Studies on the Turnover of Myocardial Actomyosin in Dogs with Experimental Aortic Insufficiency and the Effect of Vitamin B₁₂ on Actomyosin Metabolism

YASUSHI YOKOTA

The turnover rate of actomyosin of myocardium and skeletal muscle was studied in control dogs and in those with experimentally produced aortic insufficiency. Based on the concept that vitamin B₁₂ is an activating drug for myocardial metabolism, a large dose of it was given some of the dogs with aortic insufficiency to study the effect of vitamin B₁₂ on the turnover of actomyosin in the overloaded heart. During the early period, when there was an acute increase in the load on the heart, the turnover rate of myocardial actomyosin in the dogs with aortic insufficiency increased and that of dogs administrated vitamin B₁₂ increased remarkably. However, during a relatively stable period (from 9th—30th day after production of aortic insufficiency), the turnover rate of myocardial actomyosin in both dogs with aortic insufficiency and those administrated vitamin B₁₂, approached control levels. From these observations it seems that increase in turnover rate is an important compensatory factor in the diastolic-overloaded heart and administration of vitamin B₁₂ is useful in treatment of the overloaded heart.

Since the study of MEERSON¹ on changes in protein metabolism in cardiac hypertrophy, interest has been taken in it as making clear a triggering mechanism on the development of cardiac failure.

The study on dogs with aortic stenosis by MEERSON¹ indicates that a heart faced with increased outflow resistance passes through the following three stages: (1) during the first or damage stage, which occurs immediately after severe outflow obstruction, protein synthesis is increased, (2) during the second stage or stage of relatively stable hyperfunction, which lasts about one and a half to two years, the degree of protein synthesis and breakdown approaches normal levels, and (3) during the third stage, which is characterized functionally by gradual exhaustion, protein synthesis is diminished. BING et al.² also suggest that in the heart of rabbits with cardiac hypertrophy protein synthesis increases but there is no increased protein turnover rate; in chronic heart failure the relative incorporation of glycine-2⁻¹⁴C into heart muscle protein is diminished. However, these observations on heart muscle protein are concerned with changes of total protein metabolism of heart muscle, not with those in the metabolism of contractile protein or "actomyosin" itself. Although many studies concerning the physiochemical changes in properties of actomyosin in failing heart have been reported by BENSON et al.³, OLSON et al.⁴, JAMES et al.⁵, and MIYAHARA⁶, little is known about the dynamic metabolism of myocardial actomyosin. Recently OHTSUKA⁷ has observed increased incorporation of glycine-2⁻¹⁴C into myocardial

(Received for Publication, November 6, 1967)
† The Third Department of Internal Medicine (Director: Prof. N. Kimura), Kurume University School of Medicine, KURUME.
actomyosin in cases of an acutely increased load on the heart.

The purpose of this paper is to observe the turnover of myocardial actomyosin extracted from control dogs as well as from those with experimentally produced aortic insufficiency, and the effect of vitamin B_{12} on the turnover of myocardial actomyosin in the latter.

**MATERIAL AND METHODS**

Experiments were performed on 24 adult mongrel dogs of both sexes, with an average weight of 10.4 kg. The dogs were fed with a diet containing adequate salts and vitamins for 20 days. Aortic insufficiency was produced in 16 dogs, anesthetized with 5 per cent thiopental natrium in the dosage of 25 mg per kg body weight, by inserting a improved straight grasping forceps through left carotid artery and perforating the leaflets of aortic valves. In order to estimate the severity of the aortic insufficiency the blood pressure gradient across the aortic valve was recorded before and after the operation.

The evidence of the aortic insufficiency was noted by a marked fall of the aortic diastolic pressure with a typical murmur heard over base of the heart.

The dogs were divided into three groups; (1) control group, (2) A1 group (dogs with experimentally produced aortic insufficiency), (3) A1 + VB_{12} group (in this group, 500 γ of vitamin B_{12} was given by intramuscular injection daily after production of aortic insufficiency during the experimental period). DL-leucine-1-^{14}C (Specific activity 13.5 mc/mM, Daiichi-Kagaku Co., Japan) was used in all experimental dogs; 40 μc/Kg body weight of DL-leucine-1-^{14}C were injected intravenously three days after the operation.

At the 1st, 5th, 20th and 30th day after injection of DL-leucine-1-^{14}C, all the dogs were intravenously anesthetized with 5 per cent thiopental natrium, and then each chest was opened by removing the 3rd and 4th ribs under artificial respiration of room air, quickly beating hearts and specimens of skeletal muscle in the femoral region were removed and washed with cold isotonic NaCl solution. Ten grams of the skeletal muscle, and the right and left ventricles were used for extracting actomyosin, respectively.

The extraction of actomyosin was performed by the method of STRAUB-FEUFER modified by OHSAWA and ASAKURA shown in figure 1, in which twice extraction was carried out to wash out the contamination of free ^{14}C-amino acids by using OHITSUKA’s method. In order to examine the purity of actomyosin extracted by above described method, observation was made on superprecipitation, changes in velocity after addition of ATP, ATPase activity, and electrophoresis of actomyosin. As the result, neither denaturation nor contamination was found in the actomyosin solution used in this study.

Radioactivity of the actomyosin was determined, using a Shimadzu LSG3 type liquid scintillation counter, in a dioxane scintillator based on the composition of Bray. This scintillator consists of 12 g PPO (2.5-diphenyl-oxazole), 600 mg POPOP, (1,4-bis-2 (5-phenyloxazolyl) benzene) 200 ml methylcellulose and 40 g thiotropic gel powder in one liter dioxan. Actomyosin solution of 0.2 ml was suspended in 10 ml of the scintillator and determined for 10 minutes. The efficiency, as determined with an internal standard, was 50 per cent or more. Protein concentration of actomyosin was determined by the method of BIURET.

**RESULTS**

All the dogs were sacrificed at the 1st, 5th, 20th and 30th day after administration of DL-leucine-1-^{14}C. Radioactivities of actomyosin are summarized in tables I, II and III. Figure 2 shows the disappearance of the labelled material from heart and skeletal muscle actomyosin. Radioactivity of actomyosin of the left and right ventricular muscles in all the

*Japanese Circulation Journal* Vol. 34, December 1967


### Table I  Specific Activity of Actomyosin of Left Ventricle and Effect of Vitamin B₁₂ (dpm/10mg)

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th>AI</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>AI±VB₁₂</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
</tr>
<tr>
<td>1</td>
<td>3101</td>
<td>4143</td>
<td>4093±87</td>
<td>3097</td>
<td>4317</td>
<td>4568±444</td>
<td>3103</td>
<td>4952</td>
<td>5063±149</td>
<td>3105</td>
<td>5121</td>
<td>3105</td>
<td>5121</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3098</td>
<td>4044</td>
<td>3703±35</td>
<td>3100</td>
<td>3819</td>
<td>3762±200</td>
<td>3092</td>
<td>3808</td>
<td>3869±198</td>
<td>3094</td>
<td>3584</td>
<td>3094</td>
<td>3584</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2095</td>
<td>3723</td>
<td>3200±191</td>
<td>2089</td>
<td>3649</td>
<td>3137±15</td>
<td>2085</td>
<td>3088</td>
<td>3167±140</td>
<td>2087</td>
<td>3247</td>
<td>2087</td>
<td>3247</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3099</td>
<td>3683</td>
<td>2511±16</td>
<td>2082</td>
<td>3128</td>
<td>2478±220</td>
<td>2077</td>
<td>2632</td>
<td>2576±98</td>
<td>2081</td>
<td>2521</td>
<td>2081</td>
<td>2521</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1080</td>
<td>2511</td>
<td>2520±16</td>
<td>1078</td>
<td>2354</td>
<td>2457±220</td>
<td>1077</td>
<td>2632</td>
<td>2576±98</td>
<td>1081</td>
<td>2521</td>
<td>1081</td>
<td>2521</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1075</td>
<td>2530</td>
<td>2520±16</td>
<td>1072</td>
<td>2603</td>
<td>2478±220</td>
<td>1072</td>
<td>2632</td>
<td>2576±98</td>
<td>1076</td>
<td>2521</td>
<td>1076</td>
<td>2521</td>
<td></td>
</tr>
</tbody>
</table>

### Table II  Specific Activity of Actomyosin of Right Ventricle and Effect of Vitamin B₁₂ (dpm/10mg)

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th>AI</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>AI±VB₁₂</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
</tr>
<tr>
<td>1</td>
<td>3101</td>
<td>3722</td>
<td>3571±266</td>
<td>3097</td>
<td>3147</td>
<td>3097±88</td>
<td>3103</td>
<td>3400</td>
<td>3338±109</td>
<td>3105</td>
<td>3276</td>
<td>3105</td>
<td>3276</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3099</td>
<td>3421</td>
<td>3106±175</td>
<td>3099</td>
<td>3007</td>
<td>2909</td>
<td>2930</td>
<td>2771±280</td>
<td>2092</td>
<td>2594</td>
<td>2580±196</td>
<td>2094</td>
<td>2469</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2095</td>
<td>3205</td>
<td>2641±413</td>
<td>2082</td>
<td>2554</td>
<td>2438±150</td>
<td>2085</td>
<td>2454</td>
<td>2550±77</td>
<td>2087</td>
<td>2507</td>
<td>2087</td>
<td>2507</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2096</td>
<td>2875</td>
<td>2365±72</td>
<td>2078</td>
<td>2300</td>
<td>2141±108</td>
<td>2081</td>
<td>2300</td>
<td>2216±148</td>
<td>2081</td>
<td>2132</td>
<td>2081</td>
<td>2132</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1080</td>
<td>2365</td>
<td>2324±72</td>
<td>1078</td>
<td>2080</td>
<td>2141±108</td>
<td>1077</td>
<td>2300</td>
<td>2216±148</td>
<td>1081</td>
<td>2132</td>
<td>1081</td>
<td>2132</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1075</td>
<td>2283</td>
<td>2324±72</td>
<td>1072</td>
<td>2202</td>
<td>2141±108</td>
<td>1072</td>
<td>2202</td>
<td>2216±148</td>
<td>1076</td>
<td>2132</td>
<td>1076</td>
<td>2132</td>
<td></td>
</tr>
</tbody>
</table>

### Table III  Specific Activity of Actomyosin of Skeletal Muscle and Effect of Vitamin B₁₂ (dpm/10mg)

<table>
<thead>
<tr>
<th>Days</th>
<th>Control</th>
<th></th>
<th></th>
<th></th>
<th>AI</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th>AI±VB₁₂</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
<td>Mean ± S.D.</td>
<td></td>
<td>Case No.</td>
<td>dpm</td>
</tr>
<tr>
<td>1</td>
<td>3101</td>
<td>1782</td>
<td>1954±304</td>
<td>3097</td>
<td>1383</td>
<td>1346±30</td>
<td>3103</td>
<td>686</td>
<td>656±53</td>
<td>3105</td>
<td>626</td>
<td>3105</td>
<td>626</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3098</td>
<td>2126</td>
<td>1977±378</td>
<td>3099</td>
<td>1340</td>
<td>1410±40</td>
<td>3092</td>
<td>1404</td>
<td>1673±477</td>
<td>3094</td>
<td>1943</td>
<td>3094</td>
<td>1943</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2095</td>
<td>1764</td>
<td>1690±160</td>
<td>2096</td>
<td>1672</td>
<td>1424±81</td>
<td>2085</td>
<td>1651</td>
<td>1616±62</td>
<td>2087</td>
<td>1581</td>
<td>2087</td>
<td>1581</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2096</td>
<td>1600</td>
<td>1600±160</td>
<td>2082</td>
<td>1387</td>
<td>1298±88</td>
<td>2077</td>
<td>1477</td>
<td>1392±150</td>
<td>2081</td>
<td>1307</td>
<td>2081</td>
<td>1307</td>
<td></td>
</tr>
</tbody>
</table>

*Japanese Circulation Journal  Vol. 31, December 1967*
groups showed a maximum on the first day and in AI and AI + VB₁₂ groups it decreased during the following five days more rapidly than in controls. But afterwards, since its diminution became slower, there was no difference between the control and experimental groups in the turnover rate of actomyosin. Although only in controls radioactivity of actomyosin of skeletal muscle showed a maximum on the 1st day, its maximum in AI and AI + VB₁₂ groups was obtained between the 1 and 5 days after injection. After reaching the maximum values, the rate of its disappearance was about the same in every group.

The biological half-life of actomyosin can be estimated from the semilogarithmic function of the specific activity of actomyosin versus time. Figure 3 shows this function for actomyosin of heart and skeletal muscles in every group. In controls the half-life of actomyosin was about 48 days for left and right ventricles, about 110 days for skeletal muscle. while in AI group, the half-life of actomyosin of left and right ventricles and skeletal muscle after 5th day was the same value as in controls, it was, during the first 5 days, about 31 days for left and 28 days for right ventricle. Although this was also the case in AI + VB₁₂ group, it was, during the first 5 days, shorter than in AI group, that is, it was about 11 days for both the left and right ventricles.

**DISCUSSION**

Although a considerable number of papers concerning the turnover of total protein in heart muscle in animals have been published, little is known about that of "actomyosin" in it. So far as the author knows, the work reported here is the first designed to elucidate the turnover of actomyosin of heart muscle in dogs. Dreyfus et al. reported that myosin of skeletal muscle of rats was in no dynamic equilibrium and had a 30-day-life span. On the contrary, Funabiki described that since radioactivity of myosin or actin was very similar to that of the total myofibril protein, the curve of radioactivity of myosin or actin seemed to reflect that of myofibril turning over very slowly.

This is in agreement with the author's results. As shown in figure 2, radioactivity of actomyosin which declined slowly in every group shows that actomyosin has a turnover.

*Japanese Circulation Journal Vol. 34, December 1967*
sults that half life of actomyosin was 48 days for both the left and right ventricles and 110 days for the skeletal muscle show that actomyosin metabolism of heart muscle is more active as compared with that of skeletal muscle.

When the heart is subjected to functional strain by a overload on the heart, compensatory hyperfunction takes place, and this compensatory hyperfunction of the heart is an important factor in the development of the general compensation observed in the overloaded hearts. As shown in figure 2, during the early period, which was characterized by an acute increase in the load on the heart (from 1st—8th day after production of aortic insufficiency) there was a marked increase in the turnover rate in the left and right ventricles. During the successive period of a relatively stable stage, which was characterized by absence of cardiac failure (from 9th—30th day after production of aortic insufficiency), the turnover rate approached the control levels in heart muscle. KIMURA and OHTSUKA reported that the incorporation of 14C-aminio acid into the myocardial actomyosin in an early stage of diastolic overload was as twice as incorporation observed in relatively stable stage showing about the same value as in controls.

Since the study of CARBERA and MONROY on the overloaded hearts, overload on the heart generally have been classified into a systolic and diastolic types. MEERSON also suggests that hyperfunction of the heart is classified into a mainly isometric and isotonic types and that the events developing in the myocardium in the process of hyperfunction of the heart depend upon the type of the load on the heart. As reported in detail by Meerson and Bing et al., the heart with systolic overload may be compensated by increase in the protein synthesis (that is hypertrophy). However, it is thought that the compensatory mechanism in the heart with diastolic overload characterized by aortic insufficiency is different from that of a systolic overloaded heart. From the results obtained here, it may be assumed that increase in the turnover rate of actomyosin of heart muscle may be an important compensatory factor on the heart with diastolic overload.

Almost at the beginning of the work on vitamin B₁₂ in animal nutrition, it was suggested that this vitamin plays a role in protein metabolism. Since the first experiments of Hoagland, the evidence obtained in many laboratories indicates that the sequence of protein synthesis reactions begins with an amino acid activation. This activation is carried out by specific enzyme (pH5 enzyme) and requires ATP according to the following reaction:

\[
\text{Amino acid} + \text{ATP} \rightarrow \text{Activating Enzyme} \rightarrow \text{Enzyme} \rightarrow \text{Amino Acid} + \text{PP}
\]

also, WAGLE et al. reported that vitamin B₁₂ was a co-factor of pH5 enzyme for the incorporation amino acids into protein biosynthesis. Based on these observations, it is thinkable that vitamin B₁₂ has the effect on the turnover of muscle protein. These observations however, was obtained by the studies on animals fed with vitamin B₁₂ deficient diet.

On the other hand KIMURA has reported that a much large dosage of vitamin B₁₂ than usual is useful in treatment of cardiovascular diseases, particularly of those with disturbance of protein metabolism. Based on this concept, it was given in large quantities to the dogs with aortic insufficiency to examine its nonspecific effect on protein metabolism in this study.

In AI + VB₁₂ group, increase in the turnover rate of heart muscle actomyosin during the early period was most marked. This finding shows that if increase in the turnover rate is an important compensatory factor for diastolic overloaded heart, a large dosage of vitamin B₁₂ is useful in its treatment.

**Summary**

The turnover rate of actomyosin of myocardium and skeletal muscle was studied in control dogs and in those with experimentally produced aortic insufficiency. A large dose of Vitamin B₁₂ was given some of dogs with aortic insufficiency to study the effect of vitamin B₁₂ on the turnover of actomyosin in the overloaded heart. In controls the biological half-life of actomyosin was 48 days for the left and right ventricles, 110 days for skeletal muscle. During the early period, when there was an acute increase in the load on the heart (from the each of 1st—8th day after production
of aortic insufficiency), the biological half-life for the left ventricle in AI group was shorter than that in controls, and that in AI+VB12 group was the shortest of the three, but both of them approached the control levels during the relatively stable period (from 9th—30th day after production of aortic insufficiency). Whereas radioactivity of actomyosin in the skeletal muscle reached the maximum between the 1st and 5th day after injection of the labelled amino-acid, and then declined at the same rate as in controls. It is not clear why maximal incorporation into skeletal muscle is delayed. Based on the obtained data, it may be said that increase in the turnover rate is an important compensatory factor in the cardiac overload caused from experimentally produced aortic insufficiency and that administration of vitamin B12 is useful in the treatment of the overloaded heart.

Acknowledgement
The author wishes to express his appreciation to Prof. Noboru Kimura and Dr. Shunichi Kodama for their helpful suggestions throughout the course of this work and in reviewing the manuscript. Drs. Y. Ohtsuka, Y. Ohi and T. Arima have also contributed greatly to success of this experiment.

REFERENCES