The Effect of Diabetes on Performance and Metabolism of Rat Hearts

SOMSONG PENPARGKUL, THOMAS SCHAIBLE, TADA YIPINTSOI, AND JAMES SCHEUER

SUMMARY To explore the effects of diabetes on myocardial function and metabolism we injected male rats with streptozotocin and studied their hearts 8 weeks later. Blood sugar levels in the treated rats were about 600 mg/100 ml. Body and heart growth rates were diminished. When studied in an isolated working rat heart apparatus using 5.5 mM glucose, hearts of diabetic animals showed diminished cardiac output and stroke work at high filling pressures. There also were significant depressions in peak left ventricular systolic pressure, peak aortic flow rate, maximum negative dP/dt, myocardial oxygen extraction, myocardial lactate production, and effluent lactate:pyruvate ratios. Myocardial glycogen stores, calculated glycogen utilization, and pyruvate production were increased in hearts of diabetics, and myocardial oxygen consumption was the same as in control hearts. The end-diastolic pressure-volume relationship was shifted to the right in hearts of diabetics. Most of the abnormalities observed in hearts of diabetic rats persisted when insulin and 15 mM glucose were included in the perfusion medium. Hearts from young rats or from age-matched food-restricted rats with heart weights similar to those of diabetics did not show depressed function or a pressure-volume shift. Our findings indicate that streptozotocin diabetes in rats results in abnormal myocardial performance. This is not due to restrictions in coronary flow or myocardial oxygenation and is not correctable by the provision of high glucose plus insulin in the perfusion medium.


RECENT experimental evidence indicates that chronic diabetes may lead to myocardial dysfunction (Regan et al., 1974). Studies in humans also support the concept that diabetes affects the heart independently of involvement of large coronary vessels (Ahmed et al., 1975; Hamby et al., 1974; Regan et al., 1977; Rubler et al., 1972, 1978; Sanderson et al., 1978; Seneviratne, 1977). It has been postulated that this is due to a specific diabetic cardiomyopathy or that it may result from diabetic microangiopathy. Reagan et al. (1974) reported abnormal diastolic pressure-volume relationships which accompanied depressed ventricular function in the hearts of diabetic dogs; however, it is unclear from those studies whether the abnormalities of cardiac function resulted from intrinsic myocardial dysfunction or from other factors such as abnormal compliance of the ventricular wall or peripheral and neurohumoral influences.

The purpose of the present investigation was to study hearts of rats made diabetic by the intravenous injection of streptozotocin and to determine cardiac function and certain aspects of myocardial metabolism using the isolated working rat heart preparation.

Methods

Male Wistar rats weighing 180–200 g were made diabetic (D) by injecting streptozotocin, 50 mg/kg, dissolved in 0.05 M citrate, pH 4.5, into the tail vein. Control rats (C) from the same initial group were injected initially with diluent but no streptozotocin. All rats were allowed to ingest glucose in water for the next 24 hours and then were fed normal Purina Rat Chow until they were killed 8 weeks later. At weekly intervals, blood was drawn from the tail vein of ether-anesthetized control and diabetic animals for measurement of glucose. Urine was tested for ketone bodies using Ketostix. No ketone bodies were found. When the rats were killed, blood was taken from the tail vein of ether-anesthetized control and diabetic animals for measurement of glucose. Urine was tested for ketone bodies using Ketostix. No ketone bodies were found. When the rats were killed, blood was taken from some for analysis of serum sodium, potassium, chloride, and urea by autoanalyzer. In addition, plasma T4 and T3 were analyzed in selected animals. T4 was analyzed by radioimmunoassay (Corning-Immophase) and T3 by the method of Surks et al. (1972).

Experimental Groups

The experiments were performed in the following sequence:

Experiment 1

The first question asked was whether there was any effect of the streptozotocin-induced diabetes on heart function. Therefore rats treated with streptozotocin (D1) were compared with control rats (C1) which had received only diluent.

In the original group of animals treated with streptozotocin, five failed to develop diabetes (ND).
Hypoglycemic-induced Changes in Ventricular Function in Diabetic Rats

Their blood sugar levels without ether anesthesia averaged 118 ± 18 mg/100 ml. Hearts from these rats were analyzed and compared with the controls.

Experiment 2

The next question was whether the depressions in ventricular function observed in the diabetic animals could have been due to the lack of insulin in the perfusion medium. To answer this, a group of diabetic (D2) and control (C2) rats was studied with insulin, 10 milliunits/ml, in the perfusate.

Experiment 3

When insulin failed to normalize ventricular function in diabetic hearts, an additional group of hearts from diabetic (D3) and from control animals (C3) was studied in the isolated working heart apparatus in the presence of 15 mM glucose and insulin, 10 milliunits/ml.

Experiment 4

In another group of studies, rats were chosen to be younger than the other control groups to evaluate whether the smaller hearts from the young rats (YR) had different performance than hearts of the same size from diabetic rats. The effect of insulin and 15 mM glucose on performance also was evaluated by perfusing half the hearts with 5.5 mM glucose and no insulin (YRi) and the other half with 15 mM glucose and insulin 10 μ/ml (YRi).

Experiment 5

Finally, because the body and heart weights in diabetic animals were lower than in the control animals, the next question that was asked was whether caloric deficiency alone could be responsible for the changes in ventricular function that were observed in Experiment 1. Therefore, additional control animals were treated with a food-restricted diet for 8 weeks with caloric intake adjusted to keep their body weights similar to those of the diabetic animals in Experiment 1. These are called control-pair weighted rats (C-PW). Calories were restricted by approximately 40%. The hearts of these animals were studied and compared with hearts from animals allowed food ad libitum (C-FE).

Heart Perfusions

Hearts were studied during the 8th–9th week of diabetes in an isolated working rat heart apparatus. This system has been modified to measure left ventricular ejection fraction by a dye-dilution technique, and left ventricular end-diastolic volume could be calculated from the directly measured stroke volume. Left ventricular pressure was measured through a 2.5-cm polyethylene catheter attached to a Statham P23dB strain gauge pressure transducer. This system had a frequency response flat to 30 Hz. Coronary flow was measured as right heart outflow. Instantaneous aortic flow was measured using a 2.5-mm diameter Statham flow probe.

A full description of this modified apparatus and the validation of the techniques for measuring ventricular volumes have been published previously (Bersohn and Scheuer, 1977; Schaible and Scheuer, 1979). The perfusate, which was not recirculated, was a modified Krebs-Henseleit buffer maintained at 37°C and containing 5.5 mM glucose (unless otherwise stated), and 2 mM calcium with 0.5 mM EDTA yielding 1.5 mM free calcium. All hearts were paced from the right atrium at a rate of 340 beats/min. Oxygen tension was measured in the perfusate from the left atrial inflow line and from a pulmonary artery cannula. Arteriovenous differences in oxygen tension were converted to oxygen consumption by multiplying arteriovenous PO2 by coronary flow and the appropriate Bunsen coefficient and dividing by left ventricular dry weight. Samples were taken from the atrial reservoir and right ventricular outflow for analysis of lactate and pyruvate. Whenever possible, one heart from each experimental group and one from the control group were perfused on a given day.

After 10 minutes of retrograde perfusion, during which catheters for LV pressure and dye injection were positioned, antegrade perfusion was begun and the heart was allowed to equilibrate for a period of 15 minutes with the height of the left atrial reservoir at 10 cm. The height of the aortic column was 80 cm at all times. Preload was varied by raising or lowering the reservoir filling the left atrium (left atrial filling pressure). After an initial recording of data at 10 cm H2O, the filling pressure was lowered to 5 cm H2O, then raised in sequence to 10, 15, and 20 cm H2O, and finally lowered to 10 cm H2O, all for periods of 7.5 minutes. The total period of perfusion was 1 hour. After 5 minutes at each filling pressure, hemodynamics and multiple dye-dilution curves were recorded, and coronary flow and cardiac output were measured during a 1-minute period.

All analog data (dye concentration, aortic flow, left ventricular pressure) were stored on magnetic tape for later computer analysis. The dye recordings were digitized at 200 samples/sec on a Mod Comp Max II/2 digital computer. Beginning one beat after the injection of the dye bolus into the LV, digitized points for dye concentration during each aortic flow diastole were averaged until a factor of baseline noise was reached. For each ejection fraction, three to seven dye curves were analyzed and then averaged. Ejection fraction was calculated from the equation: EF = 1–K where K = Cn + 1/Cn and C is the dye concentration. End-diastolic volume was calculated by dividing the directly measured stroke volume (cardiac output divided by heart rate) by the ejection fraction. Flow and pressure analog data were digitized at 660 samples/sec and the measurements were determined as previously described (Bersohn and Scheuer, 1977; Schaible and Scheuer, 1979).
At the end of each perfusion the atria and great vessels were dissected free and the right ventricular free wall was removed. The left ventricle (including the septum) was weighed to determine wet weight. A small piece (weighing approximately 0.2 g wet) was dried to constant weight in an oven to calculate left ventricular dry weight. When glycogen was measured, the rest of the ventricle was placed in 30% KOH and kept at -70°C until ready for analysis.

Chemical analyses

Glucose was determined using hexokinase and glucose-6-phosphate dehydrogenase (Bergmeyer et al., 1974). Lactate was measured as described by Hohorst (1963) and pyruvate by the method of Segal (1963). Myocardial glycogen was determined by the method of Walaas and Walaas (1950).

Statistical Analysis

Since the experiments were set up independently each to answer a specific question as outlined above, unpaired t-statistics were used in comparing each group of experimental hearts with its corresponding group of control hearts. Statistical comparisons were made between control and experimental hearts only when hearts from each group were perfused in the same apparatus during the same experimental period, and usually with control and experimental hearts being perfused on the same day. When adjacent points within a set of hearts were compared, paired t-test was used. Statistical comparisons were not made between different control groups because they were perfused during different periods of experimentation and, at times, using different perfusate conditions.

Results

Heart and Body Weight Relationships

Table 1 presents the heart and body weight values for these experimental groups. In Experiment 1, the body weights, wet heart weights, and left ventricular dry heart weights were significantly less in diabetic than control animals. The hearts of streptozotocin injected non-diabetic animals were not significantly different from controls. The heart weight to body weight ratios were slightly higher in the diabetic rats than in controls. Diabetics failed to gain weight and the difference between diabetics and controls in body weight increased progressively until they were killed. In Experiments 2 and 3, relationships observed between the heart and body weights of diabetics and controls were similar to those in Experiment 1.

In Experiment 5, the body weight, wet heart and dry left ventricular weights were lower in C-PW than in C-FE.

Plasma Values

Plasma glucose and electrolyte and thyroid hormone values are shown in Table 2. Blood glucose rose to diabetic values within 24 hours and remained fairly stable for the duration of the experiment. At the end of the experimental periods, glucose was increased about 4-fold in diabetics compared with controls. Plasma sodium and chloride were significantly lower and plasma urea higher in diabetic rats than in controls. T4 and T3 levels were both found to be lower in diabetic animals than in controls.

Effect of Diabetes on Cardiac Performance

Figures 1 and 2 show the effect of diabetes on cardiac performance in Experiment 1. Data for

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<th>Table 1  Heart Weight and Body Weight Values</th>
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* P < 0.001; † P < 0.05 comparing each experimental group with its corresponding control group.

Results are mean ± SE. BW = body weight; WHW = wet heart weight; LV DW = left ventricular dry weight; D/W HW = dry to wet heart weight ratio; HW/BW = heart weight to body weight ratio. C1, C2, and C3 and D1, D2, and D3 refer to paired control and diabetic groups. ND refers to non-diabetic streptozotocin-treated rats. YR refers to young rats from which hearts were perfused without insulin. YR2 refers to young rats from which hearts were perfused with insulin. C-FE signifies free-eating control animals and C-PW, the control food-restricted, pair-weighted animals.
Table 2  Plasma Chemistry Values

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<td>Diabetic (8)</td>
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<td><strong>Experiment 2</strong></td>
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<td>Control (9)</td>
<td>148 ± 7</td>
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<tr>
<td>Diabetic (9)</td>
<td>602 ± 44†</td>
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<td><strong>Experiment 3</strong></td>
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<td>Control (8)</td>
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<td>Diabetic (8)</td>
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<table>
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<th>Chloride (meq/l)</th>
<th>Urea (mg/100 ml)</th>
<th>T₄ (mg/100 ml)</th>
<th>T₃ (μg/100 ml)</th>
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<td>Control (39)</td>
<td>142 ± 0</td>
<td>5.2 ± 0.1</td>
<td>99 ± 0</td>
<td>20 ± 0</td>
<td>5.5 ± 0.3 (23)</td>
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<tr>
<td>Diabetic (45)</td>
<td>136 ± †</td>
<td>5.5 ± 0.2</td>
<td>93 ± †</td>
<td>32 ± †</td>
<td>3.6 ± 0.2 (28)</td>
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Results are mean ± SE. Numbers in parentheses indicate the number of experiments.
* Plasma glucose was measured after ether anesthesia.
† P < 0.001; ‡ P < 0.05.

small control hearts (5.5 mM glucose without insulin) from Experiment 4 are also plotted to demonstrate how normal hearts that are the same size as hearts of diabetic animals perform in the perfusion apparatus. Table 3 shows data for perfusions in all the experiments. Data from the 15 and 20 cm atrial pressure levels only are presented in Table 3 because measurements made at high filling pressures provide the most pertinent information. Coronary flow, cardiac output, left ventricular volume and stroke work were corrected for heart weight because in these isolated heart experiments we were interested in heart performance per se, not integrated performance related to body weight in the intact animal. Values for these weight-related measures in the diabetic hearts tended to be or were significantly higher than for the hearts of the control animals. However, as shown in Figure 1, cardiac output in hearts of diabetics declined (P < 0.05) and coronary flow and stroke work tended to fall (P < 0.10) as atrial pressure was elevated from 15 to 20 cm, whereas these functions continued

Figure 1  Comparisons of flow-related data in Experiment 1 for hearts of control and diabetic rats. Hearts were perfused with 5.5 mM glucose without insulin in the medium. The hearts from the control group of young rats in Experiment 4 are plotted to show how small hearts from non-diabetic rats perform in the perfusion apparatus. Results are mean ± se. CF = coronary flow; CO = cardiac output; SW = stroke work. Asterisks indicate significant differences between diabetic and age-matched control hearts at each filling pressure. *P < 0.05; **P < 0.01.

Figure 2  Pressure and velocity data from heart perfusions in Experiment 1. Hearts from the control group of young rats in Experiment 4 are again plotted. PLVSP = peak left ventricular systolic pressure; max + dP/dt = maximum rate of ventricular pressure rise; max - dP/dt = maximum rate of left ventricular pressure fall; ***P < 0.001. All other symbols are the same as in Figure 1.
The page contains a table titled "Dynamic Performance at 15- and 20-cm Filling Pressure" with columns for "Atrial pressure (cm H2O)" and "CF (ml/g per min)". The table includes data for experiments 1 to 5, with columns for different variables such as "C" and "D" with standard deviations, and "ND*". The table also includes "Ejection fraction" and "Stroke work index" with corresponding values.

The text also mentions "CF = coronary flow; CO = cardiac output; PLVSP = peak left ventricular systolic pressure; dP/dt max = maximum rate of left ventricular pressure rise; LVEDV = left ventricular end-diastolic pressure; LVEDV = left ventricular end-diastolic volume; dQ/dt max = maximum rate of change of flow."

Additional notes in the text indicate that ventricular volume data were obtained in only three hearts in this series and are not included in this table. Symbols like * Ventricle volume data were obtained in only three hearts in this series and are not included in this table. P < 0.001; § P < 0.01; ¶ P < 0.05.
to rise with increasing atrial pressures in aged-matched controls \((P < 0.05)\) and tended to increase also in heart weight-matched young rats. In fact, in most diabetic hearts, but not in controls, pulsus alternans was observed at the 20-cm H\(_2\)O filling pressure.

Peak left ventricular systolic pressure was significantly lower in all three diabetic groups \((D_1, D_2,\) and \(D_3)\) than in respective control groups \((C_1, C_2,\) and \(C_3)\) but, in small hearts of YR\(_1\) (Fig. 2), peak systolic pressure was similar to that of age-matched controls. Maximum positive dP/dt tended to be less in diabetics but was significantly so only in the presence of high glucose and insulin (Experiment 3). Thus, although flow per gram left ventricle might be considered to be higher in the diabetic hearts, pressure development was depressed. Peak aortic flow and peak rate of change of flow (flow acceleration) were diminished in all three diabetic groups compared to respective controls (Fig. 2; Table 3).

Maximum negative dP/dt was lower in each diabetic group than in respective controls.

As shown in Table 3, left ventricular end-diastolic volumes expressed in milliliters were significantly smaller in each diabetic group than in corresponding control groups, but volumes normalized for left ventricular weight tended to be or were significantly higher in diabetics for the same comparisons. Hearts from C-PW also tended to have higher normalized end-diastolic volumes compared to hearts from C-FE (Experiment 5), but this trend was not of the same magnitude as observed between hearts from diabetics and controls in Experiment 1.

Figure 3 shows the end-diastolic pressure-normalized volume relationships for diabetics from Experiment 1, two age-matched control groups (controls for Experiment 1 and C-FE for Experiment 5) and a heart size-matched control group (YR\(_1\) from Experiment 4). All of these hearts were perfused with 5.5 mM glucose without insulin. The data point for diabetics in Experiment 1 at the 20 cm H\(_2\)O filling pressure is not included since these hearts were in failure at that point.

Unlike the diabetics, peak left ventricular systolic pressure and maximum positive dP/dt were the same in small hearts of C-PW and larger hearts of C-FE. Also unlike the smaller diabetic hearts, small hearts of pair-weighted control animals had the same peak flow and maximum rate of flow as the hearts from C-FE. However, as in hearts from dia-

**Effect of Heart Size on Performance**

Table 3 shows that when small hearts of C-PW were compared with hearts of C-FE (Experiment 5), flow-related measurements were increased (coronary flow, cardiac output, and stroke work) when corrected per gram dry heart weight. However,
betics, negative dP/dt was depressed. Hearts from young rats which were perfused with 5.5 mM glucose alone (YR, in Experiment 4) had flow-related performance that was similar to that of diabetics when expressed per gram heart, but they did not exhibit deterioration of function at higher filling pressures. Also, there was no depression of left ventricular systolic pressure or positive or negative dP/dt in these hearts (Fig. 2).

**The Effect of Glucose and Insulin**

Table 3 shows that diabetic hearts perfused in the presence of 5.5 mM glucose and insulin (Experiment 3) tended to have the same abnormalities in pressure and dP/dt as diabetic hearts perfused with 5.5 mM glucose alone. Therefore, neither insulin alone nor insulin plus high glucose appeared to be associated with a higher oxygen extraction, and this was confirmed by the comparison of the normal hearts perfused in the presence of high glucose and insulin in Experiment 4.

Also, there was no depression of left ventricular systolic pressure or positive or negative dP/dt in these hearts (Fig. 2).

**Metabolic Findings**

Table 4 and Figure 5 demonstrate the values for myocardial oxygen extraction and consumption, and lactate and pyruvate production.

The oxygen extraction was less in each diabetic group than in its respective control group (Experiments 1, 2, and 3). The smaller hearts of C-PW also had lower oxygen extraction than the heavier hearts of C-FE (Experiment 5). Insulin treatment appeared to be associated with a higher oxygen extraction, and this was confirmed by the comparison of the normal hearts perfused in the presence of high glucose and insulin in Experiment 4.

Oxygen consumption expressed per gram dry heart weight was similar in hearts of diabetics and controls, and the only significant difference was in the hearts from C-PW animals in Experiment 5 which had higher oxygen consumption per gram than the hearts of control free-eating animals.

Lactate production by hearts from diabetics was lower and pyruvate production higher than con-

### Table 4 Metabolic Findings at 15- and 20-cm Filling Pressure

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<th>C1</th>
<th>ND</th>
<th>C2</th>
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<th>D3</th>
<th>YR1</th>
<th>YR2</th>
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<th>C-PW</th>
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Results are mean ± se. Ext. = extraction; prod. = production; L/P = lactate/pyruvate.

* P < 0.001; † P < 0.01; ‡ P < 0.05 comparing each experimental group with its corresponding control.
trols, resulting in a much lower lactate pyruvate ratio in the effluent from the hearts of diabetic rats. This was true in the absence of insulin and in the presence of both insulin and a high glucose concentration plus insulin. Insulin per se increased lactate and pyruvate production and tended to be associated with higher lactate:pyruvate ratios than perfusions in absence of insulin. Hearts of pair weight animals had decreased lactate production and a lower lactate:pyruvate ratio similar to diabetics, but this was due to lower lactate production with no difference in pyruvate production.

External cardiac efficiency (external cardiac work expressed in calories divided by the caloric oxygen equivalent of carbohydrate metabolism (Bersohn and Scheuer, 1977) generally was not different in hearts of diabetics and controls, although efficiency appeared to be modestly enhanced in Experiment 2. The lighter pair weight hearts in Experiment 5 also had a higher external efficiency than hearts of their heavier free-eating controls.

Table 5 shows the myocardial glycogen values. Heart glycogen levels were generally higher before and after perfusion in diabetics than in controls. Apparent heart glycogen use (the difference between pre- and postperfusion values) was appreciable in the absence of insulin both in hearts of diabetics and non-diabetic animals, but appears to have been much greater in diabetics. Provision of insulin or insulin plus high glucose resulted in a net gain in glycogen in control hearts and little or no glycogen use in diabetic hearts. Final values were more nearly equal in hearts of diabetics and controls in the presence of insulin or of insulin plus a high glucose concentration.

**Discussion**

The present investigation on the hearts of male rats made chronically diabetic for 8 weeks with intravenous streptozotocin demonstrates several abnormalities. The major mechanical defects relate to left ventricular pressure development, velocity of ejection, and rate of relaxation, as measured by negative dP/dt. Regan's studies in dogs (Regan, 1974) with milder diabetes also demonstrated depressed ventricular functional responses to increasing preload and also suggested decreased diastolic compliance. Investigations in humans mainly employing noninvasive techniques also suggest that relaxation is impaired in hearts of diabetic humans (Rubler, 1978; Regan, 1977). However, careful investigations of dynamics in which preload and afterload are known have not been reported previously. A study parallel to ours, using papillary muscles from female diabetic rats to study muscle mechanics, described little alteration in resting or developed tension but prolonged time to peak tension and slower relaxation (Fein et al., 1980). There appeared to be no abnormalities in extent of isotonic shortening at various loads in muscles from diabetic animals. However, decreased peak velocities of shortening and decreased rates of lengthening were observed. Therefore studies on papillary muscles, in which the vascular bed was not perfused, provided results similar to those found in the perfused heart studies in which there was perfusion of the coronary bed.

The major difference found in the present studies compared with those using rat papillary muscles or intact dogs is that contraction, as indicated by left ventricular pressure development, was clearly depressed in the hearts of diabetic male rats. The possibility must be considered that this difference may have resulted from the smaller size of hearts from diabetic rats. However, it has been reported...
that smaller hearts perform better than larger hearts in the working heart apparatus (Neeley et al., 1967). Based on studies from rats of different ages, we would expect a 17% higher cardiac output with no change in pressure performance in the smaller diabetic hearts than in controls (T. Schäible, unpublished data observations). To control for differences in heart size in the present investigation, studies were conducted in two sets of small hearts designed to be similar in weight to hearts of diabetic animals, hearts of food-restricted C-PW rats (same age as diabetics), and hearts of younger rats. Unlike hearts of diabetic animals, these two sets of hearts from nondiabetic rats did not exhibit depressed pressure performance or declines in pump function at high filling pressures in the isolated rat heart apparatus. Thus it appears that the small size of hearts of streptozotocin-treated animals did not account for the depressed contraction and relaxation. Caloric deprivation may have contributed to the slow relaxation observed in diabetic hearts.

Hearts of diabetic rats had enhanced performance as measured by flow-related data normalized for heart weight (coronary flow, cardiac output, and stroke work). That these increased values were not due to the diabetic state is apparent from the studies with the two sets of smaller hearts which also had relatively high flow-related values. Ventricular function clearly fell in hearts of diabetics at 20-cmH2O atrial pressure, whereas no such declines were observed in any of the control groups or in the small hearts of young rats or food-restricted rats. The decline in ventricular function frequently was associated with ventricular alternans and, together, these findings are taken as evidence that the hearts of diabetic rats were failing at the high pressures.

It must still be questioned whether the diabetes induced by streptozotocin treatment caused the findings or whether some other effect of streptozotocin could be responsible. In Experiment 1, the presence of a group of rats that did not develop diabetes despite injection of the same dose and batch of streptozotocin that caused diabetes in other rats provides insight into this question. In this group, left ventricular performance in response to increasing filling pressure was similar to that of controls in that coronary flow, cardiac output, and peak left ventricular systolic pressure did not decline from 15 to 20 cm H2O filling pressure. Values for PLVSP, and dP/dt, -dP/dt and LVEDP tended to be higher in nondiabetic than diabetic animals. Also, in the series of studies of Fein et al. (personal communication) 3-O-methyl glucose was infused prior to the injection of streptozotocin, preventing the diabetogenic effect of streptozotocin. Papillary muscles from hearts of those animals demonstrated muscle mechanics similar to controls. We have observed that depression of actomyosin ATPase activity in cardiac muscle is a sensitive indicator of streptozotocin-induced diabetic effects on the heart (Malhotra et al., 1979). However the fall of acto-

myosin ATPase activity induced by streptozotocin injection was also blocked by the prior injection of 3-O-methyl glucose (A. Malhotra, unpublished observations). Thus it appears unlikely that streptozotocin alone without hyperglycemia would reproduce the effects observed in the present study.

The diabetes produced in the current investigation resulted in a failure to grow which is probably due to caloric wasting, suggesting a state of chronic undernutrition in diabetics that might have been responsible for some of the alterations observed in cardiac function. This was the major reason for conducting Experiment 5, in which food was restricted in one group of animals. Food restriction led to heart and body weight relationships similar to those found in the diabetic animals. Food restriction caused the diabetogenic effect of streptozotocin treatment to disappear in rats. Unlike hearts of similar size from the diabetic animals, hearts of the food-restricted rats maintained their ventricular pressure performance and pump function responses to increasing preload like freely eating control animals with larger hearts. However, like hearts of diabetic animals, negative dP/dt was lower in hearts of food-restricted animals than in hearts of controls. Fein et al. (1980) also have observed decreased velocities of relaxation in isometrically contracting papillary muscles from food-restricted rats. Nutter et al. (1979) have reported that severe protein and caloric deprivation in rats is associated with increased peak tension development and time to peak tension of isometrically contracting papillary muscles with prolongation in the one-half relaxation time. Therefore, it appears unlikely that myocardial dysfunction in hearts of diabetic animals in the present studies can be accounted for entirely by impaired nutrition.

It previously has been reported that insulin-requiring diabetics have low levels of circulating thyroid hormones compatible with chronic disease (Saunders et al., 1978). In the present studies, serum T3 and T4 levels were depressed in diabetic animals. Profound hypothyroidism does depress ventricular function and papillary muscle performance (Buccino et al., 1967; Taylor et al., 1967). Fein et al. (1980) studied a group of streptozotocin-treated diabetic rats in which serum thyroid hormone levels were similar to values in control animals. They found delayed relaxation only in the muscles from the diabetic animals. Thus streptozotocin diabetes alone prolongs relaxation. Also, we have observed that rats made hypothyroid and with free serum thyroxine levels that are similar to levels found in our diabetics had much lower values of cardiac actomyosin ATPase activity than found in hearts of diabetics (40% depression in diabetic and 61% depression in hypothyroidism (P < 0.05) (Malhotra, unpublished observations) indicating that the low levels of thyroid hormone in diabetic animals do not have the same cardiac effects as in primary hypothyroidism. Serum TSH levels were not elevated in the diabetic animals, whereas they were in a hypothyroid group. A further argument against a
primary hypothyroid effect is the finding of normal oxygen consumption values in hearts of diabetic rats. Gold et al. (1967) found myocardial oxygen consumption to be markedly depressed in hypothyroid dogs. Thus it appears unlikely that the effects of streptozotocin diabetes on the heart can be attributed to the mildly depressed thyroid hormone levels. The possibility that hypothyroidism might be a minor contributing factor will have to be investigated further.

In the current studies end diastolic volume corrected for heart weight was larger in hearts of diabetic animals than in hearts of controls for any given left ventricular filling pressure. This is quite different from the results of Regan et al. (1974) in which the pressure-volume relationship in hearts of diabetic dogs and humans suggested decreased diastolic compliance. In the studies of Fein et al. (1980), resting length-tension relationships of papillary muscles from hearts of diabetic rats were similar to those of controls. These contradictory findings related to diastolic properties of the ventricle or of papillary muscles require further investigation. Although Regan et al. (1974) concluded from their studies that abnormalities in ventricular performance might be accounted for by the diastolic properties of the ventricles, the present findings with opposite pressure volume relations more strongly support conclusions that impaired contractile properties may be responsible.

It has been postulated that a limitation in coronary flow and oxygen delivery might lead to impaired myocardial function in diabetes because of abnormalities in the myocardial vasculature (Hamby et al., 1974; Rubler et al., 1972). This does not appear to be a likely mechanism in the present investigation because, in contrast to the alloxan diabetic newborn lamb (Lee and Downing, 1979), coronary flow per gram heart was increased in hearts of diabetics, and myocardial oxygen consumption was similar in hearts of diabetics and controls. When coronary flow is inadequate and oxygenation limits performance, myocardial oxygen extraction and effluent lactate:pyruvate ratios usually become elevated. In the present studies, these values were lower in hearts of diabetics than of control animals.

Another possibility is that a limitation of substrate availability was responsible for depressed function in the hearts of diabetic animals. Miller (1979) perfused hearts of acute alloxan diabetic rats and found decreased ventricular function associated with low ATP stores. These findings were reversible with insulin, high concentrations of glucose, or octanoate in the perfusion medium. Allison et al. (1976) also report depressed ATP concentrations in hearts of diabetic rats 48 hours after treatment with alloxan. However, Opie et al. (1971) found normal glucose utilization and glycolytic rates in isolated working hearts of rats made diabetic with streptozotocin and perfused with 11 mM glucose. In the presence of glucose and insulin, ATP and creatine phosphate levels were normal. Thus if low ATP stores were responsible for the decreased function in the present studies, insulin and a high glucose concentration should have abolished that abnormality.

In the present study of chronic streptozotocin diabetes, the sum of lactate and pyruvate production by the hearts of diabetics was lower than by hearts of controls, and this was only partially corrected with a high glucose concentration and insulin. This finding may reflect a decrease in carbohydrate flux through the glycolytic pathway. However, if exogenous glucose utilization was limited, then partial compensation could occur by increased glycogenolysis. Glycogen utilization was increased in diabetics in the absence of insulin. However, in the presence of a high glucose concentration and insulin, it appears that net glycogenesis occurred, arguing against a deficit in total carbohydrate availability. It is possible that pyruvate entry into the Krebs cycle was restricted by the depression in mitochondrial pyruvate dehydrogenase activity reported in hearts of rats made acutely diabetic with alloxan (Kerbey et al., 1977). Since, under aerobic conditions, the heart derives very little of its energy metabolism from glycolysis, the similarity of oxygen utilization values between hearts of diabetic and control animals argues for similar rates of energy turnover, the hearts of diabetics probably relying more on stored lipids for energy (Kreisberg, 1966).

The fact that depressed heart function persisted in diabetics despite the presence of a high glucose concentration and insulin in the perfusion medium suggests that a defect in energy utilization might be present. Preliminary evidence indicates that both contractile protein ATPase activities and calcium binding and uptake by isolated sarcoplasmic reticulum are impaired in preparations from hearts of diabetic animals (Penpargkul et al., 1979; Malhotra et al., 1979).

The present study indicates that streptozotocin diabetes of several weeks' duration profoundly alters myocardial function. The resultant effects appear to be due to the diabetogenic action of streptozotocin. It is not known whether a milder form of diabetes, or diabetes induced by other techniques or in other species, would yield the same results. However, the present findings suggest that broader studies into these phenomena might lead to a better understanding of diabetic cardiomyopathy in humans.

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