Arrhythmia susceptibility and myocardial composition in diabetes. Influence of physical conditioning.

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Research Article

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Arrhythmia Susceptibility and Myocardial Composition in Diabetes

Influence of Physical Conditioning

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Abstract

Abnormal myocardial composition in diabetes mellitus has been described, but the effects on ventricular vulnerability have not been defined. We have assessed the susceptibility to arrhythmias in a canine model after 1 yr of mild diabetes induced by alloxan. Since physical conditioning can affect metabolic abnormalities in diabetes, this intervention has also been evaluated. Group 1 served as controls and groups 3 and 4 were diabetic. Animals in the latter group as well as nondiabetic controls of group 2 were exercised on a treadmill for the last 8 mo of the experiment. After 1 yr, anesthesia was induced with chloralose for vulnerability studies. The ventricular fibrillation threshold of 24.4±1.9 mA in group 3 was significantly less than in normals (45.1±2.2). Spontaneous arrhythmias were also more prevalent in diabetics during acute ischemia (group 3-A). Increased ventricular vulnerability after epinephrine infusion was present in the sedentary diabetes despite normal ventricular function responsiveness. In a superfused preparation of myocardium, resting membrane potential and action potential amplitude were normal in diabetics, and beta-adrenergic stimulation shortened repolarization more than in controls. Myocardial collagen concentrations, which included an interfibrillar distribution on morphologic examination, were increased in group 3. In the trained diabetics of group 4 the basal vulnerability thresholds and responses to epinephrine were normal. While myocardial collagen levels were normal, cholesterol and triglyceride increments persisted. Thus, in mild experimental diabetes, enhanced susceptibility to arrhythmias exists; this susceptibility may be based on a combination of nonhomogenous collagen accumulation affecting local conduction and increased electrophysiologic sensitivity to catecholamines.

Introduction

Abnormalities of the myocardium have been described in animals with spontaneous (1) or experimental diabetes (2, 3) as well as in human diabetics (4). Under conditions of mild diabetes without ketoacidosis, modest impairment of left ventricular function was present (2). Since myocardial composition was found to be abnormal particularly in terms of collagen accumulation, the question of an associated alteration in electrical properties has been examined.

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We have undertaken a study of electrical vulnerability in the myocardium of a canine model after 1 yr of alloxan-induced diabetes. Since altered cardiac cell action potentials have been described in experimental diabetes with substantial hyperglycemia (5), the action potential characteristics in this model of mild diabetes have also been examined. In addition, we have assessed the occurrence of spontaneous arrhythmias during acute myocardial ischemia.

Chronic exercise is known to improve glucose tolerance (6) and has been shown to increase the fibrillation threshold in the basal state as well as during ischemia in nondiabetics (7). However, it is not known whether the cardiac complications of diabetes are affected by endurance exercise. In these experiments, altered myocardial composition has been related to observations on ventricular vulnerability.

Methods

We studied healthy male mongrel dogs, 2–4 yr of age, weighing 22–26 kg. The dogs had no clinical evidence of disease during the initial 8-wk period. Hematocrit, serum albumin, and electrolytes as well as glucose tolerance tests were normal before admission to the study groups. All the animals were fed the same diet consisting of 8% fat, 22% protein, 58% carbohydrate, 9% ash, and 3% crude fiber. Individual animals were placed sequentially in the four groups up to the limit of the number planned for the given group.

For studies of ventricular vulnerability and composition, the following groups were formed. Group 1 consisted of 11 nonexercised controls, and group 2 had five normal controls that were exercised for 8 mo. The latter group was limited in size due to time required for daily exercise of a total of 14 dogs. Diabetics of group 3 (n = 9, nonexercised) and group 4 (n = 9, exercised) received serial low doses of alloxan. Supplemental studies to further define the sedentary diabetics were performed in additional animals as indicated below to assess action potential characteristics and arrhythmias during ischemia.

Diabetic model. To produce mild diabetes, alloxan monohydrate in sterile saline was administered intravenously in a dose of 20 mg/kg at 3-mo intervals. Larger doses of alloxan were avoided to prevent ketosis. Glucose tolerance was measured before and at 4-mo intervals after the initial alloxan in the unanesthetized dog and serial blood samples were taken as previously described (2). Plasma glucose was analyzed by the glucose-oxidase method (8) to calculate the disappearance rate from the vascular compartment.

This diabetic model exhibits reduced glucose tolerance and plasma insulin levels after intravenous glucose (2) as well as abnormal postprandial glucose concentrations (9). Reduced ventricular compliance and histochemical changes in the renal mesangium (2) are consistent with changes in human diabetes (10, 4). Hemoglobin A_{1c} levels (11) in this model were found to rise from 2.41±0.31% to 3.95±0.33 ($P < 0.004$; n = 7) (unpublished studies).

As to the question of a direct toxic effect of alloxan on myocardium, we have previously observed that myocardial abnormalities were not present when the pancreatic effects of alloxan and resultant diabetes were prevented (2). For this current study, three animals separate from groups 1–4 received a bolus injection of glucose to block the pancreatic

effects of alloxan. Ventricular vulnerability was assessed initially and after 1 yr when collagen content was also determined. These were compared with four separate normal controls 9–12 mo after the initial vulnerability assessment, which also provided information on long-term reproducibility.

Physical conditioning consisted of running on a treadmill (Warren E. Collins, Inc., Braintree, MA) at a rate of 8 mi/h on an 18° incline for 30 min/d. The animals exercised 5 d/wk for 32 wk after an initial 1–2-wk period of graded exercise (12). Two dogs were excluded from the project due to inadequate performance in this initial period. Basal heart rate was measured on at least two separate occasions before beginning the study and toward the end of the training period with the dog lying in a quiet, relaxed state. At these same periods blood was taken through intracath tubing for analyses of plasma lactate (13), before and at the termination of exercise to assess development of the conditioned state. All dogs were housed in pens large enough to permit free movement, with ambulation outside the pens for 10–15 min/d.

Ventricular vulnerability. Animals were tested under sterile conditions for susceptibility to arrhythmias under chloralose anesthesia, 100 mg/kg, with supplements as needed. Ventilation was regulated by a Harvard pump (Harvard Apparatus Co., Inc., S. Natick, MA) via a cuffed endotracheal tube, and 40% oxygen was used to prevent hypoxic episodes. Arterial pH was maintained within the range of 7.36–7.42 by adjustment of respiratory rate. Body temperature was monitored with a rectal thermocouple, and when below 37°C, was corrected by use of an infrared lamp focused on the ventral surface of the animal.

All the animals in the four groups had studies of the repetitive ventricular response threshold as an index of vulnerability after 1 yr. When it became apparent that this vulnerability index does not always parallel the fibrillation threshold (14), determinations of the ventricular fibrillation threshold were also performed. This measurement was made in eight of 11 animals in group 1, two of five in group 2, seven of nine in group 3, and seven of nine in group 4.

In addition to intergroup comparisons, control measurements before induction of diabetes were made in some of the diabetics. Four animals of group 3 and three in group 4 were evaluated before alloxan administration and 1 yr later.

At least 1 h elapsed after anesthetic induction before the formal study began (15). Electrical testing was accomplished with an intracavitary lead system, consisting of a transvenous tripolar catheter (platinum electrode with an interelectrode distance of 1 cm and pole width of 3 mm; N23-7466; Electro-Catheter Corp., Rahway, NJ) and electrocardiogram recording probe (semifloating probe No. 567; Electro-Catheter Corp.). The catheter system was positioned via jugular vein under fluoroscopic control at the apex of the right ventricle, with the distal cathodal electrode localized in the trabeculae. A pacemaker (Medtronic, Inc., Minneapolis, MN) was employed to deliver rectangular pulses, 2 ms in duration, to maintain a constant heart rate of 200 beats/min, adjusting pacemaker current to twice the middiastolic threshold. Test pulses were generated with an electrically isolated square-wave pulse generator (S44; Grass Instrument Co., Quincy, MA) and an operational power supply to produce constant stimuli. The electrical output of this unit was isolated and calibrated with an oscilloscope current probe (P6021; Tektronix, Woodbridge, NJ). The pulse generator was equipped with appropriate circuitry to shut off the output of the pacemaker for 3.0 s after delivery of the test stimulus. The test impulse was delivered after every 10th–15th paced beat.

A single bipolar stimulus rather than a train of pulses was used in view of the evidence that the latter elicits local release of norepinephrine (16). Electrical scanning was conducted at 3 ms intervals beginning at the end of the effective refractory period and terminating 10 ms after the T-wave. Using electric pulses of 2 ms duration the stimulus intensity was increased in 2 mA steps and scanning continued until repetitive extrasystoles were elicited. The lowest stimulus intensity that evoked repetitive extrasystolic beats in two of three trials defined this threshold. To determine the ventricular fibrillation threshold we used a test stimulus duration of 5 ms, since the shorter pulse duration in preliminary studies of normals occasionally required an unusually high current to produce fibrillation, resulting in a high variance. In addition, in recent published studies, the 5-ms duration has been in common use in determinations of fibrillation threshold that do not involve acute ischemia (17). Defibrillation was accomplished with a direct current pulse of 50–100 W/s, delivered through a pair of copper plates (150 cm^{-2}). This was usually effective within 10–15 s and a 1-h interval was allowed when the animal was retested.

After establishing the thresholds for each animal in the basal state, the repetitive extrasystolic response to *l*-epinephrine was assessed since this hormone is known to affect vulnerability (18), and can be present in enhanced concentrations in diabetics under some circumstances (19). The hormone was infused intravenously at a rate of 0.05 to 0.1 μg/kg per min. Metabolic responses were compared by assay of plasma free fatty acids (20) and K^+ concentrations. At the end of the above studies, clots were removed, all vessels were sutured, and the skin was closed.

Conduction intervals. An electrode catheter was passed under fluoroscopy via a carotid artery to the aortic root immediately below the valve to record a His bundle electrogram before the vulnerability studies (21). Bipolar recordings were made with a multiple electrode switch box. The output was led into the A-C input of an electrocardiogram preamplifier, filtered at 40–500 cps, and recorded on photographic paper at a speed of 200 mm/s with an Electronics for Medicine recorder (White Plains, NY). The first rapid component of the His impulse was taken as its onset. His-Q times have been reproducible over 1–2 h of observation in the dog (21) and during acute atrial pacing (22). In addition to the P-R and corrected Q-T times, the QRS duration was taken as the maximum observed value in the standard leads. Mean values were obtained by hand to the nearest 0.5 ms from three representative beats in the basal state.

Hemodynamics. 2 wk later, the left ventricular mechanical response to catecholamines was determined for comparison with the prior vulnerability changes during a repeat infusion of *l*-epinephrine in the anesthetized state. While vulnerability was tested in the right ventricle, we have assumed that the hemodynamic response compared with controls would be similar in both ventricles of the diabetic animals, particularly since the compositional changes vs. controls described below were comparable. We have previously found that the *l*-epinephrine dose range of 0.05–0.1 μg/kg per min elicits a minimal to moderate positive inotropic response in the left ventricle (23). Since the peak maximal rate of rise of ventricular pressure (dP/dt)[1] in the right ventricle, in contrast to the left, occurs after the isovolumic period (24), the use of dP/dt_{max} is associated with somewhat greater error in the right as compared with the left ventricle. Thus, the latter site was considered preferable, particularly when testing at low intropic doses.

No. 8 Goodale-Lubin catheters (U.S. Catheter Div., C. R. Bard, Inc., Billerica, MA) were placed in the left ventricular chamber and proximal aorta from the carotid arteries under sterile conditions. Statham transducers were used for the measurement of left ventricular (P23Gb) and aortic (P23Db) pressures, which were recorded on an amplifier-recorder system (Electronics for Medicine). The transducers were placed at the midthoracic level and balanced for equal sensitivity. The maximal rate of rise of left ventricular pressure (dP/dt) was obtained using a resistance-capacitance differentiating circuit. The system had a linear frequency response from 0–30 cycles/s. Systolic ejection period was measured from superimposed aortic and left ventricular pressure pulse tracings. End-diastolic pressure was measured over at least three respiratory cycles and averaged. These measurements have been found to correlate well with the pressure measurements obtained using a micromanometer tip (Millar) catheter (25). As an index of myocardial contractility we used the velocity of contractile element (Vce) at peak dP/dt, calculated as the ratio of maximal dP/dt related to the simultaneous left ventricular pressure (26).

1. *Abbreviations used in this paper:* APA, action potential amplitude; APD_{50}, action potential duration at 50% repolarization; APD_{80}, action potential duration at 80% repolarization; dPdt, maximal rate of rise of ventricular pressure; MDP, maximum diastolic potential; Vce, velocity of contractile element.

Myocardial composition. 2 wk were allowed for recovery and the animals were reanesthetized. 1 h later the thorax was incised and the heart rapidly arrested with iced Ringer's solution. Transmural samples from both ventricles were placed in liquid nitrogen for glycogen assay (27). Both ventricles and septum were separately weighed. Samples were taken from the inner and outer halves of left ventricle, the septum, and right ventricle for hydroxyproline analyses to estimate collagen concentration (28). Transmural samples of left ventricle were extracted for assay of cholesterol (29), phospholipid (30), and triglyceride (31). For cation analysis the samples were homogenized and extracted for 48–72 h in distilled water, sufficient time for complete extraction. Potassium and sodium were determined on an Autoanalyzer system (Technicon Instruments Corp., Tarrytown, NY) with flame attachment. We determined water content of the myocardium by drying the tissue samples in an oven at 100°C to constant weight.

For a histochemical assessment, transmural samples were taken as unknowns from the mid-left ventricle of three animals per group. These examinations included trichrome stain for connective tissue (32, 33). For electron microscopy, transmural samples were fixed in a 2.5% cold glutaraldehyde buffered with phosphate, washed, postfixed in osmium, exposed to lead and uranyl acetate, and embedded in epon. Eight 1-μm sections were prepared for each dog. Approximately ten micrographs were obtained from each section to acquire a longitudinal orientation at an appropriate magnification.

Acute myocardial ischemia. After completion of the above studies, we proposed to determine whether spontaneous arrhythmias during acute myocardial ischemia had a different prevalence in diabetic animals than controls. Eight male mongrel dogs received alloxan, as above. These sedentary diabetics (group 3-A) were compared with normal controls (group 1-A), matched for age and sex.

After 11–12 mo the dogs were anesthetized with chloralose. Oxygenation, body temperature, and blood pH were maintained as described above. With chest intact, a double lumen No. 5 balloon-tipped catheter was positioned via the carotid artery. Coronary angiography was performed before ischemia to determine the distribution and course of the left anterior descending coronary artery. Two normal dogs and one diabetic dog were excluded because the anterior descending artery did not course to the apex or a major oblique vessel originated close to the origin of the anterior descendens, thus diminishing the area at risk. After balloon inflation in the proximal 1.5 cm of the left anterior descending coronary artery (34), transmural ischemia was evidenced by a reduction of mean peripheral coronary artery pressure to ~25 mmHg and the appearance of an injury potential on standard lead 1. No cardioactive agents were given.

The animals were observed up to 15 min, a risk period for arrhythmias. This limit was imposed since this diabetic model exhibits a greater degree of left ventricular dysfunction during ischemia over time than do normals (34), which may represent an additional variable affecting outcome. Arrhythmias were recorded at a 25 mm/s paper speed on a recorder (Electronics for Medicine) with automatic developer.

Ventricular tachycardia was defined as a series of six or more consecutive ventricular ectopic complexes (35). We examined the total incidence of ventricular tachycardia and fibrillation in the two groups as well as the sum of ventricular ectopic complexes (35, 36). In those that survived 15 min, the heart was cold-arrested with iced Ringer's solution, and Evan's Blue dye was simultaneously administered via the coronary artery catheter at aortic-diastolic pressure levels to determine the area at risk. In animals that fibrillated, the injection pressure was that of the residual aortic pressure.

Electrophysiological studies in vitro. To determine whether this model of diabetes was associated with altered cell action potentials, we recorded intracellular potentials (37), and the response of these potentials to isoproterenol in four normals (group 1-C) and three diabetics (group 3-C) prepared as above. After ~1 yr the heart was cold-arrested, and transmural samples were rapidly taken from the free wall of the left ventricle 2.5 cm above the apex and placed in cool oxygenated physiologic Tyrode's solution with the following millimolar composition: NaCl, 135; KCl, 4.0; dextrose, 5; $CaCl_2$, 2.0; $MgCl_2$, 1.0; $NaHCO_3$, 15; Na_2HPO_4, 1.0. After removal of the epicardial two-thirds of the ventricular slice, the endocardial tissue was pinned with surface up in a 30-ml chamber and superfused at 10 ml/min of Tyrode's equilibrated with 95% O_2/5% CO_2. Temperature was maintained at 37.0±0.5°C and the pH at 7.3 to 7.4.

The isolated myocardium was stimulated through bipolar silver-wire electrodes at a rate of 60 min^{-1} beginning 30 min after superfusion was begun. The stimulating electrode was located 5–10 mm from the recording electrode. Rectangular pulses, 1–5 ms in duration and twice threshold voltage, were generated by a stimulator (Grass Instrument Co.) isolated from ground. Transmembrane action potentials were recorded through glass capillary microelectrodes filled with 3 M KCl at a tip resistance of 5–15 megohms. Microelectrodes were coupled by a silver-silver chloride wire to a high input impedance amplifier with capacitance neutralization. The depth of the fluid over the preparation was maintained at a minimal level to reduce capacitance in the recording system. The output of the amplifier was displayed on an oscilloscope and photographed with an oscilloscope camera (C4; Grass Instrument Co.). Action potential amplitude (APA), maximum diastolic potential (MDP), and action potential duration at 50% (APD_{50}) and 80% repolarization (APD_{80}) were analyzed from this film. After an initial 30-min equilibration, action potential characteristics were documented by recording Purkinje type action potentials from the endocardial surface and ventricular muscle potentials from beneath the subendocardial layers; 7–15 cells were sampled from each animal.

After control recordings, incremental doses of freshly prepared isoproterenol were superfused, 30 min for each dose, at which time steady state measurements were taken. Doses ranged from 10^{-8} to 10^{-5} M. Subsequently, drug-free solution was superfused to check for reversibility of isoproterenol effects.

Statistics. The data are expressed as mean±SE. When only one statistical comparison was employed between a control and intervention, Student's t test for paired data was used. To assess significance between groups, analysis of variance was performed using Duncan's multiple range test for comparisons when F values were significant, at an α level of <0.05 (38). Fisher's exact test was used to evaluate arrhythmias in the acute myocardial ischemia study. Action potential duration responses to graded doses of isoproterenol were compared by a t test for differences in regression line elevation (38).

Results

Body weight in animals of the four groups was maintained over the 12 mo of observation while plasma albumin and electrolytes remained within the normal range (Table I). Diabetics of groups 3 and 4 exhibited a reduction of glucose tolerance that was similar in extent at 4 mo after the initial alloxan with a G_k of 1.91±0.27 and 1.82±0.14, respectively. Fasting normoglycemia was not affected by the exercise regimen in group 4 diabetics, but at 1 yr the glucose clearance constant was increased to 2.40±0.15 ($P < 0.05$), without a change in group 3.

Evaluation of the models. To examine the potential cardiotoxic effects of alloxan, we prevented the development of glucose intolerance in three animals after pretreatment with 50 g of glucose before each alloxan dosage. The fibrillation threshold was unchanged after 1 yr in these animals as well as in four normals followed for 9–12 months without such treatment (Table II). Collagen concentrations in transmural samples of left ventricle, as a marker of diabetic changes, were 2.14±0.19 μg/mg dry weight in the glucose-alloxan group vs. 2.09±0.11 in normals. Thus, a cardiotoxic effect of this compound appears unlikely.

Adaptation to chronic exercise was indicated in groups 2 and 4 by a reduction of basal heart rate in the conscious state and the failure of blood lactate to rise during acute exercise (Table I). In addition, left ventricular weight, as a measure of hypertrophy, increased similarly in these two groups.

Ventricular vulnerability in diabetics. In control animals for the diabetic series (group 1), the repetitive extrasystolic threshold

Table I. A. Plasma Composition Before and During Diabetes

	Fasting glucose		Glucose clearance constant*		Albumin		Potassium		Sodium		Calcium		Phosphate	
Group	C‡	E‡	C	E	C	E	C	E	C	E	C	E	C	E
	mg/dl	*mg/dl*			*g%*	*g%*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mEq/liter*	*mg/dl*	*mg/dl*
1 (n = 11)	83±5.9	81±4.9	3.58±0.36	3.49±0.38	3.3±0.11	3.4±0.05	4.1±0.14	4.0±0.31	143±3	139±2	5.7±0.22	5.2±0.41	3.6±0.5	3.4±0.8
2 (n = 5)	78±4.7	72±5.8	3.49±0.42	3.61±0.32	3.4±0.32	3.2±0.21	4.2±0.12	3.9±0.26	141±1	138±2	5.5±0.17	5.4±0.27	3.3±0.28	3.5±0.24
3 (n = 9)	84±5.4	92±6.6	3.71±0.31	1.82±0.20§	3.5±0.20	3.4±0.15	4.3±0.07	4.4±0.11	142±2	138±3	5.4±0.18	5.5±0.30	3.5±0.25	3.3±0.27
4 (n = 9)	87±6.1	80±4.3	3.66±0.29	2.40±0.16§	3.7±0.19	3.5±0.14	4.4±0.15	4.3±0.10	143±2	143±2	5.6±0.12	5.5±0.17	3.4±0.19	3.6±0.21

B. Responses to Physical Conditioning

	Body weight		Basal heart rate			Plasma lactate	
Group	C	E	C	E	Heart weight E	B**	A
	kg	*kg*	*min*	*min*	*g/kg*	*mM/liter*	*mM/liter*
1	23.8±0.6	24.9±0.8	73±4	75±6	4.30±0.29	1.98±0.13	12.8±1.9§
2	25.1±1.0	24.6±0.7	78±5	59±3§	5.50±0.35‖	2.31±0.24	3.0±0.27
3	24.8±0.7	26.2±0.9	80±4	83±6	4.23±0.25	2.26±0.15	11.8±3.2§
4	23.9±0.5	24.6±0.8	82±4	62±3§	5.34±0.41‖	1.59±0.13	2.0±0.34

* C, control; E, value for last month of study. ‡ Obtained from a semi-logarithmic plot of plasma glucose decrements after an i.v. bolus of glucose expressed as units. Clearance constant is reduced in groups 3 and 4 vs. respective controls. § Paired t test; $P < 0.01$. ‖ Unpaired t test; $P < 0.05$. ** B, venous plasma lactate before acute treadmill exercise; A, after treadmill exercise. Lactate rise was significant at 12 mo only in the untrained dogs ($P < 0.01$) and group 4 vs. 3 ($P < 0.01$).

*Table II. Serial Ventricular Fibrillation Threshold in Normals**

Animal no.	Initial	12 mo
	mA	*mA*
1	48*	46
2	53*	55
3	42*	41
4	39	43
5	45	39
6	38	35
7	49	48
Mean±SE	44.85±2.09	43.86±2.47

* Indicates animals that received glucose-alloxan after the initial determination of fibrillation threshold. The other four animals did not receive glucose or alloxan.

was 45.8±2.0 mA in the basal state after 1 yr (Fig. 1). The threshold was significantly lower in the nontrained diabetics of group 3 at a level of 27.5±3.8 mA. By contrast, in the trained diabetics of group 4, this threshold at 12 mo was significantly higher than in the sedentary diabetics, did not differ from group 1 controls, but was less than the level in the conditioned normals (Fig. 1).

Evaluation of the ventricular fibrillation threshold in the basal state revealed a significant reduction in the nontrained diabetics of group 3 compared with controls of group 1 (Table III). In the trained diabetics of group 4 the threshold was significantly higher than that of the sedentary diabetics and approximated that of the normal controls. Only two of the exercised normals of group 2 were tested and had fibrillation thresholds of 51 and 53 mA.

In the subset of group 3 animals with measurements before induction of diabetes as well as 1 yr later, the extrasystolic and fibrillation thresholds were significantly reduced at the terminal studies (Table IV). The group 4 subset showed no change in either parameter from the prediabetic period.

To assess the response to adrenergic stimulation, epinephrine was infused at a rate of 0.05 and 0.1 μg/kg per min. The extrasystolic threshold was reduced to a significantly greater degree in the nontrained diabetics vs. group 1 controls (Fig. 2). In the trained diabetics, on the other hand, the threshold at the higher dose was significantly greater than in the nontrained diabetic, did not differ from the controls of group 1, but was significantly less than the trained normals.

During epinephrine infusion mean plasma K^+ levels declined ~10% from the initial normal levels but there was no difference

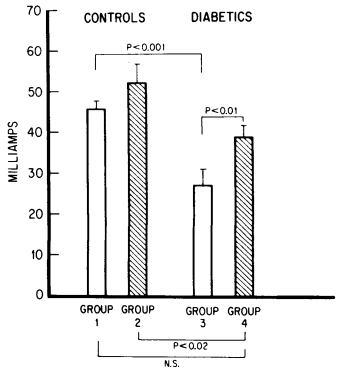

Figure 1. Thresholds for repetitive ventricular ectopic responses. ▨, exercised. Group 3, the nonexercised diabetics (n = 9), had a significant reduction of threshold compared with group 1 controls (n = 11). In the trained diabetics of group 4 (n = 9), the threshold was significantly higher than in group 3 diabetics, lower than group 2 (n = 5), and similar to group 1.

*Table III. Ventricular Fibrillation Threshold**

Group 1	Group 3	Group 4
mA	*mA*	*mA*
38	27	41
42	31	38
44	18	43
45	22	36
43	20	37
48	31	46
49	22	48
52	—	—
45.1±1.6	24.4±1.9	41.3±1.7

* Threshold for individual animals as determined after the repetitive ventricular response. Unpaired t test: group 1 vs. 3, $P < 0.005$; group 1 vs. 4, N.S.; group 3 vs. 4, $P < 0.01$.

between controls and diabetics (Table V). Basal plasma free fatty acid (FFA) levels were also similar between group 1 and the two diabetic groups, with similar elevations during the infusion of epinephrine. Group 2 controls differed from group 1 with a higher basal FFA level. Neither plasma K^+ decreases nor elevation of plasma FFA appeared to contribute to the vulnerability differences in untrained and trained diabetics.

During the hemodynamic study 2 wk later, left ventricular performance during administration of epinephrine was compared in terms of velocity of contractile element (Vce) and filling pressure (Table V). The responses in diabetics of groups 3 and 4 did not differ from those of the normals.

Conduction times. In the basal state, P-R and QRS on lead

Table IV. Serial Vulnerability Thresholds

	Group 3		Group 4	
	Control	12 mo	Control	12 mo
	mA	*mA*	*mA*	*mA*
A. Repetitive ventricular extrasysoles				
	35	25	37	36
	47	22	41	45
	43	27	46	44
	36	28	—	—
Mean±SE	40.3±2.8	25.5±1.3†	41.3±2.6	41.7±2.8
B. Fibrillation threshold				
	48	22	41	37
	42	20	43	46
	47	31	49	48
	38	22	—	—
Mean±SE	43.8±2.3	23.7±2.4‡	44.3±2.4	43.7±3.4

* Individual animals that were studied before induction of diabetes and 1 yr later.
‡ Paired t test, $P < 0.025$ (one-tail): $P < 0.05$ (two-tail).
§ Paired t test, $P < 0.0025$ (one-tail); $P < 0.005$ (two-tail).

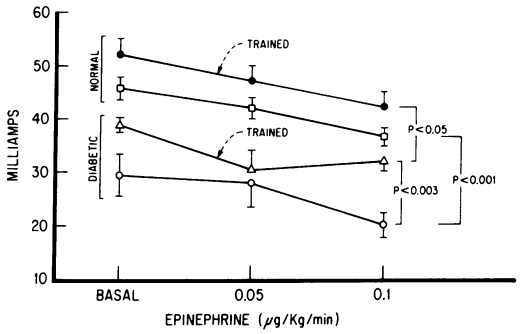

Figure 2. Repetitive ventricular response after epinephrine. The nonexercised group 3 diabetics (○) had a lower threshold at the higher epinephrine dose than the exercised diabetics of group 4 (△) and group 1 controls (□). Group 2 was significantly higher than group 4.

II of the EKG times did not differ in the diabetic groups vs. control (Table VI). In the exercised controls of group 2, P-R was significantly longer. The H-Q time was significantly prolonged in diabetics of group 3 compared with the nonexercised controls of group 1. Exercised diabetics had H-Q times that did not differ from the exercised controls of group 2.

Myocardial composition. The concentrations of collagen in the left and right ventricles as well as septum were significantly increased in group 3 diabetic animals (Table VII). These increments above controls were proportionately similar in each tissue, although concentrations were highest in the right ventricle of controls and diabetics. Collagen increments in the left ventricle were more evident in the subendocardium but this was not significantly greater than in the outer layers. Similar increments were not observed in the exercised diabetics of group 4 in which collagen concentrations approximate the normal control levels. To assess the effects of the hypertrophy of exercise on collagen accumulation, the content per total ventricle was calculated from muscle weight and collagen concentration (Table VII). Content was significantly greater in both the left and right ventricles as well as septum in group 3 but group 4 did not differ from controls.

Morphologic studies were performed to determine the distribution of collagen. Histologic sections of left ventricle stained with trichrome were negative in groups 1 and 2 (Fig. 3 *A*). However, in the sedentary diabetics of group 3, accumulation of blue-stained material was present around intramural vessels and accretions were present throughout interstitium (Fig. 3 *B*), which was negligible in group 4. On electron micrography, ultrastructure of cell organelles in the sedentary diabetic appeared to be within normal limits (Fig. 4). The interstitium contained fibrils with the periodicity of collagen and amorphous material around the capillary and nerve endings, as well as between adjoining cardiac cells.

Left and right ventricular glycogen concentrations were elevated in groups 2 and 4 but not in the sedentary diabetic (Table VIII). In groups 3 and 4, cholesterol and triglyceride concentrations were increased in both ventricles. Phospholipid concentrations in left ventricle for groups 1 through 4, respectively, were 16.8±0.7 μM/gm, 17.2±0.9, 16.7±0.8, and 17.0±0.6. There was also no significant difference in right ventricular concentrations between these groups. Sodium was also increased but potassium concentrations were normal in both diabetic groups (Table VIII). Water content as percent of left ventricular weight did not differ significantly between the four groups. In group 1 the value was 78.1±0.06%; group 2, 78.3±0.04; group 3, 79.8±0.07; group 4, 78.5±0.05.

Myocardial ischemia. To assess the incidence of spontaneous ventricular arrhythmias, we prepared additional animals to determine the response to a 15-min period of myocardial ischemia. The sedentary diabetic animals had a glucose clearance rate of 2.05±0.19, comparable to the levels in group 3 diabetics. After anesthetic induction, heart rate and aortic pressure were at similar levels in the control and diabetic animals before the onset of ischemia, and plasma electrolytes were normal. During coronary occlusion the aorta to peripheral coronary artery pressure gradient was 88±4.9 mmHg in the controls and 91±6.4 mmHg in the diabetics, and the sinus rate response was comparable (Table IX). The number of ectopic beats as ventricular tachycardia was significantly greater in the diabetic animals (Table IX). Fibrillation occurred in four of the seven diabetics and in two out of 12 in the normal group. When those with fibrillation and those with ventricular tachycardia alone were compared with nondiabetics, the difference was significant at $P < 0.05$. The area of

Table V. Responses to Epinephrine Infusion in Diabetics

	K^+	Free fatty acid	Heart rate	Aortic pressure mean	Left ventricular end-diastolic pressure	Vce*
	mEq/liter	*mEq/liter*	*beats/min*	*mmHg*	*mmHg*	
Group 1						
Basal	3.9±0.16	776±86	122±7	138±8	5.1±0.6	6.8±0.7
Epinephrine (0.1 μg/kg per min)	3.6±0.16	1,478±234	127±9	126±10	3.9±1.3	10.6±1.9
Group 3						
Basal	4.1±0.12	716±96	133±10	140±4	4.9±0.7	7.5±0.5
Epinephrine (0.1 μg/kg per min)	3.9±0.32	1,331±232	145±7	136±9	2.6±0.5	9.9±1.2
Group 4						
Basal	4.1±0.17	762±82	110±12	138±9	3.4±0.7	7.7±0.4
Epinephrine (0.1 μg/kg per min)	3.7±0.22	1,460±310	117±15	124±10	3.7±1.2	12.0±1.4

Unpaired *t* test. No significant differences in response to epinephrine between group 1 vs. groups 3 and 4. *Ratio of maximal dP/dt to simultaneous left ventricular pressure in cycle lengths/s.

Table VI. Myocardial Conduction in Normals and Diabetics

	Heart rate	PR	QRS	QTc	HQ
	beats/ min	*ms*	*ms*	*ms*	*ms*
Group 1 ($n = 6$)	128±11	71±7	65±2.6	324±13.7	25.0±0.8
Group 2 ($n = 5$)	94±15	96±8*	68±1.1	310±6.5	28.5±0.7‡
Group 3 ($n = 6$)	133±10	67±6	63±0.78	312±7	29.7±0.98‡
Group 4 ($n = 6$)	100±12	84±6	64±3.3	307±10	31.6±1.6‡

Unpaired t test vs. Group 7. * $P < 0.05$. ‡ $P < 0.01$.

risk in the region of the anterior descending artery was 33±1.8 g in the normal vs. 31.7±2.1 g in the diabetic group.

Action potentials. To determine whether electrophysiologic abnormalities were present in the sedentary diabetics, group 3-A was compared with group 1-A normals (Table X). In the basal state there was no difference in the MDP or APA in the myocardial cell between normals and diabetics. However, the duration of repolarization at 50 and 80% was slightly, but significantly, longer in the diabetic group ($P < 0.02$).

After isoproterenol, normals exhibited a significant shortening of the APD_{50} or APD_{80} without a change in MDP or APA (Table X). This response was significantly greater in diabetic animals. A plot of the log concentration of the adrenergic agonist vs. the action potential duration in myocardial cells reveals greater shortening at both repolarization durations (Fig. 5). In the presence of similar slopes the regression line levels differed in diabetics vs. normals ($P < 0.05$).

In the Purkinje fibers of the diabetics the MDP, APA, and the repolarization durations were not significantly different from normal in the basal state. There was, however, significant shortening at ADP_{50} and APD_{80} after isoproterenol in the diabetic group, which did not occur in normals.

*Table VII. OH-Proline Concentrations**

	Left ventricle			
Group	Inner ½	Outer ½	Septum	Right ventricle
1 ($n = 11$)	2.15±0.11	2.09±0.15	1.72±0.09	2.88±0.10
2 ($n = 5$)	2.16±0.28	1.88±0.26	1.64±0.21	3.0±0.29
3 ($n = 9$)	3.14±0.24‡	2.63±0.27§	2.41±0.28§	3.56±0.27§
4 ($n = 9$)	2.11±0.17	2.02±0.14	1.83±0.22	3.08±0.09

OH-Proline Content, Milligrams per Region of Heart

Group	Left ventricle‖	Septum	Right ventricle
1	40.9±2.9	15.4±0.7	23.3±1.2
2	42.4±5.9	16.0±2.5	26.7±3.4
3	59.8±3.3‡	25.0±2.2‡	30.9±3.0§
4	40.7±5.0	17.6±2.4	25.6±1.7

* Concentrations in μg/mg dry weight. ‡ $P < 0.01$. § $P < 0.02$.
‖ Contents in inner and outer halves of myocardium were averaged for each animal.

Discussion

These experiments were undertaken to determine whether a mild form of diabetes, 1 yr in duration, would alter the susceptibility of the myocardium to arrhythmias. This canine model is characterized by normal coronary arteries (2) and collateral blood flow response during coronary occlusion (34), as well as preserved ultrastructure of cardiac cells (8).

Sedentary diabetics. Diabetic dogs that were not physically conditioned showed changes in electrical vulnerability, with reduction of both the thresholds for repetitive extrasystoles and ventricular fibrillation. Moreover, a greater incidence of spontaneous ventricular arrhythmias was evident during acute ischemia of the anterior left ventricle. That arrhythmogenesis was affected in both ventricles with similar compositional abnormalities is supported by this observation.

Reduction of vulnerability thresholds in the sedentary diabetics may be related to several factors. Connective tissue septa normally present within individual muscle bundles in an intermittent distribution are considered to be associated with localized dissociation of excitation (39). Accordingly, the increase of collagen content in the diabetic myocardium in a nonhomogeneous distribution may exaggerate this phenomenon. This view is supported by the local electrode study, which revealed a modest increase of His-Q time, approximating that of an ethanol model of subclinical heart disesase (22). Assuming that this at least partially reflects subendocardial collagen accumulation, a local dispersion of refractory periods would facilitate reentrant activity and arrhythmias (40). This mechanism has been considered the basis for arrhythmias during threshold stimulation (41) as well as early in acute ischemia (42) in nondiabetics. Further, under appropriate conditions, triggered activity may be elicited in the diabetic (43).

Catecholamine responses. Several observations support the view that catecholamines may influence arrhythmia susceptibility in diabetics. Exogenous epinephrine was found to reduce the threshold for repetitive ectopic beats in sedentary diabetics and to shorten the repolarization phases of the action potential relative to normals. During the vulnerability test the sympathetic system in the myocardium appears to be activated at the site of the stimulating electrode, even by the single-impulse technique (16). Thus, an enhanced sensitivity to catecholamines in diabetics may partially account for the reduced fibrillation threshold in the basal state.

During ischemia a neurohormonal contribution to arrhythmogenesis is attested by the prevention of lethal ventricular arrhythmias after beta-blockade or chemical sympathectomy in nondiabetics (44). While a similar antiarrhythmic efficacy in mild diabetes would suggest a dominant role for this system, a quantitative comparison with normals would require assessment of the dose-response relationship to beta-adrenergic blockade. Although a reduction of vagal activity can contribute to arrhythmogenesis (45, 46), in experimental diabetes the efferent limb of this nerve has been reported to be unimpaired as judged by the heart rate response to vagal stimulation (47).

Altered responsiveness to catecholamines appears to be selective since the myocardial hemodynamic response did not differ between control and diabetic animals. In a diabetic rat model,

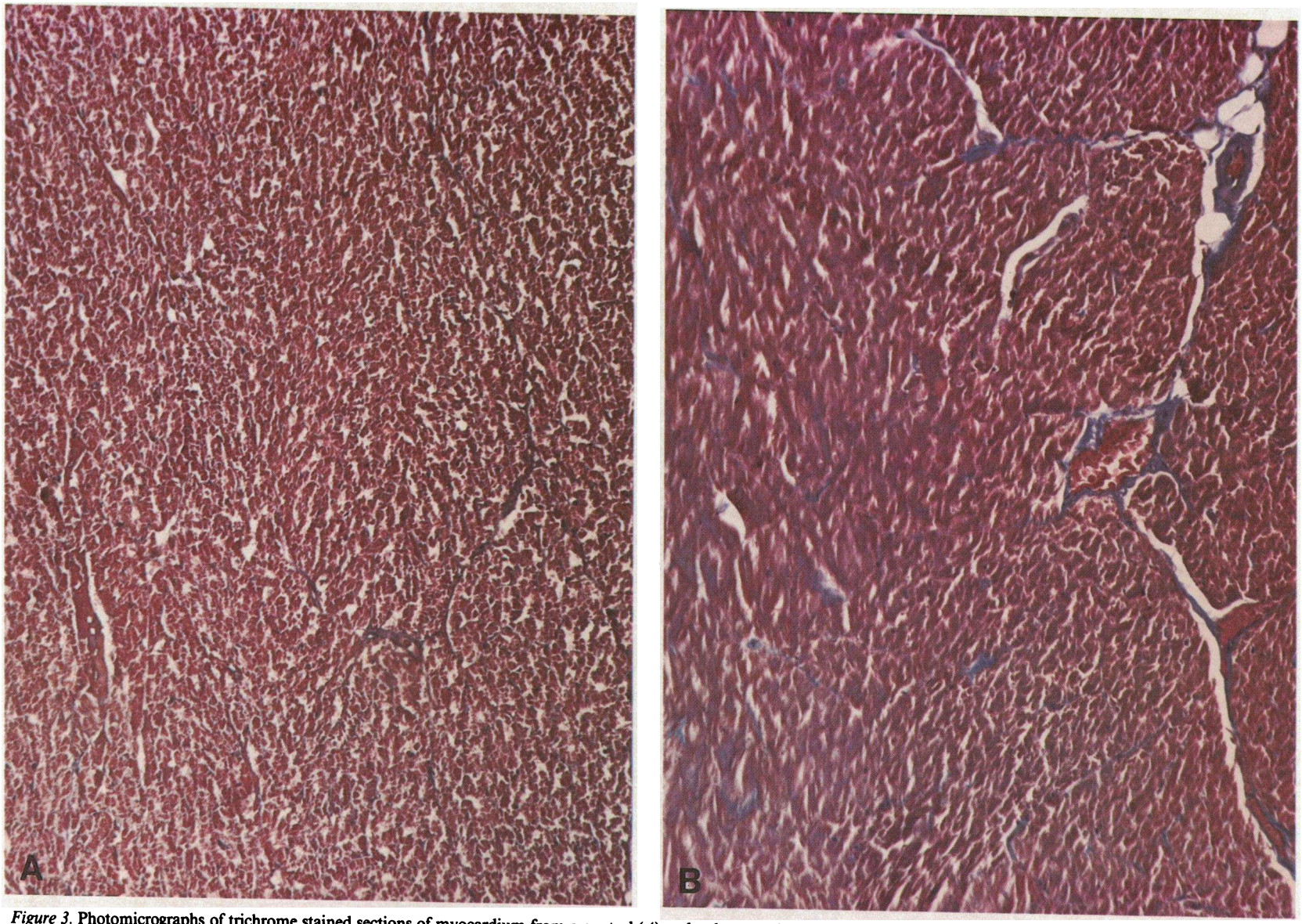

Figure 3. Photomicrographs of trichrome stained sections of myocardium from a control (*A*) and sedentary diabetic of group 3 (*B*). The control exhibits little trichrome positive material (× 140 in original before reduction). In the diabetic muscle trichrome material was present throughout the interstitium in a nonhomogeneous distribution (× 250 in original before reduction).

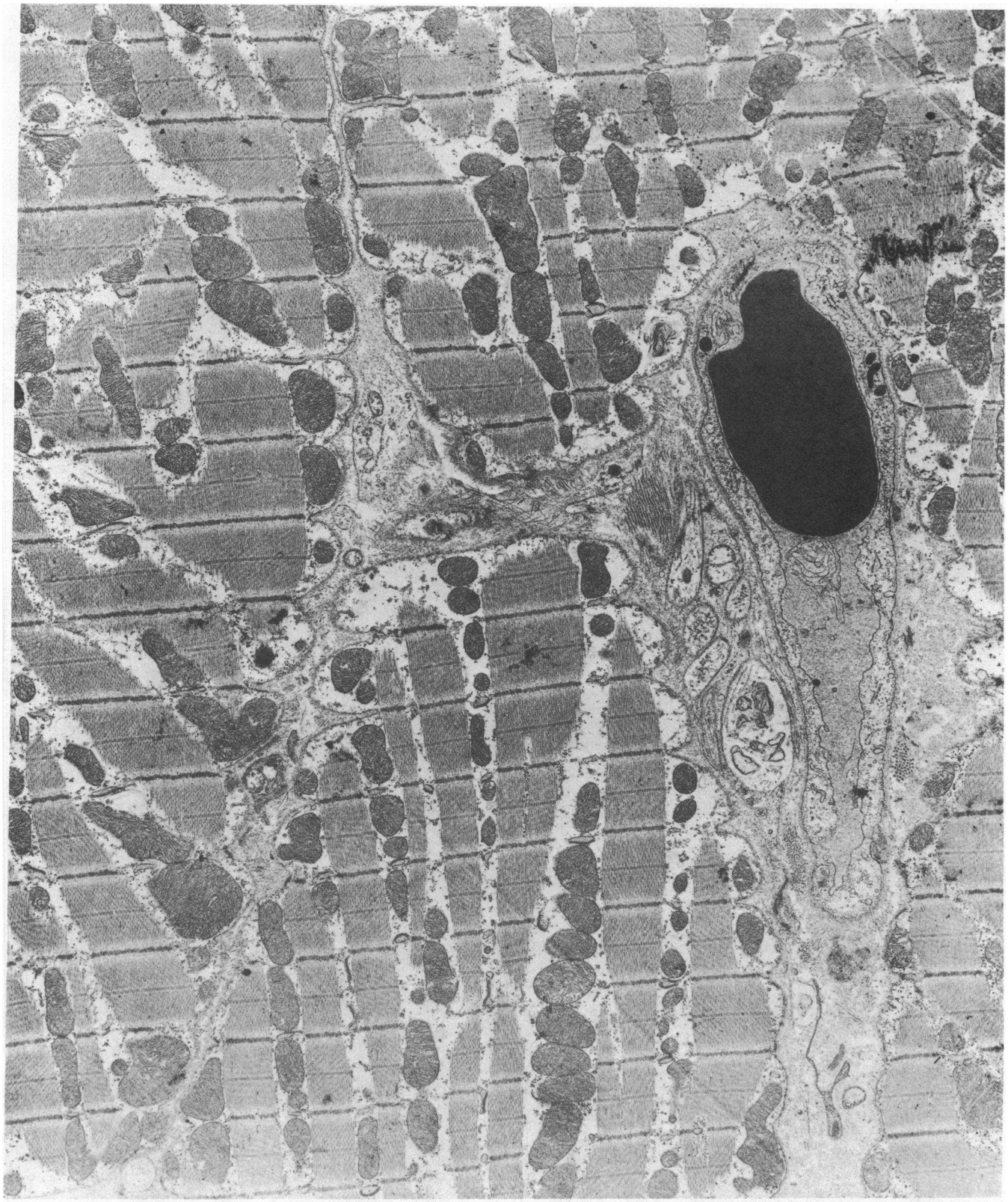

Figure 4. Transmission electron micrograph from a sedentary diabetic of group 3 (× 11,500 before reduction). Accumulation of fibrillar and amorphous material is seen throughout much of the interstitium, particularly in proximity to the capillary and nerve fibers. Some of the fibrils are seen in a plane that reveals the periodicity of collagen. Subcellular organelles appear to be normal.

Table VIII. Substrate and Cations in Transmural Left and Right Ventricular Samples

	Glycogen*		Cholesterol		Triglyceride		Sodium		Potassium	
	LV	RV	LV	RV	LV	RV	LV	RV	LV	RV
	mg/g	*mg/g*	*μM/g*	*μM/g*	*μM/g*	*μM/g*	*μEq/g*	*μEq/g*	*μEq/g*	*μEq/g*
Group 1 ($n = 11$)	3.3±0.2	3.8±0.4	3.57±0.43	3.39±0.41	2.2±0.11	1.9±0.13	34.9±1.4	31.3±1.7	72.1±2.0	73.6±1.9
Group 2 ($n = 5$)	5.4±0.4	5.0±0.6	3.78±0.56	3.45±0.61	2.0±0.13	1.8±0.27	35.2±1.6	33.6±2.0	74.0±2.9	75.4±2.3
Group 3 ($n = 9$)	3.5±0.3	3.4±0.5	4.86±0.57	4.77±0.52	4.2±0.46	3.9±0.38	42.7±2.0	40.3±0.8	76.2±3.1	76.5±2.8
Group 4 ($n = 9$)	6.1±0.3	5.8±0.4	5.68±0.82	5.39±0.73	3.9±0.41	3.6±0.49	43.2±1.2	39.5±1.2	75.8±2.7	76.7±3.2
Unpaired *t* test										
Group 1 vs. 2	$P < 0.02$	$P < 0.05$	NS	NS	NS	NS	NS	NS	NS	NS
Group 1 vs. 3	NS	NS	$P < 0.02$	$P < 0.02$	$P < 0.01$	$P < 0.03$	$P < 0.02$	$P < 0.01$		
Group 1 vs. 4	$P < 0.01$	$P < 0.02$	$P < 0.01$	$P < 0.01$	$P < 0.02$	$P < 0.05$	$P < 0.01$	$P < 0.02$		
Group 3 vs. 4	$P < 0.01$	$P < 0.02$	NS	NS	NS	NS	NS	NS	NS	NS

* These are concentrations of substrates and cations expressed per grams wet weight.

Table IX. Ventricular Arrhythmias During Acute Ischemia (15 Min)

Dog No.	Sinus rate change/min	Ectopics‡	Tachycardia‡	Fibrillation time
				min
Normals				
1	+13	14	—	—
2	+4	6	—	—
3	+2	—	—	—
4	+5	52	—	4
5	+8	15	614	—
6	+3	—	—	—
7	+12	28	—	—
8	+6	5	—	—
9	0	36	12	7
10	+7	—	—	—
11	+4	8	—	—
12	+3	14	—	—
Mean±SE	5.6±1.1	178	629	2/12§
Diabetics				
1	+8	—	1,134	—
2	+4	—	27	1
3	+5	5	48	2
4	+9	8	1,950	—
5	+11	—	745	9
6	+2	—	13	4
7	+6	12	—	—
Mean±SE	6.4±1.1	51	3,980‖	4/7§

* Sinus rate changes before arrhythmias or maximum change without arrhythmia.
‡ Number of ventricular extrasystoles, with the group total summed for each column; (—) indicates no ectopics or fibrillation.
§ Incidence of ventricular fibrillation; when combined with incidence of tachycardia in those without fibrillation, the difference in diabetics vs. normals was significant at $P < 0.05$.
‖ Unpaired t test vs. normals ($P < 0.01$).

a normal mechanical response of the heart to isoproterenol was associated with increased phosphorylase a activation (48). This supports the notion of a postreceptor locus for the selective exaggerated responses to catecholamines.

In superfused segments of ventricular muscle the action potential alteration after addition of isoproterenol was notably different in the diabetic. Both MDP and APD were normal in the basal state as well as after isoproterenol. While minimally prolonged in diabetic muscle in the basal state, after isoproterenol, repolarization at APD_{50} and ADP_{80} was shortened in myocardial cells to a greater extent in diabetics than in normals. This parallels the enhanced shortening after ouabain in the diabetic rat (5) and is of potential importance in the genesis of ventricular arrhythmias (49). Under circumstances of unequal access of the neuro-effector to cardiac cells due to patchy accumulation of collagen in the interstitium, discordant beta-adrenergic stimulation could lead to dispersion of refractoriness.

Physical conditioned state. It is assumed that normalization of the fibrillation threshold in the physically conditioned diabetics as compared to the sedentary group reflects a reduced susceptibility to arrhythmias. Despite the improved glucose tolerance this response may be nonspecific. Exercise trained nondiabetic animals have been shown to have a higher fibrillation threshold (7) and a diminished incidence of spontaneous fibrillation (50) during acute ischemia. Although the threshold response to *l*-epinephrine in our trained diabetics may also be nonspecific, the alterations of myocardial composition that occur in the trained diabetic may permit normalization of the hormonal effect as well as basal vulnerability.

Myocardial composition. The trained diabetics and normals manifested a significant increase of left ventricular glycogen in contrast to sedentary diabetics as previously reported (51). If glycogenolysis is enhanced in group 4 diabetics in response to catecholamines as occurs in trained normals (52), an increased availability of glucose to the cell may have contributed to the raised vulnerability threshold (53) after epinephrine in the conditioned state.

The reduction of myocardial collagen levels as a putative contributing factor to the normalization of vulnerability thresholds in exercised diabetics, provides support for the view that nonhomogenous accumulation of this fibrous protein in the sedentary diabetic is related to enhanced vulnerability. In contrast, triglyceride accumulation in the myocardium, consistent with diversion of fatty acid from phospholipid in the diabetic (2), was unaffected by endurance training. Similarly, cholesterol increments, presumably localized to sarcolemma (54), remained unaltered.

The normal hydroxyproline level in diabetics of group 4 was associated with minimal accumulation of trichrome positive material that was slightly more than the negligible levels of group 1 and less than the evident accumulation of group 3. A discordance between quantitative histochemistry and hydroxyproline assay has been observed (55). That trichrome can stain muccopolysaccaride (56) also suggests a degree of nonspecificity. Recently an approach to more accurately measure the area occupied by connective tissue has used composite digitized images to correct for the variation of color shades as well as for muscle fiber orientation (57).

The pathogenesis of collagen accumulation in the diabetic has not been established. This abnormality has been described in animals with experimental and clinical diabetes (9, 4), with suggestive evidence of diminished degradation (58). However, in severe diabetes hydroxyproline accumulation may not occur over the long term (59), a finding consistent with impaired synthesis of cardiac protein during sustained ketoacidosis (60).

An additional issue related to exercise is the observation that the His-Q time was increased in the physically conditioned normal as well as exercised diabetics. However, the levels would generally be considered within the normal range of up to 40 ms in this species (21). Available information in conditioned athletes with symptoms related to excess vagal tone indicates that the His-Q duration averaged 50 ms, which is near the upper limit of normal (61). Thus, this conduction interval may have been prolonged in groups 2 and 4 due to physical conditioning per se.

This study supports the view that glucose intolerance in humans can be associated with an increase in cardiac events (62), although less frequently than in frank diabetes. The influence of more severe diabetes and physical conditioning on the development of myocardial abnormalities remains to be determined.

Table X. Action Potential Parameters and Effects of the Beta Adrenergic Agonist

	Normals (group 1-A)					Diabetics (group 3-A)				
	No.	MDP	APA	ADP_{50}	ADP_{80}	No.	MDP	APA	APD_{50}	APD_{80}
		−mV	*ms*	*ms*	*ms*		*−mV*	*mV*	*ms*	*ms*
Cardiac muscle cell										
Basal	(44)	82.1±0.59	103.3±0.63	153.9±2.86	195.8±2.87	(42)	80.1±0.5	101.1±0.59	172.2±1.05*	215.0±1.59*
Isoproterenol										
$\times 10^{-7}$ M	(39)	81.9±0.86	102.8±0.92	135.4±3.39	166.5±3.63	(37)	79.4±0.98	102.3±1.18	113.3±2.74‡	143.9±2.08‡
Paired *t* test		NS	NS	$P < 0.02$	$P < 0.02$		NS	NS	$P < 0.002$	$P < 0.002$
Purkinje cell										
Basal	(23)	79.7±0.89	107.1±1.23	168.1±5.35	217.9±4.43	(19)	79.5±1.23	107.8±1.9	171.5±1.91	226.5±2.42
Isoproterenol										
$\times 10^{-7}$ M	(20)	81.2±2.9	110.4±2.35	170.3±2.67	209.4±3.2	(17)	80.1±1.56	108.7±1.15	136.8±3.64	183.6±5.52
Paired *t* test		NS	NS	NS	NS		NS	NS	$P < 0.01$	$P < 0.01$

Values are expressed as mean and SE; No. = number of impalements indicated in parentheses. * Unpaired *t* test for diabetics vs. normals in basal state: $P < 0.02$; none of the other basal parameters showed a significant difference. ‡ Statistical comparison of isoproterenol response in diabetics vs. normals was obtained from the dose-response differences in Fig. 5.

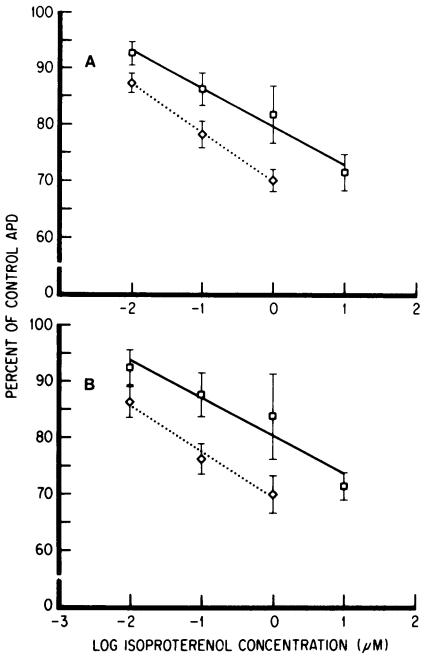

Figure 5. Plot of action potential durations versus concentrations of isoproterenol. Repolarization at APD_{50} (*A*) and APD_{80} (*B*) was significantly shorter in the sedentary diabetics (◇) vs. controls (□) ($P < 0.05$).

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