# *Hypertensive-Diabetic Cardiomyopathy in the Rat*

## *An Experimental Model of Human Disease*

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**The authors recently described a group of diabetic patients with severe congestive heart failure, hypertension, and minimal coronary artery disease, who had significant myocardial degeneration apparently secondary to the combined effects of high blood pressure and diabetes on the heart. To evaluate the effects of hypertension and diabetes mellitus more fully, the authors studied four groups of rats with either no disease, streptozotocin-induced diabetes mellitus, renovascular hypertension, or a combination of hypertension and diabetes. They employed semiquantitative light microscopy, which revealed significantly greater replacement fibrosis in the hypertensive-diabetic rats when compared with the other three groups. Interstitial fibrosis was increased in the hypertensive-diabetic animals, though it was just below the 5% level of significance when compared with the hypertensives. Further analysis, however, revealed that those hypertensive-diabetic animals with the greatest relative cardiac hypertrophy, as measured by the heart weight/body weight ratio, had significantly increased interstitial fibrosis. Surprisingly, diabetes mellitus alone produced no morphologic light-microscopic alterations; yet 8 weeks of combined hypertension and diabetes mellitus led to myocardial degeneration similar to the human disease. These changes do not appear to be secondary to abnormalities of intramyocardial muscular vessels. Measurement of 3 parameters of vascular disease revealed that hypertensive animals with less myocardial damage had greater vascular changes than the more severely affected hypertensive-diabetics. This study provides evidence that the combination of diabetes mellitus and hypertension produces significantly greater myocardial lesions than either disease alone. The similarity of the lesions with those observed in human patients suggests that the hypertensive-diabetic rat is a useful model for elucidating the pathogenesis of clinical myocardial disease in patients with hypertension and diabetes mellitus. (Am J Pathol 1981, 102:219–228)**

HYPERTENSION, which is present in 40–80% of patients with longstanding diabetes mellitus[1] has been implicated in the development of diabetic renal and retinal complications.[1-6] There is recent evidence that lowering the blood pressure in these subjects may ameliorate or prevent diabetic nephropathy.[7,8] Paralleling these observations on the pathogenesis and possible prevention of the more common diabetic complications, there is a growing awareness of another consequence of diabetes mellitus: cardiomyopathy. Several clinical and experimental reports have shown that varying degrees of left ventricular dysfunction, occurring independently of extramural coronary artery atherosclerosis, may be associated with diabetes mellitus.[9-21] However, the potential contribution of systemic hypertension to the development of myocardial failure in diabetes has not been determined.

Recently we described a cohort of diabetic patients with severe congestive heart failure and minimal coronary artery disease, who also had longstanding hypertension.[22] A portmortem histopathological and semiquantitative study supported the concept that there is a characteristic but nonspecific cardiomyopathy in diabetes mellitus with morphologic correlates that may be secondary to the combined effects of diabetes mellitus and high blood pressure. However, the retrospective nature of this study, with at-

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tendant difficulties in controlling for multiple variables, prompted us to examine the effects of diabetes and hypertension in an experimental animal model. Accordingly, we explored this question in a series of rats with either streptozotocin-induced diabetes mellitus and/or renovascular hypertension. The following report details our light-microscopic findings.

## Materials and Methods

Six-week-old male Sprague-Dawley rats weighing 175–200 g were made hypertensive by the placement of a silver clip with an internal diameter of 0.25 mm on the left renal artery. Hypertension, defined as a systolic blood pressure greater than 150 mm Hg, occurred approximately 2 weeks after surgery. Blood pressures were measured by tail cuff.[23] They were taken every week for the first 4 weeks and every 2 weeks thereafter.

Diabetes was induced with a single 10-mg (approximately 55 mg/kg body weight) dose of streptozotocin (The Upjohn Company, Kalamazoo, Mich.) dissolved in 0.5 ml of 0.9 % saline with 0.02 M sodium citrate, pH 4.5. Injections were made into the jugular veins of nonfasted animals. Bleeding schedules and methods of blood glucose measurement have been described previously.[24] Random determinations of blood glucose from diabetic rats always were greater than 250 mg/dl, when tested with glucose oxidase reagent strips (Dextrostix, Ames). Overt diabetes mellitus ensued 3 days after injection. Acetonuria (Acetest, Ames) was uniformly absent in the diabetic rats. Those animals with combined hypertension and diabetes mellitus received their streptozotocin injection 1 week after the placement of the left renal artery clip and before the increase of systolic blood pressure.

Four groups of animals were studied: 20 normal control animals (C), 15 diabetics (D), 16 hypertensives (H), and 17 combined hypertensives and diabetics (HD). The experimental period was 8 weeks of documented hypertension and/or diabetes mellitus, with all animals the same age at the time of sacrifice. The length of the study period was chosen arbitrarily.

At the conclusion of the study period, the animals were weighed, lightly anesthetized with ether, and sacrificed by exsanguination through the abdominal aorta. The hearts were rapidly removed from the chest and were immersed in 3.7% phosphate-buffered formaldehyde for approximately 15 minutes. Following immersion the hearts continued to beat with strong ventricular contractions for over 1 minute. The hearts were removed from the fixative, blotted dry, and weighed. Rings 1–2 mm in thickness were cut perpendicular to the long axis of the ventricle and were fixed in 3.7% phosphate-buffered formaldehyde for an additional 24–48 hours. Ventricular rings were embedded in paraffin, and sections were stained with hematoxylin-eosin, Masson's trichrome stain for connective tissue, Verhoeff-van Gieson stain for elastic tissue, and periodic acid-Schiff (PAS) with and without diastase pretreatment. Two to 3 ventricular rings were examined from each animal.

### Semiquantitative Light Microscopy

#### *Left Ventricular Wall Thickness*

Histologic sections of representative mid-ventricular rings were projected onto a screen at a fixed magnification of 37.6×. Measurements were taken perpendicular to the endocardium from 2 points representing the maximum and minimum thickness of the ventricular wall and were averaged for each animal. The regions measured did not include either papillary muscles or trabeculae carneae.

#### *Myocardial Fibrosis*

Two types of myocardial fibrosis were observed and quantitated in trichrome-stained sections. Myocardial scars were defined as areas of confluent fibrosis more than 500 $\mu$ in one dimension, which replaced zones of damaged or necrotic myocardium. We individually counted the lesions to determine the total number per animal and the number within the entire group. Interstitial fibrosis was defined as collagen deposition between and around myocardial cells without an apparent relationship to larger intramural blood vessels (greater than 50 $\mu$ in size). The extent and degree of fibrosis was subjectively graded on the following scale by 2 pathologists working independently: 0–1+, absent or minimally increased trichrome positive staining in a focal distribution; 2+, moderately intense staining with prominent connective tissue deposition in focal zones; 3+, severe changes, with dense or highly cellular collagen deposition between groups of cells, widening the interstitial space, and occurring in a more diffuse distribution. The endocardial versus epicardial localization of the connective tissue was noted.

#### *Vascular Lesions*

Three parameters of vascular disease were quantified. Heart sections were scanned at low power, and the total number of intramyocardial vessels measur-

Table 1—General Characteristics of Groups

| Group (number) | Blood glucose (mg/dl) | Systolic blood pressure (mmHg) | Body weight (g) | Heart weight (g) | Heart weight/ body weight (%) | Left ventricular wall thickness (mm) |
|---|---|---|---|---|---|---|
| Control (20) | 152 ± 14 | 122 ± 9 | 462 ± 47 | 1.48 ± 0.17 | 0.32 ± 0.03 | 2.66 ± 0.23 |
| Diabetic (15) | 550 ± 86 | 128 ± 9 | 325 ± 46 | 1.14 ± 0.15 | 0.35 ± 0.04 | 2.19 ± 0.21 |
| Hypertensive (16) | 160 ± 22 | 191 ± 27 | 404 ± 65 | 1.82 ± 0.26 | 0.46 ± 0.06 | 3.38 ± 0.36 |
| Hypertensive-diabetic (17) | 564 ± 98 | 199 ± 26 | 300 ± 53 | 1.48 ± 0.36 | 0.49 ± 0.08 | 2.45 ± 0.31 |

Results are expressed as mean ± SD.

ing 50–200 $\mu$ was tabulated. Measurements were made with a calibrated eyepiece micrometer. Vessels with obvious branching or double vessels in the same interstitial compartment were counted singly. All vessels within the sections were studied at higher magnification, and we tabulated the abnormal vessels to establish a ratio between abnormal and total vessels per animal. Ratios were developed for the pathologic alterations described below and then were converted to percentages. Since each vessel often had more than one pathologic change, the same vessel could be scored more than once in several ratios. Vascular abnormalities were tabulated in 2 categories, each of which included alterations of varying severity. Questionable or minimal lesions were scored as normal.

1) *Vascular sclerosis*: Vessels with intimal and medial hyperplasia, and/or fibrosis, elastic tissue proliferation, and the deposition of PAS-positive material within the vessel wall.
2) *Perivascular fibrosis*: Vessels surrounded by concentric fibrosis, or less compact connective tissue. Since it was difficult to determine where the adventitia ended and where pathologic fibrosis began, only those vessels surrounded by connective tissue which encroached upon and appeared to involve the adjacent myocardium, were scored as positive.

In addition to these parameters of vascular disease, we also calculated a vessel wall thickness/vessel diameter ratio for 10 randomly selected intramyocardial vessels from each case. We used a calibrated eyepiece micrometer to measure the outer diameter of the vessel and the mean of wall thickness at 2 points to evolve a ratio giving an indication of luminal narrowing. The wall thinkness/diameter ratio was determined from perpendicularly sectioned vessels from noncontiguous 10× power microscopic fields.

Statistical comparison of the 4 animal groups was performed by means of the Mann-Whitney nonparametric test[25] and the Student $t$ test, where appropriate. A $P$ value less than 0.05 was considered statistically significant.

## Results

### General Characteristics of Animal Groups

Final body weights, heart weights, heart weight/body weight ratios, left ventricular wall thicknesses, serum glucose levels, and systolic blood pressures for the 4 animal groups are summarized in Table 1. Both diabetic groups (D and HD) had significantly reduced ($P < 0.001$) body weights, compared with controls, and had significant elevations ($P < 0.001$) of serum glucose. There were significant elevations ($P < 0.001$) of systolic blood pressure in the 2 hypertensive groups (H and HD), compared with normotensives. Absolute heart weights were low in the diabetic animals and were markedly increased in hypertensives, compared with controls (both $P < 0.001$). Hyperten-

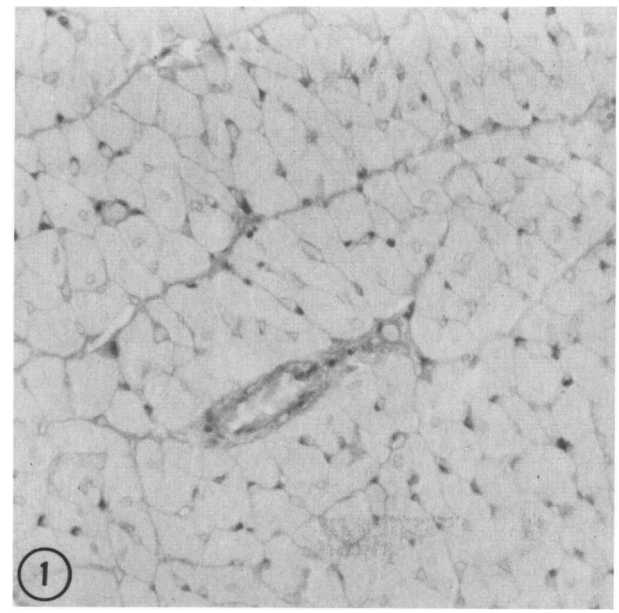



**Figure 1**—A PAS-stained section of diabetic rat myocardium following diastase pretreatment. There are fine darkly staining PAS-positive lines around individual myocardial cells, and darkly staining PAS-positive material within the small blood vessel wall in the center. No differences were noted in the amount of PAS-positive material seen in the diabetic group when compared with nondiabetic control animals. (× 150)

Table 2—Quantification of Myocardial Fibrosis

| Group (number) | Interstitial Fibrosis (Grade) | | | Myocardial Scars | | |
|---|---|---|---|---|---|---|
| | 0–1+ | 2+ | 3+ | Affected animals (number) | Affected animals (%) | Lesions (number) |
| Control (16) | 16 | 0 | 0 | 0 | 0 | 0 |
| Diabetic (15) | 14 | 1 | 0 | 0 | 0 | 0 |
| Hypertensive (16) | 10 | 5 | 1 | 3 | 19 | 4 |
| Hypertensive-diabetic (17) | 7 | 4 | 6 | 7 | 41 | 22 |

sive-diabetic heart weights were comparable to controls; however, this should not be construed as absence of hypertrophy in this group, since the diabetic and hypertensive-diabetic animals both gained body weight at a slower rate than the other 2 groups. Heart weight/body weight ratios, which correct for different body weights and are thus a measure of relative cardiac hypertrophy (Table 1), were similar in HD and H groups, both with ratios significantly greater than that of control animals ($P < 0.001$), suggesting that despite a "normal" absolute heart weight in HD animals, cardiac hypertrophy relative to body weight had in fact occurred. However, the nature of the hypertrophy in the hypertensive-diabetic animals differed from that in the pure hypertensive group in that left ventricular wall thickness was significantly different ($P < 0.001$) in the 2 groups, probably due to greater ventricular dilation in the HD animals. If absolute heart weight *and* left ventricular wall thickness are both used as the criteria of myocardial hypertrophy, then the hypertensive group clearly had significantly greater cardiac hypertrophy than the hypertensive-diabetic group ($P < 0.001$).

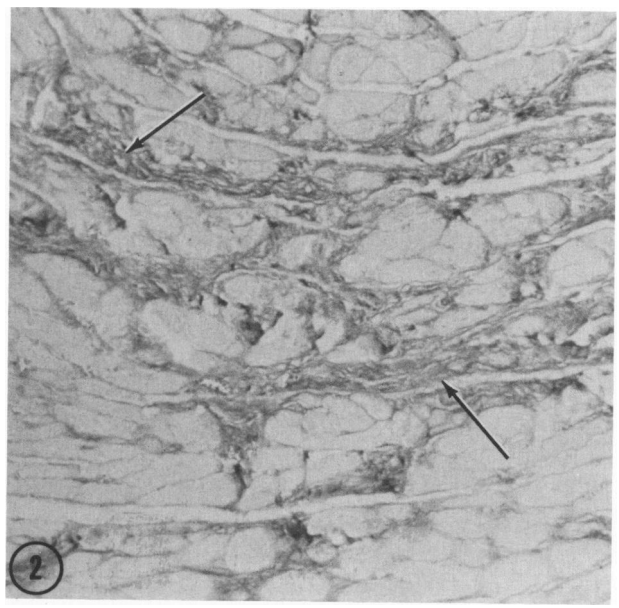


**Figure 2**—A PAS-stained subendocardial section from the most severely affected of the hypertensive animals reveals dense interstitial fibrosis (graded 3+) focally surrounding myocytes (*arrows*). Note that the fibrosis is moderately PAS-positive (darker staining) and that there is no increase in pericellular PAS-positive material in areas without fibrosis (lower right). (×150)

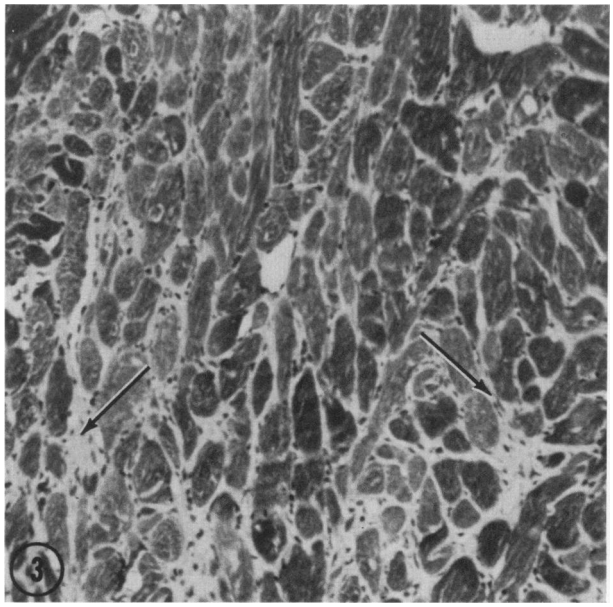
## Light-Microscopic Observations

### *Myocardial Fibrosis*

Sections from the diabetic animals showed virtually no myocardial or vascular alterations (Figure 1). In fact, controls could not be differentiated from diabetics on the basis of morphologic examination alone. Only one diabetic heart had as much as 2+ interstitial fibrosis, and no myocardial scars were observed (Table 2). Also, we did not observe the presence of abnormal accumulations of PAS positive, diastase-resistant material in this group, in either the interstitium or blood vessel walls (Figure 1).

The hypertensive animals demonstrated both myocardial and vascular lesions. Of the 16 animals in the H group, 5 had 2+ and one had 3+ interstitial fibrosis (Table 2, Figure 2). In the several animals in which interstitial fibrosis was appreciated, it was present around abnormal intramyocardial vessels, or it was localized in the subendocardium. Myocardial scars were observed in 3 animals (19%); however, there were only 4 such lesions in the entire group (Table 2). In contrast to the diabetic hearts, PAS-positive, diastase-resistant material was present in the myocardial interstitium and blood vessels of these hypertensive but nondiabetic animals, but only in areas of fibrosis or vascular sclerosis.

Fibrotic lesions were more common in the hypertensive-diabetic hearts in comparison with the other groups. A total of 6 animals had 3+ interstitial fibrosis and 4 had 2+ changes (Table 2, Figures 3 and 4). Seven animals had myocardial scars, representing 41% of the entire group, and 22 such lesions were observed (Table 2, Figure 5). The differences between the HD and H hearts with respect to the numbers of myocardial scars were significant ($P < 0.05$). As in the hypertensive animals, PAS-positive, diastase-re-



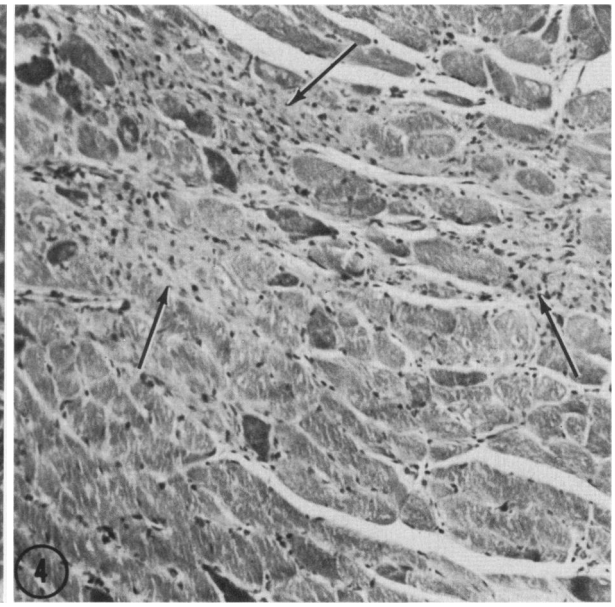


**Figure 3**—A section of myocardium from a hypertensive-diabetic animal reveals an increase in loose pericellular interstitial connective tissue and cellularity (*arrows*). This degree of interstitial fibrosis was graded 2+. (Trichrome stain, × 190) **Figure 4**—There is dense, cellular interstitial fibrosis (graded 3+) surrounding and separating myocytes in this left ventricular section from a hypertensive-diabetic rat (*arrows*). The changes are similar to those observed in one hypertensive animal (see Figure 2) but were much more frequent in this group. (Trichrome stain, × 150)

sistant material in hypertensive diabetic hearts was predominantly localized to areas of scarring and abnormal blood vessels (Figure 6).

The fibrotic lesions were more severe and occurred with greater frequency in the hypertensive-diabetic animals than in the 3 other groups. However, with regard to interstitial fibrosis, statistical comparison of HD and H animals was just slightly below the 5% level of significance. Although the trend favored the conclusion that the combination of diabetes mellitus and systolic hypertension produced severe degenerative lesions in the rat heart, 7 of the 17 HD animals had minimal or no changes. To determine if internal differences within the HD group could account for

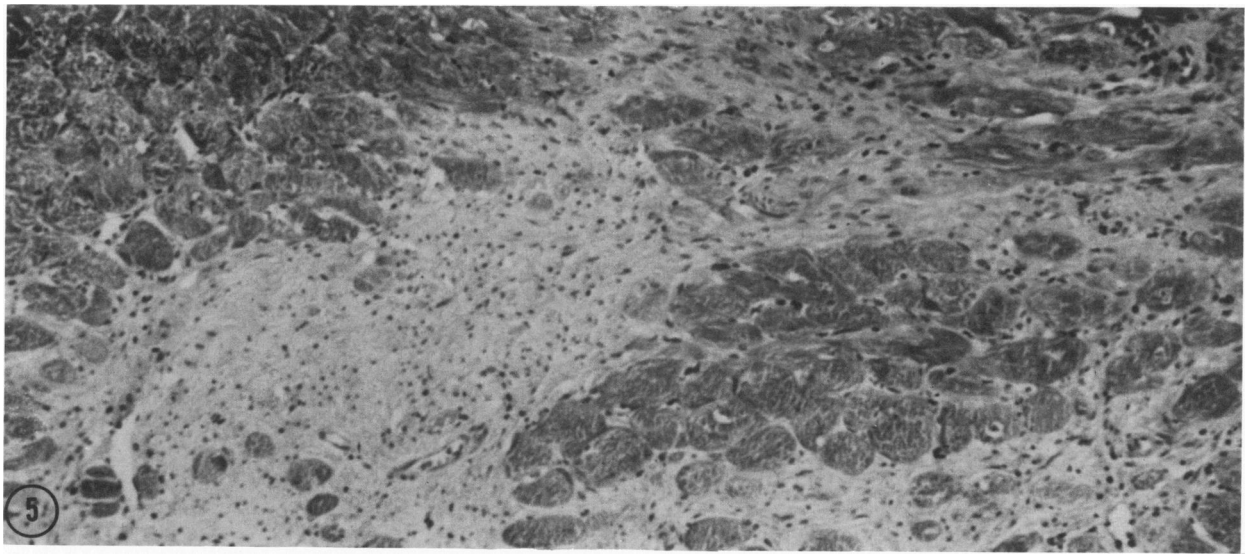


**Figure 5**—A large myocardial scar in a hypertensive-diabetic animal consists of dense collagen and moderate cellularity. This form of myocardial fibrosis differs from interstitial fibrosis, in that there has been apparent loss of myocytes in the scarred area. (Trichrome stain, × 190)

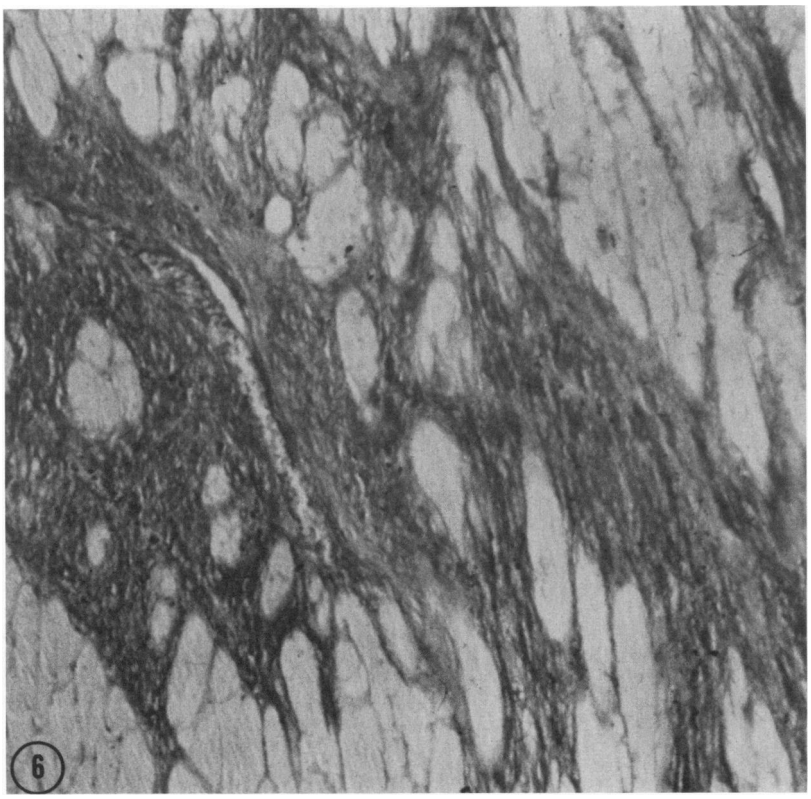


**Figure 6**—A PAS-stained, diastase-pretreated section of left ventricular myocardium from a hypertensive-diabetic rat reveals extensive interstitial and replacement fibrosis, marked by strongly positive PAS staining (dark areas in this photomicrograph). Note that a few cells in the lower portion of the field not surrounded by fibrosis have no increase in pericellular PAS staining. (×190)

the variable findings, animals were divided into those with 0–1+ changes and those with 2–3+ changes; the H group was separated in the same manner. The results (Table 3) show that HD animals with more severe histologic changes were those with greater relative cardiac hypertrophy, as judged by the heart weight/body weight ratio. Relative hypertrophy, however, may be a significant factor in the development of myocardial damage only in the setting of diabetes mellitus, since the analysis of mildly and severely affected H animals did not show a comparable correspondence.

*Intramyocardial Vascular Lesions*

Quantitation of the 3 aspects of vascular disease evaluated in this study (perivascular fibrosis, vascular sclerosis, and vascular wall thickness/vascular diameter ratio) revealed significantly greater ($P < 0.05$) wall thickness to vascular diameter ratios in the control versus diabetic animals, with no differences noted in the degree of perivascular fibrosis or vascular sclerosis between these two groups (Table 4). In contrast, vessels from both the hypertensive and hypertensive-diabetic hearts were severely affected (Table 4, Figures 7 and 8). Furthermore, H vessels had significantly more perivascular fibrosis and vascular sclerosis than HD vessels, despite similar blood pressure levels during life. It therefore seems likely that morphologic alterations in intramyocardial vessels are not causally related to the measured in-

Table 3—Comparison of Mildly and Severely Affected Hypertensive-Diabetic and Hypertensive Animals

| | Grade of interstitial fibrosis | Blood glucose (mg/dl) | Systolic blood pressure (mmHg) | Body weight (g) | Heart weight (g) | Heart weight/ Body weight (%) |
|---|---|---|---|---|---|---|
| Hypertensive-diabetic | 0 – 1+ (6) | 550 ± 49 | 189 ± 25 | 272 ± 44 | 1.16 ± 0.09 | 0.43 ± 0.08 |
| | | NS | NS | $p < 0.05$ | $p < 0.01$ | $p < 0.05$ |
| | 2+ – 3+ (11) | 566 ± 128 | 204 ± 30 | 321 ± 55 | 1.64 ± 0.35 | 0.52 ± 0.08 |
| Hypertensive | 0 – 1+ (10) | 161 24 ± 24 | 177 ± 19 | 430 ± 51 | 187 ± 0.27 | 0.53 ± 0.04 |
| | | NS | $p < 0.01$ | $p < 0.05$ | NS | $p < 0.05$ |
| | 2+ – 3+ (6) | 158 ± 23 | 214 ± 25 | 360 ± 73 | 174 ± 0.24 | 0.49 ± 0.07 |

Results are expressed as mean ± SD. Numbers in parenthesis represent numbers of animals with grade of interstitial fibrosis.

crease in total myocardial fibrosis in the HD animals (including interstitial and replacement fibrosis), since the H animals with less total fibrosis had, in fact, more severely involved vessels.

## Discussion

Primary myocardial degeneration occurring in the absence of extramural coronary artery atherosclerosis has been recognized with increasing frequency during the past decade as a significant complication of diabetes mellitus.[9-21] Clinical and pathologic studies have supported the association of diabetes mellitus with the development of congestive heart failure[9,15,16,18-21,26,27]; as yet, the pathogenesis of diabetic cardiomyopathy is unknown. Several investigators have proposed that this syndrome may be secondary to small vessel (intramural) coronary artery disease,[15,16,20,21,28-30] accumulations of glycoprotein and collagen in the myocardium,[17,18] and metabolic alterations of the diabetic heart.[14,17] Fein et al,[10,11] in mechanical studies of nonhypertensive streptozotocin-diabetic rats, have demonstrated prolongation of isometric papillary muscle relaxation, which is independent of the weight loss characteristic of severe diabetes. Regan and colleagues, in an experimental study employing alloxan to produce a moderately diabetic canine model, have correlated clinical dysfunction with altered myocardial metabolism and PAS-positive glycoprotein infiltration.[17] More recently, Giacomelli and Wiener have reported primary myocardial cell damage and vascular abnormalities in a genetically diabetic mouse.[13] The different animal models employed in these studies may account for the variability of the morphologic descriptions. Since human diabetes mellitus is most likely a multifactorial disease,[31] it is not surprising that both clinical and experimental investigations have identified several myocardial defects in diabetic cardiomyopathy.

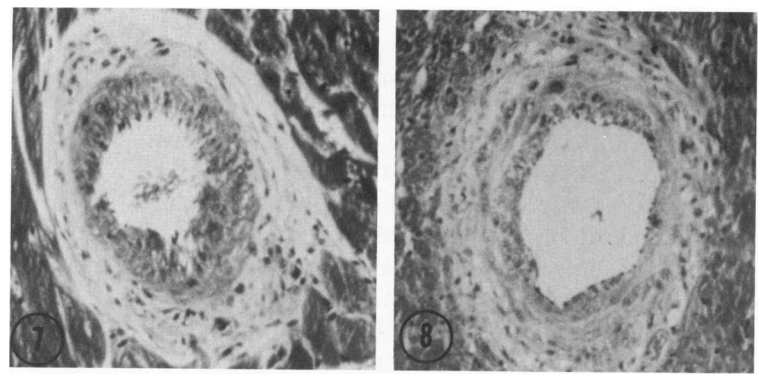
Table 4—Quantification of Intramyocardial Vascular Alterations

| Group (number) | Perivascular fibrosis | Vascular sclerosis | Vascular wall thickness vascular diameter |
|---|---|---|---|
| Control (16) | 3.6 ± 2.7 | 4.3 ± 5.7 | 0.156 ± 0.033 |
| | NS | NS | $P < 0.05$ |
| Diabetic (15) | 3.8 ± 6.9 | 2.4 ± 5.6 | 0.124 ± 0.031 |
| Hypertensive (14) | 45.7 ± 11.4 | 42.0 ± 17.3 | 0.186 ± 0.033 |
| | $P < 0.01$ | $P < 0.05$ | NS |
| Hypertensive-diabetic (17) | 27.8 ± 17.9 | 30.2 ± 20.2 | 0.199 ± 0.035 |

Perivascular fibrosis and vascular sclerosis are expressed as the mean of the vessels with the specific abnormality divided by the total number of vessels evaluated per animal, in percent ± SD. Vascular wall thickness/vascular diameter is expressed as mean ± SD.

We previously reported that in a group of patients with diabetic cardiomyopathy studied at autopsy, there was a positive correlation between congestive heart failure and hypertension.[22] This study revealed significant differences in the extent of myocardial fibrosis in the hypertensive diabetic patients when compared to controls, with no differences in either extramural or intramural vascular disease, or PAS positive glycoprotein deposition. Since hypertension has an increased prevalence in diabetes mellitus,[1,32-34] the possibility that high blood pressure plays a significant role in the development of diabetic cardiomyopathy is an important consideration. The present study is the first to investigate this relationship in a systematic fashion employing an experimental animal model.

The most striking histologic abnormality in this study was the presence of extensive interstitial and replacement fibrosis in the hypertensive-diabetic animals. It is noteworthy that these lesions occurred within a relatively short period of diabetes and hypertension (8 weeks) and that they closely resembled the



**Figures 7 and 8**—**Figure 7** is an intramyocardial muscular artery from a hypertensive animal, and **Figure 8** is a similar vessel from a hypertensive-diabetic rat. Both vessels have prominent smooth muscle cells with thickening of their walls (slightly greater in the hypertensive vessel), and both are surrounded by dense perivascular fibrosis. The artery in **Figure 8** has an increased amount of connective tissue within its wall. (Trichrome stain, **Figure 7**, ×190; **Figure 8**, ×190)

lesions described in our human HD group.[22] Of interest, we were unable to identify histologically prominant degenerative lesions in the diabetic group; nor did we observe PAS-positive interstitial glycoprotein deposition. It could be argued that the animals were not sufficiently diabetic or that the study period was too short. However, serum glucose was uniformly elevated (and to the same level as the HD group), and the animals demonstrated polydipsia, polyuria, and weight loss consistent with a severely diabetic state. Furthermore, after 24 weeks of diabetes mellitus in a similar model, the myocardium showed no evidence of further myocardial damage (unpublished observations, 1979).

Myocardial fibrosis, often with associated PAS-positive material, was identified in both the hypertensive and hypertensive diabetic animals; however, these alterations were much more severe in the HD group. This could not be explained simply by differences in cardiac hypertrophy, since absolute heart weights and left ventricular wall thicknesses were greater in the H animals, while heart weight/body weight ratios, a measure of relative hypertrophy, did not differ. Similarly, this difference in myocardial fibrosis between the HD and H groups could not be attributed to intramural vascular disease, which was different in neither type nor severity in the two groups. The lack of difference in intramural arteriopathy of the 2 experimental groups corresponds to our observations in human hypertensive and hypertensive-diabetic heart disease.[22]

A major concern in this study is whether the observed differences between the groups can be accounted for by metabolic perturbations (particularly malnutrition and weight loss in the diabetic animals) or the direct effects of the drug streptozotocin on the myocardium. These issues are addressed partly by the study design and also by recent studies performed by colleagues at the Albert Einstein College of Medicine.

In regard to malnutrition, it is apparent that both the diabetic and hypertensive-diabetic animals had equally low body weights at the time of sacrifice, yet only the HD rats showed significant pathologic changes. Despite the striking weight loss in the HD group, the *relative* cardiac hypertrophy was comparable to that seen in the heavier H animals and was much greater than that observed with diabetes alone. Furthermore, in a recent mechanical study of diabetic myocardium, Fein et al[35] underfed control rats to equal the diabetic rat body weight and were able to show that malnutrition was not a primary cause for the changes they observed. Therefore, it appears likely that weight loss per se cannot account for the observed differences between the groups in our study.

That streptozotocin itself may be directly cardiotoxic is not supported by the study data. Since the essentially unaffected diabetic animals and the severely affected hypertensive-diabetic animals received the same streptozotocin dose and attained the same degree of diabetes mellitus, it is not likely that the drug could account for the pathologic changes. Further support for this interpretation comes from the previously cited study by Fein et al,[35] who gave the strepotozotocin inhibitor 3-0-methyl glucose to a series of rats receiving the drug and showed that mechanical abnormalities in papillary muscle function were not observed in the nondiabetic animals given streptozocin plus inhibitor. Final proof of this issue will require the induction of hypertension in a group of genetically diabetic animals receiving no drugs.

The pathogenesis of the prominent fibrotic lesions in the HD rat model is unknown; however, it is likely that interstitial collagen deposition and dense scar formation have different underlying mechanisms. Interstitial fibrosis may be secondary to disease of the microvasculature, which is not easily evaluated in histologic sections. It is generally agreed that microscopic scars are secondary to replacement of necrotic myocytes.[36] In our study of human hypertensive-diabetic cardiomyopathy we were able to trace the evolution of these scars from healed discrete foci of myocytolysis.[22] In the rat model, though myocytolysis was observed on occasion, the transition from these necrotic zones to fibrous scarring was less apparent.

Myocyte degeneration and interstitial fibrosis cannot be attributed solely to larger intramyocardial vessel alterations in view of the similarity in degree of arterial and arteriolar disease in the hypertensive and hypertensive-diabetic groups (this is also supported by the quantitative analysis of vascular lesions). Microangiopathy could be responsible for interstitial fibrosis, particularly if it resulted in altered vascular permeability. In this regard, we recently described capillary microaneurysms and other abnormalities of the microcirculation in human diabetic hearts[37]; preliminary studies of the present rat models have revealed similar changes (unpublished observations, 1980). Williamson and Kilo[38,39] have proposed that leakage of plasma collagenase inhibitors $\alpha^1$-antitrypsin and $\alpha^2$macroglobulin could inhibit breakdown of basement membrane, whereas leakage of other plasma constituents might stimulate synthesis of basement membrane and collagen. Alternatively, Vracko and Benditt[40,41] have postulated that increased cell necrosis in diabetes mellitus may lead to a

progressive increase of basal lamina scaffolding. The combined effects of both hypertension and diabetes mellitus may act to increase capillary permeability far more than in either condition alone.

Clinical and experimental evidence obtained by others supports the view that hypertension adversely affects the cardiovascular complications of diabetes mellitus. In particular, Christlieb and coworkers[2] have noted an association between diabetic retinopathy and high blood pressure. Similarly, long-term follow-up of diabetic patients who were spared severe complications showed a low prevalence of hypertension.[42] Patients with diabetic nephropathy seem to have the progression of their renal disease slowed after antihypertensive treatment.[7,8] Mauer and associates[43] have shown that the clipped kidneys of streptozotocin diabetic rats with Goldblatt hypertension were protected from diabetic damage, while the unclipped kidneys exposed to high blood pressure had accelerated mesangeal alterations.

The present report of myocardial degeneration in the hypertensive-diabetic rat model, along with our description of a similar constellation of findings in humans, suggests that hypertension produces significant alterations of the diabetic myocardium. Morphologically, this disease is primarily manifested by interstitial accumulations of collagen and focal replacement fibrosis, which may result in a functionally restrictive form of cardiomyopathy. Further studies will be required to define precisely how high blood pressure mediates this alteration, with a particular focus on the microcirculation of the diabetic myocardium.

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