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 Ca\textsuperscript{2+}-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. Ca\textsuperscript{2+}-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 in selected animals. T₃ was analyzed by radioimmunoassay (Corning-Immunonuclear) 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 in selected animals. T₃ was analyzed by radioimmunoassay (Corning-Immunonuclear) and T₃ resin uptake by the method of Surks et al. (1972). Free T₄ index was calculated as the product of total T₄ and T₃ resin uptake, divided by normal T₃ resin uptake (31.4 in humans in our institution).

Because of low serum thyroid hormone values observed in diabetic animals, hypothyroidism was also studied as a control for the diabetes. Thyroidectomy was performed in one group of animals at the same time as diabetes was induced in another group. The thyroidectomized animals received 1% CaCl₂ in their drinking water. These animals were studied 10 weeks later. Another group of female rats had thyroidine administered intraperitoneally daily beginning 2–3 days after STZ injection. Diabetic groups received zero, 1.5, 3.0, or 4.5 μg/100 g. Control animals received zero or 4.5 μg/100 g. Animals were killed at 4–5 weeks.

Another control group consisted of male animals in which food was restricted (FR-C) for 9–10 weeks so that their body weights were the same as diabetics throughout their course. Calories were restricted by approximately 40% in these animals. 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 46% (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 (1965). 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 isoenzyme patterns by the method of Hob 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°C. Actin-activated Mg\textsuperscript{2+}-ATPase activity 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°C. 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°C.

Statistical differences between mean values for two groups were evaluated by \textit{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).

**Figure 1** Time course for plasma glucose levels in male control and streptozotocin injected animals. Results are mean ± se. Numbers in the parentheses indicate the number of animals. *P < 0.05. Zero time is the time of streptozotocin injections.

**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><strong>Females</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-6 weeks</td>
<td>228 ±6 (9)*</td>
<td>188 ±6 (9)**</td>
</tr>
<tr>
<td>10 weeks</td>
<td>266 ±8 (9)</td>
<td>221 ±9 (11)</td>
</tr>
<tr>
<td><strong>Males</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 days</td>
<td>249 ±1 (4)</td>
<td>215 ±6 (4)</td>
</tr>
<tr>
<td>1 week</td>
<td>257 ±7 (4)</td>
<td>202 ±6 (4)</td>
</tr>
<tr>
<td>2 weeks</td>
<td>272 ±2 (4)</td>
<td>183 ±5 (4)</td>
</tr>
<tr>
<td>3 weeks</td>
<td>355 ±23 (8)</td>
<td>235 ±7 (8)</td>
</tr>
<tr>
<td>4-5 weeks</td>
<td>367 ±10 (7)</td>
<td>267 ±17 (7)</td>
</tr>
<tr>
<td>8-10 weeks</td>
<td>435 ±12 (8)</td>
<td>280 ±17 (8)</td>
</tr>
<tr>
<td></td>
<td>FRC</td>
<td>FRC</td>
</tr>
<tr>
<td>9-10 weeks</td>
<td>451 ±10 (8)</td>
<td>296 ±11* (8)</td>
</tr>
</tbody>
</table>

Results are mean ± se. C = control; D = diabetic; 3-OMG = 3-O-methyl glucose; H = hypothyroid; FRC = food-restricted controls * P < 0.001 b P < 0.005 c = P < 0.05 vs. control; d = P < 0.005 vs. diabetic.

* 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 the 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 significantly less than values found in control hearts. 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 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 were depressed in the serum of diabetic animal...
TABLE 3

Actomyosin ATPase Activity in Hearts of Male and Female Rats

<table>
<thead>
<tr>
<th></th>
<th>Females</th>
<th></th>
<th>Males</th>
<th></th>
<th></th>
<th>3-OMG</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>4-6 weeks</td>
<td>10 weeks</td>
<td>2 days</td>
<td>1 week</td>
</tr>
<tr>
<td>Control</td>
<td>0.738 ± 0.022 (11)</td>
<td>0.647 ± 0.021 (8)</td>
<td>0.653 ± 0.016 (7)</td>
<td>0.657 ± 0.010 (7)</td>
<td>0.551 ± 0.001 (4)</td>
<td>0.602 ± 0.016 (8)</td>
</tr>
<tr>
<td>Diabetic</td>
<td>0.465 ± 0.041* (11)</td>
<td>0.421 ± 0.037 (6)</td>
<td>0.611 ± 0.011 (7)</td>
<td>0.525 ± 0.024* (7)</td>
<td>0.285 ± 0.021* (4)</td>
<td>0.291 ± 0.023* (8)</td>
</tr>
<tr>
<td>3-OMG</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Results are μmol Pi/mg per min at 30°C, mean ± se. 3-OMG = 3-O-Methyl glucose. Numbers in parentheses are number of preparations. a = P < 0.001—experimental compared with control; b = P < 0.001—3-OMG compared with diabetic.

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 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 in either diabetics or control animals. Calculated 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 Ca²⁺ 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,
the fall in actomyosin ATPase activity was not prevented when serum-free T₄ values were normalized, although the extent of the ATPase decline with T₄ treatment was not as great as without exogenous T₄.

Discussion

In the present investigation, when severe diabetes was induced in rats by the intravenous injection of streptozotocin, profound alterations occurred in the enzymatic activity of the cardiac contractile proteins. This was evidenced by depressed ATPase activities of actomyosin, myosin, and actin-activated myosin. Dillman (1980) has recently published a brief report of a similar depression of myocardial ATPase activity when severe diabetes was induced in rats by the intravenous injection of streptozotocin. The findings appear to be related to the diabetogenic effects of streptozotocin, because when diabetes was prevented by the injection of 3-O-methyl glucose before streptozotocin, cardiac contractile protein ATPase activity was not affected.

The abnormal myocardial function observed in hearts of diabetic rats probably is related in part to the depressed contractile protein function. Barany (1967) has observed a close relationship between ATPase activity and contractile function of skeletal muscle (Scheuer and Bhan, 1979). The earliest finding of depressed contractile function of heart muscle using the streptozotocin model was at 4 weeks (Fei et al., 1978), but earlier studies have not been performed. Diminished cardiac performance has been observed in diabetes as early as 48 hours after injection of alloxan, but this was most likely due to depressed high energy phosphate levels (Allison et al., 1976, Miller, 1979). Evidence in the more chronic forms of streptozotocin-induced diabetes suggests that energy availability is not limited (Opie et al., 1971, Penpargkul et al., 1979).

It is of interest that K⁺-EDTA ATPase was found to be elevated in the myosin from diabetic animal. K⁺-EDTA ATPase has not been found to correlate with physiological function in several condition (Scheuer and Bhan, 1979).

In the present studies, SDS gel electrophoresis and isoelectric focusing failed to demonstrate a alteration in the heavy or light chain patterns. SDS gel electrophoresis and isoelectric focusing failed to demonstrate a alteration in the light chain composition, if present, but further studies using two-dimensional electrophoresis may be necessary for that analysis. The electrophoretic patterns on pyrophosphate gels are compatible with a alteration in isoenzyme patterns of myosin an agrees with the preliminary report by Dillma.

The abnormal myocardial function observed in hearts of diabetic rats probably is related in part to the depressed contractile protein function. Barany (1967) has observed a close relationship between ATPase activity and contractile function of skeletal muscle, and numerous studies suggest a similar parallel relationship between contractile function and contractile protein ATPase activity of heart muscle (Scheuer and Bhan, 1979). The earliest finding of depressed contractile function of heart muscle using the streptozotocin model was at 4 weeks (Fei et al., 1978), but earlier studies have not been performed. Diminished cardiac performance has been observed in diabetes as early as 48 hours after injection of alloxan, but this was most likely due to depressed high energy phosphate levels (Allison et al., 1976, Miller, 1979). Evidence in the more chronic forms of streptozotocin-induced diabetes suggests that energy availability is not limited (Opie et al., 1971, Penpargkul et al., 1979).

It is of interest that K⁺-EDTA ATPase was found to be elevated in the myosin from diabetic animal. K⁺-EDTA ATPase has not been found to correlate with physiological function in several condition (Scheuer and Bhan, 1979).

Table 5 Thyroid Data: Female Rats Treated with T₄

<table>
<thead>
<tr>
<th>T₄ (µg/100 ml)</th>
<th>Control (10)</th>
<th>Control + T₄ (10) (1.5 µg/100 g)</th>
<th>Diabetic (11)</th>
<th>Diabetic + T₄ (9) (1.5 µg/100 g)</th>
<th>Diabetic + T₄ (9) (3 µg/100 g)</th>
<th>Diabetic + T₄ (9) (4.5 µg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T₃ (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>T₃ 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 T₄ 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 ± 0.6*</td>
</tr>
<tr>
<td>Blood glucose (mg/100 ml)</td>
<td>177 ± 12</td>
<td>171 ± 6</td>
<td>507 ± 21*</td>
<td>460 ± 19*</td>
<td>574 ± 30*</td>
<td>590 ± 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>298 ± 7*</td>
<td>228 ± 6</td>
<td>233 ± 7</td>
<td>225 ± 3*</td>
</tr>
<tr>
<td>Actomyosin Ca** ATPase (µmol Pi/mg per min)</td>
<td>0.662 ± 0.013</td>
<td>0.709 ± 0.016</td>
<td>0.246 ± 0.034b</td>
<td>0.352 ± 0.029bc</td>
<td>0.394 ± 0.018bc</td>
<td>0.385 ± 0.029bc</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 + T₄ compared with diabetic alone.
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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