The Effect of Streptozotocin-Induced Diabetes in Rats on Cardiac Contractile Proteins

ASHWANI MALHOTRA, SOMSONG PENPARGKUL, FREDERICK S. FEIN, EDMUND H. SONNENBLICK, AND JAMES SCHEUER

SUMMARY In order to determine whether diabetic cardiomyopathy in rats is associated with altered contractile proteins, male and female rats were made diabetic with intravenous streptozotocin (STZ). Calcium ATPase activity of cardiac actomyosin was significantly decreased after 1 week of diabetes and was depressed by 60% by 2 weeks. Rats pretreated with 3-O-methyl glucose to prevent the hyperglycemia caused by STZ had normal Ca²⁺-actomyosin ATPase activities, and non-diabetic rats whose food was restricted to keep their body and heart weights similar to those found in diabetic animals had only a slight fall in actomyosin ATPase activity. Ca²⁺-ATPase and actin-activated ATPase activities of pure myosin were similarly depressed in preparations from hearts of diabetic animals. Sodium dodecylsulfate gel electrophoresis and isoelectric focusing failed to reveal differences in the patterns of contractile proteins or light subunits between diabetics and controls, but pyrophosphate gels showed a shift in the myosin pattern. Because of depressed circulating thyroid hormone levels in diabetic animals, cardiac contractile proteins were also studied in preparations from thyroidectomized rats. Calcium activities of actomyosin and myosin ATPase were lower than values found in hearts of diabetic rats. When diabetic animals were kept euthyroid with thyroid replacement, actomyosin ATPase activity was still depressed. Thus STZ diabetes causes a significant decrease in cardiac contractile protein ATPase activity. This may be related to altered proportions of myosin isoenzymes.


SEVERAL investigations in experimental animals and in humans suggest that diabetes is associated with a specific cardiomyopathy (Ahmed et al., 1975; Hamby et al., 1974; Regan et al., 1974, 1977; Rubler et al., 1972, 1978; Seneviratne, 1977). Some authors have postulated that this is related to myocardial microvascular disease leading to cardiac hypoxia (Rubler et al., 1972; Hamby et al., 1974) or to substrate limitation causing inadequate energy availability. Fein et al. (1980) and Penpargkul et al. (1980) have documented that myocardial performance is impaired in the hearts of rats made chronically diabetic with streptozotocin. Abnormalities found in well-oxygenated papillary muscles from female diabetic rats included increased time-to-peak isometric tension, decreased velocity of isotonic shortening, and delayed rates of relaxation. Abnormalities found in isolated perfused hearts from male diabetic rats included blunting of the Starling effect, decreased left ventricular pressure development, and diminished rates of left ventricular pressure decline. In the latter experiments, oxygen consumption by the hearts of diabetic rats and effluent lactate pyruvate ratios were normal, suggesting that oxygen availability was not limiting. When insulin and high glucose were added to the perfusion medium, the abnormalities in performance of diabetic hearts persisted despite evidence of enhanced carbohydrate metabolism. High glucose also failed to correct papillary muscle abnormalities. Since oxygen and substrate limitation did not appear to be responsible for depressed myocardial function in the above experiments, the possibility must be considered that abnormalities exist in the energy utilization pathways.

The purpose of the present experiment was to investigate cardiac contractile proteins in the hearts of rats made diabetic with streptozotocin. Hearts of these animals were analyzed from 2 days to 14 weeks after induction of diabetes. The results indicate that the adenosine triphosphatase (ATPase) activity of the contractile proteins becomes depressed as early as one week after the onset of this type of diabetes, and this abnormality may be related to alterations in the proportions of myosin isoenzymes. The results have been published in abstract form (Penpargkul et al., 1979; Malhotra et al., 1979).

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Methods

Experimental Animals

Male and female Wistar rats weighing 170–200 g were made diabetic (D) by injecting streptozotocin 40–60 mg/kg dissolved in a citrate-saline solution, pH 4.5, into the tail vein. Control rats (C) from the same initial group were injected with diluent but no streptozotocin. In a separate group of female rats 1.65 mg of 3-O-methyl glucose was injected intravenously immediately prior to streptozotocin (60 mg/kg) in order to prevent the latter’s diabetogenic effect (Ganda et al., 1976). All rats were allowed to ingest glucose in water for the 24 hours after injection and were then fed normal Purina Rat Chow until they were killed. To determine the severity of diabetes, blood was drawn from the tail vein of non-fasted ether-anesthetized control and diabetic animals for measurement of glucose. Urine was tested for ketone bodies using Ketostix. No ketone bodies were found. In addition, plasma T4 and T3 resin uptake was analyzed by radioimmunoassay (Corning-Immuno). Their hearts were analyzed along with hearts from male controls and diabetic rats.

Studies of Contractile Proteins

In some studies, hearts were immediately prepared for extraction of contractile proteins. Other hearts were stored at −80°C in 50% glycerol containing 50 mM KCl and 10 mM KPO4− (pH 7.0) prior to preparation of the extracts. There was no difference in values for ATPase when hearts were prepared immediately or after a period of storage. Hearts of experimental animals were always extracted and analyzed simultaneously with hearts of controls, using the same reagent and incubation conditions.

The methods for preparing and analyzing cardiac actomyosin from individual hearts have been described previously (Bhan and Scheuer, 1972). The ventricles were minced and homogenized in 0.05 M KCl, 0.01 M KPO4− (pH 7.0) and centrifuged. The pellets were further treated with 0.05 M KCl, 0.01 M KPO4−, and 2 mM EGTA (pH 7.0), followed by washing with buffer containing triton X-100. Actomyosin was extracted and isolated from the myofibrils with 10 volumes of 0.6 M KCl, 10 mM imidazole, 1 mM dithiothreitol (DTT) (pH 7.0) for 20 hours.

After extraction, the brei was centrifuged at 10,000 g for 30 minutes. The supernatant was diluted 10-fold with cold de-ionized water containing 10 mM imidazole and 1 mM DTT (pH 7.0). The precipitated actomyosin was redissolved in 1 M KCl-20 mM imidazole (pH 7.0), and the volume adjusted to bring the KCl concentration to 0.6 M. The dilution-precipitation cycle was repeated one more time. The final precipitate was dissolved in 0.6 M KCl, 10 mM imidazole, 1 mM DTT (pH 7.0) and used as actomyosin. All the homogenization, centrifugation, and extraction procedures were carried out in the cold (4°C).

Myosin was purified as described earlier (Bhan and Malhotra, 1976). Four or more hearts were pooled in each group. Myofibrils washed with Triton X-100 were extracted with 10 volumes of buffer (0.47 M KCl, 0.02 M Na-pyrophosphate, 0.01 M KH2PO4, pH 6.8) for 25 minutes. This was followed by fractionation with a solution of saturated (NH4)2SO4, pH 7.0, containing 10 mM EDTA. The fraction precipitating between 35 to 45% (NH4)2SO4 was collected, dissolved in 0.6 M KCl, and dialyzed against 0.4 M KCl, 1 mM EDTA, pH 7.0 to get rid of (NH4)2SO4. All steps except the final dialysis were carried out in the presence of 1 mM dithiothreitol (DTT). Myosin obtained in this manner has been shown by SDS gel electrophoresis to be free of actin, troponin, and tropomyosin and to be without evidence of proteolytic degradation of myosin. It has a A280/260 ratio of 1.7–1.75, indicating absence of nucleotide material. In a few preparations, myosin was further purified by Sepharose 4B column chromatography by eluting with buffer (0.5 M KCl, 15 mM Tris-Cl, pH 7.5, and 2.5 mM DTT) as described by Adelstein et al. (1976). This myosin was used for actin activation studies.

SDS gel electrophoresis of actomyosin and myosin preparations was performed using 5% and 7.5% cross-linked gels containing 0.1% SDS according to the method of Weber and Osborn (1969). Isoelectric focusing was performed using a Brinkmann-Desaga instrument as described by Siemenkowski and Driessen (1978). Samples of protein in 9 M urea (Schwarz/Mann, ultrapure), 1 mM DTT, 3 mM NaN3 were run on slab gels in pH range 4–9. Pyrophosphate gels were analyzed for actomyosin patterns by the method of Hoh et al. (1977).

ATPase activity measurements were performed in a final volume of 2 ml at pH 7.6 and 30°C. For
he Ca\textsuperscript{2+}-dependent ATPase of actomyosin and myosin, the reaction mixture consisted of 0.3 M KCl, 0.05 M Tris-Cl, pH 7.6, 0.01 M Na\textsubscript{2}ATP, and 75-100 \mu g of contractile protein. K\textsuperscript{+}-EDTA ATPase activity of myosin (75-100 \mu g) was measured in 0.6 M KCl, 0.01 M EDTA, 0.05 M Tris-Cl pH 7.6 and 0.005 M Na\textsubscript{2}ATP at 30\degree C. Actin-activated Mg\textsuperscript{2+}-ATPase activity was measured in 0.0 mM KCl, 20 mM imidazole (pH 7.0), 2 mM MgCl\textsubscript{2}, 0.5 mM Tris-ATP, 100 ng of myosin, and varying mounts of actin. Actin was extracted from rabbit skeletal muscle acetone powder and purified by a combination of procedures of Bailin and Barany (1972) and Spudich and Watt (1971). The reaction was initiated by the addition of the substrate and terminated after 10 minutes by the addition of 1.0 ml of cold 10% trichloroacetic acid. Inorganic phosphate (Pi) was determined by the method of Fiske and Subbarow (1925). Protein concentration was determined by the biuret technique using bovine serum albumin as a standard. Results are expressed as micromoles of Pi liberated per minute per mg protein at 30\degree C.

Statistical differences between mean values for two groups were evaluated by t-test. For comparing three or more groups, an analysis of variance was used to determine differences within the population and Newman Keuls rank test was used to compare individual groups (Zar, 1974).

**Results**

After the injection of streptozotocin, blood glucose rose to greater than 400 mg/100 ml within 24 hours and remained fairly stable during the duration of the experiment (Fig. 1). In female diabetics, blood glucose was in the same range (160 ± 8 mg/100 ml, mean ± SE) in controls and 453 ± 41 in

**Table 1** Body and Heart Weight Relationships in Diabetic Rats

<table>
<thead>
<tr>
<th></th>
<th>Body wts (g)</th>
<th>Heart wts (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>Females</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-6 weeks</td>
<td>228 (9)</td>
<td>188</td>
</tr>
<tr>
<td>±4 (9)</td>
<td>±6 (9)</td>
<td>251</td>
</tr>
<tr>
<td>±8 (5)</td>
<td>±8 (9)</td>
<td>10 weeks</td>
</tr>
<tr>
<td>±8 (9)</td>
<td>±9 (11)</td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>249</td>
<td>215</td>
</tr>
<tr>
<td>±1 (4)</td>
<td>±9 (4)</td>
<td>1 week</td>
</tr>
<tr>
<td>±7 (4)</td>
<td>±6 (4)</td>
<td>2 weeks</td>
</tr>
<tr>
<td>±2 (4)</td>
<td>±5 (4)</td>
<td>3 weeks</td>
</tr>
<tr>
<td>±23 (8)</td>
<td>±7 (8)</td>
<td>4-5 weeks</td>
</tr>
<tr>
<td>±10 (7)</td>
<td>±17 (7)</td>
<td>8-10 weeks</td>
</tr>
<tr>
<td>±12 (8)</td>
<td>±17 (8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>451</td>
<td>296</td>
</tr>
</tbody>
</table>

*Results are mean ± SE. C = control; D = diabetic; 3-OMG = 3-O-methyl glucose; H = hypothyroid; FRC = food-restricted controls a = P < 0.001 b P < 0.006 c = P < 0.006 vs. control; d = P < 0.006 vs. diabetic.

*a These are weights calculated from combined data from nine pools of animals, each used in biochemical studies, and represent means for over 60 rats or animals. Parentheses otherwise indicate number of individual animals and hearts studied.
diabetics at 4-6 weeks and 183 ± 16 in controls and 609 ± 26 in diabetics at 10 weeks (both \( P < 0.001 \)).

In animals treated with 3-O-methyl glucose and studied 5 weeks later, blood glucose was 197 ± 4 mg/100 ml, but in the corresponding control group, mean blood glucose was 188 ± 10 (not significantly different).

Table 1 shows that animals with diabetes had lower body weights and generally had lighter hearts than control animals. This was true throughout the study for male rats. At 10 weeks, despite lesser body weights in diabetic female rats, their hearts were not lighter than in the control group. In rats pre-treated with 3-O-methyl glucose, heart and body weights were similar to those of controls. In food-restricted animals, body and heart weights were similar to the diabetic animals of the same age.

Table 2 shows myosin ATPase activity from myocardium from females with diabetes 4-6 weeks duration and males 2-10 weeks duration. Ca\(^{2+}\)-ATPase activity of myosin was significantly depressed in preparations from the hearts of both male and female diabetic animals, whereas K\(^{-}\)-EDTA ATPase activity was significantly elevated.

Figure 2 shows the actin activation of magnesium ATPase activity of myosin. The results represent the mean of three control and diabetic experiments. The activation was lower in preparations from diabetic animals than from controls.

Table 3 shows actomyosin ATPase activity of preparations from hearts of control and diabetic animals beginning 2 days after the onset of diabetes. Actomyosin was used in this and subsequent studies because analysis of each individual heart is possible using this more crude preparation, whereas we must pool hearts in order to study pure myosin. Comparison of the data in Table 2, Table 3, and in Figure 2 show the general parallelism between calcium ATPase activity of pure myosin, of actomyosin and actin-activated magnesium ATPase of myosin. Actomyosin ATPase activity was significantly depressed in preparations from hearts of both groups (males and females) of diabetic animals. Actomyosin ATPase activity in preparations from the hearts of animals treated with 3-O-methyl glucose was not significantly depressed. The studies in male rats were conducted over a 14-week period. Although actomyosin ATPase activity was significantly depressed at the end of one week, it fell further in the second week and then remained stable from the second to the 14th week. Actomyosin ATPase values for the food-restricted controls are also listed in Table 3 and are slightly but not significantly less than values found in control. However, the activity or preparations from diabetic hearts were greatly depressed at that time.

Figure 3 shows electrophoretic gel patterns of actomyosin and myosin from the hearts of control and diabetic animals. There appeared to be no significant proteolytic degradation or differences in the myosins when analyzed electrophoretically. Similarly, isoelectric focusing studies shown in Figure 3 failed to demonstrate a difference in the light chain between cardiac myosin from control or diabetic hearts. Figure 4 shows that the patterns of myosin on pyrophosphate gels are shifted from a predominant V\(_1\) pattern to a predominant V\(_2\) pattern in preparations from diabetic hearts.

Table 4 shows that circulating thyroid hormone was depressed in the serum of diabetic animal

### Table 2 Myosin ATPase Activity

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Diabetic</th>
<th>Control</th>
<th>Diabetic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females 4-6 weeks*</td>
<td>1.21 ± 0.03 (10)</td>
<td>0.93 ± 0.04* (10)</td>
<td>0.69 ± 0.02 (6)</td>
<td>0.94 ± 0.04* (6)</td>
</tr>
<tr>
<td>Males 2 weeks*</td>
<td>1.10 ± 0.08 (3)</td>
<td>0.82 ± 0.12 (3)</td>
<td>0.48 (2)</td>
<td>0.60 (2)</td>
</tr>
<tr>
<td>3 weeks*</td>
<td>1.20 ± 0.04 (4)</td>
<td>1.01 ± 0.02* (4)</td>
<td>0.70 ± 0.01 (3)</td>
<td>0.98 ± 0.05* (3)</td>
</tr>
<tr>
<td>4-5 weeks*</td>
<td>1.06 ± 0.02 (4)</td>
<td>0.74 ± 0.05* (4)</td>
<td>0.60 ± 0.04 (3)</td>
<td>0.98 ± 0.06* (3)</td>
</tr>
<tr>
<td>8-10 weeks*</td>
<td>1.00 ± 0.02 (5)</td>
<td>0.75 ± 0.03* (5)</td>
<td>0.73 (2)</td>
<td>0.97 (2)</td>
</tr>
<tr>
<td>12-14 weeks*</td>
<td>1.03 ± 0.04 (3)</td>
<td>0.72 ± 0.006 (3)</td>
<td>0.73 (2)</td>
<td>0.97 (2)</td>
</tr>
</tbody>
</table>

Results are \( \mu \)mol Pi/mg per min at 30°C, mean ± se. Numbers in parentheses are number of preparations; \( a = P < 0.001; b = P < 0.006 \).

* Pools of four-six hearts each
therefore, animals subjected to thyroidectomy were also studied. Hypothyroid animals had body weights that were lower than controls but similar to diabetics, and their heart weights were less than in both controls and diabetics (Table 1). Serum thyroxine levels were lowered to similar degrees in the diabetic and the hypothyroid animals, compared to controls. However, tri-iodothyronine resin uptake was not depressed in the diabetics but was significantly lower in the hypothyroid animals than either diabetics or control animals. Calculated free thyroxine index was similar in the diabetic and hypothyroid groups but was significantly lower than in control animals. The heart muscle was analyzed from random animals for actomyosin or was pooled for studies of myosin. These studies revealed depressions in actomyosin and myosin C₂⁺ ATPase activities in preparations from both hypothyroid and diabetic animals. However, the activities were significantly lower in hypothyroid than diabetic preparations. K⁺-EDTA ATPase was elevated in preparations from both the diabetic and hypothyroid animals. Thus, for similar levels of circulating thyroid hormones, the preparations from hypothyroid animals had more severely altered actomyosin and myosin ATPase activities.

Table 5 shows the results of thyroid replacement studies. Treatment of control rats with 4.5 µg/100 g of thyroxine per day resulted in elevated serum thyroid hormone values but not in ATPase activity. Serum TSH levels were depressed by thyroid hormone replacement in the diabetic groups treated with 3 and 4.5 µg of thyroxine. Despite this proof of complete replacement with T₄ in diabetic animals,
### Table 4 Thyroid Data: 10-Week Females

<table>
<thead>
<tr>
<th></th>
<th>Control (10)</th>
<th>Control + T&lt;sub&gt;4&lt;/sub&gt; (10) (1.5 μg/100 g)</th>
<th>Diabetic (9)</th>
<th>Diabetic + T&lt;sub&gt;4&lt;/sub&gt; (9) (1.5 μg/100 g)</th>
<th>Diabetic + T&lt;sub&gt;4&lt;/sub&gt; (9) (3 μg/100 g)</th>
<th>Diabetic + T&lt;sub&gt;4&lt;/sub&gt; (9) (4.5 μg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (μg/100 ml)</td>
<td>3.2 ± 0.2</td>
<td>6.2 ± 0.0*</td>
<td>2.1 ± 0.1*</td>
<td>3.4 ± 0.3</td>
<td>3.6 ± 0.03*</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; resin uptake (μg/100 ml)</td>
<td>55.0 ± 0.6</td>
<td>57.7 ± 0.7</td>
<td>45.8 ± 2.0*</td>
<td>52.4 ± 1.9*</td>
<td>56.6 ± 0.6*</td>
<td>56.2 ± 0.5*</td>
</tr>
<tr>
<td>Free T&lt;sub&gt;4&lt;/sub&gt; index</td>
<td>5.7 ± 0.4</td>
<td>11.6 ± 1.1*</td>
<td>3.2 ± 0.3*</td>
<td>5.9 ± 0.6*</td>
<td>6.6 ± 0.5*</td>
<td>8.0 ± 0.5*</td>
</tr>
<tr>
<td>Blood glucose (mg/100 ml)</td>
<td>177 ± 12</td>
<td>171 ± 6</td>
<td>507 ± 21*</td>
<td>460 ± 18*</td>
<td>574 ± 30*</td>
<td>560 ± 19*</td>
</tr>
<tr>
<td>Heart wt (g)</td>
<td>0.68 ± 0.01</td>
<td>0.71 ± 0.02</td>
<td>0.60 ± 0.03</td>
<td>0.64 ± 0.02</td>
<td>0.66 ± 0.02</td>
<td>0.70 ± 0.02*</td>
</tr>
<tr>
<td>Body wt (g)</td>
<td>239 ± 4</td>
<td>248 ± 5</td>
<td>228 ± 7*</td>
<td>228 ± 6</td>
<td>233 ± 7*</td>
<td>226 ± 3*</td>
</tr>
<tr>
<td>Actomyosin Ca&lt;sup&gt;2+&lt;/sup&gt; ATPase (μmol Pi/mg per min)</td>
<td>0.662 ± 0.013</td>
<td>0.709 ± 0.016</td>
<td>0.246 ± 0.034&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.352 ± 0.029&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.354 ± 0.018&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.388 ± 0.029&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Results are mean ± se. Numbers in parentheses are number of animals or preparations. a = P < 0.001; b = P < 0.01; c = P < 0.05 for experiment groups compared with control; d = P < 0.001; e = P < 0.01; f = P < 0.05 for any given diabetic + T<sub>4</sub> compared with diabetic alone.

the fall in actomyosin ATPase activity was not prevented when serum-free T<sub>4</sub> values were normalized, although the extent of the ATPase decline was increased when serum-free T<sub>4</sub> values were normalized. Actomyosin ATPase (μmol Pi/mg per min) 

### Table 5 Thyroid Data: Female Rats Treated with T<sub>4</sub>

<table>
<thead>
<tr>
<th></th>
<th>Control (10)</th>
<th>Control + T&lt;sub&gt;4&lt;/sub&gt; (10) (1.5 μg/100 g)</th>
<th>Diabetic (9)</th>
<th>Diabetic + T&lt;sub&gt;4&lt;/sub&gt; (9) (1.5 μg/100 g)</th>
<th>Diabetic + T&lt;sub&gt;4&lt;/sub&gt; (9) (3 μg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T&lt;sub&gt;4&lt;/sub&gt; (μg/100 ml)</td>
<td>3.8 ± 0.2</td>
<td>2.2 ± 0.2*</td>
<td>2.2 ± 0.2*</td>
<td>39.6 ± 2.0&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.6 ± 0.2*</td>
</tr>
<tr>
<td>T&lt;sub&gt;3&lt;/sub&gt; resin uptake (μg/100 ml)</td>
<td>53.5 ± 1.0</td>
<td>50.2 ± 1.2</td>
<td>3.0 ± 0.3*</td>
<td>2.6 ± 0.2*</td>
<td>1248 CIRCULATION RESEARCH VOL. 49, NO. 6, DECEMBER 198</td>
</tr>
</tbody>
</table>

Results are mean ± se. Numbers in parentheses are number of animals or preparations. a = P < 0.001; b = P < 0.01; c = P < 0.05 for experiment groups compared with control; d = P < 0.001; e = P < 0.01; f = P < 0.05 for any given diabetic + T<sub>4</sub> compared with diabetic alone.
levels but not increased cardiac actomyosin ATPase of T4 resulted in elevated serum thyroid hormone.

In the current experiments, the diabetic animals generally weighed less and had lighter hearts than controls. The possibility was considered that caloric deficiency and the lighter hearts were responsible for the depressed ATPase values. However, based on age vs. heart size studies, ATPase activity would be expected to be higher in lighter hearts (Malhotra, unpublished observations; Scheuer and Bhan, 1979). Also, after 10 weeks of diabetes, hearts of female rats had weights similar to those of control animals, yet the ATPase values for actomyosin were 35% lower than control. Also in pair-weighted, food-restricted rats (FRC), actomyosin ATPase values were much less depressed than in hearts of diabetics. We have also observed that myocardium from male and female food-restricted rats do not appear to have impaired contractile performance (Fein et al., 1980, Penpargkul et al., 1980). Thus it appears that the streptozotocin-induced diabetes, and not the caloric restriction was responsible for the lower enzymatic activities.

The diabetic animals had depressed values of circulating thyroid hormones. Similar diminished thyroid hormone levels have been observed in humans with diabetes (Saunders et al., 1978). Thus it was considered that the contractile protein abnormalities might be due to hypothyroidism. However, diabetic animals did not appear to have primary hypothyroidism, since their serum TSH values have been found to be normal (unpublished data from Surks et al.). To explore the role of hypothyroidism, we studied a group of thyroidectomized animals. Their serum free thyroxine values were similar to those found in diabetic rats (Table 4), but their contractile protein ATPase values were significantly lower than levels found in the diabetic and control groups. If the decreased contractile protein ATPase values observed in diabetics were due to diminished thyroid activity, much lower ATPase values would have been expected in the diabetics. When low thyroid hormone levels were prevented by the treatment with thyroxine, the decrease in actomyosin ATPase activity was still observed (Table 5). This is consistent with the finding of Fein et al. (1980), who observed that mechanical function of myocardium from a group of diabetic rats with normal serum thyroidal levels was diminished when compared to myocardium from control hearts.

Treatment of control animals with 4.5 μg/100 g of T4 resulted in elevated serum thyroid hormone levels but not increased cardiac actomyosin ATPase activity. This is consistent with the report of Yazaki and Raben (1975) who did not find increased myosin ATPase activity in hyperthyroid rats, but did find elevated enzyme levels in hyperthyroid rabbits.

The present observations of depressed ATPase of contractile proteins provide one explanation for the diminished contractile function of hearts of diabetic rats. It is unlikely that the altered ATPase activity totally explains the mechanical abnormality. The decreased rates of relaxation observed in the myocardium from these animals (Fein et al., 1978; Penpargkul et al., 1979) indicate a change at the level of the sarcoplasmic reticulum, and Penpargkul et al. (1981) have reported such an abnormality. Similarly, it is possible that sarcolemmal dysfunction might contribute to diminished function of diabetic hearts.

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