IMPAIRMENT OF MITOCHONDRIAL RESPIRATORY ACTIVITY OF NON-INFARCTED MYOCARDIUM UNDER SEVERE PUMP FAILURE IN ACUTE MYOCARDIAL INFARCTION

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We studied alterations in respiratory activity of mitochondria (Mt) in non-infarcted myocardium (NIZ) under severe pump failure complicated by acute myocardial infarction (AMI). Dogs in which AMI was induced were divided into two groups; one in which left ventricular systolic pressure (LVPs) was maintained higher than 70% of preligation level (ND group); and one in which LVPs was diminished to less than 70% (D group). Regional myocardial blood flow (MBF) in NIZ reduced significantly in proportion to decreases in LVPs and cardiac output (CO). State -III activity and RCR decreased in proportion to reductions in MBF, LVPs, and CO in Mt from NIZ of D group. Complex-I and DNP-stimulated ATPase activities were also reduced in NIZ of D group. Morphologic studies revealed slight swelling and fusion of mitochondria in NIZ cells of D group, but no changes such as the appearance of a dense deposit indicating ischemic damage were seen. Pump failure in AMI is likely to be caused partly by impaired function of Mt in NIZ induced by hypoperfusion. Improvement of metabolic impairment in NIZ is important in the treatment of pump failure.

THE prognosis of acute myocardial infarction (AMI) has improved remarkably in recent decades with the establishment of coronary care units. However, the mortality of patients in which AMI is complicated with severe pump failure remains higher, even with such measures as intra-aortic balloon pumping (IABP) and ventricular assist devices. The etiology of this severe pump failure has not yet been completely elucidated. Cardiac dysfunction in AMI is known to be related to the size of the myocardial infarction¹⁻³. Therefore the relationship be-

tween the size of the infarcted myocardium and the occurrence of pump failure has long been discussed. According to Page et al¹, cardiogenic shock is a complication when the infarct size is larger than 40% of left ventricular muscle; other researchers^{2,3} have reported similar findings. However since Kurland et al⁴ denied any correlation between extension of the infarcted myocardium and pump failure, it is difficult to attribute pump failure directly to the size of infarction alone. In this paper, we investigated metabolic impairment of the non-infarcted myocardium in AMI under severe pump failure. In our previous paper⁵, Ca⁺⁺-stimulated ATPase activity of the sarcoplasmic reticulum (SR) and tissue ATP content in the non-infarcted myocardium were recognized to decrease

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in proportion to reduction in left ventricular systolic pressure (LVPs) in experimental myocardial infarction. These pieces of evidence suggest that in the non-infarcted myocardium, both the energy producing and consuming systems of the cardiac myocytes are impaired in cardiac dysfunction. In the present study, we focused our attention on the possibility of mitochondrial dysfunction by measuring respiratory and electron transporting enzymatic activities in the non-infarcted myocardium.

MATERIALS AND METHODS

Animal experiment: Thirty adult mongrel dogs weighing 8 to 14 kg were anesthetized with intravenous injection of urethane (450 mg/kg), α -chloralose (45 mg/kg) and diazepam (10 mg). A left thoracotomy was performed under positive pressure respiration with room air using a Harvard respirator. The circumflex branch of the left coronary artery (LCX) was isolated and ligated completely at 1 cm from its origin. Production of severe transmural myocardial ischemia was checked by continuous ST segment elevation in leads II, III, and aV_F on the electrocardiogram. A pig tail catheter and a Swan-Ganz catheter were inserted into the femoral artery and saphenous vein, respectively, for monitoring hemodynamic changes such as left ventricular systolic pressure (LVPs) and pulmonary artery pressure (PAP) and cardiac output (CO). Regional myocardial blood flow (MBF) was determined by the H_2 gas clearance method using platinum electrodes inserted into the subendocardial muscles of the infarcted (IZ) and non-infarcted myocardia (NIZ). Sixty minutes after ligation the dogs were divided into two groups, those in which LVPs was maintained higher than 70% (ND group) and those in which LVPs was reduced lower than 70% of the preligation level (D group), using the view of development of cardiogenic shock according to the report of Mizutani et al.⁶ One hundred and twenty minutes after ligation the beating heart was removed, washed briefly with ice-cold saline solution, and 3g of NIZ was excised from the area perfused by the left anterior descending coronary artery (LAD), simultaneously with IZ from LCX area, and each of them was separated at the middle of

the ventricular wall into the subendocardial (Endo) and subepicardial muscles (Epi).

Preparation of mitochondria: All the procedures were carried out at 4 °C unless otherwise mentioned, and all the reagents were the reagent grade. Mitochondria were prepared by the modified method of Sordahl et al.⁷ utilizing alkaline protease (Nagarse, Nagarse Co., Ltd.) as follows. Briefly, 3g of heart muscle was minced with scissors and homogenized with 9 volumes of a solution containing 0.25M sucrose, 0.01M glycoetherdiaminetetraacetic acid (GEDTA), 0.03M tris-HCl (pH-7.2), with a Polytron homogenizer and centrifuged at $27,000\times G$ for 10 min. One mg of Nagarse per gram of starting heart tissue was added to the pellet and incubated at 4 °C for 8 min, and 40 ml of a solution containing 0.18M KCl, 0.01M GEDTA, 0.5% (wt/vol) bovine serum albumin (BSA), and 0.03M tris-HCl (pH-7.2) was added, and centrifuged at $500\times G$ for 5 min. The supernatant was centrifuged at $12,000\times G$ for 10 min twice, and the pellet was suspended in a solution containing 0.18M KCl, 0.5% BSA and 0.03M tris-HCl (pH-7.2) at a final concentration of approximately 15 mg/ml. A part of the mitochondria was sonicated by a sonifier (Branson Co., Ltd.) for 60 sec, and designated as sub-mitochondrial particles. Protein determination was made by a biuret method using BSA as a standard.

Measurement of oxidative phosphorylation: Mitochondrial oxidative phosphorylation was measured with an oxygen electrode (Oxygen Consumption Recorder PO-100A, Yanagimoto Mfg. Co. Ltd.), according to the method of Hagihara.⁸ The reaction medium contained 0.01M KCl, 0.0001M ethylenediaminetetraacetic acid (EDTA), 0.002M $MgCl_2$, 0.25M sucrose and 0.02M potassium phosphate buffer (pH-7.2) at 25 °C. Succinate and glutamate plus malate were used as substrates and ADP was added to estimate oxidative phosphorylation. Oxygen consumption rates in states-III and IV and the respiratory control ratio (RCR) were determined from a polarographic record by the method of Chance and Williams.⁹

Measurement of NADH-coenzyme Q (CoQ) reductase (complex-I) activity: Complex-I activity was assayed spectrophotometrically by a decrease in absorbancy at

Fig. 1. Hemodynamic changes of ND and D groups.

A: left ventricular systolic pressure, B: left ventricular max dp/dt, C: cardiac output and D: regional myocardial blood flow. ○: ND group, and ●: D group, in which LVPs was maintained higher than 70% of preligation level and was not, respectively. Values are expressed as the percent of preligation levels. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$

340 nm as a function of time according to the method of Hatefi and Rieske¹⁰ at 37 °C, in a reaction medium containing 0.03 mM NADH, 0.04 mM CoQ₃, 0.003M NaN₃, and 0.08M tris-HCl (pH-8.0). The reaction was started by the addition of 0.4 mg of submitochondrial particles per tube.

Measurement of succinate dehydrogenase-CoQ reductase (complex-II) activity: Complex-II activity was assayed spectrophotometrically by measuring the rate of reduction of 2, 6-dichloroindophenol sodium (DCIP) at 600 nm at 37 °C according to the method of Ziegler.¹¹ The reaction medium contained 0.02M sodium succinate, 0.01 mM EDTA, 0.01% (wt/vol) triton X-100, 0.0017% DCIP, 0.04 mM CoQ₃, and 0.1M potassium phosphate (pH-7.0), and the reaction was started by the addition of 0.05 mg of submitochondrial particles.

Measurement of cytochrome oxidase

(complex-IV) activity: Complex-IV activity was assayed spectrophotometrically at 550 nm at 37 °C using bovine cytochrome C (reduced form) as a substrate according to the modified method of Oriei et al¹² and the reaction was started by the addition of 0.05 mg of mitochondria.

Measurement of ATPase activity: Dinitrophenol (DNP)-stimulated ATPase activity of mitochondria was examined by the modified method of Pullman et al¹³ in a reaction medium containing 0.21M sucrose, 0.02M KCl, 0.02M tris-malate (pH-7.2) and 5 mg of mitochondria. The reaction was started by the addition of 0.001M DNP and 0.03M ATP at 25 °C, and the amount of phosphate liberated in the reaction was assayed by the method of Fiske and Subbarow.¹⁴

Ultrastructural observation: Portions of the infarcted and non-infarcted heart muscles were minced and prefixed with a solu-

Fig.2. Correlation between MBF in the non-infarcted myocardium and LVPs (A), and CO (B).

○: ND group, and ●: D group. Abbreviations: MBF; regional myocardial blood flow, LVPs; left ventricular systolic pressure, and CO; cardiac output. Positive correlations were noted between MBF and LVPs, CO.

Fig.3. Oxygen consumption rate in state III of mitochondria.

A: succinate and B: glutamate plus malate. Bars with slant lines and dotted bars indicate the activities of endocardial and epicardial muscles, respectively. Abbreviations: NIZ; non-infarcted myocardium zone, and IZ; infarcted myocardium zone.

In NIZ, the activity of state III of the D group reduced significantly. Values are expressed as mean $\pm$ S.D. ** $p < 0.01$, *** $p < 0.001$

tion containing 2% glutaraldehyde and 0.1M Na-cacodylate (pH-7.4) for 2 h at 0°C. Then the samples were rinsed with 0.1M cacodylate and sucrose, and fixed with 1% OsO₄, and embedded in Epon. The blocks thus obtained were ultrathin-sliced by a Sorvall MT-2 ultramicrotome, and the slices were double-stained and observed with a Hitachi H-300 electron microscope.

Statistics: Results were expressed as

mean $\pm$ S.D. of the number of experimental indicators. Statistical analyses were carried out using Student's t-test, where p values less than 0.05 were considered significantly different.

RESULTS

Hemodynamic changes: Fig. 1-A shows chronologic changes in LVPs. In the ND

Fig.4. Correlations between RCR and MBF (A), and RCR and LVPs in the non-infarcted canine myocardium.

Succinate is used as a substrate. Abbreviations: RCR; respiratory control ratio of mitochondria, MBF; regional myocardial blood flow, and LVPs; left ventricular systolic pressure. Positive correlations were noted between RCR and MBF, LVPs.

group, LVPs was maintained at 93.3% of the preligation level, but in the D group it decreased significantly to below 70% that of the preligation level 60 min after ligation and remained in this shock level, being reduced to 58.6% at 120 min. No differences were noticed in the heart rate between the ND and D groups.

With respect to left ventricular max dp/dt (LV max dp/dt), there were no significant changes until 120 min after ligation in the ND group, but in the D group, it reduced to 64.8% of the preligation level at 120 min after ligation in a similar manner to that of LVPs, as shown in Fig. 1-B. Cardiac output (CO) was maintained around 87.3% of the preligation level even 120 min after ligation in the ND group. However, in the D group it reduced significantly 60 min after ligation, and after 120 min it diminished to 53.9% of the preligation level (Fig. 1-C).

Myocardial regional blood flow (MBF) in the subendocardial layer is shown in Fig. 1-D. In the infarcted portion, MBF dropped remarkably in both of the groups to about 10% of the preligation level. In the non-infarcted myocardium (Fig. 1-D), MBF in the ND group was preserved as high as 98.8% of the preligation level until 120 min, but in the D group it began to decrease significantly at 30 min after ligation, and reduced further to

61.8% at 120 min.

Figure 2 shows the relationship between MBF in the non-infarcted myocardium and LVPs, and CO. Positive correlations were noticed between MBF and both LVPs and CO. That is to say, in the non-infarcted myocardium, MBF decreased in proportion to reductions in LVPs and CO.

Mitochondrial respiratory activity: Fig. 3-A presents the oxygen consumption rate in state-III of mitochondria, using succinate as a substrate. In the non-infarcted myocardium, the activity of the ND group was 202.9 ± 49.6 and 176.1 ± 28.7 μ atoms O/mg protein/min in Endo and Epi, respectively. But the activity of the D group reduced significantly to 127.9 ± 30.8 (63.0%) and 108.4 ± 27.8 (61.6%) in both Endo and Epi ($p < 0.001$), respectively. In the infarcted myocardium, the activities of state-III in both of the ND and D groups decreased markedly as described previously^{15,16,17}. Similarly, Fig. 3-B shows the oxygen consumption rate in state-III in which glutamate plus malate were utilized as substrates. In the non-infarcted myocardium, the activity of the ND group was 203.7 ± 24.9 and 178.8 ± 30.7 in Endo and Epi, respectively.

In the D group, the activity decreased significantly to 133.6 ± 16.2 (65.6%) and

Fig. 5. NADH-CoQ reductase (complex I) activity of submitochondrial particles in the acute infarcted myocardium in dogs. Bars with slant lines and dotted bars indicate the activities of endocardial and epicardial muscles, respectively.

Abbreviations: NIZ; non-infarcted myocardium zone, and IZ; infarcted myocardium zone. In NIZ, complex I activity of the D group reduced significantly. Values are expressed as mean $\pm$ S.D. ** $p < 0.01$, *** $p < 0.001$.

Fig. 6. DNP-stimulated ATPase activity of mitochondria in the acute infarcted myocardium in dogs. Bars with slant lines and dotted bars indicate the activities of endocardial and epicardial muscles, respectively.

Abbreviations: NIZ; non-infarcted myocardium zone, and IZ; infarcted myocardium zone. Reduction in ATPase activity was noted in NIZ of the D group. Values are expressed as mean $\pm$ S.D. * $p < 0.05$, ** $p < 0.01$.

116.5 $\pm$ 16.4 (65.2%) in Endo and Epi, respectively ($p < 0.001$ and $p < 0.01$), similar to that using succinate as a substrate. In the infarcted myocardium, the activity of state-III decreased remarkably both in the ND and D groups. The oxygen consumption rate in state-IV was not altered significantly in either the non-infarcted and infarcted myocardia or the ND and D groups. Consequently, the respiratory consumption ratio (RCR) followed the similar process to that of the oxygen consumption rate in state-III. When succinate was utilized as a substrate, RCR of mitochondria from the non-infarcted myocardium of the ND group was determined to be 4.81 $\pm$ 1.19 and 4.17 $\pm$ 0.75 in Endo and Epi, respectively. RCR decreased significantly in the D group to 3.25 $\pm$ 0.63 ($p < 0.01$) in Endo and 2.79 $\pm$ 0.53 ($p < 0.001$) in Epi. When glutamate plus malate were used as substrates, RCR of the non-infarcted myocardium in the ND group was measured to be 7.48 $\pm$ 0.79 and 6.43 $\pm$ 1.01 in Endo and Epi, respectively, and it decreased signifi-

cantly in the D group to 4.83 $\pm$ 0.59 ($p < 0.001$) in Endo and 4.39 $\pm$ 0.87 ($p < 0.01$) in Epi. The correlations of RCR with MBF and LVPs in the non-infarcted myocardium are shown in Fig. 4-A and 4-B, respectively, in which succinate was used as a substrate. Both of them exhibited positive significance, and these correlations were also similarly positive with glutamate plus malate. RCR and CO also exhibited positive correlations in the non-infarcted myocardium.

NADH-COQ reductase (complex-I) activity is shown in Fig. 5. In the non-infarcted myocardium, complex-I activity in the ND group was 0.065 $\pm$ 0.012 and 0.055 $\pm$ 0.007 μ moles NADH/mg protein/min in Endo and Epi, respectively, but in the D group it decreased significantly to 0.038 $\pm$ 0.012 and 0.035 $\pm$ 0.004 in Endo and Epi, respectively ($p < 0.01$, $p < 0.001$). In the infarcted myocardium this activity decreased markedly in both of the ND and D groups. Succinate dehydrogenase-CoQ reductase (complex-II) and cytochrome oxidase (complex-IV) activi-

Fig.7. Electron micrographs of myocardial cells in the acute infarcted myocardium.

A: non-infarcted myocardium in ND group. Fine structures are well preserved.

B: non-infarcted myocardium in D group. Slight swelling and fusion of mitochondria are observed, however, no such irreversible changes are noted as appearance of dense deposit.

C: infarcted myocardium. Ischemic necrotic changes such as dense deposits (arrows), severe intracellular edema and derangement of myofibrils are apparent. Mt: mitochondria, Mf: myofibrils, T: T-system. Bars indicate 1 μ m

ties were not altered in either the ND or D groups, nor in the non-infarcted and infarcted myocardia

DNP-stimulated ATPase activity is shown in Fig. 6. In the non-infarcted myocardium the activity was 12.91 ± 2.21 and 10.54 ± 1.70 μ moles Pi/mg protein/hr in Endo and Epi, respectively in the ND group, but the activity in the D group was significantly reduced to 8.87 ± 2.56 and 8.40 ± 2.09 in Endo and Epi, respectively ($p < 0.01$, $p < 0.05$).

Fine structural alterations: Fig. 7 shows electron micrographs of myocardial cells from the non-infarcted and infarcted subendocardia in the ND and D groups. In the non-infarcted myocardium of the D group, such slight ultrastructural changes as slight swelling and fusion of mitochondria were observed. But in all of the specimens obtained from the non-infarcted myocardium there were no irreversible necrotic changes such as the appearance of a dense deposit which was observed in the infarcted myocardial cells as shown in Fig. C.

DISCUSSION

In the previous papers of our study group,^{15,16,18-20} we reported a reduction in Ca^{++} -stimulated ATPase activity of the sarcoplasmic reticulum (SR), together with degradation of the major ATPase protein and of phospholipids of SR, and irreversible decreases in mitochondrial respiratory activities in experimental acute myocardial ischemia in the dog, as early as 20 to 30 min after coronary occlusion in Endo. With respect to the non-ischemic portion, metabolic impairment was also suspected to occur with the complication of severe pump failure such as cardiogenic shock. Cardiac dysfunction after coronary occlusion has been attributed to mechanical factors in the ischemic area, and the etiology of severe pump failure has been emphasized for a long time in relation to the size of infarction¹⁻³ But some factors other than size of infarction should be taken into consideration in the genesis of pump failure. These include differences in the coronary artery, distribution of compensated catecholamine secretions, the presence of asynergy in the infarcted myocardium and severe ventricular arrhythmias, and differences in compliance in the infarcted myocardium. Wyatt

et al²¹ described how the depression of left ventricular function after coronary occlusion may be related to depression of function in the non-ischemic myocardium. They demonstrated that functional depression in the non-ischemic myocardium is potentially important for the understanding of the mechanisms regulating cardiac performance. Further, in morphology, Viskert et al²² found mitochondrial damage including swelling, opening of the matrix and a decrease in the number of cristae in the non-occluded regions of the rabbit myocardium.

In clinical practice, the fact that catecholamine and IABP are effective to a good extent in the treatment of pump failure is thought to be due to the increase in contractility of the non-infarcted cardiac muscle as a result of the recovery of blood flow. Thus, in the clinical treatment of acute myocardial infarction, improvement of the function of the non-infarcted myocardium has been thought to be the first good for the treatment of pump failure rather than the salvage of the infarcted myocardium by intracoronary arterial thrombolytic therapy.

In this study, we examined the changes in the mitochondrial function from non-infarcted myocardium in severely depressed myocardium. There have been many reports^{15–17,23,24} describing a decrease in mitochondrial respiratory activity, especially reduction of the oxygen consumption rate in state-III in the infarcted myocardium of acute ischemic hearts. Further, the electron-transport system was examined by Geshi et al¹⁶ who described a reduction in complex-I activity as the earliest signs of irreversible cell damage; similar findings have been reported by Rouslin and co-workers.¹⁷ With regard to the function of mitochondria in non-infarcted myocardium, there are few papers and no agreement has been achieved. In the present paper, it is suggested that according to reductions in LVPs and CO, MBF decreases not only in the infarcted myocardium but also in the non-infarcted myocardium, and that mitochondrial respiratory impairment and energy producing disturbance are likely to occur as a result of coronary hypoperfusion in the non-infarcted myocardium. This leads to the suggestion that pump failure may occur as a result of

the development of a vicious cycle, that is a decrease in mitochondrial function triggered by hypoperfusion causes impairment of the energy producing system, and that in turn causes low perfusion due to decreased cardiac contractility.

With reference to the metabolism of non-infarcted myocardium in the acute ischemic heart, there are some papers^{25,26} in which creatine phosphate and ATP were reported to decrease in relation to the depletion of cardiac function.

Similarly, in the energy consumption system, Ca^{++} -uptake and Ca^{++} -ATPase activity in non-infarcted myocardium were reduced during coronary hypoperfusion.^{27,28}

Furthermore, not only in the case of the coronary ligation model but also in the experimental shock model, there are reports²⁹ that ATP synthesis and calcium transport rates of mitochondria decline during circulatory shock. Increases in the contents of free fatty acids and Ca^{++} of mitochondria and a decrease in adenine nucleotide translocase activity were shown by Chaudry et al³⁰ during hypoperfusion. Turning to morphologic observations, there are many reports concerning infarcted myocardial cells. Similar changes to these reports were observed in our ultrastructural research; these include severe cellular edema, degradation of mitochondrial cristae with appearance of dense bodies and extreme distension of myofibrils. In the non-infarcted myocardium, Schumer et al³¹ demonstrated swelling, dissolution of the internal architecture in experimental endotoxin shock in rats with injection of *E. coli*. In our observations, slight swelling and fusion of mitochondria defined as reversible changes were seen in non-infarcted myocardial cells of the D group.

In the present study, mitochondrial respiratory activity and complex-I activity decreased in the non-infarcted myocardium in proportion to the reductions in LVPs, CO, and MBF, and these decreases would result in further myocardial injury and irreversible damage.

In conclusion, metabolic impairment occurs even in the non-infarcted myocardium according to the reductions in LVPs, CO and MBF, and the recovery of these dysfunctions should be important for the treatment of pump failure.

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REFERENCES

- PAGE DL, CAULFIELD JB, KASTOR JA, DE-SANCTIS RW, SANDERS CA: Myocardial changes associated with cardiogenic shock. *N Engl J Med* 1971; **285**: 133
- SWAN HJC, FORRESTER JS, DIAMOND G, CHATTERJEE K, PARMLEY WW: Hemodynamic spectrum of myocardial infarction and cardiogenic shock. *Circulation* 1972; **45**: 1097
- ALONSO DR, SCHEIDT S, POST M, KILLIP T: Pathophysiology of cardiogenic shock. Quantification of myocardial necrosis, clinical, pathologic and electrocardiographic correlation. *Circulation* 1973; **48**: 588
- KURLAND GS, WEINGARTEN C, PITT B: The relation between the location of coronary occlusions and the occurrence of shock in acute myocardial infarction. *Circulation* 1965; **31**: 646
- MOCHIZUKI M, KONNO N, UMETSU K, KATAGIRI T: Metabolic changes in the non-ischemic myocardium under pump failure complicated in acute myocardial infarction. *J Showa Med Assoc* 1988; **48**: 95
- MIZUTANI T, HAZEKI T: A study of system and regional hemodynamic changes in experimental myocardial infarction, with reference to the effects of catecholamine. *Jpn Circ J* 1974; **38**: 479
- SORDAHL LA, STEWART ML: Mechanism (s) of altered mitochondrial calcium transport in acutely ischemic canine heart. *Circ Res* 1980; **47**: 814
- HAGIHARA B: Techniques for the application of polarography to mitochondrial respiration. *Biochem Biophys Acta* 1961; **46**: 134
- CHANCE B, WILLIAMS GR: Respiratory enzymes in oxidative phosphorylation. I. Kinetic of oxygen utilization. *J Biol Chem* 1955; **217**: 383
- HATEFI Y, RIESKE JS: Preparation and properties of DPNH-coenzyme Q reductase (complex I of the respiratory chain). In *Methods in Enzymology*, vol x, oxidation and phosphorylation, ed by ESTABROOK RW, PULLMAN ME, Academic Press, New York, 1967, p235
- ZIEGLER D, RIESKE JS: Preparation and properties of succinate dehydrogenase-coenzyme Q reductase (complex II). In *Method in Enzymology*, vol x, oxidation and phosphorylation, ed by ESTABROOK RW, PULLMAN ME, Academic Press, New York, 1967, p231
- ORII Y, OKUNUKI K: Studies on cytochrome a. XV. Cytochrome oxidase activity of the Okunuki preparation and its activation by heat alkali and detergent treatment. *J Biochem* 1965; **58**: 561
- PULLMAN ME, PENEFSKY HS, DATTA A, RACER E: Partial resolution of the enzymes catalyzing oxidative phosphorylation. I. Purification and properties of soluble dinitrophenol-stimulated adenosine triphosphatase. *J Biol Chem* 1960; **235**: 3322
- FISKE CH, SUBBAROW Y: The colorimetric determination of phosphorus. *J Biol Chem* 1925; **66**: 375
- MINATOBUCHI H, SEKITA S, YOKOYAMA M, KATAGIRI T: Regional changes in mitochondrial respiration in acute myocardial ischemia. Comparison of the inner and outer muscles. *Jpn Heart J* 1984; **24**: 609
- GESHI E, KONNO N, YANAGISHITA T, KATAGIRI T: Impairment of mitochondrial respiratory activity in the early ischemic myocardium. With special reference to electron transport system. *Jpn Circ J* 1988; **52**: 535
- ROUSLIN W: Mitochondrial complex I, II, III, IV, and V in mitochondrial ischemic and autolysis. *Am J Physiol* 1983; **13**: H-743
- TOBA K, KATAGIRI T, TAKEYAMA Y: Studies on the cardiac sarcoplasmic reticulum in myocardial infarction. *Jpn Circ J* 1978; **42**: 447
- KONNO N, YANAGISHITA T, GESHI E, KATAGIRI T: Degradation of the cardiac sarcoplasmic reticulum in acute myocardial ischemia. *Jpn Circ J* 1987; **51**: 411
- YANAGISHITA T, KONNO N, GESHI E, KATAGIRI T: Alterations in phospholipids in acute ischemic myocardium. *Jpn Circ J* 1987; **51**: 41
- WYATT HL, FORRESTER JS, LUZ PL, DIAMOND GA, CHAGRASULIS R, SWAN HJC: Functional abnormalities in nonoccluded regions of myocardium after experimental coronary occlusion. *Am J Cardiol* 1976; **37**: 366
- VIKHERT AM, CHERPACHENKO NM: Changes in metabolism of undamaged sections of myocardium following infarction. *Circ Res* 1974; **35**: 182
- SCHWARTZ A, WOOD JM, ALLEN JC, BORNET EP, ENTMAN KL, GOLDSTEIN MA, SORDAHL LA, SUZUKI M, LEWIS RM: Biochemical and morphologic correlates of cardiac ischemia. 1. membrane systems. *Am J Cardiol* 1973; **32**: 46
- JENNINGS RB, GANOTE CE: Mitochondrial structure and function in acute ischemic injury. *Circ Res* 1976; **38** (Suppl I): I-80
- GUDBJARNASON S, PURI PS, MATHES P: Biochemical changes in non-infarcted heart muscle following myocardial infarction. *J Mol Cell Cardiol* 1971; **2**: 253
- JONES CE, THOMAS JX, PARKER JC, PARKER RE: Acute changes in high energy phosphate, nucleotide derivatives, and contractile force in ischemic and nonischemic canine myocardium following coronary occlusion. *Cardiovasc Res* 1976; **10**: 275
- DHALLA NS, DAS PK, SHARMA GP: Subcellular basis of cardiac contractile failure. *J Mol Cell Cardiol* 1978; **10**: 363
- KRAUSE S, HESS ML: Characterization of cardiac sarcoplasmic reticulum dysfunction during short-term, normothermic, global ischemia. *Circ*

- Res* 1984; **55**: 176
29. MELA-RIKER L, TAVAKOLI H: Mitochondrial function in shock. *Am J Emerg Med* 1982; **2**: 2
30. CHAUDRY IH: Cellular mechanisms in shock and ischemia and their correction. *Am J Physiol* 1983; **245**: R117
31. SCHUMER W, GUPTA TKD, MOSS GS, NYHUS LM: Effect of endotoxemia on liver cell mitochondria in man. *Ann Surg* 1970; **171**: 875