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Hypertensive Diabetic Cardiomyopathy in the Rat: Ultrastructural Features

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Summary. We previously described a cohort of diabetic patients with typical congestive cardiomyopathy, in whom myocardial lesions were related to concommitant high blood pressure. To evaluate the association of diabetes mellitus and hypertension in more detail, we studied 4 groups of rats with either no disease, streptozotocin-induced diabetes mellitus, renovascular hypertension, or a combination of hypertension and diabetes. Analysis revealed significant myocardial fibrosis and degeneration in the hypertensive-diabetic group when compared to controls, without an obvious relationship to small vessel lesions. The myocardial alterations appeared similar to those observed in patients with hypertension and diabetes mellitus. Of note, although hypertensive animals had focal moderate lesions, diabetic animals had no pathological changes.

To further characterize these histological changes, we performed electron microscopy on the 4 animal groups, which we are reporting in this study. Our analysis of the ultrastructural alterations confirms the previous histological observations. Diabetic animals only had increased cellular lipid, and mild, focal areas of myofibrillolysis, with no significant increases in perivascular and perisarcolemmal basal lamina. Consistent with our light microscopic finding that PAS positive material was associated with interstitial or replacement fibrosis, we noted basal lamina proliferation in the hypertensive and hypertensive-diabetic groups, particularly in areas of scarring. Pericapillary basal lamina was increased to the greatest extent in the hypertensive-diabetics. Qualitative alterations of myocardial cells and muscular blood vessels were similar in both the hypertensive and hypertensive-diabetic animals; however, there were more extensive changes in the latter group.

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This study provides further evidence that the combination of diabetes mellitus and hypertension produces significantly greater myocardial lesions than with either disease alone, not only at the light microscopic level, but ultrastructurally as well. Although the pathogenesis of this cardiomyopathy is unknown it may be related to abnormalities of the cardiac microcirculation. The prevalence of hypertension in the diabetic population suggests that greater attention should be paid to the combination of these 2 conditions and their effects on the heart.

Key words: Diabetes mellitus – Hypertension – Cardiomyopathy – Myocardial fibrosis – Myocardial microcirculation

Diabetes mellitus has been associated recently with clinical (D'Elia et al. 1979; Hamby et al. 1974; Kannel et al. 1974; Regan et al. 1975 and 1977; Ruben et al. 1972; Sohar et al. 1970) and experimental (Fein et al. 1980 and 1981; Feuvray et al. 1979; Giacomelli and Wiener 1979; Haider et al. 1977; Penpargkul et al. 1981; Regan et al. 1974) myocardial degeneration and dysfunction in the absence of significant large coronary artery obstruction. This has given rise to the concept of diabetic cardiomyopathy. Since diabetes mellitus is a multifactorial disease (Craighead 1978), it is likely that the pathogenesis of diabetic cardiomyopathy is complex and may be dependent, at least, on species susceptibility, duration and type of diabetes, and associated cardiovascular conditions. Among the frequently mentioned causes of this cardiomyopathic syndrome, small vessel (intramural) coronary artery disease (Blumenthal et al. 1960; Hamby et al. 1974; Kannel et al. 1974; Ledet 1968 and 1976; Rubler et al. 1972; Sohar et al. 1970), accumulations of glycoprotein and collagen in the myocardium (Regan et al. 1974 and 1975), and metabolic alterations of the diabetic heart (Haider et al. 1977; Regan et al. 1974) have attained prominence.

Recently, we suggested that some forms of diabetic cardiomyopathy may result from the combined effects of hypertension and diabetes mellitus. These conclusions were based initially on post-mortem observations in a group of 9 patients with severe congestive heart failure and minimal extramural coronary artery disease, all of whom had long-standing diabetes mellitus and high blood pressure (Factor et al. 1980a). Since the publication of that report we have seen an additional 5 cases with the identical syndrome (unpublished observations, 1981). Subsequently, we investigated an animal model of the human disease and confirmed our clinical observations (Factor et al. 1981). Controlled studies in rats with streptozotocin-induced diabetes mellitus and renovascular hypertension revealed significant myocardial scarring and interstitial fibrosis independent of lesions in small intramural vessels or interstitial glycoprotein deposition. Diabetes mellitus without hypertension produced virtually no myocardial or vascular changes, whereas hypertension alone led to intermediate alterations. We concluded that there were additive effects of high blood pressure on the diabetic heart which could produce cardiomyopathic changes. These observations are particularly relevant to clinical disease because of the prevalence of hypertension in the diabetic population (Christlieb 1973).

In the present study we report our ultrastructural findings in the hypertensive-diabetic rat model, and in the appropriate control groups. The observations support our previous conclusions based on light microscopy, regarding the significant effects of hypertension on the diabetic heart.

Material and Methods

A detailed description of the methods has been published previously (Factor et al. 1981). Briefly, 6 week old male Sprague-Dawley rats were used in these studies. Hypertension, defined as a systolic blood pressure greater than 150 mm Hg measured by tail cuff, was induced with a silver clip placed on the left renal artery; blood pressure elevation occurred approximately 2 weeks after surgery. Diabetes was induced with a 55 mg/kg dose of streptozotocin (Upjohn Co. Kalamazoo, MI, USA). Overt diabetes mellitus ensued 3 days after injection; random determinations of blood glucose always were greater than 250 mg/dl when tested with glucose oxidase reagent strips (Dextrostix; Ames). Those animals with combined hypertension and diabetes mellitus received their stretozotocin injection one week after the placement of the renal artery clip and before the increase of systolic blood pressure.

Four groups of animals were studied: 20 normal controls (c), 15 diabetics (D), 16 hypertensives (H), and 17 combined hypertensive-diabetics (HD). The experimental period was 8 weeks of documented hypertension and/or diabetes mellitus.

At the conclusions 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 min. Following immersion the hearts continued to beat with strong ventricular contractions for over one 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. A mid-ventricular ring was selected, trimmed of right ventricle, and diced into one mm cubes while immersed in fixative. Tissues were fixed for 4–6 h in 3% 0.1 M cacodylate buffered glutaraldehyde at 4° C, and then maintained in cacodylate-sucrose buffer overnight at 4° C.

The cubes of left ventricular myocardium were post-fixed for 1 h in 1% osmium tetroxide, dehydrated in progressively increasing concentrations of alcohol and propylene oxide, and embedded in epoxy resin. One micron sections were prepared and stained with toluidine blue. Areas identified by light microscopy as abnormal, with degenerated myocardium, interstitial fibrosis, or sclerotic intramyocardial blood vessels were selected for thin sectioning. Histologically unremarkable myocardium and blood vessels were selected routinely for comparison. Thin sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined in a Siemens 1A electron microscope. Three to 5 grids were examined from a minimum of 5 animals randomly selected from the 4 study groups.

Results

The general characteristics of the 4 experimental groups were presented in our previous description of the light microscopic findings (Factor et al. 1981). The diabetic animals (D and HD) had significant elevations of blood glucose (>550 mg/dl), and the hypertensive animals (H and HD) had significant elevations of systolic blood pressure (>190 mmHg) when compared to the appropriate controls. Although absolute heart weights varied among the 4 groups, there were no significant differences between the 2 hypertensive groups (H vs HD) and the 2 normotensive groups (D vs C) when heart weights were normalized to body weight. The heart weight/body weight ratio was significantly greater when the H and HD animals were compared to the D and C animals.

Ultrastructural Observations

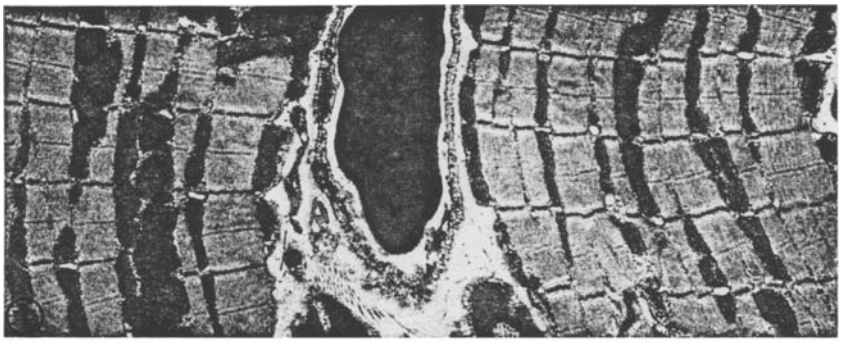
Control Animals. Myocardial cells appeared unremarkable, with sarcomeres in register, normal complements of mitochondria and ribosomes, and no

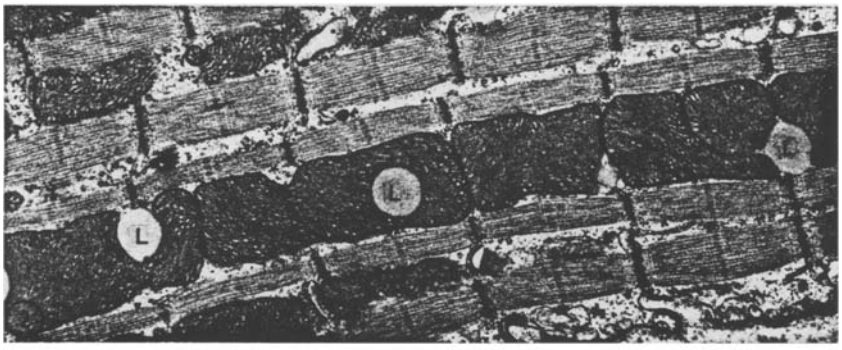
irregularities of Z-band material (Fig. 1). Only rare lipid droplets were noted. Perisarcolemmal basal lamina was thin and regular in contour, and scattered bundles of collagen fibers were present in the interstitium. Capillaries and small arterioles had widely patent lumina, intact cellular elements, and thin basal lamina. Larger arteries had no smooth muscle cell degeneration, and no increase of collagen, elastin, or basement membrane material.

Diabetic Animals. The most prominent feature of diabetic myocardium was the presence of numerous lipid droplets, frequently associated with mitochondria (Fig. 2). Myocardial cells had their sarcomeres in register, with normal numbers of mitochondria and ribosomes, and focally dilated T-tubules. Rare myocytes had areas of myofibrillolysis with loss of contractile elements over one or several sarcomeres (Fig. 3). Some cells with myofilament loss had increased numbers of mitochondria and ribosomes, haphazardly arranged groups of myofibrils, and disorganized Z-band material. The sarcolemmal basal lamina was not prominent and interstitial collagen was not increased over controls. Capillaries and arterioles had intact cellular elements, although endothelial cells had prominent and perhaps increased numbers of pinocytotic vesicles. Focal increases in width of the pericapillary basal lamina was seen infrequently (Fig. 4). Muscular arteries appeared similar to control vessels.

Hypertensive Animals. Myocytes showed several alterations, but only infrequently. Intercalated discs were markedly tortuous and irregular, with focal areas of myofibrillolysis in these zones (Fig. 5). Rare streaming of Z-bands was noted, usually in association with subsarcolemmal accumulations of Z-band like material (Figs. 6, 7). Continuity between these subsarcolemmal mats and Z-bands could be appreciated occasionally. The basal lamina surrounding myocytes was more prominent than in either control or diabetic animals, and interstitial collagen was increased, particularly in the vicinity of abnormal muscular degeneration, widening of the subendothelial space with the focal presence of fibrin, and accumulation of collagen, elastin, and basement membrane material. The only evident abnormalities of capillaries were focal increases of pericapillary basal lamina (Fig. 8).

Hypertensive-Diabetic Animals. Numerous myocardial cells demonstrated areas of myofibrillar disarray, and increased numbers of mitochondria and polyribosomes (Fig. 9). These cells, and even less affected ones, often had marked disorganization of sarcomeres, with loss of register and streaming (Fig. 10A), together with subsarcolemmal mats of Z-band-like material (Fig. 10B). Intercalated discs were hyperplastic and tortuous. The basal lamina around myocardial cells was abnormally thickened focally, particularly in regions of interstitial fibrosis (Fig. 10B). Both interstitial and replacement collagen deposition were prominent features of this model. Capillaries had an apparent increase in endothelial pinocytotic vesicles, and marked focal thickening of the pericapillary basal lamina (Figs. 11, 12).





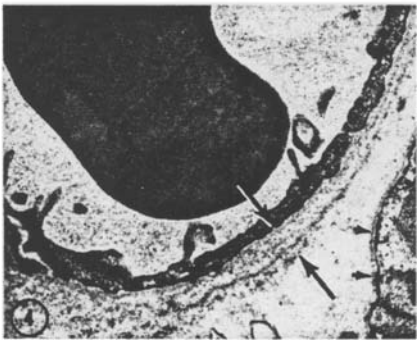


Fig. 1. Several myocardial cells and the interstitium from a control animal. The myocytes have sarcomeres in register, and a normal complement of mitochondria. Neither the capillary in the center, nor the myocytes are surrounded by a prominent basal lamina. A small number of collagen fibers are present in the interstitial compartment. × 8,000

Fig. 2. A myocyte from a diabetic rat. The sarcomeres are generally in register with straight and unthickened Z-bands. The most prominent feature is the presence of lipid droplets (*L*) intimately associated with mitochondria. × 17,250

Fig. 3. A portion of a myocardial cell from a diabetic rat. There is a focal loss of myofilaments (*arrows*) extending over two sarcomeres, with normal sarcomeres in the immediate vicinity. × 18,700

Fig. 4. A rare example of pericapillary thickening of the basal lamina is seen in the interstitium of this diabetic animal. Between the two arrows, the basal lamina is increased in width and clearly has a multilayered appearance. The basal lamina surrounding the sarcolemma of the adjacent cell (*arrowheads*), is of normal thickness. × 15,750

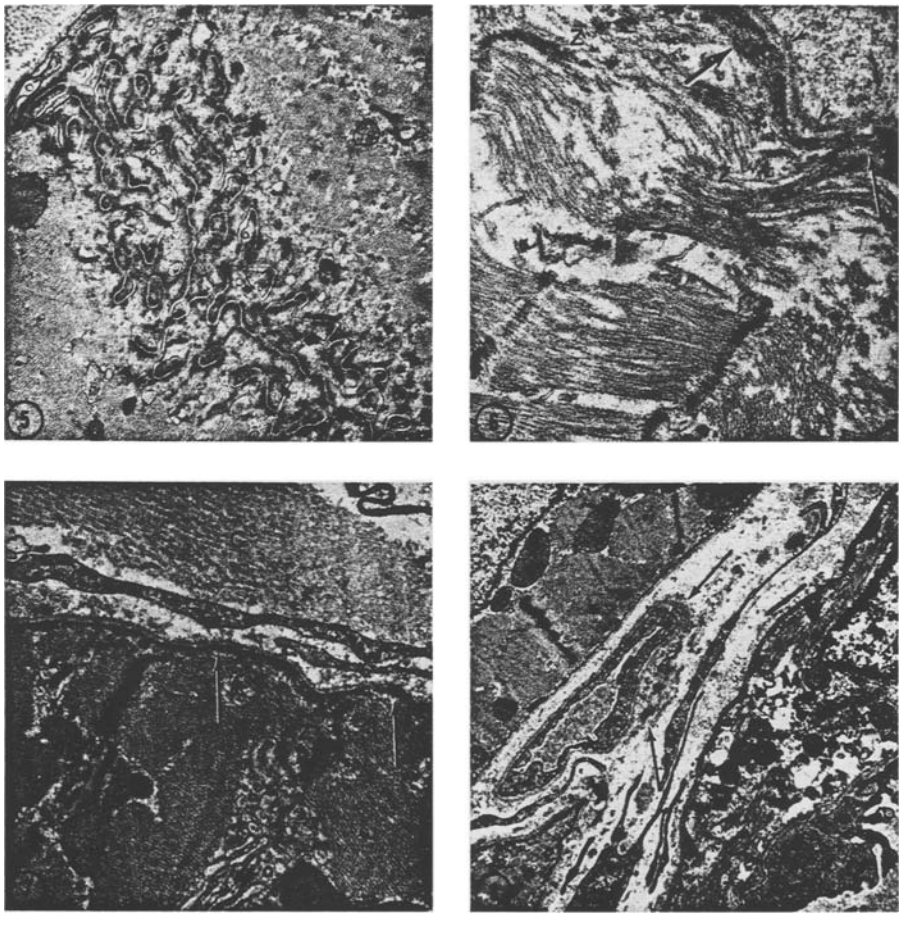


Fig. 5. An intercalated disc from a hypertensive animal reveals marked tortuosity and increase in size. There is slight rarefaction of the myofibrils in the immediate vicinity of the disc. ×14,000

Fig. 6. A portion of a myocardial cell from a hypertensive rat. There is irregularity and streaming of Z-band material (*Z*), with prominent rarefaction of myofilaments in the subsarcolemmal portion of the cell. The *thick arrow marks* a subsarcolemmal accumulation of Z-band like material; while at the *thin arrow* there appears to be continuity between this material and the streaming Z-band seen below. The basal lamina (*arrowheads*) of this cell is mildly thickened. ×17,500

Fig. 7. A portion of a myocardial cell from a hypertensive animal. There is streaming of the Z-band (*Z*), and accumulation of subsarcolemmal Z-band like material (*arrows*). The interstitial space contains dense collagen fibers (*C*). The basal lamina of the myocyte is mildly thickened. ×9,500

Fig. 8. The interstitial space from hypertensive rat myocardium. The myocardial cell on the left is unremarkable. The capillary in the center has focally thickened basal lamina (*arrows*). To the right, a portion of a small muscular vessel can be seen, with focal degeneration of a smooth muscle (*SM*) cell. ×28,000

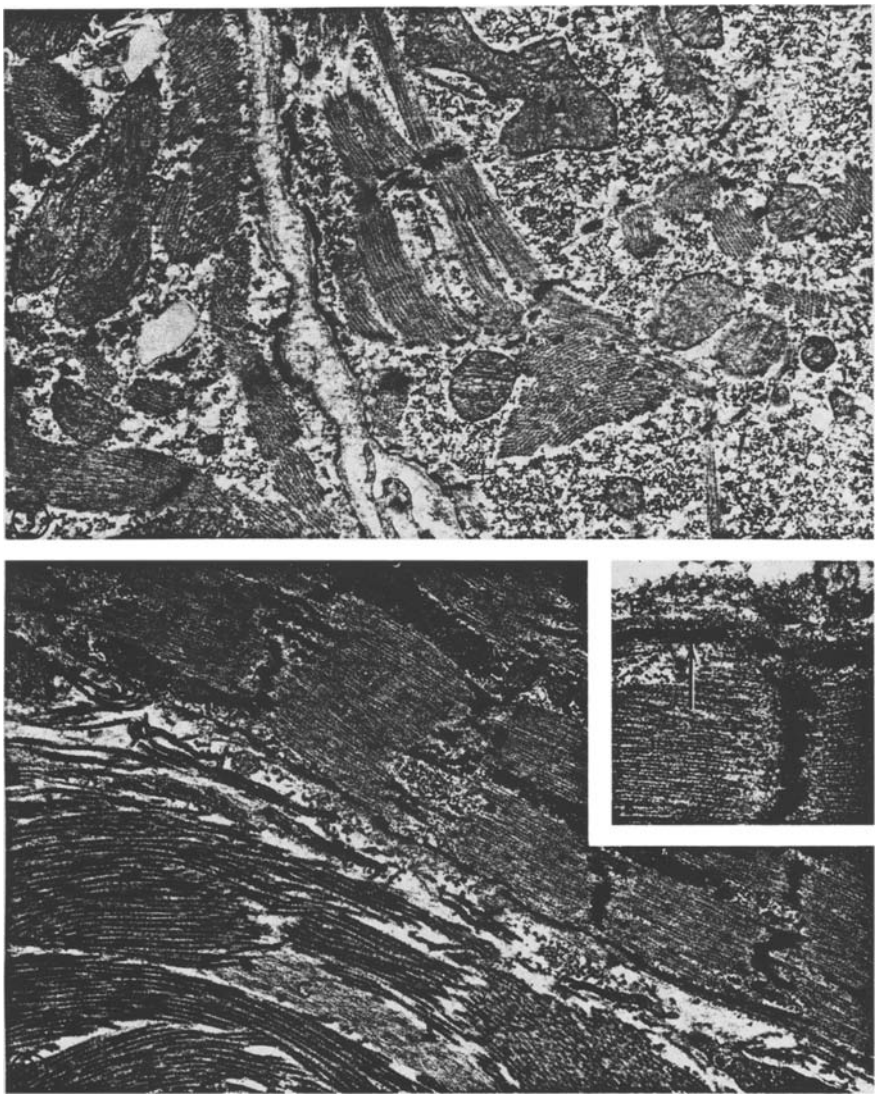


Fig. 9. Portions of two myocardial cells from a hypertensive-diabetic rat. Both cells, but particularly the one on the right, reveal extensive disarray of myofibrils (*MF*), with absence of the usual parallel arrangement of contractile elements. Large numbers of ribosomes (*R*) are present in this region, suggesting that these changes may be secondary to myofibrillogenesis. The mitochondria (*M*) in this zone are irregular in size and shape. × 24,000

Fig. 10. A, B A myocardial cell from a hypertensive-diabetic rat. There is marked streaming and irregularity of Z-band material (*Z*) involving all sarcomeres in this field. The interstitial space is filled with compact collagen (*C*) fibers which abut the myocyte. The *inset* (**B**) reveals subsarcolemmal accumulations of Z-band like material (*arrow*). The basal lamina (*BL*) of the cell is thickened, and is composed of both granular and filamentous maerial. (**A**) × 21,000 (**B**) × 36,000

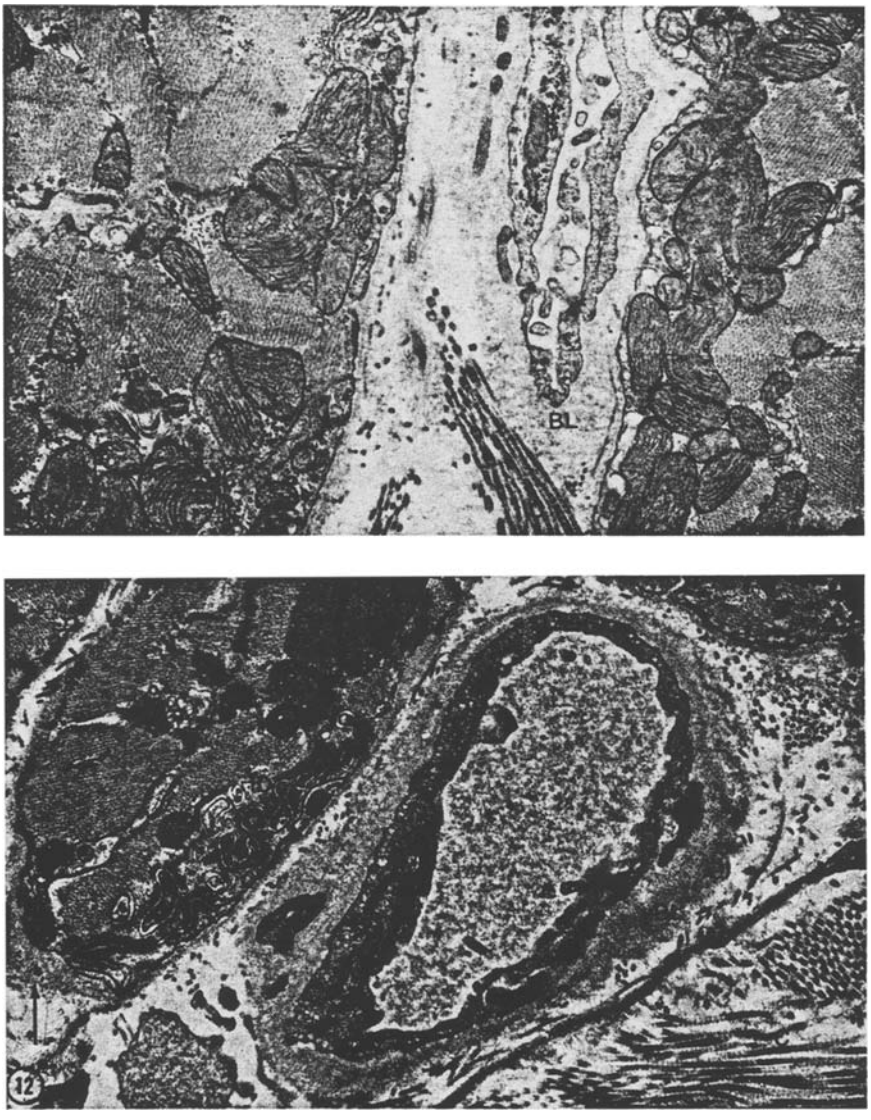


Fig. 11. Two myocytes and the interstitial space from a hypertensive-diabetic animal. The capillary in the center of the field is surrounded by a thickened, but somewhat loosely arranged basal lamina (*BL*). Prominent collagen fibers (*C*) are present in the interstitium. × 21,000

Fig. 12. A myocyte and interstitial space from a hypertensive-diabetic animal. There is marked thickening of a compacted basal lamina (*BL*) surrounding the capillary in the center of the field, with numerous collagen fibers (*C*) peripheral to the vessel. The adjacent myocyte has a hyperplastic intercalated disc (*ID*) apparatus along the sarcolemma, and a focal increase of basal lamina (*arrow*) surrounding the cell. × 15,000

Extensive degenerative changes found in both muscular arteries and arterioles did not differ appreciably from those seen in animals with hypertension alone.

Discussion

A large body of epidemiologic (Kannel et al. 1974), clinical (Regan et al. 1975 and 1977; Rubler et al. 1972; Sohar et al. 1970; Seneviratine 1977) and experimental (Haider et al. 1977; Regan et al. 1974) evidence has accumulated over the past decade implicating diabetes mellitus as a cause of a unique congestive cardiomyopathy. Despite these and other studies, diabetic cardiomyopathy has not been well characterized either clinically or pathologically. Specifically, the pathogenesis of this cardiomyopathy is unknown; nor is there much information concerning whether it is diabetes mellitus itself, or the effects of a commonly associated condition such as hypertension which leads to the development of myocardial failure.

Our studies of clinical (Factor et al. 1980a) and experimental (Factor et al. 1981) diabetic heart disease have suggested that normotensive diabetes has relatively few effects on the light microscopic appearance of the heart. In contrast, relatively short-term systolic hypertension is associated with evidence of focal myocardial degeneration (interstitial and replacement fibrosis) of a moderate degree. When the stress of elevated blood pressure is combined with diabetes mellitus, however, significant myocardial alterations are found which appear very similar to clinical cardiomyopathy studied at post-mortem (Factor et al. 1980a). The knowledge that hypertension and diabetes mellitus produce greater myocardial damage than either disease alone, is re-inforced by recent data suggesting that hypertension is also associated with diabetic retinopathy (Christlieb et al. 1976), and diabetic renal disease (Mogensen 1976 and 1977). The converse is also true; long-term follow-up of diabetic patients free of severe complications, has revealed a low prevalence of hypertension (Oakley et al. 1974).

Although light microscopy has the advantage that relatively large volumes of myocardium may be examined to evaluate degenerative features, it always is possible that significant ultrastructural abnormalities could be present which might be unappreciated with routine histology. The present report suggests however, that the ultrastructural and light microscopic observations in experimental hypertensive-diabetic cardiomyopathy are consistent with each other: severe myocardial alterations were limited to those animals with hypertension and diabetes mellitus, whereas the other animal groups had only minimal or at most moderate changes.

Diabetic myocardium had a few, mild alterations. Predominant among these was the presence of osmiophilic lipid droplets in many cells, often associated with mitochondria. This was similar to changes observed in the genetically diabetic mouse (Giacomelli and Wiener 1979), and skeletal muscle of streptozotocin diabetic rats (Chao et al. 1976), and may reflect an alteration in lipid metabolism by the diabetic myocardium (Murphy and

Shipp 1977; Neely et al. 1972; Nikkila et al. 1963). Curiously, lipid droplets were not seen with any frequency in the severely altered HD myocardium, despite the same degree of hyperglycemia in this group. Focal myofibrillolysis occurring within otherwise intact myocardial cells was also observed in the diabetic animals. This may be an early manifestation of more severe myocardial degeneration, such as is seen at later stages in the diabetic mouse (Giacomelli and Wiener 1979), or it may be a relatively insignificant form of self-limited degeneration. Ultrastructural studies at later time periods will be required to clarify this issue.

In agreement with the absence of light microscopic evidence of PAS positive material in the interstitium (Factor et al. 1981), increased thickening of pericapillary or perisarcolemmal basal lamina in the diabetic group was rarely seen at the ultrastructural level. Correspondingly, the absence of glomerular capillary basement membrane thickening in kidneys of streptozotocin diabetic rats has been noted by others; however, deposition of basement membrane-like material within the mesengial matrix was seen (Weil et al. 1976). In contrast, Osterby and Gundersen (1980), using sensitive morphometric analysis, did demonstrate slight glomerular basement membrane thickening in 47-day streptozotocin diabetic rats. Species differences, time courses, and the nature of diabetes induction must be considered in comparing various studies, as must the methodology employed. For example, capillary microaneurysms in human diabetic hearts seen by us after use of vascular injection technique, could not be appreciated in uninjected specimens (Factor et al. 1980b).

Hypertensive animals had striking degenerative and proliferative perivascular fibrosis, alterations similar to those previously published (Bhan et al. 1978; Giacomelli et al. 1976; Greditzer and Fischer 1978). Myocytes had focal areas of myofibrillolysis, particularly around tortuous and hyperplastic intercalated discs, and irregular Z-band streaming with subsarcolemmal accumulations of Z-band-like material. These changes have been considered indicative of a degenerative process (Maron et al. 1975), or alternatively, to be evidence of sarcomerogenesis (Bishop and Cole 1969; Kawamura et al. 1976; Legato 1970); indeed, both processes could be occurring simultaneously.

The ultrastructure of hypertensive-diabetic myocardium revealed much more extensive myocyte alteration than that seen with hypertension alone. The degeneration can not be attributed solely to larger vessel alterations in view of the similarity in degree of arterial and arteriolar disease in the 2 groups supported by the quantitative analysis of vascular lesions (Factor et al. 1981). Microangiopathy could be responsible, particularly if it resulted in altered vascular permeability. We did observe a greater degree of capillary and perisarcolemmal basement membrane thickening and interstitial collagen deposition in this group compared to the others which may result from leakage of plasma collagenase inhibitors, as proposed by Williamson and Kilo (1976 and 1977). We have presented recent evidence that the microcirculation of the HD rat has dynamic abnormalities which may account for the development of myocytolytic necrosis and subsequent scarring in this

model (Factor et al. 1980c; Factor 1982a). Identification of microvascular changes including spasm in this experimental cardiomyopathy, links this acquired disease to the genetic cardiomyopathy of the Syrian hamster, in which we have found preventable microvascular hyper-reactivity and associated myocardial scarring similar to that of the hypertensive-diabetic rat (Factor et al. 1982b).

The present report describing the ultrastructural changes in hypertensive diabetic cardiomyopathy, re-affirms our previous conclusion that the combination of diabetes mellitus and systolic hypertension leads to significant myocardial damage. Morphologically, this damage is primarily manifested by interstitial accumulations of collagen, basement membrane material, and focal replacement fibrosis in association with evidence of cellular hypertrophy. The focal nature of these alterations, and the absence of diffuse degenerative changes in the myocardial cells suggests that at least some of these features may be mediated by an abnormal microcirculation. Further studies will be necessary to define how the combination of diabetes mellitus and high blood pressure affects the myocardial cell and/or the microcirculation to produce significant cardiac degeneration.

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