" Effects of endurance exercise and L-thyroxine administration on cardiac muscle "

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Contents

Abbreviations in the text, figures, and tables

A-band = anisotropic band

AMP = adenosine monophosphate

ADP = adenosine diphosphate

ATP = adenosine triphosphate

Cr = creatine

CrP = creatine phosphate

CPK = creatine (phosphate) kinase (EC., 2.7.3.2)

EDTA = ethylen diamine tetra acetic acid

EGTA = glycol ethylen diamine tetra acetic acid

HC = heavy chain

HMM = heavy meromyosin

I-band = isotropic band

LC = light chain

LDH = lactate dehydrogenase (EC., 1.1.1.27)

LMM = light meromyosin

Myo = myosin

Mt = mitochondria

NAD = nicotinamide adenine dinucleotide

NADH = nicotinamide adenine dinucleotide (reduced form)

NAM = natural actomyosin

NEM = N-ethylmaleimide

PAGE = polyacrylamide gel electrophoresis

PCMB = para-chloromericuric benzoic acid

Pi = inorganic phosphate

PMSF = phenylmethyl-sulfonylfluoride

Ppt = precipitation

S-1 = subfragment-1

S-2 = subfragment-2

SDS = sodium dodecyl sulfate (detergent)

Sup = supernatant

SR = sarcoplasmic reticulum

TN = troponin

TM = tropomyosin

Tris = tris(hydroxymethyl)aminomethane

Vol = volume

Introduction

The heart is an important muscular pump to propel blood to the whole body through the vasculature. The blood carries metabolic substances, hormones, and other materials.

The heart of mammal is composed of four pumping chambers: the right and left atria and the right and left ventricles. They contain mainly working myocardial cells which represent functionally specialized striated muscles like skeletal muscle. The pumping of cardiac muscle is caused by the contractile proteins which are called myosin and actin. The cardiac contractile proteins have similar biochemical and physiological properties with slow-twitch skeletal muscle contractile proteins. The contraction of the heart, at molecular level, has been explained by the "sliding theory" with myosin (thick) and actin (thin) filaments[43] like skeletal muscle[42]. Of course, the details of the contractile mechanism remain unknown yet. Also, the explanation of excitation-contraction coupling (E-C coupling)[33] in cardiac muscle is more difficult than skeletal muscle. (Fig.-1.)

The heart must adapt its performance to various needs of the body from the minimum work during sleep to the maximum demands on heavy exercise, in order that each organ will take enough blood to support its metabolic requirements.

The metabolic process in the heart is devided into two phases: (1) energy production and (2) energy utilization. The process of the energy production includes two metabolic

pathways: aerobic and anaerobic reaction for ATP synthesis. Furthermore, energy-rich phosphates of ATP and creatine phosphate (CrP) are also synthesized directly from AMP and ADP by the catalysis of myokinase and from creatine (Cr) and ATP by that of creatine phosphate kinase (CPK), respectively. On the other hand, the main process of energy utilization in the heart is break down of ATP for the contraction.

The ATP hydrolysis during vigorous heart contraction requires rapid resynthesis of ATP for the maintenance of the contraction. This rapid resynthesis of ATP is derived from the reaction catalyzed by CPK (ADP + CrP $\rightleftharpoons$ ATP + Cr) and results in local store of Cr via the reaction like skeletal muscle. Therefore, the CPK activity and Cr concentration give an important information about the performance of the heart[57]. Furthermore, it has been found that the increased intracellular Cr concentration correlated to the proliferation of cardiac muscle cell[15,45], the hypertrophy of the heart, the increased rate of synthesis of myosin heavy chain (HC) and actin, and the increased muscle-specific isoenzyme of CPK[44,61]. The adaptability of the heart to various circumstancial conditions and stimulation may be evaluated by the biochemical assay of these metabolic factors.

Many investigators reported the following effects of exercise on the heart of human and rodent: a cardiac hypertrophy by the stimulation of hemodynamic overload[26,109]: an increased cardiovascular function[13,75]: an increased function of cardiac contractile system[5,59,95]: and the positive changes of some metabolic enzymes[36,37,41].

Recently, Bhan et al. (1972[6], 1975[7], 1976[55]) showed the increased activities of contractile proteins (myosin ATPase and actomyosin ATPase) and the increased rate and extent of the superprecipitation by prolonged swimming exercise. The ATPase activities and superprecipitation are models *in vitro* to explain the muscle contraction. The ATPase activities have been reported to correlate to the contractility of both cardiac muscle (Chandler et al., 1969[11], and Takauji et al., 1972[92]) and skeletal muscle (Barany, 1967[4]). Myosin ATPase activity had something to do with the property and the number of sulfhydryl (SH) groups [71,78], the number of light chain (LC) of myosin[100], and the phosphorylation of LC[72,89,103]. Particularly, SH groups are contained in many enzymes, and sometimes locate at or near the active site of the enzymes and regulate the important function of them. For example, skeletal myosin has been found to contain about 31-36 moles SH groups per molecule of myosin[62]. The chemical modification of myosin with para-chloromeric-benzoic acid (PCMB) and N-ethylmaleimide (NEM) caused some changes in biochemical properties of myosin[78]. Similar observations were reported also in cardiac myosin [2,105]. In general, the exercise has been thought to be good for health. Therefore, cardiac muscle may change reasonably in the meaning of the cardiac adaptation. And the changes may appear in the cardiac contractile proteins and metabolic enzymes.

On the other hand, Thyrum et al.[94] (1970) and Yazaki et al. (1975[105]) reported that the L-thyroxine induced

alterations of enzymic properties and amino acid composition of myosin, and the increased Ca^{2+}-ATPase activity of myosin as the result of cardiac hyperfunction owing to the hormonal action. Thyroxine (T_4) is a component of thyroid hormone which cause the metabolic hyperfunction of the body[86]. Basedow's disease is one of the examples. The heart which is treated with L-thyroxine pumps the blood even at rest as vigorously as the non-treated heart on the endurance exercise[38]. The direction of changes in contractile proteins seems to be same in the two cases of exercise and thyroxine administration. For example, the two cases often caused the hypertrophy of the heart[64]. Finally, however, the chronic thyrotoxism results in the pathological changes of the body. Whereas the physical exercise seems to keep the good conditions of the heart or to promote the inotropic performance of it[20]. This difference is very interesting problem to the investigators for the evaluation of the effectiveness of exercise on heart.

The changes of cardiac myosin in thyrotoxic animals suggest the appearance of isoenzymes of cardiac myosin[60,69]. It is also a very interesting phenomenon and probably significant to the biochemistry because it may explain the essential character of contractile proteins for the physiological role of the heart.

As the author is interested in the essential function of myosin, which is one of the most important proteins of muscle activity, he tries to explain the difference between the effects of endurance running and L-thyroxine injection

on cardiac contractile proteins as the energy utilizing system.

Furthermore, the effects on lactate dehydrogenase (LDH) and CPK of energy producing system are studied in order to obtain the informations on adaptability of cardiac metabolic function. LDH was selected as the index of aerobic and anaerobic metabolism in the myocardial cell[22,36,37,107].

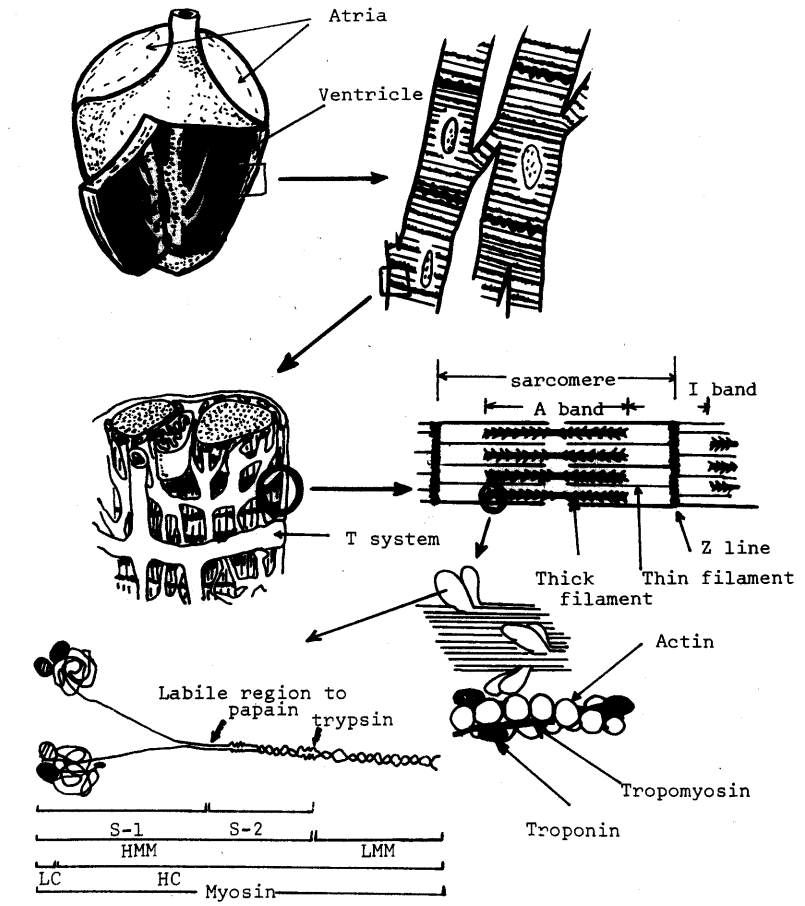


Fig-1. Model of cardiac muscle.

Materials and Methods

(Materials)

Twenty-four male albino rats (specific pathologen free Sprague-Dawley strain of the same venter), with initial body weight (B.W.) close to 40g at 2-week-old, were used in this study. They were obtained from Matsumoto Lab. (Chiba) and housed separatly in 3 appropriate groups until 4-week-old. After then they were placed in individual cages. All rats were given a diet of solid called MF (Oriental Yeast Co., Chiba) and water ad libitum during the whole experiment. They grew well without apparent specific diseases. Their B.W. got 300-450g at the end of the experiment.

The animals were put into three groups at random at the age of 4-weeks (80-100g) following that: (1) Control group (n=8), (2) Running group (n=8), (3) Thyroxine group (n=8).

(Program of training and methods of thyroxine injection)

Running group was trained to run on a motor-driven treadmill (T-804B type, Tokiwa Co., Osaka) at night. It consisted of two endless belts on metal rollers, and they were driven at a selected rate by a variable-speed control system. Two Lucite boxes, composed of individual compartments 7.5 cm high, 51 cm long by 14 cm wide, were suspended over the belts, providing the sepatate running area for each animal. A shock grid was located at the rear of the each compartment; the animals learned to avoid being shocked by keeping pace with the belt movement, particularly the shock grid was required only during the initial training phase. The treadmill was set at 2° incline.

The rats were trained for 2 months (30 min/day, 5 days/week), and the running velocity was progressively increased to about 45 m/min every week for about 4 weeks and then was maintained at this level finally. The final intensity was corresponded to 70-80% of maximal oxygen uptake ($\dot{V}O_2$ max) according to the results of Shepherd et al.[79](1976[80]).

In Thyroxine group, L-thyroxine was injected intraperitoneally 15μg/100g B.W. daily at 10-week-old and the administration was maintained for 3 weeks.

(Preparation of samples)

The animals were killed by decapitation after anesthetized with inhalation of ethyl ether. The heart was removed, trimmed of visible fat, rinsed in Ringer's solution, blotted with moist filter paper, and weighed. Then it was divided into left and right ventricles and atria. The interventricular septum and residue were discarded. All subsequent steps were carried out at 4°C except where indicated.

Samples for LDH and CPK were prepared essentially according to Sjödin (1976[84]). Each muscle was minced with stainless scissors in 5 volume of the ice-cold preparation medium of slightly modified Chappel-Perry medium[12,69] containing 0.3 M sucrose, 0.1 M KCl, 5 mM $MgCl_2$, 1 mM EDTA, 0.5 mM PMSF (protease inhibiter)[63], and 50 mM Tris-HCl buffer (pH 7.4). The minced muscle was homogenated with a motor-driven glass-Teflon homogenizer. Care was taken to minimize foaming and heating. The homogenate was subjected to some centrifugation according to the scheme of Sjödin[84]. First, it was centrifuged at 600 x g for 10 min. The supernatant (sup.) was done again at 14,000 x g for 10 min to remove mitochondria, and further the sup. was centrifuged at 105,000 x g for 60 min. Finally, the centrifuged sup. at high-speed was used for the assay of LDH, CPK, and soluble proteins.

The contractile proteins were prepared essentially by the method of Schiverick et al. (1975[81]) from the homogenate of left ventricles in each group. The precipitation (ppt.) at 600 x g was stirred in 4 vol. of the cold solution

containing 0.1 M KCl, 1 mM $NaHCO_3$, 0.5 mM PMSF, and 2 mM β-mercaptoethanol (β-ME.) and centrifuged at 14,000 g for 10 min. This step was repeated more 4 times until red disappeared from ppt. The contractile proteins were extracted in 4 vol. of the modified Hasselbach-Schneider solution, consisted of 0.3 M KCl, 1 mM $MgCl_2$, 0.15 M K_2HPO_4, 10 mM $Na_4P_2O_7$, 0.5 mM PMSF, and 2 mM β-ME. (pH 6.8) for 2 hrs. at 0°C. The mixture was centrifuged at 12,000 x g, for 10 min. The sup. was centrifuged again at 14,000 x g for 4 hrs. The sup. was diluted with 5 vol. of cold 1 mM $NaHCO_3$, and centrifuged at 14,000 x g for 10 min. The ppt. was dissolved in 0.6 M KCl and 1 mM $NaHCO_3$ finally. The solution was used as myosin B or natural actomyosin (NAM), which contains mainly actomyosin and regulatory proteins: troponin (TN), tropomyosin (TM). Myosin B has calcium-regulating mechanism by TN-TM system on actin (thin) filaments. Actomyosin, which does not contain regulatory proteins, was prepared from the myosin B by the repeated washing-technique under high and low ionic strength conditions.

Myosin was prepared from myosin B. Myosin B was diluted with 9 vol. of cold 1 mM $NaHCO_3$ and centrifuged at 14,000 x g for 10 min. The ppt. was dissolved in 5 mM ATP, 4 mM $MgCl_2$, 1 mM EGTA, 0.6 M KCl, and 10 mM Tris-HCl (pH 8.0) and centrifuged at 200,000 x g for 2 hrs. The sup. was fractionated with 40-50% saturated $(NH_4)_2SO_4$ containing 10 mM EDTA. The fractionated ppt. was dialized against 0.6 M KCl and 1 mM $NaHCO_3$ in a cold room at 4°C and sometimes the solution for dialysis was replaced with fresh one. The

absence of $(NH_4)_2SO_4$ was confirmed by the disappearance of the complex with barium (Ba). The purity of the samples was determined in every step by the sodium dodecyl sulfate (SDS)-gel electrophoresis according to Weber and Osborn[98] (1969). Every step of preparation was summarized in Fig.2.

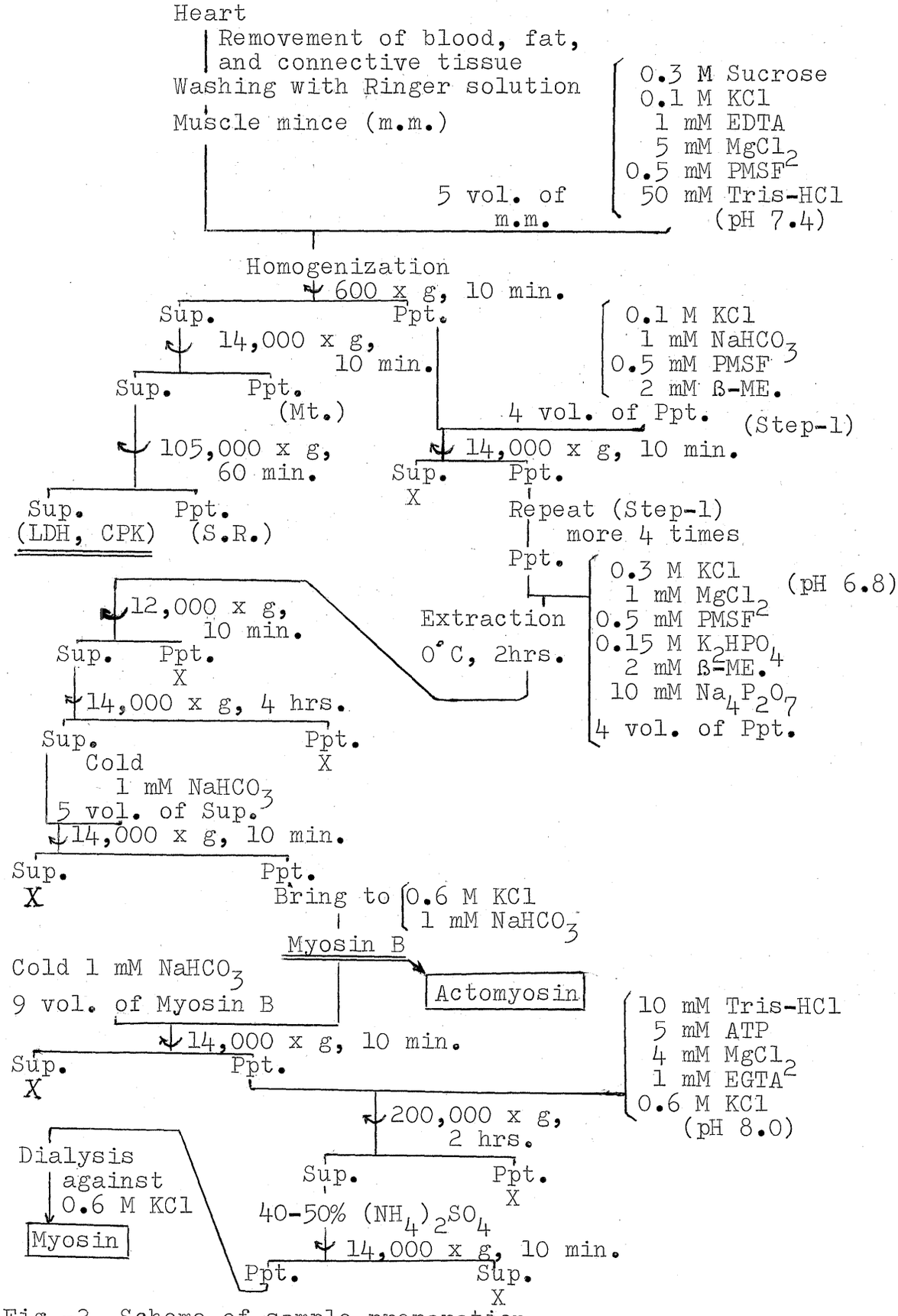


Fig.-2. Scheme of sample preparation

(Assay of ATPase activity and chemical modification)

Calcium activated ATPase activity of myosin (Ca^{2+}-ATPase activity) was determined at 25°C in a reaction medium containing 0.6 M KCl, 5 mM $CaCl_2$, 1 mM ATP, and 20 mM Tris-maleate buffer (pH 6.5). Potassium and EDTA activated ATPase activity of myosin (K^+-EDTA-ATPase activity) was determined at 25°C in a solution containing 0.6 M KCl, 1 mM EDTA, and 20 mM Tris-maleate buffer (pH 7.0). The pH dependency of myosin ATPase was determined at 25°C in the same reaction medium described above with substitution of each adequate buffer. The used buffers were 20 mM Tris-maleate (below pH 7.2) and 50 mM Tris-HCl (above pH 7.2).

In the study of effect by N-ethylmaleimide (NEM), the chemical modification and myosin ATPase activity was determined according to Yamaguchi et al. (1970[104]) About 5 mg of the cardiac myosin was incubated at 0°C with 0.1 μmole of NEM in 1 ml of a solution containing 0.5 M KCl and 20 mM Tris-HCl buffer (pH 7.0). After the incubation of an adequate period, an aliquot of the reaction mixture was diluted with 10 vol. of 0.5 M KCl containing 0.05 μmoles of β-ME. The myosin ATPase activity was determined at 37°C by the released inorganic phosphate (Pi) with the method of Fiske and SubbaRow (1925[31]). The reaction medium contained 0.5 M KCl, 20 mM histidine buffer (pH 7.6), 1 mM ATP, either 1 mM EDTA (K^+-EDTA-ATPase) or 5 mM $CaCl_2$ (Ca^{2+}-ATPase).

Magnesium activated ATPase activity of actomyosin (Mg^{2+}-ATPase activity) was assayed at 25°C in 60 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, and 20 mM Tris-maleate buffer (pH 6.5).

The reaction was started by the addition of either ATP or sample, and terminated by the addition of 5% trichloroacetic acid (TCA). The mixture was centrifuged at 4,000 x g for 10 min to remove of proteins in cold room. The liberated Pi from ATP was determined by the method of Fiske and SubbaRow (1925[31]). The specific activity was expressed in μmoles Pi liberated per min per mg protein.

(Determination of SH groups)

SH groups were determined by the method of Ellmann[29] (1959) in the presence of SDS[96] or absence. Myosin (2 mg/ml) was incubated at 25°C in a solution containing 0.08 mM 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB), 50 mM Tris-HCl buffer (pH 8.0), either 0.1% SDS or no. SH content was calculated from the extent of absorbance at 412 nm with molar absorbance coefficient 13,600 of 2-nitro 5-mercapto-benzoic acid which was product of DTNB. In the present study, the difference of the value between in the presence and absence of SDS was negligible. The SH content was calculated in the presence of SDS and expressed with moles SH per mole myosin. Molecular weight of myosin was estimated as 45×10^4 dalton.

(Measurement of superprecipitation)

Superprecipitation of actomyosin was measured at 25°C according to Ebashi (1961[27]). The reaction mixture contained 60 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, and 20 mM Tris-maleate buffer (pH 6.5). About 1 mg/ml of actomyosin was added.

The change of absorbance at 660 nm was recorded with Hitachi recording spectrometer Type EPS-3 (Hitachi Co., Tokyo). From the time-course of increase in optical density, the increase of optical density after completion of super-precipitation (the extent of superprecipitation) and the time required to attain half the maximum ($\tau_{1/2}$, the reciprocal of the specific rate of superprecipitation) were determined.

(Determination of LDH activity and isoenzymes)

LDH activity was determined at 25°C by the method of Wroblewski and La Due (1955[102]) with the amount of NAD produced from NADH. The reaction medium contained 50 mM potassium phosphate buffer (pH 7.5), 0.6 mM pyruvate, and 0.18 mM NADH. The reaction was started by the addition of an aliquot of the sample. The amount of sample for assay was adjusted so as to give a change in absorbance of 0.150-0.300 per min. The activity was calculated from the changing rate of absorbance at 340 nm with Hitachi recording spectrometer Type EPS-3. Molar absorbance coefficient for NADH is 6.22×10^6 at 340 nm. One unit of enzyme activity oxidizes NADH at the rate of 1 μmole/min at 25°C and international unit (I.U.) was described in μmoles NADH per min. The results were shown with I.U./mg protein.

LDH isoenzyme pattern was analysed by the slightly modified method using polyacrylamide gel electrophoresis (PAGE) of Dietz and Lubrano (1967[25]). An aliquot of the sample was diluted with an equal volume of 40% sucrose and applied on 7.5% polyacrylamide gel (PAG) (c=2.6%) in 5 x 60 mm tube and 2 x 100 x 136 mm slab-gel plate. The prepared PAG solution was overlayed again on the sample-sucrose layer and polymerized chemically. For the detection of migrating front of PAGE, 0.1 ml of 0.05% bromphenol blue (BPB) was added into 200 ml of cathode chamber. The electrophoresis was run in 50 mM Tris-glycine reservoir buffer (pH 9.3) for 2 hrs. at 3 mA a tube and 40 mA a plate in the cold room. After the activity staining with a nitroblue tetrazolium staining

solution (Springell and Lynch, 1976[88]), Simon et al. 1977[83]), the five isoenzymes' bands were quantitated with the light absorbance at 570 nm with Fujiox AD-FIV densitometer (Fuji Co., Tokyo). The relative value of muscle (M)-type and heart (H)-type was calculated according to Thorling and Jensen (1966[93]). A part of slab gel was subjected to second dimensinal gel electrophoresis in the presence of SDS.

(Determination of CPK activity)

CPK activity was assayed by the modified method of Kuby et al. (1954[49]) and Okinaka et al (1964[65]). The reaction medium contained 20 mM $MgCl_2$, 10 mM cystein, 5 mM ATP, either 80 mM creatine or no, and 50 mM Tris-HCl buffer (pH 9.0). The reaction was started by the addition of ATP after preincubation at 30°C for 5 min and stopped with an equal volume of 10% TCA after the reaction at 30°C for 30 min. The Pi transphosphorylated from ATP to Cr by the catalysis of CPK was determined by the method of Fiske and SubbaRow (1925[31]) like ATPase activity. One unit of enzyme was defined as 1 μmole of transfered phosphate from ATP to Cr per min. The specific activity was shown in unit per mg protein.

(SDS gel electrophoresis)

SDS continuous gel electrophoresis (T=7.5 or 10%, c=2.6%) was carried out according to Weber and Osborn (1969[98]) using sodium phosphate buffer system (pH 7.1). Sample was prepared for the electrophoresis by mixing of protein with an equal volume of a solution containing 1% SDS, 1% -ME and 10 mM Na-phosphate buffer (pH 7.0). The mixture was immediately heated to 100°C for 3 min to inactivate residual proteolytic enzymes. After an equal volume of a mixture containing BPB tracking dye and 50% glycerine was added into the heated sample, the electrophoresis was run at 8 mA/tube for about 3 hrs. at room temperature (20-25°C). A tube was 5 mm inner diameter and 60 mm high. The gel was immersed in 30% TCA for fixation of small proteins.

SDS second dimensional gel electrophoresis for the study of comigrating proteins with LDH was carried out according to Laemmlie (1970[50]) employing SDS discontinuous Tris-glycine buffer system. After first dimensional electrophoresis, a part of PAG was overlayed on the stacking gel layer in the presence of SDS. The electrophoresis was run at 10 mA/plate for about 16 hrs. at room temperature. Coomassie brilliant blue R (CBB-R) was used to stain the components of proteins in the gel. Molecular weights were estimated from a plot of the logarithm of molecular weights versus mobilities, using cytochrome c, hemoglobin, serum albumin, aldolase, and skeletal myosin as standards. The staining intensity of the different each band were analysed by the densitometoric procedure like LDH isoenzymes' pattern.

(Determination of protein concentration)

Protein concentration was determined by the biuret method[39]. In some cases, proteins were precipitated with 5% TCA for removal of obstacles, and the precipitated proteins were redissolved with 0.1 M NaOH. Bovine serum albumin was used as the standard of protein concentration.

(Chemicals)

ATP, Bovine serum albumin, phenylmethyl-sulfonylfluoride (PMSF), Coomassie Brilliant Blue R, and Glycol ethylen diamine tetra acetic acid were purchased from Sigma Chemical Co (St. Louis, Mo.). The reagents required for assay of LDH activity was obtained from Boehringer Mannheim GmbH (Mannheim). N-ethylmaleimide, DTNB, Acrylamide, N,N′-Methylene-bis(acrylamide), Ammonium sulfate, Ammonium persulfate, and other chemicals were biochemical research grade from Wako pure chemical Ind., Ltd (Osaka).

(Statistical analysis)

Statistical analysis was accomplished using the unpaired Student's t test to Control, Running, and Thyroxine groups.

Results

At the time of final study the body weight of the rats in the Control, Running, and Thyroxine groups were 397.6 ± 17.1 g (means ± S.D.), 338.9 ± 36.7 g, and 360.7 ± 30.8 g, respectively. At the time of the excising myocardium the mean (± S.D.) heart-to-body weight ratios (1,000 x H.W./B.W.) of three groups were 2.663 ± 0.325 (Control g.), 3.005 ± 0.433 (Running g.), and 3.120 ± 0.190 (Thyroxine g.). The results were described in Table-1. A significant difference ($P<0.01$) was observated in the heart-to-body weight ratios between Control and Thyroxine groups. There was no significant difference between Control and Running groups. But the body weight of Running group was significantly lower than Control group ($P<0.01$). The rate of increased body weight in Running group was lower than other two group but the body weight increased constantly every day throughout the exercise period. Whereas, after a week of thyroxine-injection the body weight of Thyroxine group showed a fixed or decreased tendency, which was a clear symptom of the thyrotoxism[57,64]. The tendency of increased heart-to-body weight ratios suggested that running-exercise caused a little heart hypertrophy, while the thyroxine administration showed a marked heart hypertrophy.

The ATPase activities of contractile proteins were shown in Table-2. There was no clear difference among three groups. But Ca^{2+}-ATPase activity of Thyroxine group was 0.44 μmoles Pi/min/mg myosin and it was a little higher than Control group (0.29 μmoles/min/mg). That of Running

group was 0.32 μmoles/min/mg and it was not different from Control group. K^+-EDTA-ATPase activities were 0.44 μmoles/min/mg myosin (Control g.), 0.46 (Running g.), and 0.49 (Thyroxine g.). These values showed no difference among them. Also Mg^{2+}-ATPase activities of three groups were 0.29 μmoles Pi/min/mg protein (Control g.), 0.28 (Running g.), and 0.31 (Thyroxine g.). These differences among three groups were negligible.

The pH dependency of myosin ATPase activity was shown in Fig.-4. Myosin Ca^{2+}-ATPase had an optimal region at or near pH 6.5 for the enzymatic activity in every group. Fig.-4 showed that the Thyroxine group had a little higher activity than other two groups on Ca^{2+}-ATPase activity. No clear difference was found in K^+-EDTA-ATPase activity of each group. The peak values were obtained at near pH 7.5. At pH 7.5, K^+-EDTA-ATPase activities were 0.67 μmole/min/mg myosin (Control g.), 0.68 μmole/min/mg (Running g.), and 7.2 μmole/min/mg (Thyroxine g.). The basic profile of myosin pH dependency was same in every group.

SH content of myosin was also shown in Table-2. In the present study, rat cardiac myosin had about 32 moles SH per mole myosin. The differences of SH content in Table-2 were negligible and could not explain the different properties of cardiac myosin on Ca^{2+}-ATPase activity.

The effect of NEM modification on myosin was shown in Fig.-5. By the chemical modification of myosin SH_1 with NEM, Ca^{2+}-ATPase activities increased in early stage of the reaction and decreased soon. This change of ATPase activity

Table-1. Anatomical data

	Control (n=8)	Running (n=8)	Thyroxine (n=8)
Body weight (g)	397.6 ± 17.1	338.9 ± 36.7**	360.7 ± 30.8*
Heart weight (g)	1.057 ± 0.119	1.035 ± 0.186	1.129 ± 0.153
(H.W./B.W.) x 1,000	2.663 ± 0.325	3.005 ± 0.433	3.120 ± 0.190**

Heart weight (H.W.) is shown by wet weight and the ratio of the heart to body weight (B.W.) is calculated with the equation of $\left(\frac{\text{wet heart weight (g)}}{\text{body weight (g)}}\right) \times 1{,}000$. Values are means ± S.D. n = number of animals in a group.

** Significantly different from Control at $P<0.01$.

* Significantly different from Control at $P<0.02$.

Table-2. ATPase activity and SH content

	(1) Control group	(2) Running group	(3) Thyroxine group
Ca^{2+}-ATPase (μmoles/min/mg)	0.29	0.32	0.44
K^{+}-EDTA-ATPase (μmoles/min/mg)	0.44	0.46	0.49
Mg^{2+}-ATPase (μmoles/min/mg)	0.29	0.28	0.31
SH content (SH mol./mol.myo)	32.6	32.8	32.0

Values were obtained from the collected left ventricular contractile protein of each group. SH content was determined according to Ellman. The details are described in "Materials and methods".

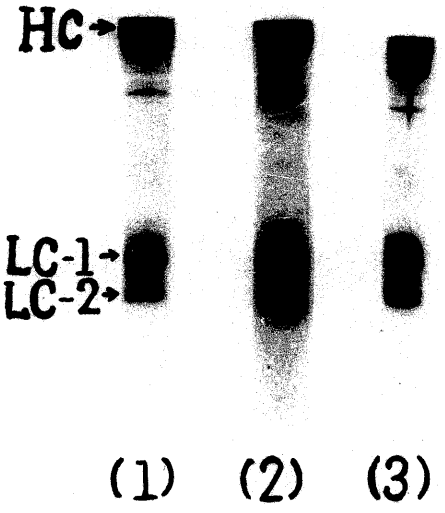


Fig.-3. SDS polyacrylamide gel electrophoresis of myosins of three groups.

The arrows show each component of prepared myosins. (1), Control group; (2), Running group; (3), Thyroxine group; HC, heavy chain; LC-1, light chain 1 (MW=2.7 x 10^4 dalton); LC-2, light chain (MW=2.0 x 10^4 dalton). The details are described in "Materials and methods".

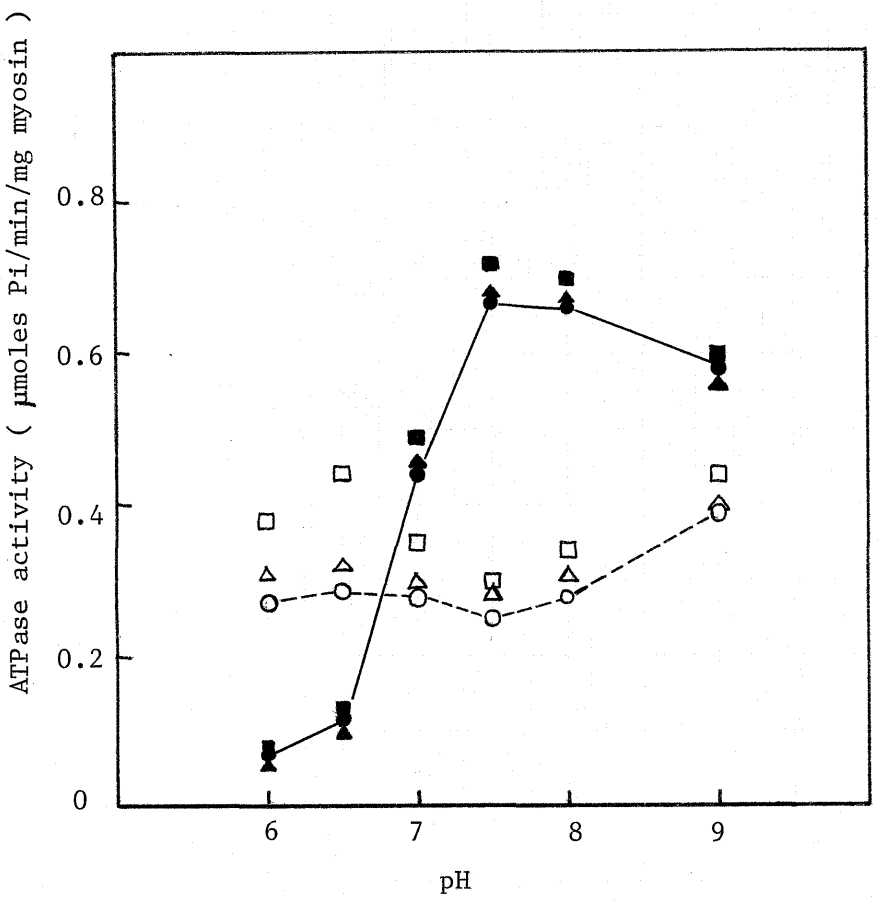


Fig.-4. Effect of pH on myosin ATPase activity.

ATPase activity was determined in the reaction medium containing 0.6 M KCl, 1 mM ATP, either 1 mM EDTA or 5 mM $CaCl_2$, and buffers. The used buffers were 20 mM Tris-maleate (below pH 7.2) and 50 mM Tris-HCl (over pH 7.2). The reaction was started by the addition of ATP at 25°C. The details are described in "Materials and methods". Open symbols, Ca^{2+}-ATPase; Closed symbols, K^{+}-EDTA-ATPase; o●, Control group; △▲, Running group; □■, Thyroxine group. The lines indicate the values of Control group.

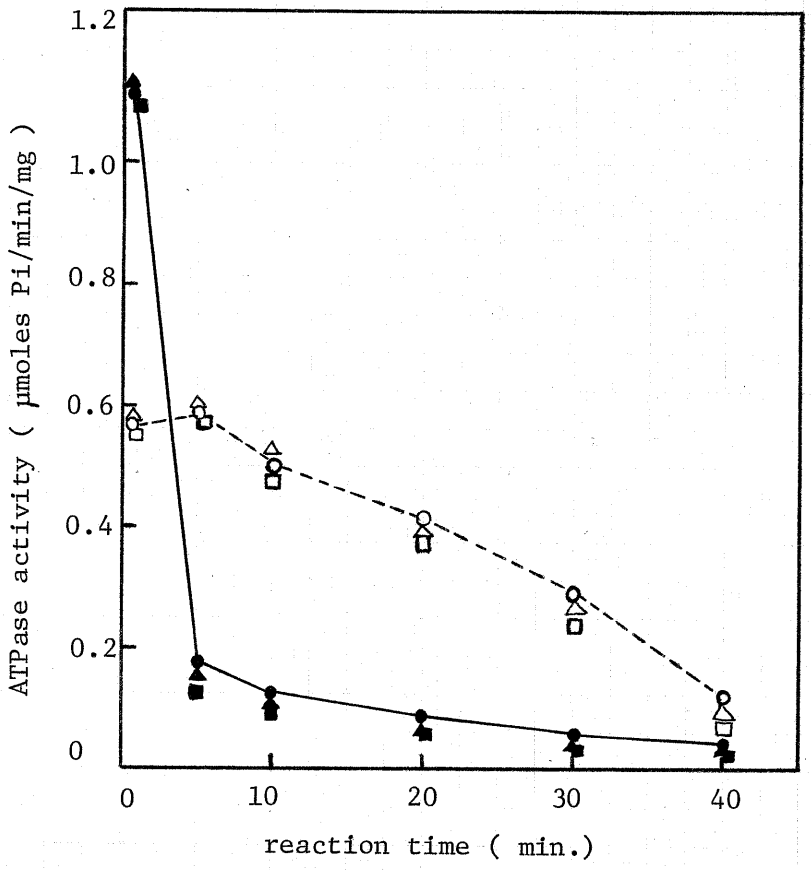


Fig.-5. Effects of NEM on myosin ATPase activity

Myosin was preincubated with NEM at 0°C in 0.5 M KCl and 20 mM Tris-HCl (pH 7.0). ATPase activity was determined in a reaction medium containing 0.5 M KCl, 20 mM histidine buffer (pH 7.6), 1 mM ATP, either 5 mM $CaCl_2$ or 1 mM EDTA. Ordinate indicates ATPase activity and abscissa shows the reaction time (minute) of myosin with NEM. Open symbols, Ca^{2+}-ATPase; Closed symbols, K^{+}-EDTA-ATPase; o●, Control group; △▲,Running group; □■,Thyroxine group. The lines indicate the values of Control group.

was similar in every group. K^+-EDTA-ATPase activity was decreased remarkably by the NEM modification. This profile of time course on K^+-EDTA-ATPase activity was similar to that of skeletal myosin. But Ca^{2+}-ATPase activity showed the faster change of itself than skeletal myosin Ca^{2+}-ATPase activity. The significant differences of effects by the NEM modification were not observed in myosin ATPase activities of three groups.

The subunit compositions of myosin in each group were shown in Fig.-3 using SDS-PAGE. Fig.-3 showed typical electrophoretograms on 7.5% acrylamide gel with 0.1% SDS of myosin. Every gel showed no significant contamination with actin or other proteins and indicated a high molecular weight band (20×10^4 dalton) and two low molecular weight bands (2.7×10^4 dalton and 2.0×10^4 dalton). These bands were corresponded to HC, LC-1, and LC-2. The amount of LC and the proportion of subunits were almost same in three groups. There were two light chains, and myosin contained about 10-13% of total light chains. No significant difference in the number of LCs and the proportion of the subunits was found among three groups. These observations were similar to the results reported by Klotz et al. (1975[47]) and Murakami et al (1976[62]).

The recorded results of superprecipitations were shown in Fig.-6. No significant difference was found in the extent and the reciprocal of the rate ($\tau_{1/2}$) of superprecipitations in three groups. The $\tau_{1/2}$ were 1.7 (Control g.), 1.7 (Running g.), and 1.6 (Thyroxine g.). These observations

were different from the results of Bhan and Scheuer (1975[7]). This confliction was might be due to the different way of sample preparation or the conditions for assay except the different effect of exercise on contractile proteins.

LDH activity and isoenzyme-distribution were shown in Table-3, Fig.-8. Table-3 indicated the significant increased activity of LDH in Thyroxine group's left ventricle ($P<0.02$) and atria ($P<0.01$). On the other hand, The LDH activity of right ventricle was markedly lower in the Thyroxine group than other two groups. The LDH specific activities in left ventricles of three groups were 8.585 ± 0.706 (Control g.), 9.031 ± 0.526 (Running g.), and 10.156 ± 1.405 (Thyroxine g.). The values were shown in I.U./mg protein. The activities of right ventricles were 7.770 ± 0.324 (Control g.), 7.657 ± 0.636 (Running g.), and 4.654 ± 0.624 (Thyroxine g.). And the activities of atria were 1.130 ± 0.151 (Control g.), 1.191 ± 0.195 (Running g.), and 1.528 ± 0.177 (Thyroxine g.). The percentages of H-type isoenzyme were 64.8 ± 3.8 % (Control g.), 63.3 ± 5.4 % (Running g.), and 59.9 ± 2.8 % (Thyroxine g.) in each left ventricle. The percentage of the left ventricle was significantly decreased by the thyroxine administration ($P<0.02$). The percentage of right ventricle in Thyroxine group was 60.2 ± 2.2 % and significantly lower ($P<0.01$) than Control group (65.0 ± 5.8 %) and Running group (63.0 ± 6.6 %). In atria, Control group had 54.8 ± 4.3 % of the percentage of H-type; Running group had 54.8 ± 2.5 % of that; Thyroxine group had 54.1 ± 3.0 %. No significant difference was not found among these values.

Although the percentage of H-type in Running group was not significantly different from Control group, a little decreased LDH specific activity and the unchanged percentage of H-type isoenzyme (Table-3, Fig.-8) suggested the increased zymogram of H-type in right ventricle. Because the LDH activity under used conditions for assay reflected mainly the activity of M-type. The transformation from H-type to M-type of LDH indicated the anaerobic metabolism in the left ventricle and right ventricle of Thyroxine group. The predominant M-type showed anaerobic metabolism in the tissues. The anaerobic metabolism suggested the deficiency of oxygen in the cells. Whereas, the predominant H-type suggested aerobic metabolism like the effect of endurance exercise on skeletal muscle, particularly slow-twitch muscle[36,37,85,107]. The present observation suggested a similar effect of exercise on cardiac muscle.

The present results on CPK activity was shown in Table-4. A significant increases were found in right ventricle of Thyroxine group (0.776 ± 0.153 unit/mg protein, $P < 0.01$) and that of Running group (0.460 ± 0.100 unit/mg, $P < 0.02$). The activities of left ventricle and atria showed slight increases in Running group and Thyroxine group. The increased activity of CPK suggested the increased concentration of Cr in the cell.

No significant difference was found in Fig.-9 of the comigrating proteins with LDH among three groups. The assay was achived to obtain new informations on effects of the treated or stimulated heart. The difficulty of evaluation

on second dimensional gel electrophoresis might cause no clear difference.

SDS gel electrophoretograms of soluble proteins were shown in Fig.-10. In Thyroxine group, a low molecular weight protein (0.9×10^4 dalton) of right ventricle was less than other two groups, and 3.0×10^4 dalton component was more than other groups. The marked difference was found in the component of 3.0×10^4 dalton. There was no clear difference between Controll and Running groups in all samples. The present results of Thyroxine group as described above suggested abnormal situation of right ventricle.

Table-3. LDH activity and percentage of H-type isoenzyme

	Control (n=8)	Running (n=8)	Thyroxine (n=8)
Left vent. (I.U./mg)	8.585 ± 0.706	9.031 ± 0.526	10.156 ± 1.405**
Left vent. (%)	64.8 ± 3.8	63.3 ± 5.4	59.9 ± 2.8*
Right vent. (I.U./mg)	7.770 ± 0.324	7.657 ± 0.636	4.654 ± 0.624**
Right vent. (%)	65.0 ± 5.8	63.0 ± 6.6	60.2 ± 2.2**,⊙⊙
Atria (I.U./mg)	1.130 ± 0.151	1.191 ± 0.195	1.528 ± 0.177**
Atria (%)	54.8 ± 4.3	54.8 ± 2.5	54.1 ± 3.0

Values are means ± S.D. Numbers in parentheses represent the number of animals in each group. ** Significantly different from the Control group, $P<0.01$; * Significantly different from the corresponding Control group, $P<0.02$; ⊙⊙ Significantly different from the Running group, $P<0.01$.

Table-4. CPK activity

	Control (n=8)	Running (n=8)	Thyroxine (n=8)
Left vent. (Unit/mg)	0.307 ± 0.047	0.356 ± 0.071	0.354 ± 0.082
Right vent. (Unit/mg)	0.337 ± 0.064	0.460 ± 0.100*	0.776 ± 0.153**,⊙⊙
Atria (Unit/mg)	0.448 ± 0.071	0.559 ± 0.119	0.527 ± 0.078

Values are means ± S.D. Numbers in parentheses represent the number of animals in each group. ** Significantly different from the corresponding Control group, $P<0.01$; * Significantly different from the corresponding Control group, $P<0.02$; ⊙⊙ Significantly different from the corresponding Running group, $P<0.01$.

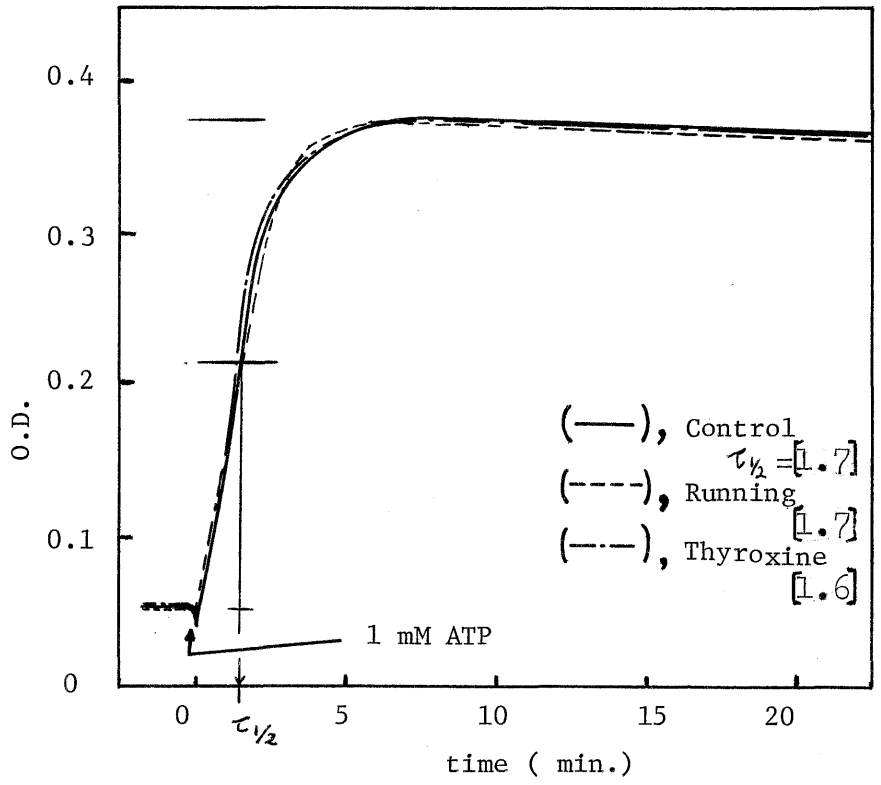


Fig.-6. Superprecipitation of actomyosin. Superprecipitation was recorded at 25 °C in reaction medium containing 60 mM KCl, 1 mM $MgCl_2$, 1 mM ATP, and 20 mM Tris-maleate buffer (pH 6.5). The details are described in "Materials and methods".

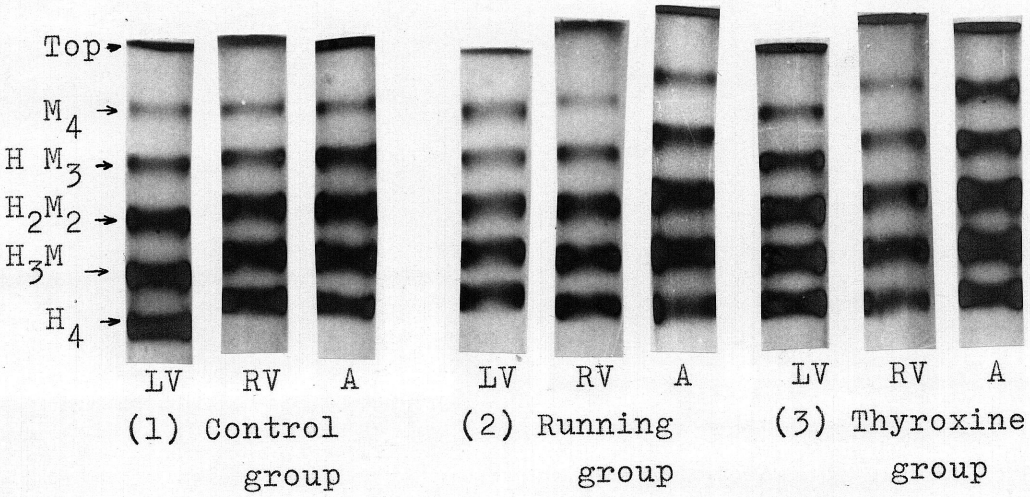
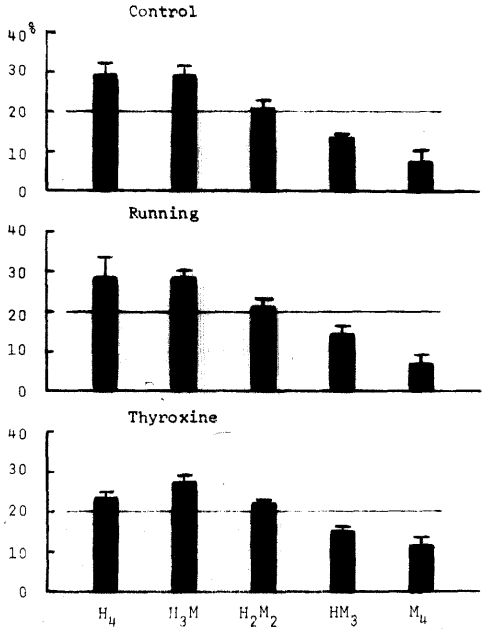


Fig.-7. LDH isoenzymes' pattern on PAGE with activity staining method.

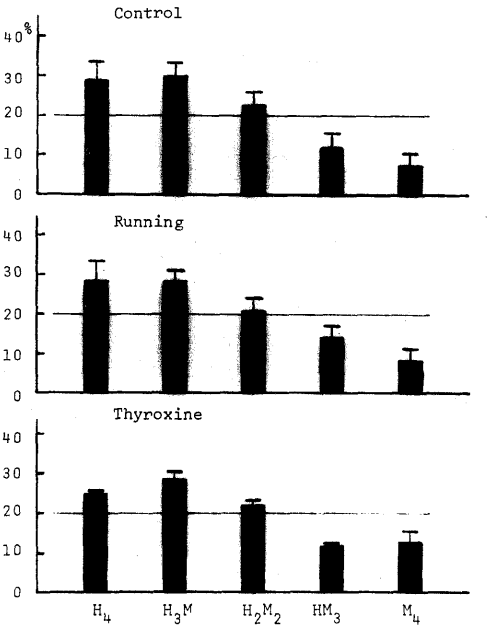
The top of gel was stained darkly, but it was due to other dehydrogenases. Because it appeared even without lactate of substrateand LDH isoenzymes' bands appeared only in the presence of the substrate. The details are described in "Materials and methods". LV, left ventricle; RV, right ventricle; A, atria; H, heart-type isoenzyme; M, muscle-type isoenzyme.

Left ventricle



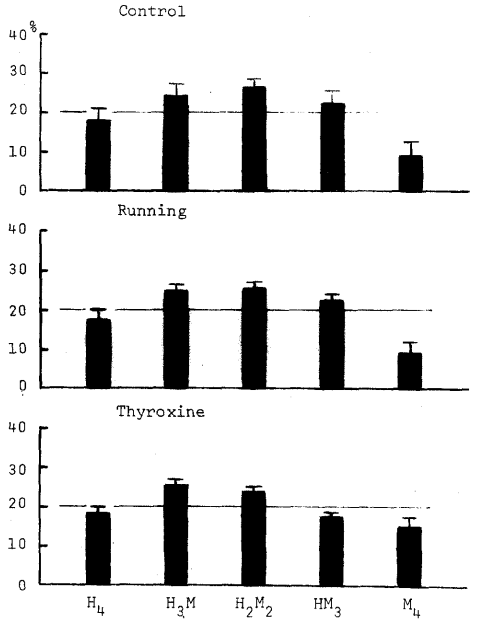
(A)

Right ventricle



(B)

Atria

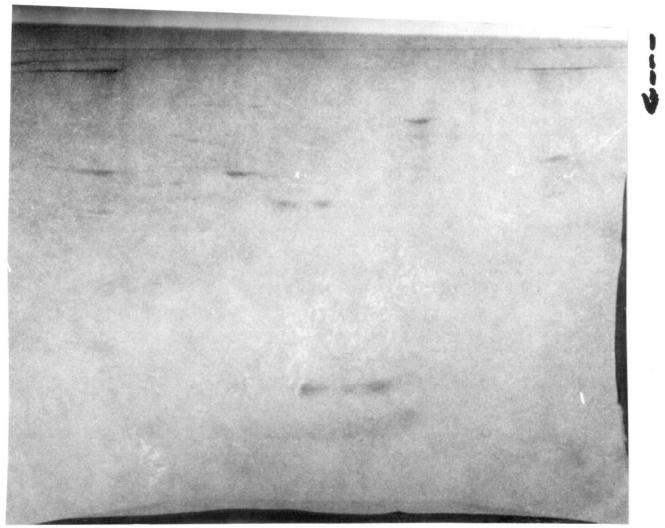


(C)

Fig.-8. Five LDH isoenzymes' distribution.

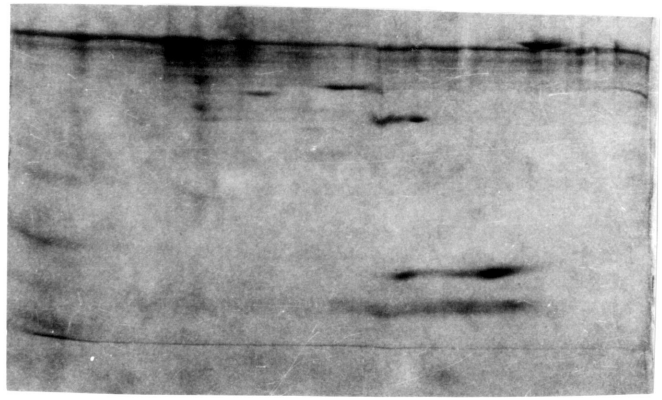
Ordinate indicates the % of each isoenzyme. The vertical lines show standard deviation (S.D.). (A), left ventricle; (B), right ventricle; (C), atria. See the details in "Methods and materials".

(A)



(1) Control group

(2) Running group

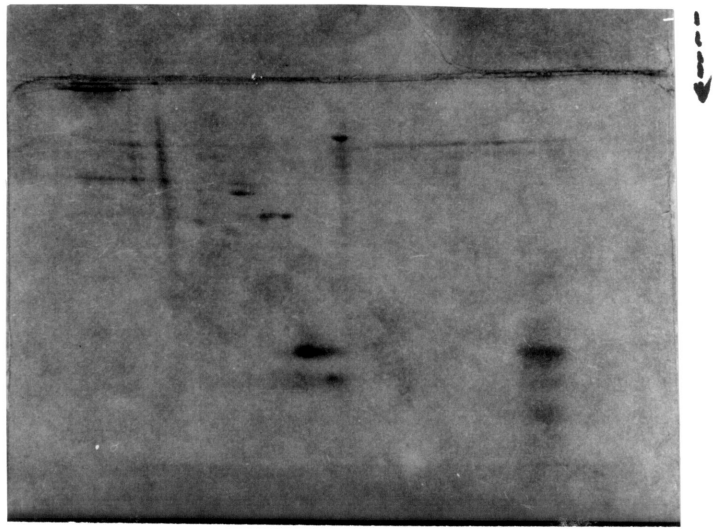


(3) Thyroxine group

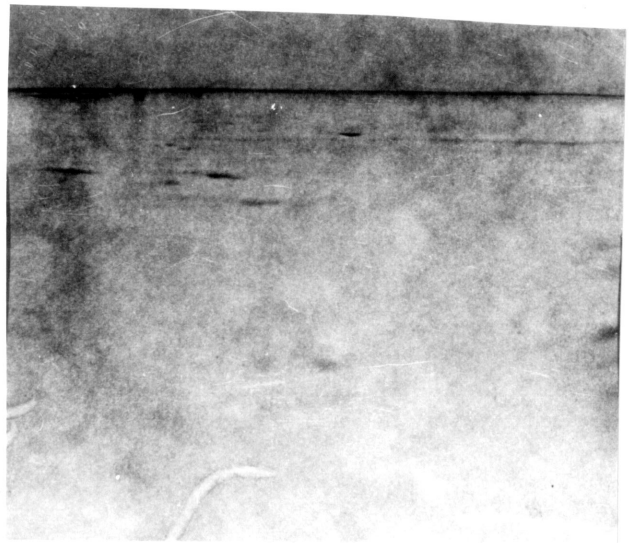
Fig.-9. The distribution of comigrating proteins with LDH.

The comigrating proteins were shown in the 2nd dimensional gel electrophoresis in the presence of SDS. The arrow (--→) indicates the direction of electrophoresis. (A), left ventricle; (B), right ventricle; (C), atria. The details are described in "Materials and methods".

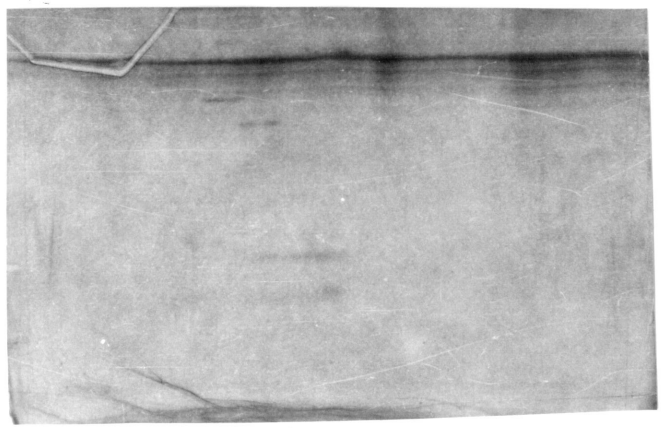
(B)



(1) Control group

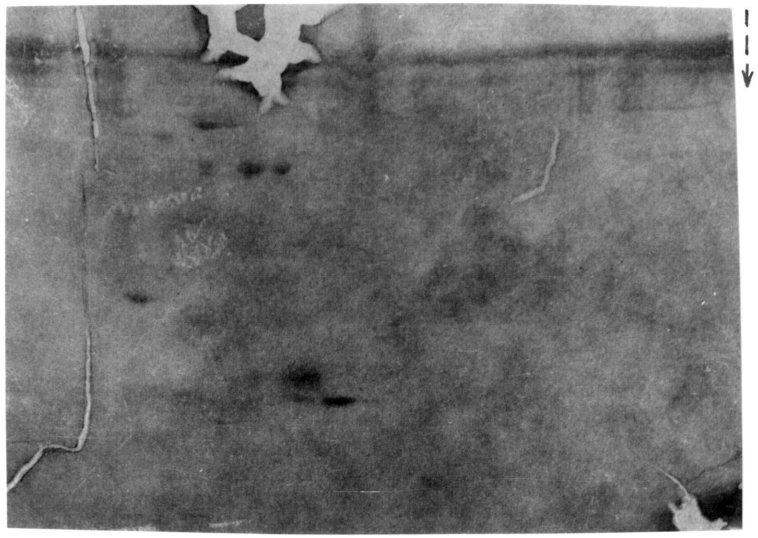


(2) Running group

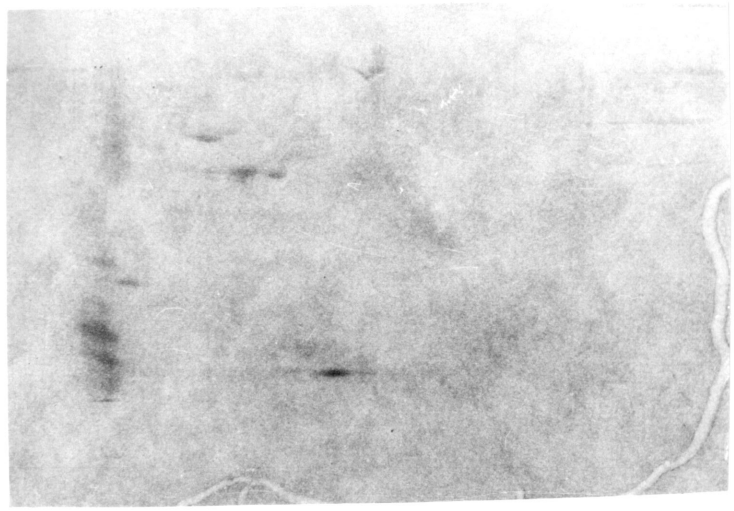


(3) Thyroxine group

(C)



(1) Control group



(2) Running group

(3) Thyroxine group

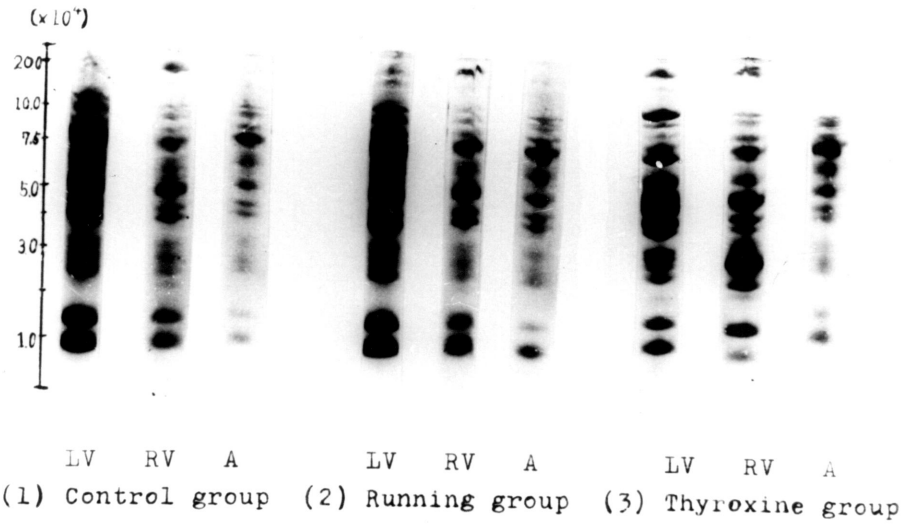


Fig.-10. Pattern of soluble prteins on PAGE with SDS.

The vertical line shows molecular weight. The details are described in "Methods and materials". LV, left ventricle; RV, right ventricle; A, atria.

Discussion

Although it is clear that myocardial mass increases rapidly and myocardial contractility changes in response to pressure or volume overload[9,10,15,23,30,38], the pattern of changes in individual myocardial components and their properties still has not been fully delineated. Furthermore, such hypertrophied hearts were sometimes accompanied with heart failure[11,20,87]. The present study was accomplished to explain the difference between the failing and non-failing hearts, which were sometimes accompanied with hypertrophy, from the biochemical point of view.

In the present study, running-exercise and thyroxine-administration were used as the stimulations that would induce the cardiac hypertrophy. The data from the present study (Table-1) showed that prolonged endurance-running induced a little hypertrophy of the heart but thyroxine-injection was accompanied with marked hypertrophy. The hypertrophied heart of athletes and rodent by exercise have been reported by several investigators[23,28,97,109], and they suggested that the hypertrophy was one of the high cardiac adaptabilities to many physiological stimulations[5]. But the endurance exercise doesn't always cause cardiac hypertrophy, but induces the increased performance as the pump of blood[13,75]. Thyrotoxism caused often hypertrophy of the heart[64]. The present observation confirmed the general phenomenon of the cardiac hypertrophy with thyroxine-administration. But the heart hypertrophy results from growth of muscle fibers, coronary vessels, connective tissue and other

constituents of the myocardial wall, and the region which is most influenced by the thyroxine-administration have not clear yet. Although there were not the direct observations in the present study, the growth of cardiac muscle fiber has been found to be associated with the following factors: the increase of the RNA polymerase activity[52], the increased rates of protein[57,91,99] and RNA [108,110] syntheses, the increases of the total DNA[40], RNA and protein contents[14,16,26,30,51,58], and the increased production and utilization of energy-rich phosphates which are essential for protein synthesis[15,45]. Only the present observation of CPK activity which was increased by running-exercise and thyroxine-administration (Table-4) suggested the changes of energy-rich phosphates. The increased myocardial mass and appearance of new isoenzyme of contractile proteins will be regulated subtly by these many factors described above, which are controlled by the hormonal level in the body[73,86]. Both the exercise and thyroxine-administration will influence the level of the hormone, so their effects on the hormonal level need to be studied further in detail.

As regards the contractility of the heart, Bhan et al. showed the increased myosin and actomyosin ATPase activities using prolonged swimming-exercise of the rats and explained the increased contractility of the heart[6,7,55]. The result of the present study showed no clear change in the following observations owing to the running-exercise: the value of myosin and actomyosin ATPase activities (Table-2), the profile of pH dependency (Fig.-4), NEM modification (Fig.-5),

and the superprecipitation of contractile proteins (Fig.-6) These observations with no change supported the result of Watras et al. (1978[97]) who showed that a long-term endurance-running program could produce muscular enlargement without changing the basic biochemical characteristics of the myosin.

The different effects of the exercise on cardiac hyper-hypertrophy and contractile proteins may be due to the following factors: the kind of used training program, the frequency, the intensity, the duration of the training sessions, the age at onset of the program, the sex of the animal[66], and the strain of animals used[33].

The results of the present study (Table-2,3,4, Fig.-8, 10) suggested the abnormality of myocardial cell by thyroxine-administration. The essential properties of contractile proteins in the pH dependency and NEM modification was maintained even by the administration of thyroxine, but the value of myosin ATPase activity showed a little high level sometimes, particularly in Ca^{2+}-ATPase activity (Fig.-4). The contractility which was increased by thyrotoxism was confirmed by the physiological method using shortening speed [38] and the biochemical method using ATPase activities of contractile proteins[60,105]. The increased ATPase activities of contractile proteins by the thyroxine-administration were not well explained. Some investigators suggested that the increased ATPase activity was due to the appearance of new active myosin isoenzymes[3,32,53,60,76,90], and others suggested the fragmentation of myosin with proteolitic enzymes[8,56,82]. The present study (Table-2, Fig.-4,10)

suggested the fragmentation of contractile proteins. Siemankowski et al. (1978[82]) suggested the change in the myosin ATPase activity during pressure overload hypertrophy might reflect denaturation[17] or other modification of myosin in vivo. Their suggestion was supported by the present observation partly. But the increased ATPase activity[18,82] was different from other reports[48,87] using aortic constriction which was same with their treatment. This confliction might be due to the different stages of the heart adaptation to some treatments and methodological differences. For example, the present study (Table-3,4, Fig.-7,8,10) suggested the difference of response in the muscle regions to the stimulation. Some investigators showed the different contractile proteins in right and left ventricles[71], atria[106], and Purkinje cell[54]. These muscles have been found to have different properties in immunochemistry[74] and ATPase activity of contractile proteins[71]. As regards the myosin activity Wikman-Coffelt et al. (1975[101]) showed right ventricle myosin had the lower ATPase activity than left. Whereas, Kleid et al. (1972[46]) reported no difference between left and right ventricle myosins. At least, sufficient care to minimize contaminating of each muscle region may be required in the preparation of the contractile proteins. The appearance of myosin isoenzyme which was suggested by Yazaki[105] was recently supported by several investigators[3,32,60]. Surely, the high level of thyroxine in the body promotes the function of the heart. But the relationship between the hormonal level and the appearance of the isoenzyme is not

clear yet.

The present observations on LDH (Table-3, Fig.-7,8) suggested the different effects of exercise and thyroxine-administration on the capacity of cardiac oxidative metabolism. The effect of the exercise which is reversible[19,35] is well explained by the high capacity of oxidative metabolism with the development of cardiovascular system[13,75] and the transformation of LDH isoenzymic pattern from anaerobic type to aerobic type[36,85,107]. Therefore the increased aerobic capacity may explain the non-failing hypertrophied heart. On the other hand, the present data (Table-3, Fig.-8) suggested the induced anaerobic condition owing to thyroxine-administration and may explain failing hypertrophied heart accompanied with the deficiency of oxidative metabolism[70]. After all, the present study as described above showed that a non-failing hypertrophied heart contained normal contractile proteins[9,10,21] and that the hypertrophy itself meaned no pathological symptom[24]. The biochemical adaptation of nonfailing heart might be seen prior to the onset of hypertrophied heart under normal physiological conditions and maintained during the exercise-period as Albin et al. suggested[1].

Conclusion

1) Prolonged endurance-running showed the normal biochemical characteristics in the ATPase activity, pH dependency, NEM modification, and superprecipitation of the cardiac contractile proteins and induced a slight hypertrophy of the heart.

2) Thyroxine administration caused a clear heart hypertrophy and showed a little increased Ca^{2+}-ATPase activity of the cardiac myosin and the basic changing profile in the pH dependency, NEM modification, and superprecipitation of the heart contractile proteins.

3) Endurance-exercise increased the aerobic capacity of the heart.

4) Aerobic metabolism decreased in thyroxine-administrated heart.

5) There was a different adaptability to various stimulation in the muscle regions of the heart and the effect of thyroxine administration was heavier in right ventricle than left ventricle and atria.

6) The low molecular weight component of soluble proteins increased in the thyroxine-administrated right ventricle of the heart and suggested the fragmentation of the proteins with proteolytic enzymes.

Summary

The present study was accomplished to compare the each effect of prolonged endurance-exercise and thyroxine-administration on cardiac muscle using biochemical procedures. Male Sprague-Dawley rats from same venter were used in the whole experiment. The animals were put into 3 following groups: (1) Control group (n=8), (2) Running group (n=8), and (3) Thyroxine group (n=8).

The rats of Running group were trained to run on a treadmill for 2 months (30 min/day, 5 days/week). The running velocity was about 45 m/min. Thyroxine was injected intraperitoneally 15 μg/100 g B.W. per day for 3 weeks. Control group was not treated with anything at all.

The present study showed following results: (1) The prolonged running-exercise maintained normal and basic characteristics of contractile proteins in ATPase activity, pH dependency, NEM modification, and superprecipitation. (2) The exercised animals had a slight cardiac hypertrophy. (3) The cardiac contractile proteins of thyroxine-administrated rats showed the basic properties in the profile of pH dependency, chemical modification (SH_1 block of NEM), and superprecipitation as Control and Running groups. But sometimes, the value of Ca^{2+}-ATPase activity in Thyroxine group showed higher level than other two groups. (4) K^+-EDTA-ATPase activity was not changed by the thyroxine-administration. It induced a cardiac hypertrophy clearly and promoted the anaerobic metabolism in the heart, while running-exercise showed the predominance of aerobic metabo-

lism in the heart of the exercised group. (5) Thyroxine-load influenced the right ventricle largely, and the effect suggested the specific response of the cardiac muscle regions : right and left ventricles and atria. (6) Thyroxine-injection induced the increased component of the low molecular weight (M.W.= 3 x 10^4 dalton) in the right ventricle.

The result of the present study suggested no appearance of the new cardiac myosin isoenzyme, and it indicated the basic biochemical characteristics of the cardiac contractile proteins which might be dependent on sufficient aerobic adaptability to enviromental stress. Because the sufficient aerobic conditions in the cell could guarantee the well energy-production system and the normal intracellular condition like pH, energy-rich phosphates and so on. Such sufficient metabolic adaptability owing to prolonged endurance-exercise may explain the good conditions of the heart function and the reversible cardiac hypertrophy without failure. Whereas, the defficient aerobic capacity owing to thyroxine administration may explain the pathological situations of the heart with irreversible hypertrophy.

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