug, 0.1 per cent in concentration, the mechanogram became the contraction type, so that the fluid level was raised in both the systolic and diastolic phases. In the integration curve the gradient and the amplitude increased. In the monophasic action current the spike height enhanced but the Q-T time was little changed. A slight increase in the pulse rate was observed.

(g) Pituitrin: A small amount of ten times pituitrin solution was dropped into the perfused fluid. There was a tendency to transfer to the contraction type in the mechanogram. The perfused fluid level was elevated in both the systolic and diastolic phases. In the integration curve there was an increase in the gradient and the amplitude. The spike height of the monophasic action current enhanced, and a slight prolongation in the Q-T time was observed. The pulse rate was unchanged.

(h) Nitrite: Perfusion with nitrite, 1mM in concentration, caused to render the mechanogram the relaxation type. The perfused fluid level fell in both the systolic and diastolic phases. There was a reduction in the gradient and the amplitude of the integration curve. In the monophasic action current the amplitude decreased and the Q-T time was shortened. A slight increase in the pulse rate was observed.

(i) Urethane: In perfusion with 1mM urethane, the mechanogram became the relaxation type,

Fig. 11 left (A): before application of renal ATPase solution. right (B): 5 min. after application of renal ATPase solution. from top downwards, mechanogram (a), integration curve (b) and monophasic action current (c) respectively.

so that the fluid level fell in both systolic and diastolic phases. There was a decrease in the gradient and the amplitude of the integration curve. In the monophasic action current a reduction in the spike height was observed, and the Q-T time was shortened. There was also a slight increase in the pulse rate.

DISCUSSION

Upon discussing on the present results, an attention should be called to the following four points: (1) The sensibility of the excised heart to drugs is different from that of the heart in situ. For instance, circus movement occurred by the application of potassium chloride in the heart in situ, while this was not the case in the excised heart. (2) The sensibility of the toad heart to drugs is different from season to season. For instance, the Q-T time was prolonged by calcium chloride in Summer, while in Winter the result was the reverse. (3) The sensibility of the toad heart to drugs is dependent upon the condition of the cardiac muscle at that time. The Q-T time was prolonged by calcium chloride in the excised heart showing a weak beat, while the result was the reverse in the excised heart showing a strong beat. (4) There is occasionally a difference in the sensibility to drugs between mammalian and amphibian hearts.

The result will be discussed, the above four points being taken into consideration.

(1) *Electrolytes (Sodium chloride, Potassium chloride, Calcium chloride, Magnesium chloride and Sodium fluoride)*

(a) Sodium chloride and Potassium chloride: A series of investigations on the effects of sodium and potassium ions on muscle and nerve was carried out by Overton^{21,22}, and a clear explanation of the ionic process was given. On the other hand, Fleckenstein²³⁻²⁶ proposed an assumption on the primary osmodynamic accumulator of energy, depending upon an asymmetrical distribution of sodium ions outside and inside of the membrane of cardiac muscle and nerve. Recently the ionic processes that occur during the excitation have been precisely studied by Hodgkin, Katz, Huxley and Keynes²⁷⁻³¹, and the so-called sodium theory was formulated. Graham & Blair³² and Hodgkin & Katz have shown that an increase in the external potassium concentration produced a reduction in the resting potential of nerve and muscle, the potential being

proportional to the logarithm of the ratio of the potassium ions on the two sides of the membrane. Thus Bernstein's membrane theory has been replaced by a new ionic hypothesis by Hodgkin and Katz upon which an interpretation of electrocardiogram has been based. Prof. Maekawa³³, however, has already pointed out that this new ionic hypothesis is not also correct. Sodium ions have a counter-action with potassium ions on the contraction and the electrical generation of cardiac muscles. This fact is not attributed to direct changes in the polarity of the membrane but is probably considered as a result of changes in the enzyme system of myocardial parenchyma, especially the ATP-actomyosin system. This consideration may be proven to be satisfactory as will be shown in the following.

(b) Calcium chloride: There is a counteraction between the toxic action of sodium ions and the inhibitory action of potassium ions in the frog heart. When the concentration of calcium ions in Ringer's solution is reduced below 0.43 mM, the contraction is completely inhibited. The site of this action has been attributed to the plasma membrane, because of its rapid change. It has been also reported that low concentration of calcium ions causes to facilitate the relaxation process, while high concentration strengthens the contraction process (Kionka, 1914). In the present results, an increase in the concentration of calcium chloride caused to augment the height of the T wave and the amplitude of the monophasic action current. The Q-T time was shortened by dropping calcium chloride solution in the heart showing a strong beat in the winter stage, while the result was the reverse in the heart showing a weak beat in summer. Generally speaking, as increase in the concentration of calcium chloride caused to enhance the contraction and the electrical generation.

(c) Magnesium chloride: It has been shown that the application of magnesium sulfate solution resulted in a reduction in the contraction and the electrical generation of mammalian hearts (Smith, Winker & Hoff and Rothberger & Zwillinger³⁵⁻³⁷). On the other hand, as shown in the present result, the toad excised heart was not at all affected by the application of magnesium chloride solution.

(d) Sodium fluoride: It has been generally accepted that sodium fluoride inhibits the anaerobic glycolysis in muscles and is similar to monoiodo-

acetic acid in the action^{38,39}). The application of sodium fluoride (10 to 20 gamma) has caused changes in the S-A and A-V conductions of the frog excised heart. On the dog, sodium fluoride, 20 to 70 mg per kg in concentration, has caused a reduction in the blood pressure and to produce the temporal relaxed state in the heart which has been often followed by the auricular flutter. These changes have been restored to the normal, though finally transferred to the sinus block, extrasystole and the ventricular flutter⁴⁰). In the present experiment, sodium fluoride caused a reduction in the contraction and the electrical generation but was somewhat different from monoiodoacetic acid in the action.

(2) *Salts of heavy metals (Copper sulfate, Silver nitrate, Ferric chloride and Zinc sulfate)*

The effect of salts of heavy metals on the cardiac muscle has not so far been reported. Immediately after the application of salts of heavy metals the mechanogram became the relaxation type. The heart showed a reduction in the contraction and the electrical generation, and was eventually transferred to the contracture state. Salts of heavy metals have also a close relation with the enzyme system. (i) When salts of heavy metals co-exist with an activated group of the SH-radical of enzyme protein, mercaptide is formed, the enzyme action being inhibited (41, 42). (ii) The enzyme is coupled with protein, and an inactivated group is formed (43). Thus, salts of heavy metals act as an inhibitory substance. Salts of heavy metals are then likely considered as a substance which acts to inhibit directly or indirectly the enzyme system relating to the contraction and the electrical generation.

(3) *Adrenaline and other drugs (Adrenaline, Nor-adrenaline, Acetylcholine, Phlorhizin, Dinitrophenol and Monoiodoacetic acid)*

It has been well known that adrenaline has different actions on the perfused frog heart at varied concentration. In 10^{-9} to 10^{-13} adrenaline, the inhibitory action has been observed, and to the contrary the action has been enhanced by adrenaline, 10^{-4} to 10^{-7} in concentration. With adrenaline more than 10^{-6} in concentration, arrhythmia or a cessation of the heart beat has been often observed. Similar effects have been also found on the strip of cardiac muscles. And the auricular muscle has been more sensitive to adrenaline than the ventricular muscle (44). In the present experiment, with

adrenaline, 10^{-6} to 10^{-7} in concentration, an increase in both the contraction and the electrical generation was observed. A most interesting investigation on the locus of action of adrenaline was carried out by Ellis⁴⁵). He has observed changes in the positive inotropic action of the frog heart by adrenaline under administration of various metabolic inhibitors, such as cyanide, azide, fluoroacetate, iodoacetate, iodoacetamide and 2, 4-dinitrophenol. Since adrenaline has well interacted with dinitrophenol, it has been reasonably concluded that the locus of action of adrenaline is between metabolically formed high-energy phosphate and the contractile process. In the present experiment it was shown that adrenaline was similar to sodium chloride in the action, and the action of adrenaline was apparently suppressed by potassium chloride which was previously given and that adrenaline interacted well with acetylcholine and temporarily with monoiodoacetic acid. Adrenaline also suppressed the inhibitory action of phlorhizin and dinitrophenol. All these findings were in good agreement with the results obtained by Ellis. The locus of action of adrenaline may thus likely be attributable to the ATP-ATPase system.

(4) *pH*

It has been generally accepted that there is almost no change in the activity of cardiac muscles within the range of pH of 6.5 to 8.3, and that in the alkaline region the relaxation of the heart becomes incomplete, and eventually the heart ceases to beat in the systolic state, while in the acid region the cessation of beating occurs in the diastolic state. In the present experiment there was little change in the contraction and the electrical generation within the range of pH of 6.5 to 10.0. Out of this range the mechanogram became the relaxation type, and there was a reduction in the contraction and the electrical generation in both the alkaline and acid regions.

(5) *ATP and ATPase*

(a) ATP: According to Wedd and Fenn⁴⁶), the effect of ATP on the cardiac muscle has been exclusively of inhibition. The application of adenine compounds to the frog heart caused a temporal decrease in excitability of the sinus with a rapid reduction of the contraction height and finally resulted in cessation of beating (Fleisch & Weger⁴⁷), Ostern & Parnas⁴⁸). Though the heart ceased to beat temporarily in low concentration of ATP, the

beating reappeared after a while, and the beating reappeared after the cessation was stronger than that before the cessation. To the contrary, as shown in the present result, by perfusion with 0.001mM ATP solution the mechanogram became the contraction type, and there was an increase in the contraction and the electrical generation. In perfusion with 0.01mM or more concentrate ATP solution there was an initial relaxation, but after a few beats an enhancement in the contraction was observed. The inhibitory action of ATP on the cardiac muscle which has often been reported up to the present was thus obtained only in high concentration or only immediately after the application.

(b) Actomyosin: In the experiment made by Prof. Maekawa and his collaborators⁴⁹⁾ an artificial hypertension was brought about by injecting the skeletal muscle actomyosin solution of the toad into the mammalia. In the present experiment the application of the actomyosin solution has caused an increase in the contraction and the electrical generation in a few minutes. Similarly the application of ATP caused an increase in the contraction, but the action of ATP was different from that of the actomyosin solution in several points. The contraction enhanced by ATP was followed by a slower pulse rate, and a prolongation of the Q-T time, while the application of the actomyosin solution caused a tachycardia and shortening in the Q-T time.

(c) Renal ATPase: The experimentally induced hypertension was also obtained by injection of the dog renal ATPase solution as well as the skeletal muscle actomyosin solution of the toad (Maekawa⁴⁹⁾). Perfusion with a small amount of the dog ATPase solution caused an enhancement in the contraction in a few minutes, though the effect was weaker than that of the toad actomyosin solution.

(6) *Vitamins and other drugs (Vitamin B₁, Vitamin C, Ephedrine, Prostigmine, Theophylline-ethylenediamine, Pituitrin, Nitrite and Urethane)*

(a) Vitamine B₁: The contraction and the electrical generation of the excised toad heart were not affected by Vitamine B₁.

(b) Vitamine C: The effect of vitamin C on activity of the cardiac muscle has not been found in the literature. In the present experiment Vitamin

C had no effect on the contraction and the electrical generation of the excised toad heart.

(c) Ephedrine: It has been so far reported that in low concentration of ephedrine (1 to 10,000 for turtles, 1 to 100,000 for rabbits), there was an increase in the pulse rate and the contraction height, and a slight increase in the concentration caused a reduction in the pulse rate, while the contraction height was not affected, and that in high concentration of ephedrine (1 to 100 for turtles, 1 to 2,000 -1,000 for rabbits), partial heart block and or cessation of beating in the relaxed state occurred (50, 51). In the present study, however, ephedrine had no effect on the contraction and the electrical generation.

(d) Prostigmine: By perfusion with prostigmine no change has been observed in the contraction and the electrical generation of the excised heart.

(e) Chlorophyllin: Perfusion with chlorophyllin solution caused to enhance the contraction and the electrical generation of the excised heart. This effect appeared in a few minutes after the application and gradually increased, approaching to the final steady state.

(f) Theophyllin-ethylenediamine (Neophyllin: It has been so far reported⁴⁴⁾ that a small amount of theophylline caused an increase in the pulse rate and the cardiac output (Voit, 1860), while a large amount caused a decrease in the pulse rate and the cardiac output (Maki) and made the heart relax (Faval). Stimulant dose by subcutaneous injection is 5 mg, and paralysis dose is 25 mg (Wagneer). In the perfused frog heart, low concentration of theophylline (0.025 to 0.2 per cent) caused an increase in contraction, while in higher concentration (0.2 to 2.0 per cent), a decrease in the contraction height and an irregular automaticity were observed (Cheney, 1935). Similarly to theophylline, theophylline-ethylenediamine (0.1 per cent) has caused an increase in the contraction and the electrical generation.

(g) Pituitrin: In the excised heart pituitary extracts made the beats strong, while in the intact animal the efficiency of the heart was reduced by pituitary extracts, because of the insufficient oxygen supply caused by constriction of coronary arteries. In the excised frog heart low concentration of pituitrin caused an increase in the pulse rate, and with higher concentration a decrease in the pulse rate and eventually the cessation of beating occurred

44). In the present experiment, the contraction and the electrical generation slightly enhanced, though the pulse rate was not affected.

(h) Nitrite: In mammalian hearts, in situ, the cardiac muscle itself has not directly been affected by nitrite, but the activity of the heart increased secondarily by the dilated coronary arteries. In the excised mammalian hearts, therefore, little change was observed, though there was occasionally a slight facilitation which followed by inhibition. In the excised frog heart, the pulse rate was reduced by low concentration of nitrite (1:10,000) and enhanced by higher concentration (1:5,000). Further increase in concentration (1:1,000) caused the cessation of beating⁴⁴). In the present experiment, perfusion with 1mM nitrite caused a slight increase in the pulse rate and a decrease in the contraction height.

(i) Urethane: The contraction and the electrical generation were decreased by urethane.

Discussion has been so far made only qualitatively from results obtained by simultaneous recording of the mechanogram, the integration curve and the monophasic action current. The relation of mechanical events with electrical events will be then considered quantitatively.

As a measure of mechanical contractile force of the toad heart, the product of the average pressure (Pcm. H₂O) by the cardiac output (Vcc) was taken. The total electrical quantity generated during excitation will be given by the area of curve of the monophasic action current (QmV. sec). Then the ratio of the electrical generation to the work externally done by the heart (Q/PV) was calculated before and after the application of several drugs and ions. This ratio, however, was almost the same before and after the administration of drugs. Thus it was clearly revealed that changes in the mechanical events were qualitatively and quantitatively in parallel with changes in the electrical events (Table I, II, III).

In the present experiment 3 M-KCl solution was used for recording the monophasic action current. In a recent investigation⁵²), it was shown that the monophasic action current recorded with intracellular electrodes was not essentially different from the monophasic action current obtained by the KCl injury, though the potential recorded was larger in the former than in the latter.

The fundamental consideration on the electrical

TABLE I

Medicament		Electric Quantity mv. × sec.	Cardiac Work gm. × cm.	Proportional Constant mv. × sec./ gm. × cm.
NaCl	before	4.32	4.50	0.96
	after	6.40	7.20	0.89
KCl	before	4.80	3.60	1.33
	after	3.50	1.80	1.94
CaCl ₂	before	4.80	3.60	1.33
	after	5.30	4.11	1.29
MgCl ₂	before	2.60	3.50	0.74
	after	2.60	3.50	0.74
NaF	before	2.88	4.05	0.71
	after	1.82	2.00	0.91
CuSO ₄	before	2.76	4.50	0.61
	after	2.04	2.40	0.85
AgNO ₃	before	3.25	3.15	1.03
	after	2.75	2.20	1.25
FeCl ₃	before	2.80	5.40	0.52
	after	1.60	4.05	0.40
ZnSO ₄	before	2.08	3.38	0.62
	after	1.53	2.72	0.56
pH 6.0	before	4.55	3.06	1.49
	after	3.49	2.09	1.67
pH 10.0	before	3.60	2.64	1.36
	after	2.00	1.11	1.80

events of the cardiac muscle was made by Prof. Maekawa in 1956³³). In order to make clear the purpose and significance of the present study, his discussion will be quoted here. He states that: "Bernstein's membrane theory has been generally accepted as the basis of the electrocardiographic study. Bernstein's theory, however, has been found to be inadequate, because a reversal of membrane potential during excitation has been shown by intracellular recording. For this reason, the Bernstein theory was replaced by a new ionic theory proposed by Hodgkin and Katz (1949). This new ionic theory, however, is not necessarily correct. The temperature coefficient of bioelectricity was revealed to be 0 by intracellular recording (Hodgkin & Katz, 1949, Woodbury et al. 1951). This fact throws doubt on the new ionic theory, because according to that theory the temperature coefficient should not be 0. The mechanism of the electrical generation will now

TABLE II

Medicament		Electric Quantity mv. × sec.	Cardiac Work gm. × cm.	Proportional Constant mv. × sec./ gm. × cm.
Adre- naline	before	1.50	4.05	0.37
	after	2.25	7.65	0.30
Norad- renaline	before	7.50	3.87	1.94
	after	8.66	5.06	1.71
Adrenaline	before	3.35	3.52	0.95
	NaCl	5.03	4.50	1.12
	Adre- naline	5.58	6.24	0.89
Adrenaline	before	24.00	6.64	3.61
	KCl	13.71	4.45	3.08
	Adre- naline	10.28	3.54	2.90
Adrenaline	before	16.43	6.11	2.69
	Acetyl- choline	9.58	4.10	2.33
	Adre- naline	15.06	6.24	2.41
Adrenaline	before	11.64	3.36	3.46
	Phlor- hizin	9.54	2.76	3.46
	Adre- naline	11.00	3.22	3.42
Adrenaline	before	6.60	3.91	1.69
	D. N. P.	5.95	2.70	2.24
	Adre- naline	9.00	4.56	1.95
Adrenaline	before	22.40	4.95	4.53
	M. I. A. A.	17.42	4.07	4.28
	Adre- naline	22.40	5.30	4.23

be re-considered in relationship to the fundamental formulation of electromotive force once given by Helmholtz (1882). Since temperature coefficient is 0, the electromotive force, E , should be equal to the free energy, U , of the system. In other words, the entity of the electrical generation should be exclusively chemical. I have proposed the contraction-electrical generation theory. And this theory has already been confirmed by finding that the contraction curve was in accordance with the integration curve of the monophasic action current in strip of the toad cardiac muscle⁵³⁾ (Fukuda, 1950), the toad heart preparation (Ono, 1954) and skeletal muscle fibers⁵⁴⁾ (Tanaka, 1953). It has been also concluded that the cell membrane is not the site of the generation of action current, but the site to be distorted or the site to change the intersurface

TABLE III

Medicament		Electric Quantity mv. × sec.	Cardiac Work gm. × cm.	Proportional Constant mv. × sec./ gm. × cm.
ATP	before	4.25	5.64	0.75
	after	5.87	8.64	0.68
ATP (Auricle)	before	0.75	0.71	1.06
	after	0.80	0.92	0.87
Acto- myosin	before	7.22	6.52	1.11
	after	6.93	6.62	1.05
ATPase of Kidney	before	5.94	3.83	1.55
	after	7.03	4.70	1.50
Chloro- phyllin	before	2.70	2.29	1.18
	after	3.01	2.50	1.20
Pituit- rin	before	7.50	4.23	1.77
	after	8.33	4.32	1.93
Neo- phyllin	before	9.00	3.22	2.79
	after	11.45	4.32	2.65
Nitrite	before	11.20	4.28	2.62
	after	7.94	2.87	2.77
Ure- thane	before	3.92	4.05	0.97
	after	3.00	3.15	0.95

polarity. This conclusion has recently been supported by investigation made by Ono and Maekawa."

SUMMARY AND CONCLUSION

The mechanogram, the intergration curve and the monophasic action current were simultaneously recorded in the excised perfused heart of the toad, and the effects of various drugs and salts were observed. It has been revealed that changes in the mechanogram were completely in accordance with changes in the integration curve qualitatively and quantitatively. The ATP-ATPase system also seemed to be affected directly and indirectly by various kinds of drugs. These findings were in agreement with the results to be expected from Maekawa's assumption that the entity of the electrical generation and the contraction is unitarily attributable to the ATP-ATPase system.

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