The Economy of Isometric Force Development, Myosin Isoenzyme Pattern and Myofibrillar ATPase Activity in Normal and Hypothyroid Rat Myocardium


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SUMMARY. Hypothyroidism was induced in Wistar-Kyoto rats by adding propylthiouracil to the drinking water (0.8 mg/ml). Initial heat, total activity-related heat, and resting heat rate were measured in left ventricular papillary muscle preparations of propylthiouracil-treated and control rats contracting isometrically at 12 beats/min (21°C), using Hill type, planar vacuum-deposited bismuth and antimony thermopiles. In the propylthiouracil preparations, relative to control, time-to-peak tension increased from 288 ± 27 (mean ± SD) to 411 ± 25 msec (P < 0.001), dp/dt max decreased from 38.3 ± 9.5 to 20.4 ± 3.5 g·mm⁻²/sec (P < 0.001), and peak developed tension decreased from 6.11 ± 1.75 to 4.64 ± 0.89 g·mm⁻² (P < 0.05). In the propylthiouracil preparations, initial heat was significantly (P < 0.001) reduced by 27 or 43% when normalized to peak twitch tension or tension-time integral, respectively. In experiments where the papillary muscles were tetanized, the slope of the linear function of total activity-related heat versus tension-time integral was decreased by 43% (P < 0.001) in the propylthiouracil preparations, indicating an improved economy of isometric tension maintenance. The predominant myosin isoenzyme of the left ventricular wall, as well as the papillary muscle myocardium, was the V₃ variety in the propylthiouracil animals, in contrast to V₁ in the controls. Myofibrillar actomyosin calcium-magnesium-stimulated adenosine triphosphatase activity was significantly (P < 0.02) decreased from 55 ± 18 (control) to 31 ± 8 nmol inorganic phosphate ion/mg-min (propylthiouracil). Correspondingly, the myofibrillar myosin calcium-stimulated adenosine triphosphatase activity was also significantly (P < 0.01) decreased from 294 ± 98 (control) to 85 ± 25 nmol inorganic phosphate ion/mg-min (propylthiouracil). The results give evidence of (1) increased economy of force generation and maintenance in propylthiouracil myocardium, which is paralleled by (2) structural changes of myosin (shifts in the isoenzyme pattern), and (3) associated changes of myofibrillar adenosine triphosphatase activity. We conclude that the observed myothermal data reflect slowed crossbridge cycling in propylthiouracil myocardium, which must not necessarily be interpreted as being detrimental, in view of the increased economy of force generation. (Circ Res 56: 78-86, 1985)

A POSITIVE correlation has been observed between contractile protein adenosine triphosphatase (ATPase) activity and shortening velocity across species in skeletal muscle (Barany, 1967) and as a function of age in the myocardium (Alpert et al., 1967). These observations were confirmed for the myocardium, using different species and a variety of temperatures (Delcayre and Swynghedauw, 1975; Hamrell and Low, 1978). The experiments relating contractile protein ATPase activity and mechanical V max were extended to include cardiac hypertrophy secondary to pressure overload (Chandler et al., 1967; Alpert et al., 1974; Medugorac and Jacob, 1976; Hamrell and Alpert, 1977; Alpert and Mulieri, 1977; Jacob et al., 1977, 1980, 1983; Maughan et al., 1979). Furthermore, increases of both parameters due to experimentally induced hyperthyroidism have been reported (Goodkind et al., 1974; Yazaki and Raben, 1975; Banerjee et al., 1976; Rovetto et al., 1972). No significant increase of myosin ATPase activity was observed when thyroxine was administered to young rats, which, under normal conditions, exhibit a high level of cardiac myosin ATPase activity (Yazaki and Raben, 1975; Szabo et al., 1979). Decreases of myocardial contractile protein ATPase activity and mechanical V max were observed in hypothyroid rats (Buccino et al., 1967; Korecky and Bezna, 1971; Yazaki and Raben, 1975; Strauer and Schulze, 1976; Hoh et al., 1977; Pope et al., 1980; Schwartz et al., 1981; Ebrecht et al., 1982).

These studies lead to the hypothesis that the intrinsic enzymatic properties of actomyosin regulate the velocity of muscle shortening, although the in vitro measured myosin ATPase activity need not necessarily reflect the true conditions in vivo. This problem was recently reviewed in detail (Scheuer and Bhan, 1979). To answer the question whether the general parallelism between biochemical and mechanical data is founded upon a causal connection, studies are required which link the biophysical and biochemical parameters in living muscle preparations.
Recently, a rapid myothermal technique was used to demonstrate that the tension-dependent heat per unit tension during an isometric contraction of rabbit papillary muscle is decreased by pressure overload and increased by thyrotoxicosis (Alpert and Mulieri, 1980, 1982; Alpert et al., 1979). In this method, measurements of liberated energy are utilized to distinguish between alterations of excitation-contraction coupling and intrinsic chemomechanical transduction economy of the contractile proteins in vivo. Thus, in the rabbit myocardium, the economy of force generation is inversely related to the contractile protein ATPase activity. The goal of the present study was to extend these observations to the rat myocardium to test the hypothesis that the economy of force development is inversely related to contractile protein ATPase activity. In these experiments, we exploit the sensitivity of the rat myocardium to the thyroid status where decreases in circulating thyroxine produce dramatic changes in contractile protein ATPase.

Because it has been suggested, recently, that changes in ATPase activity rely on structural changes of heavy chains of myosin (Hoh et al., 1977; Lompré et al., 1979; Pope et al., 1980; Rupp, 1982; Litten et al., 1982), we also analyzed the myosin isoenzyme pattern so that correlations between structural and functional data could be obtained.

Methods

Definition of Heat Terms

Total heat (T) is the sum of total activity-related heat (TA) and basal heat (B). Ta is the sum of initial heat and recovery heat (Alpert and Mulieri, 1982). Initial heat is the heat production arising from calcium cycling (TIH—the tension-independent heat) plus crossbridge cycling (TDH—the tension-dependent heat). The recovery heat (R) arises from the metabolic resynthesis of high energy compounds from ADP. The ratio of recovery to initial heat (R/I) is the recovery ratio.

Animals and Preparations

All experiments were performed on left ventricular papillary muscles of rats obtained from a colony of Wistar-Kyoto rats maintained at the University of Vermont. The experimental animals were housed individually, and hypothyroidism was induced by adding 0.8 mg/ml propylthiouracil (PTU) to the drinking water over a period of 3 weeks. The treatment was started when the rats were between 6 and 7 weeks old. In a recent paper (Holubarsch et al., 1982), the methods, procedure of sacrifice, and papillary muscle preparation are described in detail.

Thermopile and Thermal Measurements

The exact details of producing and calibrating antimony-bismuth planar thermopiles were described previously (Mulieri et al., 1977). The experiments of the present study were performed on three different thermopiles which had the same design and exhibited almost equal values of temperature sensitivity (1.238 mV/°C, 1.311 mV/°C, 1.325 mV/°C) and heat loss coefficient (0.538 mcal/°C.s, 0.546 mcal/°C.s, 0.590 mcal/°C.s). The temperature sensitivity was evaluated according to the method of Hill and Woledge (1962), while heat loss coefficient was determined by a modification (Mulieri et al., 1977) of the method of Kretzschmar and Wilkie (1972). The voltage output of the thermopile was amplified by a galvanometer (Kipp, A 80) and a twin phototube (RCA 920). The signals from the galvanometer and the capacitance-type, isometric force transducer were displayed on a Tektronix 5103N oscilloscope and a chart recorder (Gould-Brush 2400).

For heat measurements, the Krebs-Ringer solution was drained; during that procedure, premoisturized, and temperature-equilibrated gas was admitted at the top of the chamber to avoid contamination by room air.

Experimental Procedure

During the 2-hour equilibration period, the muscle preparation was stimulated at 12/min about 20% above threshold. The optimum length (L0) was found by increasing the muscle length in small steps (0.1-0.05 mm). To define resting tension, the muscle was released by 1.5 mm to establish mechanical zero. When steady state conditions were reached at L0, the chamber was drained, and temperature and tension signals were recorded synchronously during repetitive stimulation at different paper speeds for analyzing initial heat, as described below. For the analysis of total activity-related heat, stimulation was stopped, and the decline of the temperature signal (cool-off curve after activity) was recorded until the signal was constant. This baseline represents the resting temperature. Under these resting conditions, the thermopile, including the preparation and adhering Krebs-Ringer solution, was heated by infra-red emitting diodes, and cool-off curves were recorded (Mulieri et al., 1977). This procedure was repeated three times, and the time constants of the cool-off curves, after infra-red heating, were analyzed by a computerized nonlinear least squares method. These time constants were used for calculating initial heat (see below).

To analyze the basal heat rate, we partially filled the chamber to submerge the lower part of the frame of the thermopile. This allowed the achievement of temperature-equilibration. The muscle preparation remained in the temperature-equilibrated moisturized gas environment. After a steady state rest period of 20 minutes, the muscle preparation and thermopile were submerged abruptly, and the deflection in temperature trace was taken as the resting temperature. The basal heat rate was determined from this deflection and the heat loss characteristics of the muscle and system. Resting tension was determined at this point, as described above.

Heat Calculations

The recorded temperature changes were converted into heat values as follows. Initial heat was calculated as the product of the peak temperature difference, the cool off constant, τ, and the heat loss coefficient (Alpert and Mulieri, 1982). Peak temperature difference corrected for heat loss was evaluated by extrapolating the cool off curve of the preceding twitch and measuring the temperature difference between the extrapolated curve and the peak temperature signal (arrows, Fig. 1). The shape of the extrapolated line was obtained by omitting the stimulus and recording the cool-off after the last twitch response in the series and then translating that curve back to the previous falling temperature excursion (see dotted lines on Fig. 1). The time constant, τ, was evaluated by means of a nonlinear least squares exponential fitting program.
from the cool-off curves after infra-red heating. The heat loss coefficient was defined as previously described (Kretzschmar and Wilkie, 1972; Mulieri et al., 1977). The total activity-related heat was calculated from the area between temperature base line during rest and the oscillating temperature signal during the steady state period of activity for one stimulus interval (Bugnard, 1934; Alpert and Mulieri, 1982; Holubarsch et al., 1982). Since the total activity-related heat is the sum of initial heat and recovery heat, the recovery:initial heat ratio equals (TA-I)/I.

The temperature decrease when submerging the resting muscle preparation abruptly was multiplied by the heat loss coefficient and yielded the resting heat rate.

Experiments with Iodoacetic Acid (IAA) in N2-CO2 Atmosphere

In order to protect ourselves from the possibility that initial heat is contaminated with recovery heat, we performed experiments in control preparations incubated in IAA and 95% N2-5% CO2. In contrast to rabbit myocardium (Alpert and Mulieri, 1982), rat myocardium cannot tolerate long periods (90 minutes) of 0.1 mM IAA incubation. Therefore, the muscle preparations were soaked in 0.25 mM IAA and 95% N2-5% CO2 solution, for 5 minutes, and then drained and allowed to equilibrate for an additional 10 minutes. In untreated muscle in a single twitch following a 15-minute period without stimulation, the developed peak tension and initial heat per unit developed tension were not significantly different from values of paced twitches. The IAA and N2-CO2 treatment resulted in a 17 ± 9% reduction in peak developed tension. In this preparation, the recovery heat diminished by 89% ± 15%. The comparison of the ratio of measured initial heat to tension-independent initial heat.

Measurements of Myosin Isoenzymes

Pyrophosphate Gel Electrophoresis

The left ventricle (free wall and septum) from each of the hearts from which papillary muscles were taken was frozen in liquid nitrogen, powdered, and stored at −75°C. Pyrophosphate gel electrophoresis was carried on 25 mg of the left ventricle. In some instances, electrophoresis was also carried out on left ventricular papillary muscles (1–5 mg) (Fig. 4). This method is used to separate the myosin isoenzymes V1, V2, and V3 from the supernatant of crude heart extracts and is based on the procedure described by Hoh et al. (1977). The pyrophosphate gels were then scanned with a laser beam densitometer of our own design having high spatial resolution (Brayden and Halpern, 1983).

Quantification of the Relative Amounts of Myosin Isoenzymes

A special method for quantifying the relative amounts of V1, V2, and V3 from the densitometer tracing was developed because of the potential problem from the overlapping of V1, V2, and V3 peaks. The overlap may lead to erroneous evaluations of the concentration of V1 and V3. The percent V1 isoenzyme was calculated from densitometer traces using the following equation:

% V1 = Av1 / Av

where Av1 equals the area under the V1 peak and Av equals the total sum of areas under V1, V2, and V3 peaks. The V1 peak area (Av1) was determined by weighing the paper which makes up half the V1 peak area (side away
Myofibrillar ATPase Activity

Myofibrillar Preparation

Cardiac myofibrils were prepared from the remaining left ventricle according to the following modification of the method of Pagani and Solaro (1984). All solutions used in the purification procedure contained the following protease inhibitors: 2 μg/ml pepstatin A (Sigma), 2 μg/ml leupeptin (Sigma), and 4 mM dichlorvos (serine protease inhibitor) (Bodwell and Meyer, 1981) (Vapona brand of 2,2-dichlorovinyl dimethyl phosphate from Shell Development Co.). The myofibrils were stored on ice overnight in 30 mM KCl, 30 mM imidazole (pH 7.0), 2 μg/ml leupeptin, 2 μg/ml pepstatin A, and 4 mM dichlorvos and used the next day to determine the myofibrillar ATPase activities.

Myofibrillar ATPase Activity Measurements

Myofibrillar actomyosin Ca"++-Mg"++-stimulated ATPase activity was measured in 30 mM KCl, 25 mM imidazole (pH 7.0), 7.5 mM MgCl2, 5.0 mM Na2ATP, 10 mM NaN3, 1.0 mM CaCl2, 0.5 mM EGTA [free Ca"++] concentration = 3.35 × 10^-8 M, using the program of Maughan et al. (1979)]. Myofibrillar myosin Ca"++-stimulated ATPase was assayed in 450 mM KCl, 51 mM imidazole (pH 7.5), 10 mM CaCl2, 5 mM Na2ATP, and 5 mM NaN3. Assays were carried out at 21°C, and reactions were stopped by the addition of cold HClO4. Precipitated protein was removed by centrifugation, and inorganic phosphate levels were determined by modified Fiske-Subbarow method (1925). Myofibrillar ATPase assays were also carried out on myofibrils from fresh WKY rat heart (not frozen or stored). Under these circumstances, the myofibrillar ATPase activity was comparable to the myofibrils from frozen hearts.

Statistics

Unless stated otherwise, values are given as mean ±SD. Unpaired and paired Student’s t-test was used to determine significant differences. Additionally, linear regression analysis was used, and the correlation coefficient was determined (Snedecor, 1956). A P value <0.05 was taken as statistically significant.

Results

Body and Heart Weight

The body weight at the time the animals were randomly selected for the control and treatment groups was 100 ± 11 g. Both groups received water and rat chow ad libitum. After 3 weeks, the weight of the control animals was 208 ± 29 g. The body weight of the experimental animals was reduced by 29% (P < 0.001) (Table 1). The heart weight and heart-to-body weight ratio for the control animals was 0.67 ± 0.04 g and 3.26 ± 0.42 g/kg, respectively. These were reduced in the PTU animals by 40% (P < 0.001) and 15% (P < 0.05), respectively (Table 1).

TABLE 1

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Body wt (g)</th>
<th>Heart wt (g)</th>
<th>Heart wt</th>
<th>Body wt (10^-2)</th>
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</thead>
<tbody>
<tr>
<td>Control (n = 10)</td>
<td>208 ± 29</td>
<td>0.67 ± 0.04</td>
<td>3.26 ± 0.42</td>
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</tr>
<tr>
<td>PTU (n = 40)</td>
<td>147 ± 14†</td>
<td>0.40 ± 0.03†</td>
<td>2.77 ± 0.23*</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05.
† P < 0.001.
TABLE 2
Mechanical Data of Control and PTU-Treated Rat Preparations

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Cross-sectional area (mm²)</th>
<th>Optimum peak twitch length (mm)</th>
<th>Peak twitch tension (g/mm²)</th>
<th>Resting tension (g/mm²)</th>
<th>Time-to-peak tension (msec)</th>
<th>dP/dtmax (g/sec-mm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.43 ± 0.13</td>
<td>6.00 ± 1.18</td>
<td>6.11 ± 1.75</td>
<td>1.15 ± 0.21</td>
<td>288 ± 27</td>
<td>38.3 ± 9.5</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTU</td>
<td>0.46 ± 0.07</td>
<td>6.11 ± 1.10</td>
<td>4.64 ± 0.89*</td>
<td>0.99 ± 0.25</td>
<td>411 ± 25†</td>
<td>20.4 ± 3.5†</td>
</tr>
<tr>
<td></td>
<td>(n = 10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < 0.05.
†P < 0.001.

Mechanical and Thermal Measurements

Time-to-peak tension was prolonged from 288 ± 27 to 411 ± 25 msec (P < 0.001) in the PTU rats, whereas peak twitch tension was decreased from 6.11 ± 1.75 (control) to 4.64 ± 0.89 g/mm² (PTU) (P < 0.05). Maximum dP/dt decreased from 38.3 ± 9.5 in control to 20.4 ± 3.5 g/mm²/sec in the PTU preparations (P < 0.001) (Table 2; Fig. 1). The active-to-resting tension ratio was 5.45 ± 1.68 and 4.85 ± 0.96 (NS) for the control and PTU papillary muscles, respectively.

Paced Twitches

Initial heat was 2.31 ± 0.69 in the control and 1.27 ± 0.25 mcal/g in the PTU preparations (P < 0.001) (Fig. 1; Table 3). Initial heat, normalized for peak twitch tension or the tension-time integral, was significantly (P < 0.001) diminished from 3.79 ± 0.42 in control to 2.75 ± 0.32 μcal/g-cm in PTU preparations, and from 6.76 ± 1.28 (control) to 3.85 ± 0.45 μcal/g-cm·sec (PTU) (P < 0.001) (Table 3).

When recovery heat was taken into account, total activity-related heat per peak tension in the PTU preparations was not significantly different from the control (Table 3). In contrast, total activity-related heat per tension-time integral was significantly (P < 0.05) smaller in the PTU (8.60 ± 1.31 μcal/g-cm·sec) than in the control preparations (10.96 ± 2.40 μcal/g-cm·sec) (Table 3). The recovery to initial heat values were 0.63 ± 0.29 in control and 1.24 ± 0.23 in PTU preparations (P < 0.001) (Table 3).

The tension-independent heat as assessed by the hyperosmotic mannitol method was 0.154 ± 0.009 and 0.118 ± 0.025 mcal/g in the control and in PTU preparations, respectively (P < 0.02). Tension-dependent heat, normalized for peak twitch tension or tension-time integral, was depressed by 26% (P < 0.01) or 40% (P < 0.01), respectively, in the PTU preparations (Table 4).

Resting heat rate was reduced from 4.41 ± 1.19 mW/g in control to 3.68 ± 1.12 mW/g in PTU preparations (n = 10; NS).

Tetanic Contractions

The total activity-related heat was measured, along with the tension-time integral, where the tension-time integral was varied by changing the number of stimuli applied to the papillary muscle (Fig. 2). The correlation coefficients for the relation between total activity-related heat to tension-time integral varied between 0.96 and 0.99 as evaluated by linear regression analysis (Fig. 3). The slope of the linear regression equations was 43% (P < 0.001) lower in the PTU than the control preparations (Table 5).

Myosin Isoenzyme and Myofibrillar ATPase Analyses

Three-week treatment of PTU shifted the percent content of myosin V5 in left ventricular wall myocardium from 85.0 ± 5.6% (control) to 3.9 ± 2.9% (PTU), while the myosin V3 was increased from 6.6 ± 1.7% (control) to 90.4 ± 9.9% (PTU) (Table 5). Along with the shift in the myosin isozyme pattern,
TABLE 4

Tension-Dependent Heat (TDH) and Tension-Independent Heat (TIH) for Control (C) and PTU-Treated Rat Preparations

<table>
<thead>
<tr>
<th>Preparation</th>
<th>TIH (mcal/g)</th>
<th>TDH (mcal/g)</th>
<th>TDH Peak tension (μcal/g·cm)</th>
<th>TDH fο·t (μcal/g·cm·sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>0.154 ± 0.009</td>
<td>1.97 ± 0.22</td>
<td>3.60 ± 0.42</td>
<td>5.90 ± 1.51</td>
</tr>
<tr>
<td>PTU (n = 5)</td>
<td>0.118 ± 0.025*</td>
<td>1.12 ± 0.24†</td>
<td>2.65 ± 0.21†</td>
<td>3.52 ± 0.24†</td>
</tr>
</tbody>
</table>

* P < 0.02.
† P < 0.01.
‡ P < 0.001.

The myofibrillar actomyosin Ca**+-Mg**+-stimulated ATPase activity of the left ventricle was significantly (P < 0.02) decreased from 55 ± 17 (control) to 31 ± 8 nmol P_i/mg·min (PTU). Similarly, myofibrillar myosin Ca**+-stimulated ATPase activity was significantly (P < 0.01) decreased from 294 ± 98 (control) to 85 ± 25 nmol P_i/mg·min (PTU).

Before correlating the myosin isoenzyme pattern with heat data, it is necessary to ascertain that the isoenzyme pattern of the left ventricular wall myocardium corresponds to that of the papillary muscles. There is an excellent match between the percent values of all the myosin isoenzymes of the papillary muscle and the wall myocardium (Fig. 4). These data were obtained from a separate group of animals subjected to the identical treatment described in Methods.

Discussion

Comparison of Mechanics and Heat in Normal and Hypothyroid Rat Myocardium

The results of the present study demonstrate that a 3-week period of treatment by addition of PTU to the drinking water leads to an impairment of normal growth, diminished body weight, and reduced heart weight when compared to age-matched controls. The fact that growth of the heart is impaired more than that of the whole body is reflected in the reduced ratio between heart and body weight. These results are in good agreement with those of Minelli and Korecky (1969), Whitehorn (1971), and Loiselle et al. (1982), who induced hypothyroidism by hypophysectomy or application of a single dose of

The effects of thyroid hormone deficiency on the contractile properties of the myocardium are also obvious. The 24% decrease of peak developed ten-
sion in the PTU preparations is significant, and has already been shown in hypothyroidism (Buccino et al., 1967; Minelli and Korecky, 1969; Strauer and Schulze, 1976). In contrast to the latter papers, the prolongation of time-to-peak tension and the decrease of maximum rate of tension development were much more pronounced in the present study. This difference may be attributed to different experimental temperatures (37°C vs. 21°C). In the recent paper of Loiselle et al. (1982), an increase of peak-developed tension of papillary muscles from hypothyroid rats was found when hypothyroidism was induced by a single dose of \( ^{131}I \) in 1-year-old rats. These data are in contrast to all the papers cited above, and to our findings. The degree of hypothyroidism, as well as the age of the rats, may be responsible for these differences.

To acquire direct evidence on the process of ATP splitting in the intact myocardium during mechanical activity, the rapid myothermal method (Mulieri et al., 1977; Alpert and Mulieri, 1980, 1982) was applied to myocardium of normal and PTU rats. The heat liberated due to mechanical activity is analyzed in terms of \( I \) and \( T_A \) (\( T_A = \) initial plus recovery heat). Because legitimate objections may be raised against the experimental approach of separating initial and recovery heat by the Bugnard method, measurements were made using IAA treatment with an \( N_2-CO_2 \) atmosphere which, on the average, blocked recovery metabolism and heat by 89%. We found that initial heat, under these conditions, might have 3% (range 1–6%) contamination with recovery heat. This potential contamination does not influence our final conclusions.*

Heat liberated during a paced twitch was significantly reduced by PTU treatment, when I was related to peak twitch tension (Hill, 1965; Alpert and Mulieri, 1982) or tension-time integral (Gibbs and Gibson, 1970), or when \( T_A \) was related to tension-time integral. This indicates an improved economy of force generation. However, \( T_A \) per peak developed tension is not significantly different in PTU and control preparations. This lack of effect is brought about by the following two conditions: First, in control compared to PTU animals, a higher twitch tension is reached in a shorter time; thus control compared with PTU exhibits a significantly higher (24%) peak twitch tension, while there is no significant difference in the tension-time integral. Second, there was an increase in the recovery ratio from 0.63 in control to 1.24 in PTU rat myocardium, which has an off-setting effect on the decrease in initial heat. Accordingly, where \( T_A \) is normalized for peak twitch tension, there is no difference between the two groups of animals.

The difference in the R:I ratio for the normal and PTU preparations is of interest. We suggest an activity-related baseline shift, possibly due to alterations in average intracellular levels of ADP and calcium, which can explain the higher apparent R:I ratio in the PTU rat (Alpert and Mulieri, 1982). Alternatively, there may be a change in the energy cost of ATP resynthesis resulting from the treatment.

In order to evaluate how much of the observed differences between PTU and control rat myocardium are due to activation heat, i.e., excitation-contraction coupling, and to tension-dependent heat alterations, i.e., crossbridge cycling, the hyperosmotic mannitol method was used (Holubarsch et al., 1982). The 23% reduction of tension-independent heat in hypothyroid rat myocardium suggests that calcium release and uptake might be reduced. This could be explained by changed properties of the sarcoplasmic reticulum and/or sarcosommal binding sites. It is interesting to note that Nayler et al. (1971) were able to verify reciprocal alterations of calcium uptake and binding by cardiac microsomal fractions in hyperthyroid dogs.

There is also a reduction in tension-dependent heat evident when tension-dependent heat is nor-

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**TABLE 5**

The Relationship between Total Activity-Related Heat (\( T_A \)) and Tension-Time Integral during Isometric Tetanus in Muscles with Varying Myosin Isoenzyme Ratios for Control (C) and PTU-Treated Rat Preparations

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Slope of ( T_A ) vs. ( J - t ) (mcal/g/g-sec-mm(^2))</th>
<th>Intercept (mcal/g)</th>
<th>Correlation coefficient</th>
<th>( V_1 ) (%)</th>
<th>( V_3 ) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>2.09 ± 0.26</td>
<td>0.44 ± 3.90</td>
<td>0.98 ± 0.01</td>
<td>85.0 ± 5.6</td>
<td>6.5 ± 1.7</td>
</tr>
<tr>
<td>PTU (n = 5)</td>
<td>1.19 ± 0.19*</td>
<td>2.58 ± 3.00</td>
<td>0.99 ± 0.01</td>
<td>3.9 ± 2.9*</td>
<td>90.4 ± 9.9*</td>
</tr>
</tbody>
</table>

* \( P < 0.001. \)

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*One could hypothesize that the difference between the recovery ratio of \( \text{PTU} \) myocardium (1.24) and that of control myocardium (0.63) results from an alteration in the time course of recovery heat. If it is assumed that the differences in the R:I ratios for the control (63) and PTU (1.23) result from contamination of the initial heat by recovery heat in the control hearts, then to account for the differences in these rates, a contamination of 27% is needed. \( \text{R:I} = (63 + 27)/(100 - 27) = 1.23 \). This value is four and one half times higher than the largest, and nine times greater than the average measured value of recovery heat contamination in IAA/\( N_2-CO_2 \), and, thus, represents an unlikely possibility.*
normalized for developed tension. A value of $3.60 \pm 0.42 \mu\text{cal/g-cm}$ is calculated for control and $2.65 \mu\text{cal/g-cm}$ for PTU rat preparations (Table 4). These data indicate an improvement of economy of tension generation of the contractile proteins based on a change in the basic force generating process itself, i.e., crossbridge cycling. In a recent paper, Loiselle et al. (1982) show similar results by analyzing heat measurements on the basis of heat-stress relations: the slope of heat vs. stress is significantly decreased in hypothyroid myocardium when 1-year-old rats were made hypothyroid by $^{131}I$. This finding is in good agreement with the demonstrated $26\%$ decrease of tension-dependent heat in the PTU myocardium used in the present paper. However, Loiselle et al. reported increased activation heat, i.e., tension-independent heat, in their hypothyroid papillary muscles. Accordingly, the hypothyroid preparations of these authors showed an increase in peak developed tension when compared to controls. The differences in activation heat between the PTU preparations of the present paper and the hypothyroid preparations of Loiselle et al. (1982) may result from the following: (1) pharmacological vs. radio-active intervention, (2) the age of the rats, (3) the temperature at which the experiments were carried out, and (4) the method by which TIH was measured.

Heat vs. Myosin Isoenzymes and Myofibrillar ATPase

The present paper confirms the presence of the isoenzyme patterns shown by Hoh et al. (1977), Schwartz et al. (1981), and Ebrecht et al. (1982). In control myocardium of young rats, the $V_1$ variety is predominant, whereas in hypothyroid myocardium the predominant isoenzyme is the $V_3$ species. It was important to show that there were no significant differences of the myosin isoenzyme content in the myocardium of the papillary muscles and the left ventricular wall, as was also shown by Loiselle et al. (1982). This dramatic change of the isoenzyme pattern is associated with energetic changes, i.e., improved economy of force generation, on the one hand, and characteristic biochemical changes, i.e., slowed ATPase activity, on the other (see Results). The comparison demonstrates similar decreases in the PTU myocardium for heat per tension-time integral (43% reduction) and actomyosin ATPase activity (44% reduction), with associated changes in the isoenzyme pattern (shift from $V_1$ to $V_3$).

The parallels among changes in isoenzyme distribution, myofibrillar ATPase activity, and myothermal economy of the rat myocardium offer strong evidence for possible causal links between structural changes of myosin and functional changes in myosin ATPase and mechanical performance of the myocardium. Slowed mechanical $V_{\text{max}}$ and lowered ATPase activity are manifestations of a slowed crossbridge cycling rate (Buccino et al., 1967; Strauer and Schulze, 1976; Schwartz et al., 1981; Ebrecht et al., 1982). The present demonstration of accompanying improved economy of force generation can be interpreted in terms of a combination of slowed cycling rate and an increased duration of the on-time of the crossbridge (Alpert and Mulieri, 1980).

In conclusion, the fast normal rat hearts seem to sacrifice economy of tension development for ability to eject blood at high velocity. Although the decreased economy of force development might have resulted in an increased myocardial oxygen demand, this is apparently compensated for by development of a more efficient production of ATP as manifested by the smaller $R:I$ ratio in the euthyroid hearts (Table 3). The slow hypothyroid rat hearts, however, develop tension with high economy at the expense of speed. Under these conditions, the proposed oxygen demand for force development is low and can be met with a slower heart rate and velocity of shortening. Thus the normal rat heart is adapted for fast work, while the PTU rat heart is adapted for slow work.

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