Myocardial Composition and Function in Diabetes
The Effects of Chronic Insulin Use

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SUMMARY This study was undertaken in an animal model of mild diabetes to determine if provision of chronic insulin replacement during postprandial hyperglycemia may modify the abnormalities of myocardium. Group 1 served as controls with normal glucose tolerance by intravenous testing. Two additional groups were made diabetic with low doses of alloxan. Diabetic animals of Group 2 were untreated (n = 8). Group 3 animals (n = 6) received regular insulin daily to reduce postprandial hyperglycemia. After one year with maintained body weight, the animals were studied in the intact anesthetized state using the indicator dilution technique for left ventricular volume determinations. Basal left ventricular function and contractility were similar to normals in both diabetic groups. During intraventricular infusion of saline, end-diastolic pressure rose to higher levels in untreated diabetes (14.8 ± 2 mm Hg) than normals (8.8 ± 0.84), despite similar basal levels. Insulin treatment was associated with higher filling pressures than in group 1 as well as reduced end-diastolic volume response. Collagen concentrations were enhanced an average of 50% in layers from the inner to outer myocardium in both untreated and treated diabetics, associated with sodium and water accumulation. Since hypertrophy was not present, the diminished compliance appeared related to increased collagen levels. On electron microscopy, the subcellular organelles of the cardiac cell appeared normal in both diabetic groups. Thus, collagen accumulation and abnormal myocardial function in this model of diabetes is not affected by control of postprandial hyperglycemia, but a potential role for sustained hormone replacement is not excluded.

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ABNORMALITIES of the myocardium apparently independent of coronary atherosclerosis have been described in spontaneous (Giacomelli and Wiener, 1979), experimental (Regan et al., 1974), and human diabetes (Regan et al., 1977). The influence of treatment on the development and progression of the tissue complications of diabetes has been a matter of controversy. Long term use of insulin in diabetic animals has apparently reduced the incidence of retinal microvascular disease (Engerman et al., 1977), but the influence of this hormone on the myocardium is less clear.

This present study was undertaken in an animal model of mild diabetes with maintained body weight to determine whether provision of insulin replacement early in the course of the disease might modify the myocardial abnormalities. This hormone was administered at mealtime when requirements are maximal and the potential for hypoglycemia is reduced. After 12 months of observation, left ventricular function was compared in untreated and treated diabetics with similar initial levels of glucose intolerance. In terms of myocardial composition, we wished to determine whether a major protein of interstitium, collagen, was quantitatively altered in diabetes. Since tissue water has been reported to be increased in the heart of spontaneous diabetic animals (Regan et al., 1974), the content of water and cations in myocardium has also been ascertained. We have determined the influence of chronic insulin use on these parameters, as well as the altered lipid content of the left ventricle (Regan et al., 1974).

Methods

Three groups of healthy male mongrel dogs, 2-4 years old, and weighing 22-26 kg, were studied. The dogs had no clinical evidence of disease for 6-8 weeks before admission to the study groups. Hematocrit and serum albumin were initially normal and both groups received the same diet consisting of 8% fat, 22% protein, 58% carbohydrate, 9% ash, and 3% crude fiber. Group 1 served as controls with normal glucose tolerance by intravenous testing. The other two groups were made diabetic with three low doses of alloxan at monthly intervals to ensure that the reduction of glucose tolerance would be sustained over the course of 12 months (Regan et al., 1974). Diabetic animals of group 2 were untreated (n = 8). Group 3 animals (n = 6) were
treated with regular crystalline zinc beef insulin (Eli Lilly), 60 mU/kg, sc, just prior to mealtime starting 3 months after the initial alloxan. Thus, we have studied the effects of insulin therapy only after the state of chronic glucose intolerance was established.

Both groups were maintained for an average period of 12 months before study and compared with the eight normals (group 1). To assess general nutritional state, body weight, hematocrit, plasma albumin (Nishi and Rhodes, 1966), potassium by flame photometry and phosphate (Bartlett, 1956), were determined in the control and experimental state.

To produce mild normoglycemic diabetes, alloxan monohydrate in sterile saline was administered intravenously in a dose of 20 mg/kg over a 1-minute period. Two additional doses were given at monthly intervals to maintain a relatively steady state of glucose intolerance. Larger doses of alloxan were avoided to prevent ketoacidosis. The diabetic dogs and controls were observed for an average period of 9 months after the initial alloxan dose. This diabetic model exhibits reduced glucose tolerance and plasma insulin levels after intravenous glucose and diminished pancreatic levels of insulin (Regan et al., 1974). Since abnormalities of myocardial function and composition were not observed in this canine model in which the pancreatic effects of alloxan (20 mg/kg) and resultant diabetes were prevented (Regan et al., 1974), a chronic cardiotoxic effect at this dose range appears unlikely. In addition, similar abnormalities of the left ventricle, as after alloxan, were observed in animals with spontaneous diabetes (Regan et al., 1974). Moreover, in a recent study of the isolated rat heart 3 days after a 60 mg/kg dose, left ventricular function was depressed (Miller, 1979). When glucose transport was enhanced, ventricular function was normalized, supporting the view that severe diabetes and its metabolic consequences, rather than a direct toxic effect, were responsible.

Glucose tolerance was measured before and every 3 months after the initial dose of alloxan. Glucose was infused over 1 minute (1.05 g/kg, iv) through catheter tubing in the relatively relaxed anesthetized dog. Blood samples were taken at 1, 2, 4, 6, 10, 20, 30, 45, 60, and 120 minutes, and plasma glucose was analyzed by the glucose oxidase method (Hill and Kessler, 1961). For group 3, insulin was omitted on the day of the test until sampling was completed. The glucose clearance constant was calculated to estimate the disappearance rate from the vascular compartment and was derived by a semilogarithmic plot of glucose concentrations beginning with 1-minute sample for calculation of slope.

**Hemodynamic Studies**

Dogs were anesthetized with morphine sulfate (2 mg/kg) and sodium pentobarbital (12 mg/kg, iv) and studies were performed with the chest intact. Ventilation was regulated by a Harvard pump via a cuffed endotracheal tube to maintain pH and Po2 within normal range. Catheters were placed in the pulmonary artery, left ventricle, and ascending aorta and maintained patent with infusion or intermittent flushing with small volumes of saline.

Because of its high frequency response, the Statham P23Gb gauge was used for recording the left ventricular pulse. A no. 8 Goodeal-Lubin catheter was connected directly to the strain gauge and pressures recorded on an Electronics for Medicine photographic recorder. The natural frequency of this catheter system tested by rupture of a liquid-filled balloon was found to be 56 cycles/sec. Left ventricular end-diastolic pressure was recorded at high sensitivity, and the average of four to five end-expiratory pressures was calculated. To evaluate the accuracy of the fluid-filled system for measuring left ventricular pressure, four separate dogs were studied. Left ventricular pressure recordings were obtained with a no. 8 catheter-tip transducer (Micro-Tip, Millar Instruments) and compared with pressures recorded via the 50-cm Goodeal-Lubin catheter connected directly to a P23Gb Statham strain gauge transducer positioned at the midthoracic level. Thirty-two simultaneous determinations of left ventricular end-diastolic pressure during a wide range of hemodynamic interventions showed close agreement, with a correlation coefficient of 0.98, whereas the first derivative of the ventricular systolic pressure had a coefficient of 0.94.

The first time derivative of the ventricular pulse was obtained with a resistance-capacitance differentiating circuit (time constant 1.1 mm/sec) connected to the output of the left ventricular pressure channel. The amplitude of dP/dt was a linear function of frequency to 70 cycles/sec. Maximum error of the differentiator was approximately 0.9% when summing the fundamental with the 10th harmonic.

Cardiac output and left ventricular volumes were determined in duplicate by the thermal indicator dilution method (Weisel et al., 1974; Weigand and Jacob, 1965). End-diastolic volume measurements obtained with the indicator method have correlated with values obtained by angiography as detailed previously (Regan et al., 1977). The least favorable comparison of the indicator and angiographic measurements has indicated that both methods reflect the same directional change during acute interventions. Thermodilution catheter placement and techniques were as described in an earlier report (Haider et al., 1977). Left ventricular ejection fraction was obtained in duplicate. Five ml of cold saline were injected as a bolus at the inflow site of the left ventricle, and washout curves were determined from a thermistor just above the aortic valve. Adequate mixing appears to be present at this injection site, since prior studies showed good correlation of ejection fraction derived sequentially from left atrium and ventricular injection sites at the level of the inflow tract or apex (Regan et al., 1974). End-diastolic volume was calculated from the ratio of
stroke volume and ejection fraction and expressed per kilogram of body weight.

Contractility was assessed in the intact animal from an index expressing the end-isometric force-velocity relation normalized for initial fiber length. This index exhibits a relatively narrow range in normal ventricles, increases in a predictable fashion with positive inotropic interventions, and is depressed in the presence of left ventricular disease (Frank and Levinson, 1967). The formulation includes \((dP/dt \ max/MIP)^2\), where \(dP/dt\) is the maximal rate of rise of left ventricular pressure in millimeters of mercury per second, MIP the maximal isovolumetric pressure in millimeters of mercury, \(2\pi\) the end-diastolic left ventricular circumference in centimeters (Frank and Levinson, 1967). The left ventricular radius was calculated from end-diastole in centimeters (Frank and Levinson, 1967). Per kilogram of body weight.

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The left ventricular function was assessed further during volume expansion. A second catheter was introduced into the left ventricle, and saline was infused at 50 ml/min. During infusion of saline, end-diastolic pressure was continuously recorded at high sensitivity and the cardiac output and ejection fraction were determined at 3 minutes of infusion when the animals were in a steady state as judged by heart rate, aortic pressure, and ventricular end-diastolic pressure.

To determine if ventricular end-diastolic pressure increments during volume expansion are at least partially affected by enhanced intrapericardial pressure, three separate normal dogs were assessed in the intact anesthetized state. A catheter was introduced into the pericardial space by a transseptal technique described previously (Weisse, 1969). To determine ventricular end-diastolic transmural pressure, catheter tips were located within the pericardium by postero-anterio chest films. Statham pressure transducers (P23Db) were placed at the level of the spine and pressures were corrected to this reference level. Ventricular end-diastolic and pericardial pressures were recorded at high sensitivity (1 mm Hg equal to 4 mm paper) and were measured both at end-expiration and by using averaged pressure levels throughout three respiratory cycles.

After infusion of normal saline, 50 ml/min, into the left ventricular chamber for 4 minutes, intrapericardial end-diastolic pressure was \(-1.2 \pm 0.6\) mm Hg compared to the control of \(-2.0 \pm 0.7\) mm Hg, a nonsignificant change. Moreover, sampling of right heart blood before and after 4 minutes of saline infusion revealed no decrease of hematocrit or serum albumin, implying that much of the solution diffused out of the vascular system without recirculation in this time span. Thus, the filling pressure responses during infusion of saline into the left ventricle are considered to be largely independent of intrapericardial pressure.

Myocardial Ischemia

In view of the potential for small vessel disease in diabetes, myocardial oxygen demand is moderately enhanced by cardiac acceleration. A pacing catheter was placed in the right atrium and the rate was increased approximately 50 beats to between 220 and 240/min. A coronary sinus catheter was positioned in the coronary sinus to sample blood for lactate estimations (Lowry et al., 1964). In the control and pacing states, paired samples were taken from the aorta and coronary sinus for lactate analysis and the whole blood was immediately depolarized in tubes with 6% perchloric acid on ice.

Myocardial Composition

After cold arrest of the heart with iced Ringer’s solution, ventricular transmural samples were rapidly placed in liquid nitrogen for glycogen assay (Roe and Dailey, 1966). Since morphological and functional abnormalities in this diabetic animal model have suggested a change in myocardial composition, samples were taken from the inner, mid, and outer layers of myocardium for hydroxyproline analyses to estimate collagen concentration (Prockop and Underfriend, 1960). Three additional normal dogs were used as controls to determine whether heterologous insulin administered as a dose of 60 mU/kg at mealtime might affect the quantity of myocardial collagen. In addition, a sample of the ventricle was divided into inner and outer layers. For analyses of sodium and potassium concentrations, samples were homogenized and extracted for 48–75 hours in distilled water, a sufficient time for complete extraction. Potassium and sodium were determined on an Auto Analyzer system with flame attachment. Water content was determined by drying samples in an oven at 100°C to constant weight. To examine ultrastructure of the cardiac cell, specimens for electron microscopy were fixed in cold glutaraldehyde buffered with phosphate. The tissue was then washed, postfixed in osmium, exposed to lead and uranyl acetate, and embedded in Epon.

After careful trimming of epicardial adipose tissue, transmural samples were homogenized in phosphate buffer and lipids extracted in chloroform-methanol to determine triglyceride (Kessler and Lederer, 1967), cholesterol (Levine and Zak, 1964), as well as free fatty acid (Kelly, 1975) concentrations. Plasma concentrations were also determined in the terminal study for comparison with levels 12 months previous.

Statistical Analysis

The data were expressed as mean ± standard error in all cases. When only one statistical com-
TABLE 1

<table>
<thead>
<tr>
<th>Group</th>
<th>C</th>
<th>E</th>
<th>C</th>
<th>E</th>
<th>C</th>
<th>E</th>
<th>C</th>
<th>E</th>
<th>C</th>
<th>E</th>
<th>C</th>
<th>E</th>
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<tbody>
<tr>
<td>1</td>
<td>24.3 ±0.7</td>
<td>25 ±0.6</td>
<td>86 ±3</td>
<td>87 ±7</td>
<td>3.5 ±0.2</td>
<td>3.7 ±0.3</td>
<td>3.6 ±0.11</td>
<td>3.4 ±0.13</td>
<td>3.7 ±0.15</td>
<td>4.0 ±0.19</td>
<td>4.2 ±0.17</td>
<td>4.5 ±0.1</td>
</tr>
<tr>
<td>2</td>
<td>25.1 ±0.4</td>
<td>24.9 ±0.6</td>
<td>92 ±4</td>
<td>95 ±6</td>
<td>3.8 ±0.3</td>
<td>1.8+</td>
<td>3.4 ±0.07</td>
<td>3.5 ±0.16</td>
<td>4.1 ±0.21</td>
<td>3.9 ±0.14</td>
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<td>4.4 ±0.09</td>
</tr>
<tr>
<td>3</td>
<td>23.9 ±0.8</td>
<td>24.7 ±0.9</td>
<td>91 ±4</td>
<td>93 ±5</td>
<td>4.1 ±0.4</td>
<td>2.0+</td>
<td>3.5 ±0.2</td>
<td>3.6 ±0.14</td>
<td>3.8 ±0.15</td>
<td>3.8 ±0.2</td>
<td>4.0 ±0.13</td>
<td>4.1 ±0.16</td>
</tr>
</tbody>
</table>

* C = control; E = average of last two monthly values.
† Unpaired t-test, P < 0.01.

Results

The diabetic animals and controls maintained body weight throughout the 12 months of observation (Table 1). In addition, serum albumin, the hematocrit, and electrolytes were at normal levels. Both diabetic groups had significant reduction of glucose clearance rate that persisted throughout the experimental period despite fasting normoglycemia (Table 1).

The efficacy of insulin treatment in diabetics, 60 mU/kg subcutaneously, was evaluated by analyzing plasma glucose before and during meal ingestion either with or without acute insulin pretreatment. The postprandial increase in plasma glucose above the fasting level was significantly less in the animals pretreated with insulin (Fig. 1).

Left ventricular function was assessed in the intact anesthetized state after one year of observation. At comparable levels of heart rate and aortic pressure in the basal state, the end-diastolic volume was significantly lower in the animals pretreated with insulin.
and stroke volume were similar in all three groups (Tables 2-4). End-diastolic pressure was higher than controls, significantly elevated in group 3, but the latter was not different from untreated diabetics. The index of contractility in the basal state derived from the first derivative of left ventricular pressure normalized for afterload and preload was 1.4 ± 0.13 in controls, 1.31 ± 0.14 in the untreated diabetic group 2, 1.30 ± 0.19 in the insulin-treated group and 1.26 ± 0.19 in the untreated diabetic group 3, respectively. With arterial concentration unchanged during pacing, there was no evidence of diminished lactate extraction or actual lactate production. The coefficient of lactate extraction was calculated from the concentration ratio, arterial coronary venous/arterial × 100. In group 1, the level in the control state was 28.4 ± 1.6% and 27.0 ± 2.2% during pacing. For group 2, the respective

### Table 2  Hemodynamic Responses in Controls

| Group 1 Dog no. | Heart rate (beats/min) Mean aortic pressure (mm Hg) | Left ventricular end-diastolic | Mean aortic Pressure (mm Hg) Volume (ml/kg) Stroke volume (ml/kg) |
|----------------|--------------------------------------------------|-------------------------------|---------------------------------|------------------|
|                | C vs. E; P =                                      |                               |                                 |                  |
| 1              |                                                   |                               |                                 |                  |
| 2              |                                                   |                               |                                 |                  |
| 3              |                                                   |                               |                                 |                  |
| 4              |                                                   |                               |                                 |                  |
| 5              |                                                   |                               |                                 |                  |
| 6              |                                                   |                               |                                 |                  |
| 7              |                                                   |                               |                                 |                  |
| 8              |                                                   |                               |                                 |                  |
| Mean           | 143.6 ± 142.9                                     |                               | 5.8 ± 8.8                       | 3.4 ± 5.0        |

**Paired t-test**

| C vs. E; P = | NS | NS | <0.001 | <0.01 | <0.006 |

* C = basal state in terminal study in this and subsequent tables.

† E = to saline infusion in left ventricular chamber.

### Table 3  Hemodynamic Response to Saline Infusion in Untreated Diabetics

| Group 2 Dog no. | Heart rate (beats/min) Mean aortic pressure (mm Hg) | Left ventricular end-diastolic | Mean aortic Pressure (mm Hg) Volume (ml/kg) Stroke volume (ml/kg) |
|----------------|--------------------------------------------------|-------------------------------|---------------------------------|------------------|
|                | C vs. E; P =                                      |                               |                                 |                  |
| 1              |                                                   |                               |                                 |                  |
| 2              |                                                   |                               |                                 |                  |
| 3              |                                                   |                               |                                 |                  |
| 4              |                                                   |                               |                                 |                  |
| 5              |                                                   |                               |                                 |                  |
| 6              |                                                   |                               |                                 |                  |
| Mean           | 147.5 ± 142.7                                     |                               | 7.4 ± 14.8*                     | 3.1 ± 4.2        |

**Paired t-test**

| C vs. E; P = | NS | NS | <0.001 | NS | <0.001 |

* Significantly greater than response in group 1 by Duncan’s multiple range test.
TABLE 4  Insulin-Treated Diabetics

<table>
<thead>
<tr>
<th>Group 3 Dog no.</th>
<th>Mean heart rate (beats/min)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Left ventricular end-diastolic Pressure (mm Hg)</th>
<th>Volume (ml/kg)</th>
<th>Stroke volume (ml/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C</td>
<td>E</td>
<td>C</td>
<td>E</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>94</td>
<td>91</td>
<td>148</td>
<td>149</td>
<td>10.6</td>
</tr>
<tr>
<td>2</td>
<td>160</td>
<td>155</td>
<td>138</td>
<td>150</td>
<td>10.7</td>
</tr>
<tr>
<td>3</td>
<td>122</td>
<td>119</td>
<td>140</td>
<td>146</td>
<td>9.8</td>
</tr>
<tr>
<td>4</td>
<td>110</td>
<td>95</td>
<td>125</td>
<td>129</td>
<td>8.6</td>
</tr>
<tr>
<td>5</td>
<td>158</td>
<td>155</td>
<td>108</td>
<td>110</td>
<td>9.3</td>
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<tr>
<td>6</td>
<td>114</td>
<td>100</td>
<td>141</td>
<td>141</td>
<td>8.4</td>
</tr>
</tbody>
</table>

Mean ± SE  
C: 126.3 ± 11.0  E: 119.2 ± 12.0  NS  
Paired t-test  
NS  <0.02  NS  <0.03

* Significantly higher than control level in group 1.  
† Significantly less than response in group 2.  
‡ Significantly less than response in group 1.

levels were 26.3 ± 1.9% and 27.4 ± 2.2%. The respective levels for group 3 were 29.1 ± 3.0% and 26.9 ± 2.7%.

Analysis of the left ventricular free wall revealed a significant increase of collagen concentration in the inner, middle and outer layers of the untreated diabetic group (Fig. 3). This was not affected by insulin therapy in group 3 in which the collagen concentrations were similarly enhanced. A small group of three normal controls that received insulin at mealtime for 8 months had collagen levels in the normal range. The combined weight of the left ventricle and septum was assessed in the three groups. The weight was 4.4 ± 0.38 g/kg in the normal controls of group 1, 4.26 ± 0.23 in the untreated diabetics, and 4.39 ± 0.24 in the treated group. Thus, by this measure, hypertrophy did not appear to be a basis for the observed change in compliance.

Tissue water was also increased in the diabetic groups, particularly in the inner layers (Table 5). Whereas potassium concentrations were normal, sodium was significantly higher in the untreated diabetic group and was not significantly affected by insulin. By electron microscopy, no evidence of swelling in subcellular organelles (Fig. 4) was observed, so that the process was presumably extracellular in distribution.

Myocardial composition was also altered in terms of lipid concentration (Table 6). Untreated diabetics exhibited a significant elevation of myocardial triglyceride and cholesterol. Similar increments were observed in the diabetic group treated with insulin. Neither diabetic group exhibited a differ-

Figure 2 Left ventricular end-diastolic pressure-volume relationship before and during infusion of saline into the left ventricular chamber. * Both diabetic groups exhibited significantly higher levels of left ventricular end-diastolic pressure than the normals of group 1, without a corresponding end-diastolic volume increment.
ence in free fatty acid nor in glycogen concentrations of the left ventricle compared to normal controls.

**Discussion**

In terms of glucose metabolism, this model of diabetes may be considered to be relatively mild. Glucose intolerance is associated with reduced plasma insulin levels in response to a glucose challenge (Regan et al., 1974). Whereas the fasting state is marked by glucose levels in the normal range, abnormalities of myocardial function and composition, as well as PAS deposits in the renal mesangium consistent with diabetes, previously have been observed (Regan et al., 1974). Similarly, normoglycemia with abnormal glucose tolerance in man has been associated with thickened capillary basement membrane in skeletal muscle as well as segmental increase of renal mesangium and apparently significant changes in retinal vessels (Camerini-Davalos et al., 1973). Thus, abnormal glucose tolerance with normal fasting plasma glucose can be associated with some of the tissue alterations seen in chronic hyperglycemia and permits the study of chronic diabetic animals without ketoacidosis and weight loss. Conversely, tissue responses that may depend for progression upon a more severe metabolic abnormality cannot be well tested under this circumstance.

Since duration of diabetes has been described as a major factor in the development of complications such as collagen accumulation in renal glomeruli (Klein et al., 1975), we have assumed that changes in heart muscle would be progressive with time. Although plasma glucose was presumably elevated above normal in the untreated animals only during the 4-hour postprandial period, the interrelation between duration and degree of hyperglycemia required for initiation and progression of chronic tissue abnormalities is not known.

Observations on the left ventricular end-diastolic pressure-volume relationship during volume expansion indicated that there was enhanced stiffness of the myocardium in the untreated diabetic animal. This was not ameliorated by chronic insulin treatment. As indicated earlier (p. 1270), the quantity of saline infused into the left ventricular chamber does not significantly affect pericardial pressure, so that the pericardium would not appear to contribute to the abnormal rise of end-diastolic pressure. In addition, the basal end-diastolic volume was comparable in the three groups and the filling pressure increment in diabetics was associated with a volume

**Table 5** Myocardial Water and Cations in Diabetics and Effects of Insulin

<table>
<thead>
<tr>
<th></th>
<th>% Water</th>
<th>Sodium</th>
<th>Potassium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inner</td>
<td>Outer</td>
<td>Inner</td>
</tr>
<tr>
<td>Group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>78.3 ± 0.3</td>
<td>78.1 ± 0.4</td>
<td>35.6 ± 1.3</td>
</tr>
<tr>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 2</td>
<td>80.5 ± 0.8</td>
<td>78.9 ± 0.6</td>
<td>43.7 ± 2.0*</td>
</tr>
<tr>
<td>(n = 6)</td>
<td>(n = 6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group 3</td>
<td>80.2 ± 0.7</td>
<td>78.7 ± 0.5</td>
<td>42.1 ± 1.7*</td>
</tr>
</tbody>
</table>

* Significantly different from group 1.
FIGURE 4  Electron micrograph of the left ventricle representative of the diabetic animals in group 2. There are no evidence abnormalities of myofibrils, mitochondria, or sarcoplasmic reticulum. The apparent lack of edema supports the view that the enhanced tissue levels of sodium and water are extracellular in location. 12,400x.

increment that was less than in normals. Moreover, the diminished compliance observed in the diabetic animals during volume infusion was also not dependent upon the presence of hypertrophy, as judged by the weight of left ventricle.

Elasticity of normal muscle has been attributed predominantly to extracellular structures, with collagen of particular significance (Brady, 1968). This fibrous protein is considered to increase prior to the development of substantial ventricular hypertrophy in aortic banded animals and the early increase of myocardial stiffness has been ascribed to this compositional change (Mirsy and Laks, 1980). A quantitatively similar increase of collagen concentration
in the left ventricle appears to be the basis for the changed compliance observed in these diabetic animals. The basis for the collagen increment in the animal has not been defined. There is evidence from absence of hypertrophy in the chronic diabetic animal may be diminished, since insoluble collagen was enhanced in heart muscle, while the acid soluble fraction declined (Haider et al., 1978). Presumably, enhanced insoluble collagen accounts for the functional change in the left ventricle.

Although the time course of the change in myocardial collagen concentration is not known, in a rat model made diabetic with streptozotocin the quantity of hydroxyproline per mg of cardiac protein was not significantly altered after 3 weeks but was increased after 6 weeks (Modrak, 1980). That this is not an invariable response is indicated by observations at later intervals when heart weight significantly declined as an apparent manifestation of enhanced catabolism and reduced body mass. Under this circumstance, hydroxyproline accumulation did not occur, which is consistent with the impaired synthesis of cardiac protein observed during sustained ketoacidosis (Pain and Garlick, 1974).

Tissue sodium and water were also increased in diabetic animals and may have contributed to the greater stiffness of myocardiun. This alteration was not affected by insulin. Increments of glycoprotein collagen in the interstitium (Regan et al., 1974) can increase the number of anionic sites for binding sodium. Thus, the enhanced tissue levels of sodium and water may be due to this phenomenon, with cation hydration contributing to the latter. In support of this interpretation, the cardiac cell gave no evidence of swelling on electron microscopy. The subcellular structures lacked the distortion characteristic of cell edema.

We have intervened with insulin only after glucose intolerance was established to evaluate the effects of insulin in a period of putative progression of cardiac abnormalities and also because pretreatment may adversely affect carbohydrate metabolism compared to untreated controls (Frankel and Grodsky, 1979). Moreover, in a canine model, retinopathy has been effectively prevented when insulin administration was delayed up to 10 weeks after alloxan (Engerman et al., 1977).

In our model, the fasting levels of plasma insulin do not differ from those of normal controls (Regan et al., Circ Res 1974) but the insulin response after glucose administration was significantly reduced. The dosage of 60 mU/kg administered at mealtime significantly reduced postprandial hyperglycemia for the initial 4 hours. Additional hormone administration over the subsequent hours in these animals with normal fasting blood glucose levels was not used so as to avoid hypoglycemia and the confounding effects of increased blood levels of the cardiovascular hormones, epinephrine and glucagon (Rizzo et al., 1979). Although monocomponent insulin devoid of impurities would have been desirable, the normal controls that received insulin did not exhibit abnormalities of the myocardium. In addition, impure heterologous insulin has prevented the development of microvascular disease in diabetes (Engerman et al., 1977).

Enhanced activity of glucosyltransferase in renal tissue of a diabetic rat preparation has been reduced to normal after insulin (Beiswenger and Spiro, 1970). A similar response in the myocardium with resultant reduction of glycoprotein could affect accumulation of collagen. That this was not observed may be related to an influence of counter regulatory hormones such as growth hormone, reportedly elevated in diabetes (Kjeldseng et al., 1975). This hormone can intensify the collagen abnormality represented by basement membrane thickening in the insulin deficient state (Osterby et al., 1978) and promote collagen synthesis in arterial cell cultures (Ledet and Vuut, 1980). Although insulin treatment at mealtime presumably resulted in enhanced carbohydrate utilization, sustained hormone replacement over a 24-hour period under conditions where hypoglycemia is avoided might alter the counter regulatory hormone responses and thus prevent myocardial collagen accumulation. It is noteworthy that, in the setting of chronic afterload hypertrophy, collagen accumulation has been observed to persist even after regression of cardiac muscle hypertrophy with aortic band removal (Cuttilletta et al., 1978). Consequently, it has been suggested that mature collagen fibers may not be as accessible to degradation as cardiac cell components. In terms of diabetes, the effects of insulin on myocardial collagenase and its inhibitors are not known.
A distinctly different cardiac abnormality has been described in the rat model during severe, chemically induced diabetes (Miller, 1979). In the early days of ketoacidosis after a large dose of alloxan, left ventricular function and high energy phosphate levels were reduced due to impaired glucose utilization by the cardiac cell. Acute correction of this substrate deficiency in the isolated heart with insulin or addition of high levels of glucose to the perfusion media restored ATP levels and normalized left ventricular function (Miller, 1979). In a chronic preparation, impaired mechanical properties were observed in the isolated papillary muscle which was apparently unaffected by duration of diabetes (Fein et al., 1980). These models contrast with what is known of complications in human diabetes where duration is a major determinant (Klein et al., 1975).

Modest accumulations of triglyceride and cholesterol in myocardium were observed in the untreated canine diabetic model. We have previously observed that the triglyceride increase is associated with enhanced incorporation of U-14C-labeled fatty acid while incorporation into myocardial phospholipid is diminished (Regan et al., 1974). The lack of effect of insulin on lipid changes in the heart is analogous to the response of neural tissue in diabetic animals, where the reduced incorporation of myoinositol into phospholipid was unaffected by insulin (Clements and Stockard, 1980).

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