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Enzymological Studies on Myocardial Metabolism

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(Received for Publication, July 1, 1956)

Recently, since the biochemistry of muscle has been well studied, many reports concerning the myocardial metabolism have also been published. Studies done in previous years concerning the biochemistry of skeletal muscle are easily accessible through the reviews (1-5a), and monographs (6-8). But the reviews concerning the field of myocardial metabolism, especially pathological myocardial metabolism are very few at present moment (9-11).

The functions of all muscular tissues are formed of contraction, and the contractile matter is actomyosin. Since myocardial tissue must contract through a life without rest, this tissue should reconstitute the ATP which is constantly broken down in order to provide the energy for maintenance of the function of contraction. In myocardial tissue, there are less high energy phosphate stores, such as phosphocreatine and ATP, than in skeletal muscle. Therefore, there should be very active aerobic phosphorylative reaction in heart muscle. The heart is a very sensitive organ to lack of oxygen and abounds in blood flow and oxidative enzymes.

The present discussion will be dealt with particular emphasis on the role of aerobic phosphorylation, mineral metabolism and transamination, depending on the data which were obtained in recent several years by our research members, Dr. Kimura, Dr. Nakamura, Dr. Goto, Dr. Irie, Dr. Itasaka, Dr. Nishimoto, and Dr. Nawata.

METHODS

Dogs averaging about 10 kg. in weight were used as a experimental animal through the whole experiments.

Succinoxidase—The activity of this enzyme was measured manometrically chiefly by the method reported previously (12) except using 5% aqueous homogenate and phosphate buffer to give final concentration of 0.05 M.

Lactic Dehydrogenase—The lactic dehydrogenase activity was also measured manometrically as follows. Each Warburg flask contained 0.5 ml.

of 5% aqueous homogenate of myocardial tissue, 0.25 ml. of 0.2% solution of methylenblue (MB), 0.3 ml. of neutralized M potassium cyanide, 0.5 ml. of flavoprotein, DPN equivalent 1.5 mg. of pure DPN, phosphate buffer (pH 7.4) to give final concentration of 0.05 M in main chamber and 0.3 ml. of 2 M Na lactate at pH 7.0 in the side arm. The total volume was 3.0 ml. The temp. was 37°, and the gas phase was air. Enzyme unit was expressed in terms of QO_2 (N). DPN was prepared from baker's yeast by the method of Williamson and Green (13). The purity of the preparation of DPN with respect to DPN amounts to 30-40% according to the estimated values by alcohol dehydrogenase methods (14). Flavoprotein was purified from pig heart muscle chiefly by Straub's method (15), though the aluminium hydroxide treatment was omitted.

Cytochrome oxidase—The cytochrome oxidase activity was measured manometrically by the method of Okunuki and Sekuzu (16) except using homogenate instead of green brei.

Endogenous respiration of homogenate—The endogenous respiration of myocardial homogenate was measured manometrically by using the method reported by Lemley (17).

Oxidative phosphorylation of mitochondrial fraction—Dog heart muscle mitochondria were prepared in 0.25 M sucrose by the procedure of Schneider (18). Yeast hexokinase was a partially purified fraction obtained from baker yeast extract by fractionation with ammonium sulfate. The system contains 1.5×10^{-2} M phosphate buffer pH 7.4, 2.1×10^{-2} M KCl, 5×10^{-4} M $MgSO_4$, 3.0×10^{-3} M ATP, 8×10^{-3} M α -ketoglutarate or 2.6×10^{-2} M Na succinate, 2×10^{-2} M glucose, 2.5×10^{-6} M cytochrome C, 6 mg. hexokinase preparation, 1.3×10^{-2} M NaF, 0.4 mg. coenzyme I, 1.0 ml. of dog heart mitochondrial suspension.

The total volume was 3.0 ml. Two tenth ml. of 87.5% trichloroacetic acid was contained in the side arm, two tenth ml. of 20% KOH in the center well. The incubation time was 15 min. at 37°. The gas phase was air.

Tissue respiration and substrate utilization—The oxygen uptake of cardiac slice and substrate utilization was studied in conventional manner. The incubation mixtures were analyzed by using following methods: Glucose, lactate and inorganic phosphate were determined by using the methods of Somogyi and Schaffer (19), Barker and Summerson (20) and Fiske and Subbarow (21) respectively.

Analysis of phosphorylated intermediates—The dog heart was dissected quickly and were thrown into the mixture of ether and dry ice. The frozen muscles were ground into a fine powder with small pieces of dry ice in the mortar and these powder can be transferred into a ice-cold trichloroacetic acid. The fractionation of the acid soluble phosphorous compounds was performed chiefly by the method of LePage (22). The determinations of inorganic phosphate and phosphocreatine were carried out by Takahashi's method (23). The determination of total phosphate was made by the method of King (24). Particular care must be taken to insure a low temperature during the extraction and fractionation of the extract. In the tracer experiments of radioactive P_{32} , 50 μ c. of P_{32} was injected per kilogram body weight of the dogs in the form of Na_2HPO_4 into the femoral vein, then after the lapse of 30 min., the hearts were dissected and frozen as mentioned before. The various P compounds were separated from trichloroacetic acid extracts of the powdered tissues, the P converted to $MgNH_4PO_4$ and measurements of P_{32} content per milligram P were then made. At the same time, the contents of P_{32} and inorganic P in plasma were determined and all measurements have been corrected for decay to a standard time for each dog and have been recalculated to the basis of the content of plasma P and P_{32} .

ATP-ase—The activity of adenosinetriphosphatase of myocardial aqueous homogenate was determined chiefly by the method of DuBois and Potter (25), except that the reaction period was 5 min.

Transaminases—The activities of glutamic-oxalacetic transaminase (G. O. T.) and glutamic-pyruvic-transaminase (G. P. T.) of serum and myocardial homogenate were determined chiefly by the method of Karmen et al (26) except using alanin instead of aspartate in the determination of G. P. T. activity and using Toyo No. 131 filter paper

instead of Whatman No. 1.

Others—The blood glucose and lactate were measured by the method of Shaffer and Somogyi (19) and Barker and Summerson (20) respectively. The glycogen, total nitrogen of the myocardial tissue were determined by Somogyi's method (19, 27) and Kjeldahl's method respectively. The amounts of potassium, sodium, oxygen and carbon dioxide of blood plasma and whole blood, were measured flamephotometrically and manometrically by using Beckman spectrophotometer and Van Slyke manometer respectively. The electrocardiograms (ECG) of dog at the condition of opened chest, were taken by the direct leading method.

RESULTS

1. Normal metabolism

The normal values of these several enzyme activities were previously reported by us (12, 28–38).

Effects of several chemicals on the metabolism in vitro—We have reported previously the inhibitory effects of acetylcholin, coffeinum-Nabenzocum, ephedrin, aminocordin on the succinoxidase activity of myocardial homogenate (12). Among the effects of many chemicals on the succinoxidase, cytochrome oxidase, endogenous respiration, lactic dehydrogenase, respiration and substrate utilization of cardiac slices, we want to pay attention here to the effects of cardiac glycosides and g-strophantin.

In experiments with dog heart slices, we were able to confirm the findings of sustained stimulation of respiration by g-strophantin reported by several investigators (39, 40). But we were not able to confirm the stimulation of succinoxidase, cytochrome oxidase, lactic dehydrogenase and endogenous respiration of myocardial homogenates. The rate of oxygen uptake was more lineally related to time when g-strophantin concentration was at the range of 10^{-7} M, but all higher concentrations depressed the oxygen uptake markedly.

It was observed that g-strophantin has an effect like dinitrophenol which is a classical uncoupler of oxidative phosphorylation. It was assumed that adrenochrome semicarbazone had a pasteur effect, but had no effect on the succinoxidase, lactic dehydrogenase, cytochrome oxidase, and endogenous respiration.

The effects of hexokinase, and ethylendiamin

tetraacetate (EDTA) on the oxidative phosphorylation of mitochondrial fraction prepared from heart muscle, were studied. EDTA has an inhibitory effect. Lanatocide C, one of the cardiac glycosides, has no particular effect on the oxidative phosphorylation. The effect of coenzyme I concentration upon the activity of lactic dehydrogenase was studied. It was ascertained that coenzyme I was necessary to link lactic dehydrogenase to other terminal hydrogen acceptors, such as flavoprotein and MB. The optimal concentration in the determination system was found to be about 0.05–0.1 per cent under the above conditions. It was ascertained that the flavoprotein and KCN in high concentration are necessary to be present in the reaction system, when the activity of lactic dehydrogenase are measured manometrically.

II. Metabolism after coronary occlusion—Dogs weighing an average of 10 kg. were used. Intravenous lavonal (Tanabe-made) anesthesia was administered and the respiration was sustained by intermittent positive pressure oxygen through tracheal intubation. No dog was anesthetized for more than three hours. A left lateral chest incision was made, the fifth rib resected, and isolated the branches of coronary artery. All manipulations were carried out in aseptic conditions. But when it does not need to let dog to survive more than three hours after coronary occlusion, the following simplified operation method was used. After anesthetizing the dog by lavonal, both fifth ribs were resected, both A. mammae interna were tied, cut off and then the sternum was cut off. The advantage of this method is that the broad area including the coronary sinus can be easily seen, the blood from coronary sinus was easily drawn through needles under the naked eyes and it is easy to ascertain the grade and the size of infarction. Before occlusion, the dogs were given morphine sulphate subcutaneously (up to 30 mg. are usually necessary to abolish pain). Samples of heart muscle were taken from the infarcted and normal areas of dog left ventricles and other all experimental manipulations were carried out in a same way mentioned before.

a) *Succinoxidase*—This enzyme activity of myocardial homogenate from the dogs subjected to coronary occlusion decreased markedly at 24 hours after ligation (Fig. 1). It can be seen that there is a much smaller concentration of enzyme in the

injured infarcted myocardium than in normal myocardium from the same dogs. In the period of 24 hours after occlusion, no particular decreasing of this enzyme activity was observed, even though certain macroscopical and histological changes could be obtained (Fig. extra 1). After 24 hours infarc-

Fig. extra 1 Myocardial infarction of dogs. 15 hours post ligation (PAS-staining, 120×)

tion, the decrease of the activity of this enzyme was parallel to the degree of degeneration or necrosis of heart muscle, in general.

b) *Cytochrome oxidase and lactic dehydrogenase*—These enzyme activities almost continued to decrease progressively with the myocardial

Fig. 1 Several enzymes, glycogen and total nitrogen of myocardium from dogs subjected to coronary occlusion;

○—○: Succinoxidase, □—□: Endogenous respiration, ●—●: Lactic dehydrogenase, △—△: Glutamic-oxalacetic-transaminase and glutamic-pyruvic-transaminase, X—X: Cytochrome oxidase, →—→: Oxidative phosphorylation of mitochondrial fraction, ⊙—⊙: Adenosintriphosphatase

infarction until several days (Fig. 1).

c) *Endogenous respiration*—Endogenous respiration of myocardial homogenate from dogs subjected to coronary occlusion decreased markedly. Even in the myocardial ischemia of 40 min. duration, endogenous respiration decreased to about one tenth (Fig. 1).

Among the several metabolic oxidative activities which we have examined, this endogenous respiration of homogenate decreased most highly and early. This striking decrease in endogenous respiration after coronary occlusion, may be due to the earlier destruction of coenzymes which are necessary to maintain normal respiration. All attempts to recover the decreased level of respiration to normal level by addition of DPN, ATP, boiled extract of normal and infarcted myocardium and several substances, were failed.

d) *Oxidative phosphorylation*—The oxygen uptake of mitochondrial fraction isolated from the infarcted myocardium, even though the duration of myocardial ischemia was within one hour, was about 80 or 70 per cent of the oxygen uptake of mitochondrial fraction isolated from normal myocardium (Fig. 1). But P:O ratio did not decrease markedly. The technique of measuring the oxidative phosphorylation of mitochondrial fraction was difficult and the results obtained in normal mitochondria were rather variable. Although all data are expressed as a mean value of duplicate experiments, further studies are necessary to dissolve the problem in detail.

It can be seen that the individual oxidative enzyme activity of myocardial homogenate did not change so markedly until several hours after infarction, but the mitochondrial oxidative capacity as organized biological unit of whole oxidative enzymes decreased rather in shorter duration of myocardial ischemia.

e) *Respiration of cardiac slice*—Both the endogenous respiration and the oxygen uptake at the presence of substrate continued to decrease as shown in Fig. 2. Effects of the addition of Na succinate, several coenzymes, boiled extract of heart muscle were studied as shown in Fig. 3. It can be assumed that the boiled extract of normal and infarcted heart muscle should contain the substance which increase the rate of oxygen uptake of the both normal and infarcted cardiac slice. It was confirmed that ATP and DPN stimulated the rate

Fig. 2 Q_{O_2} , Q_G , and Q_P of cardiac slices after coronary occlusion

X—X: Oxygen uptake with glucose
O—O: Oxygen uptake without glucose
Δ—Δ: Q_G , ●—●: Q_P

Fig. 3 Effects of additions of coenzymes and others on cardiac slices

of oxygen uptake of cardiac slice and the phosphate uptake at the presence of glucose. Glucose-6-phosphate, however, did not produce any stimulative effect on the rate of oxygen uptake of cardiac slice. Therefore, we have not distinct idea concerning the mechanism of stimulation of oxygen uptake by ATP and DPN. It was interesting to know the difference of the rate of effects of vitamin B_1 , cocarboxylase and muscle boil extract on the oxygen uptake between normal and infarcted cardiac slice. The rate of oxygen uptake of infarcted cardiac slices increased more greatly than that of normal cardiac slices by addition of Na succinate (Fig.3).

Out of a total 12 experiments with coronary occlusion, myocardial extraction of glucose became negative or diminished in 8, those of lactate in 9 experiments in situ.

f) *Transaminases*—The G.O.T. and G.P.T. of myocardial homogenate from infarcted myocar-

dium decreased markedly as shown in Fig. 1. Then we measured these enzymes in serum obtained from coronary sinus, left ventricle and femoral vein. We obtained the results that the amounts of both serum transaminases in coronary sinus, began to rise moderately at 3 hours after ligation, later the enzymes in peripheral vessels began to rise and continued to increase up to several hundred per cent and fell to normal in 3 or 4 days as shown in Fig. 4. As a control experiment, the serum transaminases of dog operated in the same way except ligation of

Fig. 4 Serum transaminase of dogs subjected to coronary occlusion
 ○—○: Glutamic-oxalacetic-transaminase at femoral vein
 ●—●: Glutamic-oxalacetic-transaminase at coronary sinus
 △—△: Glutamic-pyruvic-transaminase at femoral vein
 ▲—▲: Glutamic-pyruvic-transaminase at coronary sinus
: control

Fig. 5 Glutamic-oxalacetic-transaminase in serum of dogs subjected to coronary occlusion

the coronary artery, was measured. It was found that the slight elevation of serum transaminase was noted during operation, but the rate of elevation was much smaller than the rate of it after coronary occlusion as shown in Fig. 4. Fig. 5 shows the relationships between the size of the myocardial infarction and the peak level of the serum G.O.T. and G.P.T. Although, the absolute linear relationships between the size of the infarction and the peak of these enzyme elevations could not be obtained, in general speaking, the larger the size of the myocardial infarction, the higher the maximum elevation of serum transaminases. It should be considered the many factors which might influence the rate of elevation of these enzymes in serum, i.e., variation in the anatomical collateral blood vessel communication, the completeness of necrosis, in the original concentrations of enzymes in the individual heart muscle, the degeneration of enzymes and other factors.

We also found the evidence for increased serum G.O.T. and G.P.T. activity following other degenerative, necrotic diseases, such as hepatitis, infectious mononucleosis and leucemic disease in man, as reported previously (38). From these results, we have the concept that transaminase of myocardial tissue is released into the blood stream from the necrotic tissue, especially myocardial infarction.

g) *ATP-ase*—The ATP-ase activity of myocardial homogenate decreased markedly at 15 hours after ligation.

h) *Analysis of phosphorous intermediates*—The high energy phosphates, such as ATP and phosphocreatine, especially phosphocreatine, began to decrease at 10 min. after ligation, and continued the decreasing up to 7th day. The inorganic phosphate increased in 24 hours after ligation because of liberation of phosphate from labile P, but began to decrease at 24 hours after occlusion and continued to decrease with the progress of infarction (Fig. 6).

It was found that the relative specific activity of ATP, phosphocreatine (PC) and inorganic phosphate began to decrease at 10 min. after ligation, especially PC (Fig. 7).

i) *others*—Glycogen of myocardium from dogs subjected to coronary occlusion decreased markedly at 30 min. after ligation, then transitory recovered, and further decreased completely (Fig. 1). Although there were individual variables in

Fig. 6 Phosphorous compounds in myocardium after coronary occlusion

Fig. 7 Distribution of P_{32} in myocardium after coronary occlusion

glycogen content of heart muscles, there were tendency of showing the decrease of glycogen content even in normal muscle of infarcted dogs.

It was ascertained that there were no direct relationships between the decrease of endogenous respiration and the changes of the glycogen content of myocardium after the coronary occlusion, but the R. Q. of endogenous respiration was approximately 1.0.

Total nitrogen of the myocardial tissues continued to decrease very slightly (Fig. 1). The difference of the potassium content between the coronary artery and the coronary sinus (A-V), became to be lower after the coronary occlusion (Fig. 8). It was assumed that potassium released into the coronary sinus due to myocardial degeneration or due to depletion of active energy production. We could not obtain the distinct tendency concerning the Na metabolism.

III. Metabolic disturbances by injection of 2, 4-dinitrophenol (DNP)

It has become likely that ATP is the primary energy carrier between the energy yielding reaction, such as oxidation, and the energy requiring reaction,

Fig. 8 Potassium and sodium in plasma of dogs subjected to coronary occlusion
○: Before coronary occlusion
●: After coronary occlusion

such as actomyosin, myosin-ATP-ase reaction. Oxidative phosphorylation, synthesizing ATP from inorganic phosphate, is closely associated with the energy producing oxidation, especially cyclophorase enzyme system, because the chain phosphorylation is coupled to the passage of electrons from a reduced carrier to oxygen.

DNP inhibits this coupling reaction between oxidation and phosphorylation, i. e., depressed the generation of energy rich phosphates which normally accompanies certain aerobic oxidation. Pinchot and Bloom (41) have reported that both crude and purified diphtheria toxin markedly depress the labile high energy phosphates of skeletal muscle in guinea pigs without apparent effect on oxygen utilization. It is likely that diphtheria toxin, like DNP, inhibits the coupling of oxidation and phosphorylation. An attempt to investigate how important the oxidative phosphorylation is in the heart function, was carried out by applying DNP for the heart muscle in vitro and in vivo together.

a) *Experiments in vitro*—Effects of DNP on the respiration of normal cardiac slices were investigated as shown in Fig. 9. It is apparent from the data obtained that DNP inhibits both oxygen uptake and phosphate uptake at higher concentration (10^{-4} M), and stimulates the oxygen uptake with inhibiting the phosphate uptake of cardiac slices at the lower concentration. DNP had no particular effect on the succinoxidase, cytochrome oxidase, lactic dehydrogenase and endogenous respiration of myocardial homogenates as shown in Fig. 9.

b) *Experiments in vivo*—DNP was administered intravenously drop by drop in the course of about 40 min. The dogs were anesthetized and

Fig. 9 Effects of 2,4-dinitrophenol on myocardial metabolism

their chests were opened under the artificial respiration as mentioned before. Approximately 20 min. were allowed for a waiting period. In general, the control sample from left ventricle, coronary sinus and other vessels before injection of DNP and the ordinary blood samples from those vessels just before dissecting heart, were drawn for measuring the blood glucose, lactate, oxygen, carbon dioxide, potassium, sodium, DNP and transaminases.

DNP was estimated spectrophotometrically by means of a double extraction method reported by Mudge and Taggart (42).

It seems likely that the myocardial extraction of glucose, and lactate diminished moderately as shown in Fig. 9.

When 0.05% DNP solution made by dissolving in H_2O were administered, the oxygen content in blood decreased markedly with a gradual elevation of plasma DNP level due to hemolysis, but 0.05% DNP solution made by dissolving in a physiological saline solution produced no particular oxygen changes.

Therefore, we used DNP as 0.05% DNP-saline solution in the following all experiments.

The average coronary arterio-venous differences (A-V) of blood glucose, and lactate in 7 dogs poisoned by DNP, became negative in 3 expts. But the utilization of glucose in cardiac slices from dogs poisoned by DNP, did not decrease so much in vitro.

The oxygen uptake of cardiac slices from dogs poisoned by DNP, was decreased, when the concentration of DNP in plasma were higher ($3.8 \times 10^{-4} M$), but was not increased even when the concentration of DNP in plasma were lower ($1.0 \times 10^{-4} M$).

Fig. 10 Potassium and sodium in blood plasma of dogs poisoned by 2,4-dinitrophenol. (): DNP conc. in plasma ($10^{-4} M$)

The activities of several enzymes, succinoxidase, cytochrome oxidase, lactic dehydrogenase, ATP-ase, G. O. T., G. P. T., endogenous respiration of myocardial homogenate of dogs poisoned by DNP, were almost within the normal range. The high energy phosphate bond stores, especially phosphocreatine, in cardiac muscle under the administration of DNP in vivo, depleted markedly. The relative specific activities of these compounds decreased particularly (Fig. 11). A finding of low levels of high energy

Fig. 11 Phosphorous compounds and distribution of P_{32} in myocardium of dogs poisoned by 2,4-dinitrophenol and sodium fluoride

phosphate stores could tend to support a concept of failure of energy production or conservation.

The ECG was studied also. And it was observed that the elevation of J point, ST, T segments and sometimes block had happened (Fig. extra 2).

For the histological study of heart muscle poisoned by DNP, all specimen taken from heart muscle, were fixed in formalin or alcohol and dyed with hematoxylin-eosin, and P.A.S. The changes of the microscopic structure consisted mainly in the enlargement of intercellular spaces as shown in Fig. extra 3. The substances extensively dyed by PAS had localized in the peripheral portion of

muscle fiber (Fig. extra 4).

IV. Metabolic disturbances by injection of NaF

0.4 M NaF solution was prepared as follows: 16.8 gm. of NaF were dissolved in 1000ml. of 0.9% saline solution. This solution was administered intravenously. In the experiments with NaF, the ventricular fibrillation was often observed. The myocardial extraction of lactate became negative in 3 expts. out of 5 expts. The rate of oxygen uptake of cardiac slices was reduced moderately as shown in Fig. 12. The phosphocreatine decreased markedly and promptly, but ATP increased or did not change or decreased (Fig. 11). No other particular change

Fig. extra. 2-I DNP (no Haemolysis)

Fig. extra. 2-II DNP (Haemolysis)

Fig. extra. 3 Myocardial tissue of dogs poisoned by DNP Hematoxylin-eosin-staining (300×)

Fig. extra. 4 Myocardial tissue of dogs poisoned by DNP P. A. S.-staining (300×)

Fig. 12 Effects of NaF on myocardial metabolism

could be observed under the conditions used.

DISCUSSION

The studies on the metabolism of heart muscle, especially on energy metabolism of it, can be investigated by use of techniques on cardiac slices or myocardial homogenates, isolated enzyme systems, mitochondrial fraction, on the isolated heart in vitro and on the intact heart in situ.

Each of these methods possesses certain advantages and disadvantages. For example, with mitochondrial fraction including cyclophorase system and isolated enzyme systems, the fundamental effects of several substances on them or fundamental mechanisms can be studied, but the use of these analytical methods does not reveal the properties of the system as a whole, and certainly the conditions under which the studies are made are highly specific and artificial. The slices are in the rather organized condition than the isolated enzyme in homogenate and are more similar to the condition which are in intact animal in situ. When the studies on the tissue utilization of substrates in vitro are attempted, slice technique is very valuable and important although the conditions under which studies are carried out are also artificial. An attempt to study the metabolism of myocardium by using the whole heart outside the body, such as isolated heart and heart-lung preparation etc., should be restricted to the observations of changes in acute process and also that under the artificial conditions. But many various conditions can be easily performed in these slice methods. Metabolic studies carried out on the intact human or animal heart in situ, have the disadvantages that only overall, not intermediary changes can be observed. But this method has the advantage that all results are obtained under the physiological conditions in natural and relatively undisturbed environment.

We used all these methods at the same time in the same dogs and could compare the results obtained from these individual methods together. We paid our attention chiefly on the processes of energy production.

We studied the metabolic abnormalities of the coronary occlusion which is the most severe cardiac ischemia and in which the metabolic changes are numerous. We studied also the metabolic changes of dog-heart muscle subjected to be explored the injection of DNP and NaF which are metabolic

inhibitors. It is well known that myocardial infarction in man is frequently followed by a fall in blood pressure. It might be expected that the coronary flow is further reduced because of diminished pressure in aorta in patients with severe fall in blood pressure.

Out of a total of 12 experiments, the average coronary arteriovenous difference in blood glucose and lactate became to decrease. In many instances, potassium appeared in increased amounts in coronary vein blood, especially at 3 hours after ligation.

In the artificial coronary occlusion, the energy producing systems, such as mitochondrial oxidative phosphorylation, became to decrease at 30 min. after ligation. It was also recognized that the intermediates of phosphorous metabolism, especially high energy bond stores, such as phosphocreatine, and ATP, became to be reduced markedly at 30 min. after ligation of coronary artery. The endogenous respiration, cytochrome oxidase, lactic dehydrogenase and succinoxidase activity of myocardial homogenate from dogs subjected to coronary occlusion were reduced markedly. The substrate utilization and oxygen uptake of cardiac slices prepared from infarcted myocardium were reduced markedly with a progress of infarction. Therefore, it was postulated that the energy producing metabolism of myocardium began to decrease at 30 min. or more faster after the ligation. It appears that studies on the mitochondrial cyclophorase system and endogenous respiration of homogenate were more beneficial and valuable than the studies on the individual oxidative enzymes to study the oxidative capacity of cardiac muscle. We could not say how closely the potassium liberation from cardiac muscle, the depletion of high energy phosphates, and the elevation of ST, T segments in ECG are related in general. But in artificial coronary occlusion, it seems likely that this relationships between them would be present.

The serum G. O. T. and G. P. T. were invariably elevated following myocardial infarction. The height of the rise of the activity of these enzymes in the serum was roughly proportional to the amount of infarcted myocardial tissue. These enzymes were elevated faster in the blood serum of coronary sinus than in the serum of femoral vein or other peripheral vein. We also found the fact that these enzymes were elevated following infectious mononucleosis, leucemic disease, and severe

hepatitis in the human being. Thoracotomy results also the elevation of these enzymes although the degree of elevation of them is very low. These findings would indicate that a rise in the serum G. O. T. and G. P. T. may result from leakage of enzymes through myocardium and the leakage may easily occur through severely damaged cell membranes.

The low activities of several enzymes studied here, may result from some kind of degeneration or insufficient synthesise of enzyme protein, probably due to depletion of high energy phosphate stores.

In experiments with DNP inhibition in situ, the distinct reduction of high energy phosphate bond stores, and the leakage of potassium from myocardium into coronary vein, had been observed with the slight effects on the utilization of substrate and respiration.

In experiments with DNP inhibition in vitro, the distinct reduction of phosphate uptake was observed without particular effect on the utilization of oxygen and substrates. It would be possible to produce a failure of energy production in dog heart by administration of DNP.

In experiments with coronary occlusion and DNP injection, three particular changes, the depletion of high energy phosphate, leakage of potassium from myocardium into coronary vein, and the elevation of ST, T segment in ECG were observed in common.

But we could not observe a distinct tendency of these metabolic changes after the injection of NaF. Further works are required to solve the definite mechanism concerning the elevation of ST, T segment in ECG.

SUMMARY

1. The myocardial metabolism of dogs subjected to coronary occlusion or poisoned by metabolic inhibitor, such as DNP and NaF were investigated by use of metabolic studies on tissue slices, homogenates, mitochondrial fraction in vitro and on the intact heart in situ.

2. In experiments with artificial coronary occlusion, at first, phosphocreatine in myocardium were reduced, then endogenous respiration, oxygen uptake of cardiac slice, oxidative phosphorylation of isolated mitochondrial fraction decreased markedly at about 30 min. after ligation. Further, cyto-

chrome oxidase, lactic dehydrogenase, glutamic oxalacetic transaminase, glutamic pyruvic transaminase, ATP-ase, and succinoxidase of myocardial homogenate prepared from infarcted heart muscle were gradually decrease with a progress of infarction. Glucose, lactate and oxygen utilization of heart muscle in situ were reduced, and the glucose utilization and oxygen uptake of cardiac slice were reduced markedly. The amount of glycogen and total nitrogen in myocardium decreased. The serum G. O. T. and G. P. T. rose sharply after myocardial infarction and there might exist some relationships between the peak serum G. O. T. and G. P. T. levels and the grade and size of the injured myocardium. The average coronary arterio-venous difference of potassium became smaller after coronary occlusion.

3. In experiments with DNP poison, only the depletion of high energy phosphate stores, slight disturbances of the myocardial extraction of glucose and lactate, leakage of potassium from myocardium into coronary vein, and the elevation of ST, T segment in ECG were observed. And the relationships between the DNP level in blood plasma and the degree of those metabolic changes were discussed.

4. In experiments with NaF poison, only the depletion of phosphocreatine and the reduction of the rate of oxygen uptake of cardiac slices were observed.

Acknowledgement

The authors wish to express their appreciation for the wishes to appoint for this opportunity to participate in the symposium to the committee members of the Japanese Circulation Society and for the advice and counsel of Prof. T. Misao, Prof. Y. Oshima and Prof. M. Shirakawa in this work.

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