MYOCARDIAL FUNCTION AND CORONARY FLOW IN DIABETES/Haider et al. 577

SUMMARY To examine the influence of preexistent diabetes mellitus on left ventricular performance and coronary blood flow responses to acute ischemia, mild normoglycemic diabetes was induced in nine mongrel dogs after three doses of alloxan, (20 mg/kg, iv), at monthly intervals. Hemodynamic measurements and coronary blood flow (85Kr clearance) were obtained before and after the onset of ischemia. This was produced by occlusion of the proximal left anterior descending coronary artery via a balloon-type catheter in nine intact anesthetized diabetic dogs and 10 nondiabetic dogs. During the 1st hour of ischemia in the diabetic group, the end-diastolic pressure rose from 7 ± 1.1 (mean ± se) mm Hg to 23.8 ± 2.3 without a significant increase of end-diastolic volume. In controls end-diastolic pressure rose from 8.6 ± 1.1 mm Hg to 15.3 ± 1.4, and end-diastolic volume was significantly increased, so that the ratio of end-diastolic pressure and volume was significantly higher in the diabetic group (P<0.005). Although indices of contractility did not differ, stroke volume and work reductions were significantly greater in diabetes, despite the fact that coronary blood flow was reduced to a similar extent. Size of the ischemic areas appeared comparable as judged by distribution of dye injected distal to the occlusion. Since potassium loss and sodium gain in the inner and outer layers of ischemic tissue did not differ between the two groups, the intensity of ischemia seemed similar. Glycogenolysis was unimpaired in the diabetic ischemic muscle but triglyceride levels remained elevated. Morphologically the diabetic myocardium was characterized by a diffuse accumulation of periodic acid-Schiff-positive glycoprotein in the interstitium, which was thought to limit diastolic filling of the ischemic ventricle and to contribute to the substantial reduction of ventricular performance.

ALTHOUGH the influence of acute regional ischemia on left ventricular function has been well defined in the previously normal animal,1,2 the response of the ventricle affected by a chronic metabolic or structural abnormality has not been described. Acute myocardial infarction has been reportedly associated with a greater incidence of pump failure and higher mortality in diabetes mellitus.3 Although the increased mortality from cardiac disease complicating diabetes mellitus has been traditionally attributed to accelerated atherosclerosis of the coronary arteries,4 this is a disputed issue since recent evidence in studies using more quantitative methods and age-matched controls has shown that the complicated lesions of atherosclerosis may occur to only a slightly greater extent in diabetics.5

In a previous study from this laboratory,6 we observed altered myocardial function in chronic diabetes mellitus in dogs, associated with accumulation of periodic acid-Schiff (PAS)-positive glycoprotein in the myocardial interstitium without coronary obstructive lesions; this morphological abnormality also has been observed in man.7,8 To examine the response of the diabetic myocardium during acute regional ischemia as compared to normal controls the following study was undertaken.

Methods

Two groups of healthy male mongrel dogs 2–4 years old and weighing 21–28 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. One group (n = 10) served as controls with normal glucose tolerance by intravenous testing. The other group (n = 9) was made diabetic with low doses of alloxan at monthly intervals. To produce mild normoglycemic diabetes, alloxan monoh-
diate 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 ketoadiposis. The diabetic dogs and the controls were observed for an average period of 9 months after the initial alloxan dose.

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, unanesthetized 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. 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 the 1-minute sample for calculation of slope. In the diabetic dogs venous blood samples were obtained in the fasting state at the onset of the study and at 3-month intervals for determination of plasma lipids. Blood was placed in chilled tubes containing ethylenediaminetetraacetic acid (EDTA); after separation in a refrigerated centrifuge, the plasma was stored at —20°C until assay. Duplicate determinations of free fatty acid, triglyceride, and phospholipid were made.

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. The 50-cm Goodale-Lubin catheters were connected directly to a Statham strain gauge transducer (P23Gb) and recorded on a multichannel oscilloscope recorder (Electronics for Medicine). The first derivative of left ventricular pressure pulse (dP/dt) was computed continuously by a resistance-capacitance differentiating circuit and converted to mm Hg per second. Left ventricular end-diastolic pressure was recorded at high sensitivity and the average of 4–5 end-expiratory pressures were calculated. To evaluate accuracy of the fluid-filled system used in this study for measuring left ventricular pressure, four separate dogs were studied. Left ventricular pressure recordings were obtained with a no. 8 catheter-tip transducer (MicroTip, Millar Instrument) and compared with pressure recorded via a 50-cm-long Goodale-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 (y = 1.019x — 0.409) in accord with a prior report.

Cardiac output and left ventricular volumes were determined in duplicate by the thermal indicator-dilution method. Cardiac output by this technique has been found to correlate well with the dye-dilution technique, and end-diastolic volume has been correlated with the angiographic method. A Swan-Ganz thermodilution catheter tip was placed in the main pulmonary artery and 10 ml of normal saline at room temperature was injected as a bolus into the right atrium through the proximal lumen. Change of temperature was detected by the thermistor in the pulmonary artery and displayed on the recorder through a Wheatstone bridge; the area of the curve was computed manually for cardiac output calculation. Left ventricular injection fraction was obtained in duplicate from the left ventricular washout curves obtained by thermodilution method. Cold saline (5 ml) was injected as a bolus at the inflow site of the ventricle and the step change of temperature was determined from a thermistor-tip catheter placed just above the aortic valve. Adequate mixing appears to be present at this injection site, since prior studies showed good correlation of ejection fractions derived sequentially from left atrial and ventricular injection sites at the level of the inflow tract or apex. The total amount of saline infused for the measurement of cardiac output and ejection fraction was approximately 150 ml in each dog during the course of study. We have previously observed that 180 ml/hour infused in dogs of equivalent size produced no significant hemodynamic effects.

End-diastolic volume was calculated from the ratio of stroke volume and ejection fraction and expressed per kilogram of body weight. The ejection fraction values were lower than the mean of 38% observed by the indicator-dilution technique in conscious sedated dogs. However, this is within the range observed after the administration of sodium pentobarbital anesthesia, apparently related to the myocardial depressant effects of the anesthetic agent.

Stroke work index in gram-meters per kilogram was calculated from the product of stroke volume per kilogram and the mean left ventricular systolic pressure minus end-diastolic pressure times 1.36. The ratio of left ventricular end-diastolic pressure and volume was used as a simple index of wall stiffness at end-diastole. To evaluate the contractile state of the ventricle in vivo we have used an index that normalizes left ventricular dP/dtmax, for (1) the maximal isovolumic pressure (MIP); (2) circumferential fiber length (2πr), assuming a spherical shape at the end of the systolic isovolumic period and deriving the radius from the end-diastolic volume. The formula is (dP/dtmax/MIP)/2πr. Using the same end-diastolic fiber length and pressure, the end-diastolic tension in dynes × 10⁹/beats was calculated as end-diastolic pressure × r² × 4,188; the latter is derived from τ × 1.36 (cm H2O/mm Hg) × 980 (cm/sec²) × 1 (g/cm³).

MODEL FOR ISCHEMIA

A double-lumen, 5F catheter with a distal lumen was positioned in the proximal 1.5 cm of the left anterior descending coronary artery under fluoroscopic control. The balloon was inflated gradually over a period of 60 seconds. Aortic pressure, peripheral coronary pressure,
and electrocardiogram (ECG) lead 1 were continuously monitored. Complete coronary occlusion was evidenced by a sustained reduction of mean coronary pressure to approximately 25 mm Hg and appearance of an injury potential on standard lead I in all the dogs studied.

To reduce mortality due to the high incidence of arrhythmias in the initial 15 minutes after ischemia, procainamide (10 mg/kg) was administered intravenously to dogs developing ventricular tachycardia (four in the nondiabetic group and three of the diabetics). No antiarrhythmic agent was administered after the initial 15 minutes. Since the circulatory effects of procainamide so administered are considered to last a matter of minutes, it is improbable that the antiarrhythmic drug contributed to the hemodynamic differences observed in these two groups. Measurement of coronary blood flow to the ischemic area was obtained by injections of 85Kr distal to the occlusion site. This inert gas method appears to give valid flow measurements over a wide range of tissue perfusion. Approximately 100 μCi of 85Kr was injected at 6- to 10-minute intervals in duplicate before ischemia and 12- to 20-minute intervals thereafter. Blood flow was calculated from the decay slopes obtained by precordial scintillation counting.

At 60 minutes of ischemia, the chest was opened and the heart was arrested with iced Ringer’s solution. To delineate the area profused by the left anterior descending coronary artery (LAD) distal to the occlusion, Evans blue dye was injected via the LAD catheter just prior to arrest. A transmural section was rapidly excised from the central ischemic area and the nonischemic posterior wall at least 1 cm from the former. Both were frozen in liquid nitrogen for glycogen assay. The remainder of the dyed area was excised, weighed, and related to the total left ventricle and septum. Sections were taken from ischemic and nonischemic areas of left ventricle for electrolyte and lipid analysis as well as histochemical examination. The ventricle was divided into inner and outer layers; the latter was carefully trimmed of epicardial adipose tissue. Samples were homogenized in phosphate buffer and the lipids were extracted in chloroform-methanol to determine free fatty acid, triglyceride, and phospholipid. Separate samples were homogenized and extracted for 48 hours in distilled water. Potassium and sodium were determined on a Technicon AutoAnalyzer system with flame attachment. Water content was obtained by drying samples in an oven at 100°C to constant weight. A group of normal intact anesthetized dogs without ischemia underwent similar tissue studies for comparison with the two experimental groups.

Histochemical examination included PAS staining after treating twice with diastase to exclude staining of glycogen. Statistical data were expressed as means ± standard errors; the paired or nonpaired Student’s t-test was applied as appropriate.

**Results**

Both groups remained healthy over the approximate 9-month period prior to the induction of ischemia. Body weight was maintained; the initial hematocrit of 45 ± 1.7 in the control group and 44 ± 2.0 in the diabetics was not significantly changed during this period. Prior to the terminal study in the controls, the mean fasting blood sugar was 83 ± 3 mg/100 ml and the mean glucose clearance constant was 3.7 ± 0.1. In the alloxan-diabetic dogs the mean fasting plasma glucose was increased significantly from a control of 84 ± 4 mg/100 ml to 104 ± 2 (P < 0.001) prior to the final study, although the mean value of plasma glucose for the group was within normal limits. The glucose clearance constant was reduced from 3.3 ± 1 prior to alloxan to a level of 2.1 ± 0.1 (P < 0.001), before the terminal study.

**HEMODYNAMIC FINDINGS DURING ISCHEMIA**

The hemodynamic measurements were made in duplicate in the control state and during the course of ischemia in both groups. Baseline hemodynamic values prior to ischemia were not significantly different in the control and diabetic groups (Tables 1 and 2). Between 30 and 60 minutes of ischemia in the 10 dogs of the control group (Table 1), the stroke volume declined an average of 4%, ejection fraction by 21%, and stroke work by 16%. The end-diastolic pressure and volume increased by 80% (P < 0.01) and 25% (P < 0.02), respectively. Changes in heart rate and arterial pressure were not significant. In this nondiabetic group, three dogs developed ventricular fibrillation between 30 and 60 minutes of ischemia and the remaining seven survived the observation period. Since the seven surviving dogs exhibited ventricular responses at 30 minutes similar to those at 60 minutes, the three that succumbed early have been included.

The hemodynamic changes during ischemia in the diabetic group are indicated in Table 2. There were two patterns of hemodynamic response in this group. The two dogs that exhibited marked hypotension (group B, Table 2) progressed to shock and developed cardiac arrest between 30 and 60 minutes of ischemia. Group A was characterized by a modest but significant decline of arterial pressure and left ventricular failure and only one of the seven dogs developed late ventricular fibrillation.

In the seven dogs of group A, the end-diastolic pressure rose more than 3-fold while end-diastolic volume did not change significantly. Stroke volume and stroke work declined by 35% (P < 0.02) and 55% (P < 0.01), respectively, a significantly greater decrease than in the nondiabetics (Table 2). In the normal control dogs, the cardiac index was maintained during ischemia and reduced insignificantly from a control of 119.9 ± 6.9 ml/kg to 112 ± 9.0. In contrast, during ischemia in the diabetic group, the cardiac index was reduced by almost one-third, (control, 114 ± 7.9 ml/kg to 78.5 ± 4.2; P < 0.01), a significantly different response from that of controls (P < 0.02).

Calculation of the normalized index of contractility to compare the relative performance of the contractile elements revealed no significant difference between normal dogs (1.10 ± 0.11) and diabetic dogs (1.26 ± 0.09) before ischemia; there was a similar small decline in both groups during ischemia to 0.86 ± 0.07 and 1.10 ± 0.19, respectively. Since the index of contractility did not differ in the
TABLE 2

<table>
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<tr>
<th>No.</th>
<th>Heart rate (beats/min)</th>
<th>Systolic/diastolic aortic pressure (mmHg)</th>
<th>Mean arterial pressure</th>
<th>Volume (ml/kg)</th>
<th>Stroke volume (ml/kg)</th>
<th>Stroke work (g-m/kg)</th>
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<tr>
<td>Mean</td>
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<td>15.3</td>
<td>4.37 5.47</td>
<td>1.98 3.03</td>
<td>0.92 0.89</td>
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</tbody>
</table>

C = control data; E = value obtained at 60 minutes of ischemia; EDP/EDVI = ratio of end-diastolic pressure and end-diastolic volume index.
* E values obtained between 30 and 60 minutes of ischemia in nonsurvivors.
† P = paired t-test comparing C vs. E.

Left ventricular function was further assessed by plotting stroke work index against end-diastolic pressure (Fig. 1A). In the control group during ischemia, the rising end-diastolic pressure was associated with a small decline of stroke work. In contrast, the diabetic group at 60 minutes of ischemia exhibited a substantial reduction of stroke work associated with a 3-fold increase of end-diastolic pressure. To determine whether the heart rate response may have affected this relationship, dogs with relatively small rate changes, within 16 beats/min of the control state, were compared in the two groups (Fig. 1B). The
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CONTROL

DIABETIC

*p < 0.01

LV END-DIASTOLIC PRESSURE (mmHg)

FIGURE 1 Left ventricular (LV) function before and during ischemia. The stroke work index is plotted against end-diastolic pressure in the nondiabetic and the diabetic group. The changes from the preischemic control are depicted by arrowhead. Panel A, which represents the data (mean ± SEM) for all the dogs in each group, illustrates that the impaired ventricular function in response to ischemia is significantly greater in the diabetic group (P < 0.01). Panel B represents the data for six dogs of the control group and four dogs of the diabetic group with heart rate change within 16 beats/min of the control state. The responses were qualitatively similar to those observed in the groups as a whole, indicating that these changes were independent of heart rate.

responses were qualitatively similar to those observed in the groups as a whole, and the same relationship of end-diastolic pressure and stroke work was evident.

Coronary blood flow to the ischemic site, measured by the 85Kr clearance technique, was similarly reduced in both groups (Fig. 2). The ischemic area, as ascertained by injection of Evans blue dye, was 31.0 ± 2.4% of the total left ventricle in the control group and 32.7 ± 1.6% in the diabetic group.

To examine the tissue response to ischemia, the transmural concentrations of potassium and sodium in the outer and inner layers of ischemic myocardium were assayed (Fig. 3). In the control group, a significant reduction of potassium and an increase of sodium concentrations were present in the inner and outer layers of ischemic tissue. Diabetic dogs exhibited a similar K+ reduction and sodium gain in the two layers of ischemic myocardium. Tissue water was 78.1 ± 0.73% in normals, and 82.9 ± 0.38% and 81.7 ± 0.44% in the inner and outer layers, respectively, of nondiabetics during ischemia; the respective values were 83.0 ± 0.63% and 82.5 ± 0.85% in the diabetic group. The nonischemic muscle exhibited small but non-significant changes of K+ and Na+ concentration in both groups with ischemia. However, tissue water was elevated in the nonischemic posterior wall of diabetics to 81.3 ± 0.42% and 81.4 ± 0.42% in the inner and outer layers, respectively, compared to 79.7 ± 0.32% (P < 0.01) and 79.0 ± 0.52 (P < 0.01) in nondiabetics. The increment of tissue water is more apparent when expressed in terms of grams of H2O per gram of dry weight. In the inner layers, where the largest changes were observed during ischemia, water increased to 4.85 ± 0.09 g/g dry weight in nondiabetics, and 4.88 ± 0.19 for diabetics, compared to 3.56 ± 0.21 in normals. The nonischemic posterior wall was increased to 4.35 ± 0.09 in diabetics and 3.92 ± 0.9 in nondiabetics (P < 0.01).

The expected decrease of glycogen levels in the ischemic tissue of the anterior wall in normals occurred to a similar extent in the diabetics (Table 3), supporting the view that the severity of ischemia was comparable in the two groups. Myocardial triglycerides were elevated in the inner and outer layers of both ischemic and nonischemic regions in the diabetics as compared to the corresponding area in the controls. Myocardial phospholipid and free fatty acid were not significantly different in the two groups. In the diabetics the plasma lipid classes in the fasting conscious state were within normal limits through the 9-month observa-
Experiments, the significantly higher ratio of end-diastolic ventricle during ischemia was not determined over a wide pressure to end-diastolic volume in diabetics is consistent (n = 10) Nondiabetic ischemia Normals (n = 8) = 7) Diabetic ischemia Nondiabetic ischemia (n = 10) Diabetic ischemia (n = 7)

<table>
<thead>
<tr>
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<th>Glycogen (µg/g)</th>
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<td>163* ±58‡</td>
<td>±0.64</td>
<td>±0.72</td>
<td>1±1</td>
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Values are expressed as mean ± SEM. Ant = anterior wall, which was ischemic in the experimental groups; Post = the posterior wall perfused by normal circumflex artery.
* P > 0.005 (nonpaired t-test vs. corresponding area in normals).
† P > 0.01 (nonpaired t-test vs. corresponding area in normals).
‡ P > 0.05 (nonpaired t-test vs. corresponding area in nondiabetes with ischemia).

Compliance period, except for free fatty acids, which increased from a control of 486 ± 48 µmol/liter to 985 ± 101 (P < 0.001) prior to the terminal study.
As previously observed, the diabetic dogs exhibited PAS-positive, diastase-resistant material in the interstitium of both the ischemic and nonischemic areas. This was absent in the nondiabetic controls.

Discussion

This diabetic dog model exhibits reduced glucose tolerance due to insulin deficiency and the mild degree of diabetes enables long-term maintenance without exogenous hormone administration. Since abnormalities of myocardial function and composition were not observed in dogs in which the pancreatic effects of alloxan and resultant diabetes were prevented, a direct cardiotoxic effect of this compound appears unlikely. Moreover, similar morphological and functional abnormalities of left ventricular myocardium were observed in dogs with spontaneous diabetes.

Occlusion of the proximal left anterior descending artery produces ischemia of approximately one-third of the left ventricle as judged from distribution of dye injected distal to the obstructed artery. Despite this sizable area of ischemia, the normal dog maintained stroke volume near distal to the obstructed artery. Despite this sizable area of ischemia, the latter process during acute global ischemia is associated with an increase of end-diastolic diameter and presumably volume, in contrast to the diabetic dogs in which end-diastolic volume did not change.

Although the pressure-volume relationship of the left ventricle during ischemia was not determined over a wide range of pressure and volume values in these in vivo experiments, the significantly higher ratio of end-diastolic pressure to end-diastolic volume in diabetes is consistent with an interpretation of reduced compliance compared to the nondiabetic group. A prior study of ischemia in dogs with a scar in the nonischemic myocardium indicated a response similar to that of these diabetic dogs. This was characterized by a 4-fold rise of filling pressure and a more profound reduction of stroke volume and work, Hypotension analogous to that in the diabetics of group B occurred in some dogs. Thus, alteration of a portion of the nonischemic myocardium may substantially affect the response to ischemia even if hypertrophy does not occur. Since the interstitial accumulation of PAS-positive glycoprotein is a diffuse process in the diabetic myocardium (cf. Fig. 4 of Regan et al.), the relative contribution of ischemic and nonischemic ventricle cannot be defined with certainty. However, the behavior of the myocardium in dogs with scar strongly supports the view that chronic compositional changes in the nonischemic zone of the left ventricle may be the basis for the observed differences in response to ischemia compared to controls.

The nonischemic area is known to undergo mechanical and metabolic alterations, associated with those of the ischemic tissue. A modest reduction in glycogen levels was similar in diabetics and nondiabetics (Table 3). Slight reductions of potassium and gain of sodium in this area of muscle previously have been shown to be related to an enlarged extracellular space. However, our diabetic dogs exhibited a significant increase in water content of the nonischemic segment. This acute change may have contributed, with the accumulated glycoprotein, to the enhanced diastolic stiffness manifested as a larger rise of end-diastolic pressure in relation to end-diastolic volume.

Although the declining blood pressure initially may have contributed to reduced end-diastolic volume by virtue of the smaller afterload, the significantly greater rise of left ventricular end-diastolic pressure suggests that this was not a major factor in altered pressure-volume relationships during ischemia. A pressure decline approximating that which occurred in diabetics has been shown to effect a relatively small (less than 4 ml) reduction of end-diastolic volume. The greater reduction of stroke output in diabetics is at least partially related to reduced diastolic filling of the ventricle rather than impaired systolic performance, since neither ejection fraction nor the index of contractility were significantly lower than in normals sub-

Table 3 Substrate Composition of Left Ventricle

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ject to ischemia. This was also manifested in the stroke work to end-diastolic pressure relationship, which was more abnormal than in nondiabetics and was independent of heart rate change (Fig. 1).

The modest reduction of aortic pressure in the diabetics was largely due to the reduction in stroke volume, since there was no significant difference in the calculated mean peripheral resistance when this group was compared to the nondiabetics. In two of nine diabetic dogs that exhibited marked hypotension, the drop in arterial pressure was in excess of the decline in stroke volume, so that the vasomotor response of the peripheral vasculature appeared to be inappropriate.

Coronary blood flow to the ischemic myocardium when the coronary artery is completely occluded is presumed to represent collateral flow. In this study the perfusion level in ischemic tissue appeared to be comparable in both normal and diabetic groups undergoing ischemia. Although morphological abnormalities have been observed in the media of intramural vessels in this animal model, this did not appear to effect luminal narrowing. The observation of collateral flow levels which were comparable to the nondiabetic group at this stage of diabetes implies a functionally similar microvascular response to the vasodilator stimulus of ischemia. The fact that the transmural distribution of potassium and sodium ions in the ischemic area was similarly altered in both groups supports the view that blood perfusion of the inner and outer wall was quantitatively similar.

In addition to the anatomical alterations of the ventricle in the diabetic dogs, the altered response to ischemia may be related to changes in energy production and utilization. Enhanced glycolysis is a feature of the metabolic response in ischemic muscle. In view of the similarly reduced glycogen levels in diabetics and in nondiabetics after 60 minutes of ischemia, the glycolytic response does not appear to be impaired by diabetes of this degree and duration. On the other hand, endogenous lipolysis does not seem to contribute significantly to substrate availability for energy production, since the elevated levels of triglyceride characteristic of the diabetic heart were not altered by ischemia. Because acute ischemia effected by a complete coronary occlusion in the normal dog results in severe depletion of high energy phosphate and inhibits the formation of calcium-myosin complexes, it remains to be shown that the diabetic state significantly intensifies these biochemical abnormalities.

Acknowledgments

We gratefully acknowledge the expert technical assistance of B. Jenkins and A. K. Gandhi and the secretarial assistance of A. Brown and A. Binetti in the preparation of this manuscript.

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Circ Res. 1977;40:577-583
doi: 10.1161/01.RES.40.6.577

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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