Coronary Microvascular Abnormalities in the Hypertensive–Diabetic Rat

A Primary Cause of Cardiomyopathy?

STEPHEN M. FACTOR, MD, TAKASHI MINASE, MD, SANGHO CHO, MD, FREDERICK FEIN, MD, JOSEPH M. CAPASSO, PhD, and EDMUND H. SONNENBLICK, MD

From the Departments of Pathology and Medicine (Cardiology), Albert Einstein College of Medicine, Bronx, New York

The authors have continued their investigation of the hypertensive–diabetic (HD) rat by evaluating changes in the myocardial microvasculature in this model. Perfusion of HD animals *in vivo* with a silicone rubber solution revealed numerous areas of microvascular tortuosity, focal constrictions, and microaneurysm formation. These alterations were present to a lesser extent in normoglycemic hypertensive (H) rats, and were distinctly rare in normotensive diabetic rats and unaffected control animals. Quantitation of these vascular lesions revealed highly significant differences between HD animals and the other three groups, with hypertensive rats intermediate between HD rats and diabetic control rats. Areas of pronounced arteriolar constriction were also identified in the HD and H animals with the use of serial sections of Epon-embedded myocardium. It is believed that these lesions represent dynamic changes in the microcirculation, which may cause segmental reperfusion injury to the myocardium, leading to focal replacement fibrosis. Interstitial scarring may result from increased leakiness of small vessels exacerbated by the combined disease. The authors propose that the additive effects of hypertension and diabetes mellitus on the myocardial microcirculation may be a primary cause of cardiomyopathy in this model of human disease. (Am J Pathol 1984, 116:9–20)

IN OUR PREVIOUS STUDIES of diabetic cardiomyopathy,[1-3] we focused on the relationship between diabetes mellitus and systemic hypertension, and the development of significant degenerative changes in the myocardium. Initially, we identified a cohort of hypertensive and diabetic patients with severe congestive heart failure, and minimal extramural coronary artery disease.[1] Subsequently, we showed that relatively short-term streptozotocin-induced diabetes mellitus and two kidney, one-clip Goldblatt renal hypertension in the rat produced additive morphological cardiac damage similar to the clinical syndrome.[2,3] The focal nature of the myocardial lesions in this disease suggested to us that the microcirculation might be abnormal, and might have dynamic alterations comparable to those we described in the cardiomyopathic Syrian hamster.[4] In the hamster, there is preliminary evidence that microvascular spasm, possibly mediated by α-1-adrenergic receptors on vascular smooth muscle, may give rise to focal reperfusion myocardial injury and subsequent discrete zones of scarring.[4,5]

Microvascular involvement is a well-recognized complication of chronic diabetes mellitus,[6-8] with both anatomic alterations and pathophysiologic dysfunction described. Generalized basement membrane thickening is probably the most common abnormality. Although not specific for diabetes,[9-11] such changes have been identified in virtually all tissues studied from diabetic patients.[12] Microaneurysm development in retinal vessels is a hallmark of the disease, and is often used as an indicator of systemic complications.[13,14] Similar diabetic microaneurysms

Supported in part by Grants HL-20426 and HL-18824 from the National Institutes of Health.

Accepted for publication January 26, 1984.

Address reprint requests to Stephen M. Factor, MD, Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461.

recently were described in renal glomeruli[15,16] and in the myocardium,[17] suggesting that this phenomenon is a generalized abnormality. Increased capillary permeability has been reported in both human and experimental diabetes,[18-20] but it is unclear whether such "leakiness" is a primary complication, is secondary to basement membrane proliferation, or is related to microaneurysm formation.[8,13] Recent evidence also has accumulated which reveals that the diabetic microcirculation *in vivo* may have abnormal patterns of reactivity to both neurohumoral and metabolic stimuli.[21,22] It is still uncertain what role these pathophysiologic alterations play in the development of anatomic microvascular lesions or tissue disease.

Many of the abnormalities characteristic of the diabetic microcirculation also are present in systemic hypertension. In particular, increased permeability changes,[23] focal constrictions and microaneurysm development,[24] and transient microvascular hyperreactivity[25] have been described in several different models of hypertension. Because of the increased prevalence of high blood pressure in the human diabetic population,[26] many of the microvascular complications of diabetes may be primarily related to or exacerbated by hypertension. This issue has not been clarified as yet, but there is suggestive clinical evidence that normotensive diabetics have less retinal or renal disease[27,28] and that control of blood pressure elevation may lessen the progression of complications.[29]

The purpose of the present report is to investigate myocardial microvascular alterations associated with combined hypertension and diabetes mellitus, which may explain the pathogenesis of cardiac lesions in the cardiomyopathic rat model. Comparison is made among lesions seen in normotensive diabetic rats and normoglycemic hypertensive rats, as well as unaffected control rats, by the use of methods which may indicate dynamic abnormalities of the microcirculation.

Materials and Methods

Animal Preparations

Female Wistar rats, originally weighing between 140–160 g, were divided into four groups of 6 animals each, comparable to those of our previous reports.[2,3] We studied diabetic (D), hypertensive (H), combined hypertensive and diabetic (HD), and control (C) animals. The two groups of rats made diabetic (D and HD) were given intravenous tail vein injections of streptozotocin, 60 mg/kg dissolved in 0.05 M citrate, pH 4.5. We weighed the animals and did blood glucose determinations weekly, using a glucose analyzer. Overt diabetes mellitus without acetonemia developed within 3 days of injection. Diabetes was defined as a blood glucose level above 250 mg/dl throughout the study. In all nondiabetic animals the blood glucose level was maintained below 130 mg/dl.

Two groups of animals (H and HD) were made hypertensive by placement of a silver clip with an internal diameter of 0.25 mm on the left renal artery. Blood pressure elevation occurred within 2 weeks after surgery and was monitored biweekly by the tail-cuff method.[30] Hypertension was defined as a systolic blood pressure of 150 mmHg, which was maintained at or above this level until the time of study. Those animals with combined hypertension and diabetes (HD) received their streptozotocin injection approximately 1 week after left renal artery clipping, before the increase of systolic blood pressure.

The animals were sacrificed 9.5 weeks following renal artery clipping and 8 weeks after streptozotocin injection. The total duration of diabetes mellitus and/or hypertension was 7–8 weeks, similar to that of the animals described in our previous light-microscopic[2] and electron-microscopic[3] studies.

Histology and Perfusion Studies

At the time of sacrifice, the animals were lightly anesthetized with ether and were weighed. The chest cavity was opened with a sternum-splitting incision exposing the beating heart. Approximately 5 ml of heparinized saline (5000 units/250 ml) was injected into the left ventricular apex before silicone rubber (Microfil; Canton Bio-Medical Products) was perfused. Microfil is an opaque, relatively low-viscosity liquid that fills the arterial and venous circulation and hardens *in situ*, producing a three-dimensional cast of the microvasculature with minimal shrinkage or distortion. Details of our perfusion technique were reported previously.[4] Briefly, the midthoracic aorta was isolated over a mosquito hemostat, and 3–5 ml of freshly prepared Microfil was gently infused retrograde into the aorta. Minimal hand pressure was required to get excellent filling of the coronary microcirculation, determined by a diffuse white blush which developed in the myocardium with the first 1–2 ml of perfusate. With further perfusion, the Microfil extended distally beyond the injection site to fill the abdominal aorta and the intraabdominal organs. Microfil was allowed to escape through the cut ends of the chest wall vessels. When resistance to further

perfusion was encountered, the procedure was terminated. The heart continued to beat for approximately 1 minute during the procedure.

After perfusion, the hearts were rapidly excised, placed on ice to "cure" the Microfil, blotted dry, and weighed. Slices of ventricular myocardium 2–3 mm thick were cut with a razor blade from the apex to the base. A small portion of a midventricular slice from 3 animals in each group was diced into 1-mm cubes under cold 2.5% glutaraldehyde with 0.1 M cacodylate buffer, pH 7.4. The tissues were fixed for 4–6 hours and then were rinsed overnight in buffer. Subsequently, the tissues were postfixed for 1 hour in osmium tetroxide, dehydrated in increasing concentrations of alcohol and propylene oxide, and embedded in Epon 812. One-micron sections were prepared with glass knives and were stained with alkaline toluidine blue. Pellets with arterioles or capillaries cut perpendicular to their long axis were selected from each animal, and approximately 100–200 serial 1-μ sections were prepared for analysis and reconstruction of the vessels along their length.

Portions of randomly selected ventricular tissue from 1–2 rings were fixed in 3.7% phosphate-buffered formaldehyde for 24–48 hours and were processed routinely for light microscopy. Paraffin sections were stained with hematoxylin and eosin (H&E) and were evaluated for the presence of replacement or interstitial fibrosis, active necrosis, cellular infiltrates, and morphologic alterations in small muscular arteries and arterioles.

For study of the microvasculature, 2–3 perfused myocardial rings were cleared by the method of Schaper.[31] Tissues were fixed in 3.7% formaldehyde for 2 weeks, bleached in hydrogen peroxide, washed, dehydrated in alcohol and benzol, and cleared in methyl salicylate. This process results in semitranslucent tissue, without cellular detail, in which the Microfil-perfused vessels stand out sharply. Cleared slices of ventricular rings were immersed in methyl salicylate and were examined with trans- and epi-illumination. With transillumination the Microfil absorbs light and appears black; with epi-illumination the color (white) and three-dimensional features of the vessels can be analyzed.

Our previous studies of the normal rodent,[4] canine,[32] and human[33] microvasculature demonstrated smoothly tapering vessels with numerous branches forming loops and arcades. Real or apparent irregularities may be noted at branch points. Because partially filled vessels or vessels viewed on end may mimic true lesions, we evaluated vessel abnormalities away from branch points with good Microfil perfusion proximally and distally. The alterations tabulated and photographed included regions of vascular constriction with or without pre- and post-stenotic dilation, focal saccular or fusiform aneurysms, and marked vascular tortuosity, or "corkscrewing." We quantitated these changes by counting the number of individual vessel segments with abnormalities seen in 10 nonadjacent fields viewed with the ×10 microscopic objective. A vessel segment with more than one abnormality was counted only once. The mean number of abnormal vessels in the 10 fields was calculated for each animal, and the group means with standard deviations were used for a statistical comparison between the groups.

Statistical comparison of the four animal groups was performed by means of analysis of variance and Scheffé multiple comparison tests. A P value of <0.05 was considered statistically significant.

Results

General Characteristics of Animal Groups

One hypertensive animal died prior to the completion of the experiment and is not included in the analysis. Final body weights, heart weights, heart weight/body weight ratios, blood glucose, and systolic blood pressure for the four groups are summarized in Table 1. The blood glucose between the two diabetic groups (D and HD) and the systolic blood pressure between the two hypertensive groups (H and HD) did not differ significantly. The body weights of the HD and H animals differed significant-

Table 1—Group Characteristics

Group (n)	Blood glucose (mg/dl)	Systolic blood pressure (mmHg)	Body weight (g)	Heart weight (g)
Control (6)	<130	<150	250 ± 12	0.90 ± 0.06
Diabetic (6)	635 ± 169	<150	188 ± 33	0.78 ± 0.11
Hypertensive (5)	<130	179 ± 23	253 ± 14	1.04 ± 0.26
Hypertensive-diabetic (6)	730 ± 282	176 ± 30	164 ± 51	0.90 ± 0.30

Results are expressed as mean ± SD.

ly ($P < 0.003$), whereas the normoglycemic hypertensives gained weight comparable to that of the control animals. The HD animals reacted like D rats and did not gain weight (HD versus D, P = NS). Absolute heart weights between the four groups showed no significant difference. These data are similar to those we observed previously.[2]

Myocardial Histology

We analyzed small segments of myocardium by routine microscopy to verify that there were pathologic changes typical of this model. Qualitatively, the findings were similar to those of our previous report.[2] Hypertensive-diabetic animals had multiple foci of interstitial (Figure 1) and replacement fibrosis (Figure 2), with the latter consistent with healed myocytolytic necrosis. Several areas of replacement fibrosis were observed in one hypertensive animal, but the H group generally manifested only focal interstitial fibrosis in the immediate area of thickened and sclerotic muscular arteries. The small intramyocardial muscular arteries appeared to be equally affected in both the H and HD groups. Diabetic animals demonstrated no evidence of morphologic damage, with the exception of one animal noted to have a small zone of loose interstitial fibrosis. The myocardium and the intramyocardial vessels could not be differentiated otherwise from those of the normal-appearing control group.

Microvascular Perfusion

The Microfil perfusion technique, followed by clearing of the tissue, allows for a three-dimensional visualization of the myocardial microvasculature. By focusing up and down in the relatively thick slices of myocardium employed, individual vessels can be followed at great length as they progressively branch into capillaries. The quality of the perfusion can be evaluated in Figure 3, which shows normal vessels from a diabetic animal. Although the relative size and branching pattern of the vessels gives some indication of their *in vivo* identification within the vascular tree, it is only possible to indicate whether a vessel is consistent with a small artery, arteriole, or capillary. The gradual tapering of the vessels can be appreciated in Figure 4, from a control animal.

Three distinctive microvascular abnormalities were observed in this study. Marked tortuosity of arteriolar-sized vessels giving rise to a corkscrew appearance was seen in the HD group (Figures 5 and 6). This pattern was not caused by shrinkage of the tissues similar to what we previously noted in perfused skeletal muscle,[33] since the surrounding vasculature did not have a corkscrew configuration. A comparable abnormality was illustrated in our previous study of human diabetic myocardium (see Figure 4 of Factor et al[17]); in that investigation subjects were not evaluated for the presence or absence of long-standing hypertension. In the rats, none of the H, D, or C animals had evidence of microvascular tortuosity.

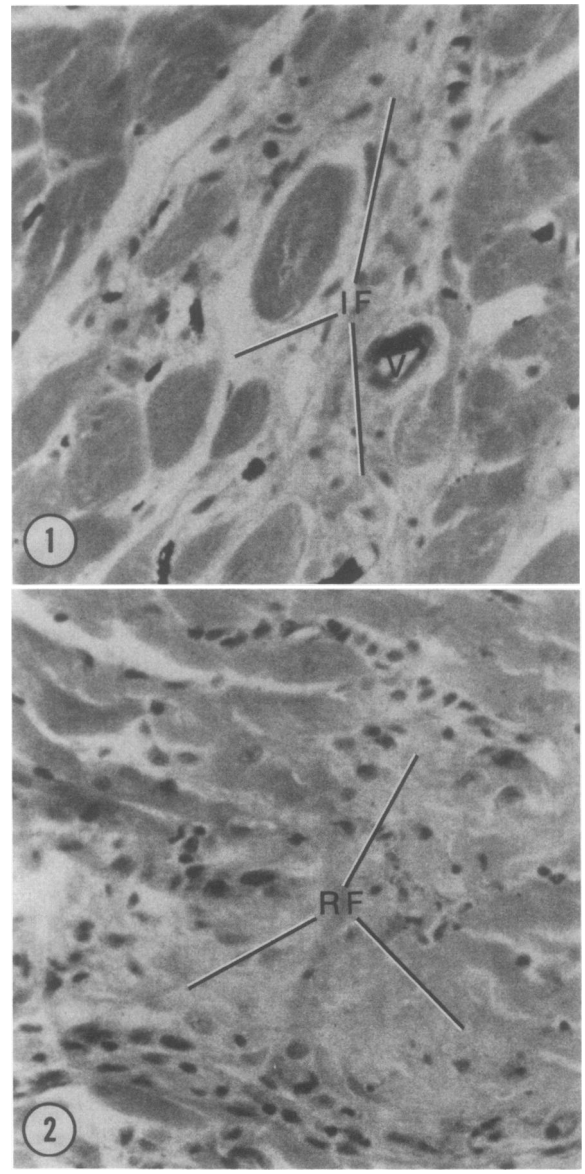


Figure 1—Myocardium from a hypertensive-diabetic animal with loose interstitial fibrosis (*IF*) around myocytes and a small arteriolar vessel (V). The lumen of the arteriole contains black silicone rubber, which has retracted during paraffin embedding and histologic sectioning. (H&E ×150) **Figure 2**—Myocardium from a hypertensive-diabetic animal with an area of subepicardial replacement fibrosis (*RF*), consisting of dense collagen and a mononuclear inflammatory cell response. Such areas, observed in different stages of formation, are consistent with organized myocytolytic necrosis. (H&E, ×150)

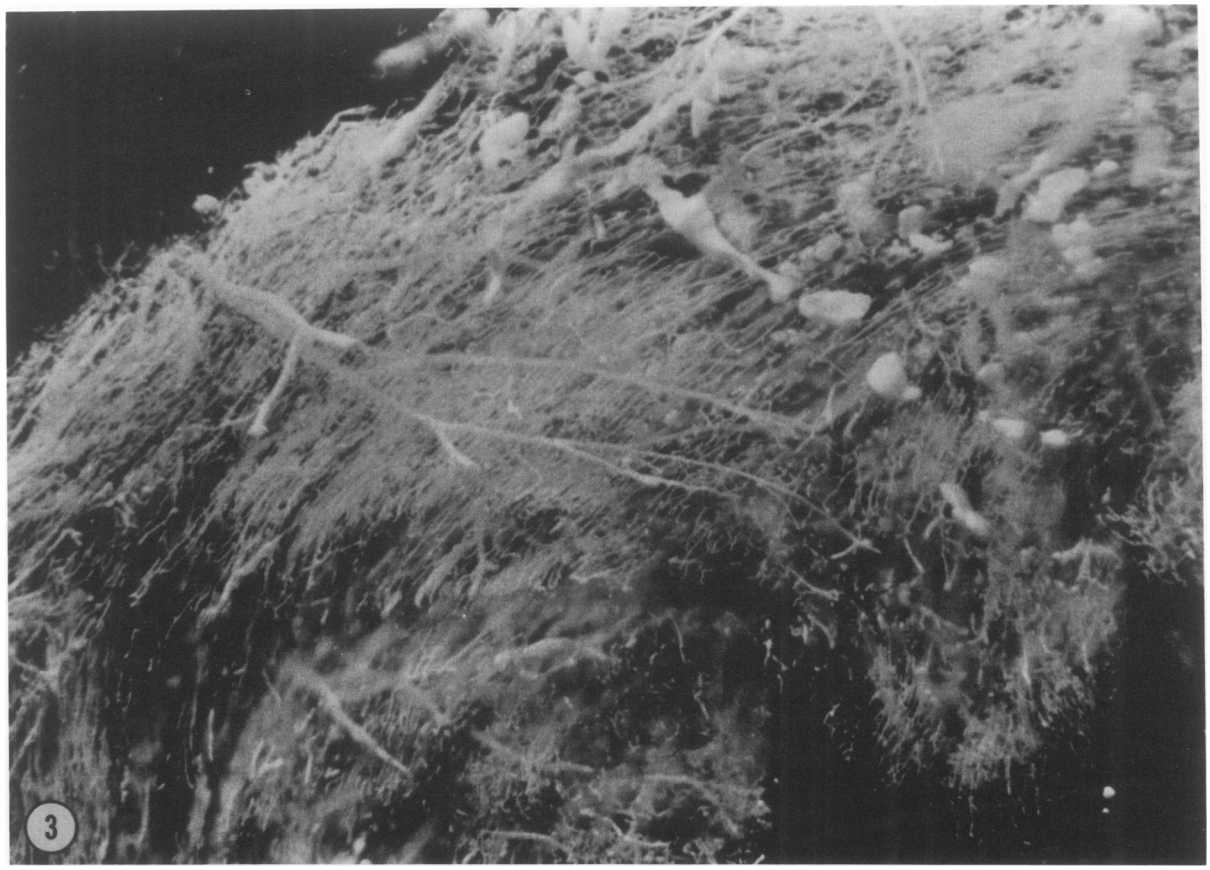


Figure 3 – A transmural view of cleared Microfil-perfused left ventricular myocardium from a diabetic animal. The epicardium is to the upper left, and the endocardium with papillary muscle is at the lower right. The 1-2-mm-thick tissue slice has been epi-illuminated to demonstrate the white silicone rubber. Both large and small vessels are perfused; this is particularly well seen in the central vessel, which runs obliquely from epicardium to endocardium. No vascular abnormalities are present in this specimen. (×60)

Focal areas of pronounced constriction were observed in arteriolar sized vessels, predominantly in the HD group (Figures 6 and 7), whereas they were occasionally noted in 2 hypertensive and 2 diabetic animals (Figures 8 and 9), and rarely in control rats. The narrowed zone appeared as a pronounced constriction of the Microfil cast of variable length, with proximal and distal reconstitution of the vessel caliber. Not infrequently, the pre- and post-stenotic segments were fusiformly dilated (Figures 6, 8 and 9). When the dilation was greater than the vascular caliber peripheral to the constriction, then these lesions were considered to be fusiform aneurysms. Similar constricted lesions with and without dilations were seen in our postmortem study of diabetic human hearts (see Figure 5 of Factor et al[17]).

As noted above, microaneurysms usually were observed in association with vascular narrowing, but they also occurred in unconstricted vessels. Both fusiform (see above) and saccular aneurysms were ob-

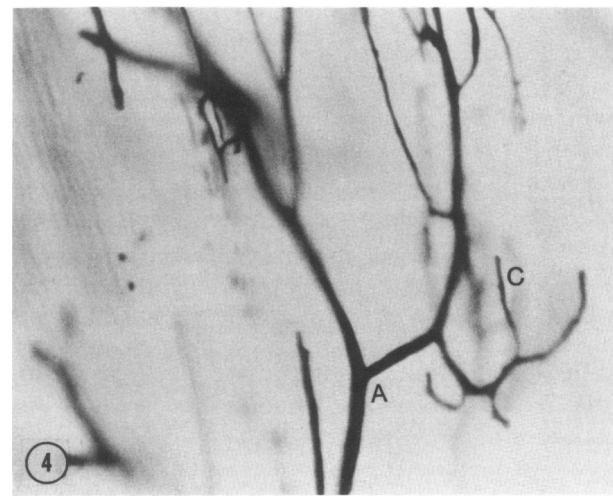
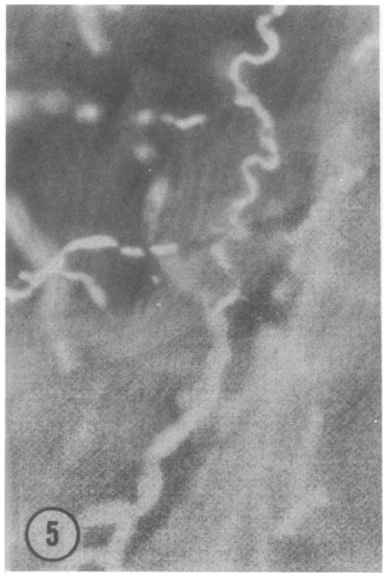


Figure 4 – Microperfused, cleared, and transilluminated tissue from a control animal which demonstrates smoothly tapering arteriolar-sized (*A*) vessels, which give rise to capillaries (*C*). Vessels appear to end abruptly as they enter or leave the plane of section. There is no evidence of vascular tortuosity, constriction, or aneurysmal dilatation. (×150)



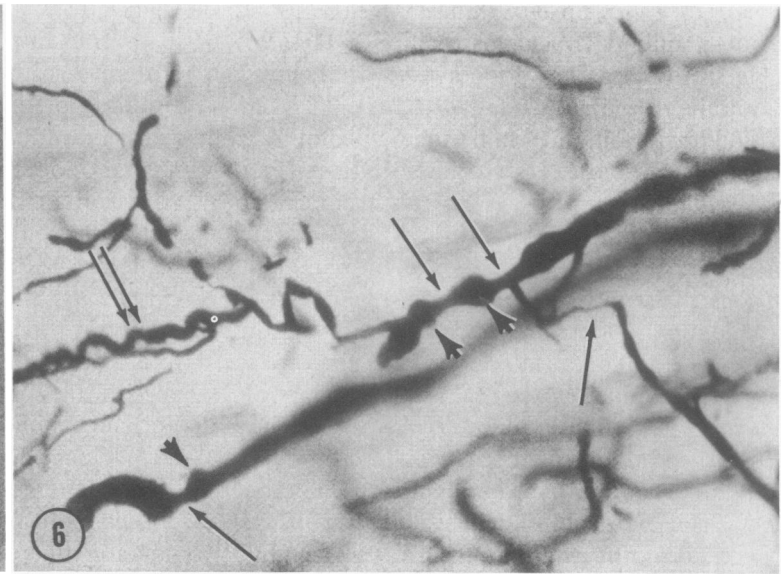


Figure 5 – An arteriolar-sized vessel from a hypertensive-diabetic rat demonstrates pronounced tortuosity. Under the microscope the vessel had a corkscrew appearance. The surrounding vessels (not shown here) did not show similar changes; thus this alteration was not due to tissue shrinkage. (× 150) **Figure 6** – Numerous vessels from this hypertensive-diabetic animal show areas of constriction (*single long arrows*). The arteriole on the right gives rise to a smaller branch, which displays marked tortuosity and focal constriction (*double long arrows*). Around the constricted zones in the larger vessels there are segments of pre- and post-stenotic dilatation, giving the appearance of fusiform microaneurysms (*arrowheads*). (× 150)

served in vessels consistent with small arteries, arterioles, and capillaries (Figures 10 and 11). They were seen in the three disease groups, but with greater frequency in the HD animals; only rare dilations were noted in unaffected controls.

Quantitation of all vascular abnormalities in these animals (see Table 2) revealed a highly significant difference ($P < 0.0001$) between the HD rats and the control or diabetic animals. The H animals were intermediate between the C or D group and the HD group; however, there was a highly significant difference between the HD versus the H group ($P < 0.0001$). Of note, was the fact that there was no difference between the diabetic and control animals (P = NS). What cannot be appreciated from the statistical comparison is that all of the HD animals had microvascular lesions, whereas in the other three groups lesions were relatively uncommon and some animals were completely normal. The quantitation of the comparative vascular abnormalities corresponded very closely to the subjective evaluation of myocardial lesions in this study: the HD rats were most severely affected, there were moderate changes in the H rats, and there were minimal to absent lesions in the diabetic and control groups. A similar distribution was apparent when we quantitated myocardial lesions in our previous study.[2]

Serial Section Study

Analysis of sequential 1-μ sections revealed focally abnormal arteriolar-sized vessels in both the HD and H groups, with no changes noted in D or C animals. This study was not designed to be quantitative, so no comment can be made about the frequency of the abnormalities between the two groups in the small volume of tissue examined. In the HD and H specimens we traced individual arterioles over a 100–200-μ length. These vessels generally had no significant

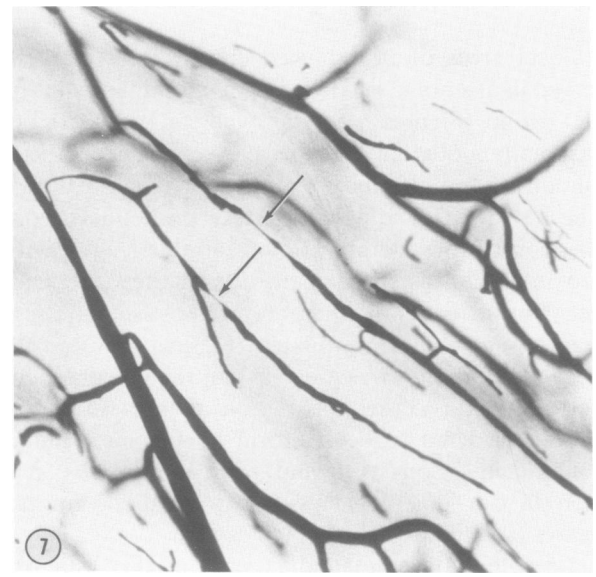


Figure 7 – A field of predominantly normal appearing vessels from a hypertensive-diabetic rat, in which two separate arterioles have relatively long segments of marked constriction, with distal restitution of the vascular caliber. (× 150)

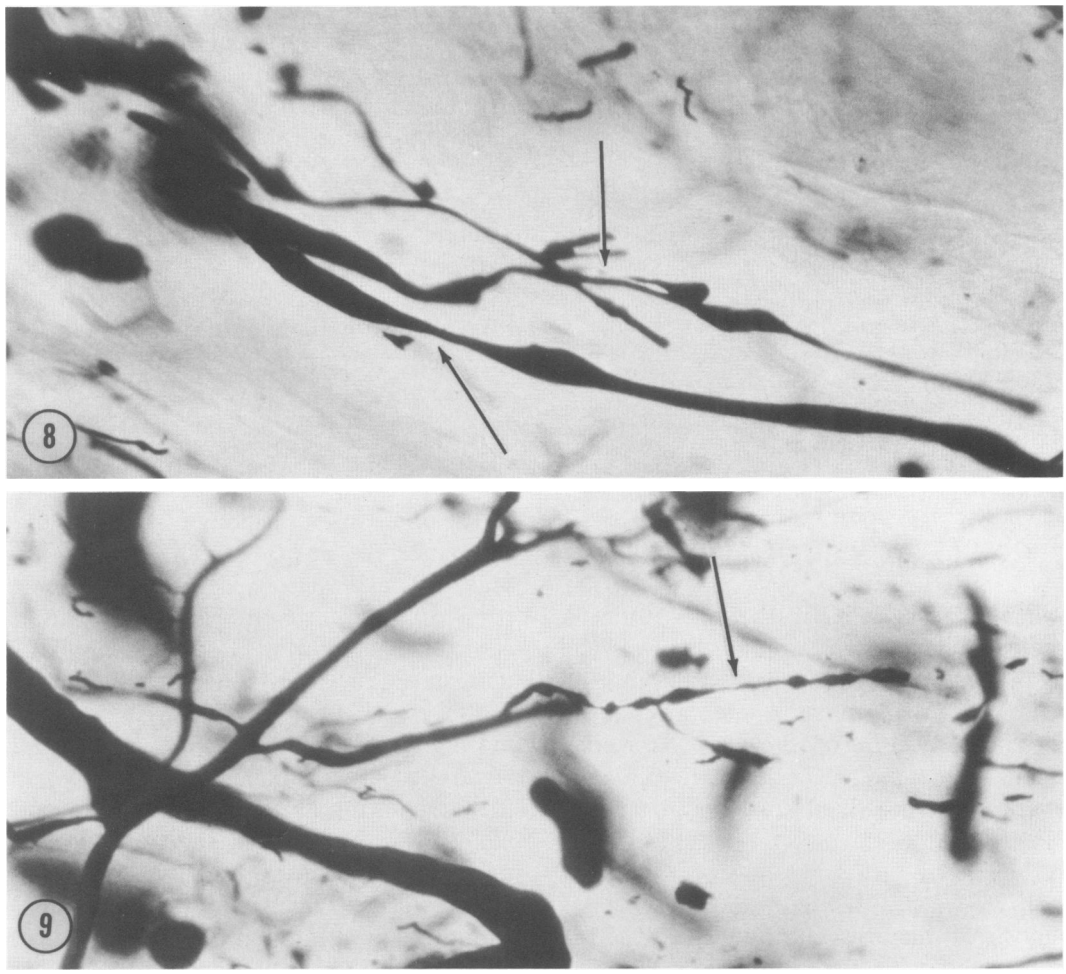


Figure 8—Two branch vessels arising from the same artery in this hypertensive rat have relatively long segments of vascular constriction (*arrows*). Note the pre- and post-stenotic fusiform dilatation, which is particularly prominent in the upper vessel. (×150) **Figure 9**—An arteriole (*arrow*) from one of the 2 abnormal diabetic animals has multiple areas of pronounced constriction and intervening dilated segments giving the vessel a beaded appearance. (×150)

anatomic abnormality; ie, they were composed of single layers of endothelium and smooth muscle without obvious connective tissue deposition, marked mural hypertrophy, or luminal compromise. We observed vessels with patent lumens, which within distances as short as 2 μ constricted to a pinpoint lumen (Figures 12 and 13). Contraction of vessels also occurred more gradually, with luminal obliteration observed in one HD arteriole within 18 μ and subsequent reconstitution of the lumen within an additional 30 μ (Figures 14 and 15). Loose perivascular fibrosis and edema were observed around vessels, often in the

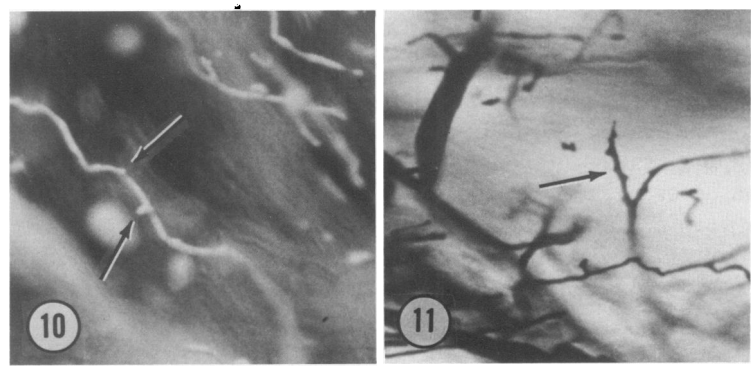


Figure 10—This epi-illuminated small vessel from a hypertensive-diabetic rat has two saccular outpouchings (*arrows*) consistent with microaneurysms. (×150) **Figure 11**—Multiple saccular outpouchings consistent with microaneurysms are present in this small vessel (*arrow*) from a hypertensive-diabetic animal. (×150)

Table 2—Microvascular Perfusion

	Number*	Microvascular abnormalities (Mean ± SD)†	P
Control	5	2.10 ± 0.66	NS; <0.01; <0.0001
Diabetic	4	2.05 ± 1.03	<0.01; <0.0001
Hypertensive	4	4.43 ± 0.64	<0.0001
Hypertensive-diabetic	6	8.42 ± 0.79	

* One control, one hypertensive, and two diabetic animals had inadequate perfusions which precluded quantitation.

† Number of individual vessels with tortuosity, constriction, or microaneurysm formation in 10 nonadjacent × 10 microscopic fields.

region proximal or distal to a constriction. Mast cells also were noted to be increased around these vessels, particularly in areas of perivascular scarring. In general, we believe that the sequential vascular changes identified in this study correspond closely to the constricted lesions demonstrated by the Microfil perfusion cast technique described previously.

Technical Considerations

The diagnosis of microvascular abnormalities, including spasm, employing the techniques of *in vivo* Microfil perfusion and serial 1-μ sections of Epon-embedded tissue is dependent on an analysis of fixed images illustrating a dynamic process. Although we have had extensive experience with these methods in multiple preparations, we cannot say with absolute certainty that the vascular lesions described in this study have only one possible interpretation. It is conceivable that what we defined as spasm is a consequence of poor silicone rubber perfusion or focal vessel collapse. Poor perfusion is not likely to produce areas of narrowing alternating with poststenotic dilatation; nor is segmental collapse of a vessel explicable hemodynamically. However, since we have not actually photographed the vessels during a period of *in vivo* reactivity, this proviso must be kept in mind. Similarly, we cannot entirely rule out the possibility that Microfil itself may induce some of the vasospastic changes demonstrated in this study, by stimulating vessels to contract. If so, we would have to postulate differential reactivity of HD vessels to explain the differences between the four groups of animals. This may, in fact, be the case; however, rather than indicating an artifact of the procedure, it may reflect vascular abnormalities present *in vivo*. Finally, it is necessary to point out that interpretation of saccular aneurysms or other focal 3-dimensional vessel alterations requires sections of cleared tissue sufficiently thick to permit adequate focusing above and below the lesion. Without such care, end-on views of a vascular branch or incomplete Microfil perfusion of a branch may be misconstrued as a microaneurysm. Although microaneurysms, such as those depicted in Figure 10, were carefully analyzed in depth, any failure to study these lesions at different levels could give an erroneously increased number of such abnormalities.

Discussion

The objective of the present study was to determine the pattern of microvascular disease in perfused *in vivo* HD rat hearts. We previously showed that the stress of hypertension in diabetes mellitus, a common clinical association,[26] leads to the development of extensive focal myocardial necrosis and scarring significantly greater than with either disease alone.[2,3] The morphologic abnormalities in the rat model are similar to those we identified at postmortem examination in human subjects with hypertension and diabetes mellitus, who died with severe congestive cardiomyopathy.[1] Recent investigations of the HD rat have shown features which suggest an even closer parallel with the clinical cardiomyopathy; a longitudinal study has revealed a high prevalence of sudden death and morphologic features consistent with acute and chronic heart failure (Fein et al, unpublished observations, 1983). In both the clinical and the experimental disease, the presence of interstitial and replacement myocardial fibrosis was independent of extramural or intramural coronary artery arteriosclerosis,[1,2] suggesting that the lesions might be secondary to microvascular disturbances.

In the present study we demonstrated highly significant differences, comparing HD rat microcirculatory abnormalities versus disturbances present in hypertensive, diabetic, or control animals. The relative frequency of vascular lesions in each group corresponds to the comparative morphologic myocardial degeneration previously observed in these four groups.[2] The microvascular alterations were similar to those we described in a postmortem study of human diabetic hearts,[17] and were qualitatively like the dynamic lesions we identified in the Syrian hamster,[4] another model of cardiomyopathy. In both the HD rat and the Syrian hamster microvascular perfusion patterns consistent with hyperreactivity (spasm) have been seen associated with focal myocardial degeneration. In the

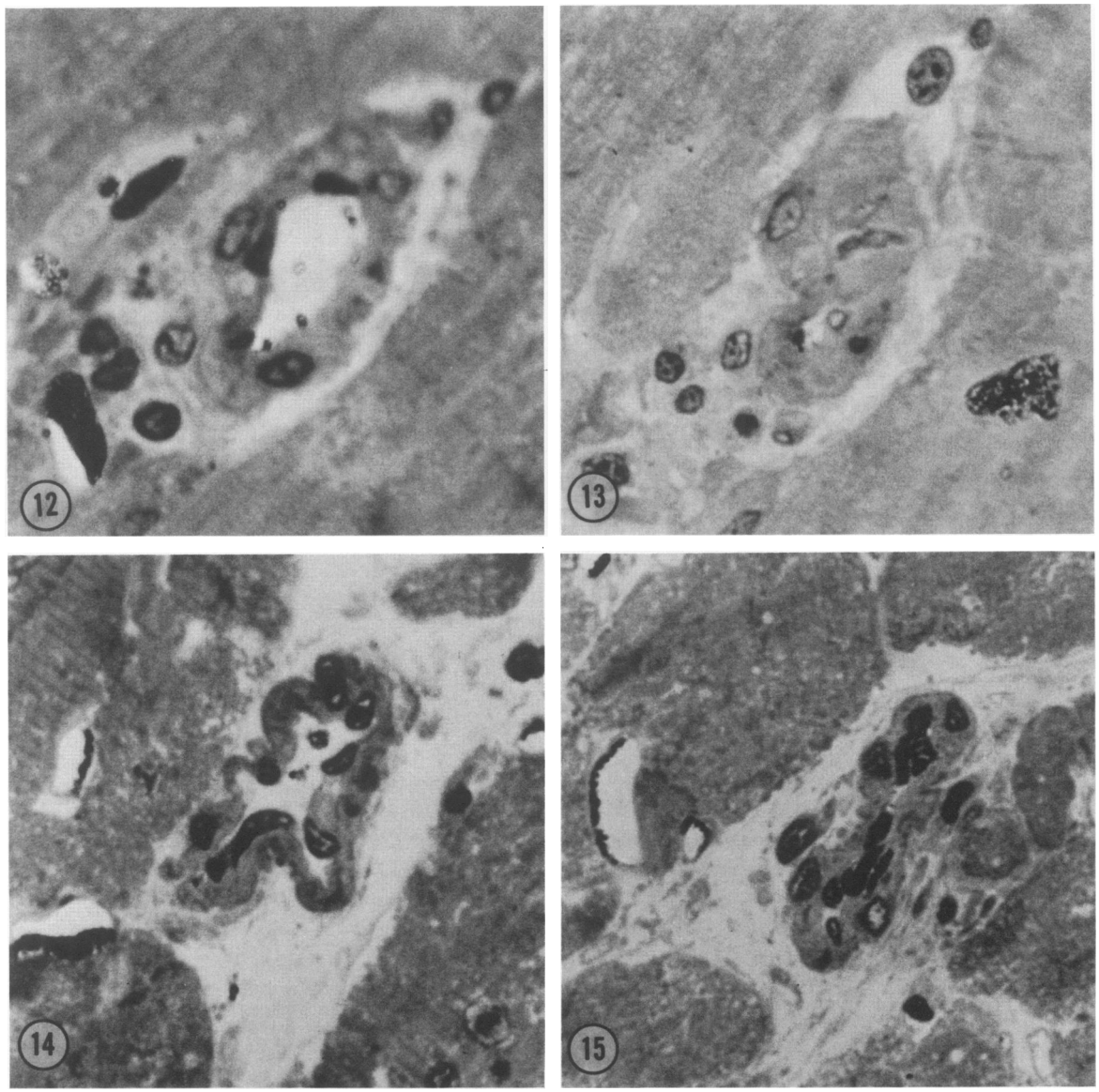


Figure 12—This 1-μ, Epon-embedded serial section from a hypertensive rat shows an arteriole composed of endothelial cells and a single layer of uncontracted smooth muscle. The lumen is patent at this level. The vessel is surrounded by a few mononuclear cells and a small amount of connective tissue. (Toluidine blue, ×400) **Figure 13**—The same vessel seen in Figure 12, sectioned 2 μ further along its longitudinal axis. At this level the vessel lumen is almost completely obliterated, and the smooth muscle is contracted or possibly degenerated. (Toluidine blue, ×400) **Figure 14**—A 1-μ, Epon-embedded serial section from a hypertensive-diabetic rat shows an arteriole with a partially contracted wall but patent lumen at this level. Note the relative absence of connective tissue or inflammatory cells in the perivascular space. (Toluidine blue, ×400) **Figure 15**—The same vessel seen in Figure 14, sectioned 20 μ further along its longtiduinal axis. The vessel lumen is almost completely obliterated at this level, with contraction of endothelial and smooth muscle cells. The perivascular space contains loose connective tissue and mononuclear cells. Within an additional 30 μ of sectioning, the vessel lumen was completely patent and the perivascular space was devoid of significant connective tissue. (Toluidine blue, ×400)

hamster, microvascular lesions have been linked to the development of cardiomyopathy[4,34]; in the HD rat it is conceivable that similar disturbances may play a role in the pathogenesis of myocardial disease.

Structural (in contrast to dynamic or transient) microvascular abnormalities are a relatively consistent feature of long-standing diabetes mellitus. The most easily identified histologic alteration described in virtually every tissue studied[12] is basement membrane thickening. Increases of myocardial pericapillary basal lamina material have been described in human diabetics[10,19] and in experimental diabetes with[3] and without associated hypertension.[35] Although Regan et al[36] implicated increased interstitial PAS-positive material in the development of diabetic cardiomyopathy in alloxan-treated dogs, observations in normotensive diabetic rats and humans suggest that there is minimal if any morphologic myocardial

degeneration consistent with cardiomyopathy in this setting.[1,2]

The other fixed anatomic microvascular abnormality characteristic of diabetes mellitus is the microaneurysm. These lesions may be fusiform dilatations or saccular outpouchings of arterioles, venules, or capillaries. Probably because they can be examined *in vivo* without special tissue preparation, they have been most extensively described in the retina,[13] where their presence is usually taken as an indicator of systemic disease severity. Like basement membrane thickening, retinal microaneurysms are not diagnostic of diabetes mellitus, because they have been described in many conditions including hypertension.[37] Our studies of diabetic hearts perfused postmortem with silicone rubber revealed typical capillary and arteriolar microaneurysms, as well as areas of constriction and tortuosity in the microcirculation.[17] The microvascular abnormalities in the autopsy material were similar to the alterations described in the present report.

In addition to fixed structural lesions, there are indications that there may be other abnormalities of the diabetic microvasculature which may contribute to tissue and vessel damage. Much of the evidence is contradictory; however, this may reflect different models of diabetes, methods of diabetes induction, and duration of hyperglycemia. Altura et al[38] have recently reviewed the question of diabetic vascular smooth muscle reactivity and have concluded that in early diabetes there may be hyporeactivity, whereas advanced diabetes may be characterized by arteriolar constriction and microangiopathy, among other abnormalities. Bohlen and colleagues[21,39,40] have focused on progressive loss of capillaries with increased duration of diabetes, in association with early microvascular dilatation and subsequent constriction. Mueller et al[22] have shown that there is marked sympathetic autonomic dysfunction of small vessels in alloxan-treated rats, with eventual hypersensitivity of the microvasculature to norepinephrine. In contrast to the latter observation, Pfaffman et al[41] showed a decreased responsiveness of arterial smooth muscle to alpha-adrenergic stimulation in streptozotocin diabetic rats. The general lack of responsiveness of the microcirculation in normotensive diabetes mellitus may explain the relative absence of significant spastic lesions that we observed in perfused diabetic cardiac vessels in this study.

In contrast to the lack of strong evidence of arteriolar constriction in diabetes mellitus, there is direct observational data to support this concept in hypertension. Prewitt et al[25] have identified arteriolar vasoconstriction in the spontaneously hypertensive rat, with functional rarefaction of arterioles, possibly leading to anatomic rarefaction of capillaries. Click and colleagues[42] have shown that arterioles in the hypertensive hamster cheek pouch have increased sensitivity to norepinephrine; they concluded that structural changes in these vessels result from rather than cause hypertension. Directly relevant to our observations in the heart, Bhan et al[43] demonstrated segmental contraction of intramyocardial vessels in angiotensin-induced hypertension, with the development of reflow-type myocardial necrosis, which they attributed to the vascular lesions. They were able to block the development of myocardial injury by treating the animals with phentolamine, but this did not prevent morphologic vascular injury. They concluded that the vascular lesions may provoke myocardial necrosis in the setting of angiotensin II hypertension. In our model of renovascular hypertension with or without diabetes, we identified focal myocardial necrosis and replacement fibrosis, which we believe to be a reperfusion type of injury.[2] The present study demonstrates marked constrictive activity of the microcirculation in these animals, which may contribute to the development of myocardial necrosis by causing localized ischemia and subsequent reperfusion upon relaxation of the spasm.

We suggested previously[4] that the presence of multiple constrictions, dilatations, and tortuosities studied in cleared tissue slices following silicone rubber perfusion of the beating heart or by serial section reconstruction are indicative of changes in the *in vivo* microcirculation. Direct support for this view is not available from our laboratory; however, the fact that we abolished similar abnormalities in the cardiomyopathic hamster microcirculation is indirect evidence that these lesions are dynamic.[4] More conclusive support comes from several older studies of hypertension and microvascular reaction patterns studied *in vivo*. Byrom,[24] in his investigation of hypertensive encephalopathy in rats, demonstrated transient spasm in cerebral vessels viewed through a cranial window. The patterns of focal constriction and dilatation identified in the brain (and in the mesentery of encephalopathic animals) are remarkably similar to the Microfil casts illustrated in this study. In another study of hypertensive vascular disease, Giese[23] showed mesenteric vessels photographed through an abdominal window, and demonstrated typical constrictions and dilatations in affected animals. By employing a vascular labeling technique, he also was able to show that the hypertensive vessels were leaky in areas of dilatation. Finally, although the perfusions were not performed *in vivo*, another technique has been used to study the microvasculature in hypertension, which

demonstrated findings comparable to ours. Hill and Heptinstall[44] studied renal hypertensive vessels with a microangiographic method after barium perfusion. They showed prominent vasoconstricted, dilated, and tortuous vessels in severely damaged kidneys and concluded that the inability of the vessel to contract led to focal dilatation and subsequent lesions. Based on these previous observations as well as our own studies, we believe that our microvascular perfusion technique enables us to identify dynamic abnormalities in the myocardial microcirculation.

We believe that the microcirculatory patterns in the HD animals are intimately related to the development of myocardial degeneration. In the cardiomyopathic Syrian hamster, where similar vascular lesions are present, the prevention of vasoreactivity with verapamil[4] or prazosin[5] prevents the development of myocardial necrosis. That spasm of the microcirculation can cause myocardial necrosis has been demonstrated by our group in another model. We recently showed that embolization of 25-μ microspheres into the coronary microcirculation produced focal myocardial necrosis of the reperfusion type essentially the same as that seen in the HD rat or cardiomyopathic hamster.[45] Of significance, however, pretreatment of animals with α_1-adrenergic blocking drugs prevented necrosis, suggesting that adrenergically mediated spasm of small arterioles rather than embolic obstruction alone can produce micronecrosis. In the HD rat, and to a lesser extent in the H rat, it is conceivable that the identifiable vasoconstrictive lesions in the microcirculation lead to myocardial damage and eventual replacement fibrosis. That myocardial necrosis is not seen in normotensive diabetic animals or controls may be accounted for by the absence of significant vasoconstrictive events in the microcirculation.

The major differences between the HD rat model of cardiomyopathy and the Syrian hamster is the presence of extensive interstitial fibrosis in the former but not the latter. A direct explanation for the interstitial scarring awaits further study. However, the demonstrated increased leakiness of the diabetic circulation[18-20] and the identification of increased vascular permeability in the dilated segments of hypertensive vessels[23] suggest that the combination of the two vascular insults in the HD rat leads to accelerated leakage of fluid into the interstitial space. Williamson and Kilo[8] proposed that extravascular plasma constituents might stimulate synthesis of basement membrane or collagen in diabetes, leading to fibrosis. Our observations of perivascular fibrosis proximal to constricted segments of serially sectioned small vessels supports a relationship between microvascular abnormalities and interstitial fibrosis.

We propose that the summation of focal microvascular spastic events in hypertension and diabetes leads to progressive multifocal myocardial necrosis and subsequent fibrosis. In addition, the effect of hypertension and diabetes on microvascular permeability may lead to interstitial scarring. Although the individual events are occurring at the microvascular level and may only eventuate in microscopic lesions, the result over time is generalized multifocal damage and ventricular dysfunction. We have previously suggested that this scenario of microvascularly determined micronecrosis may explain the development of cardiomyopathy in the Syrian hamster and may play a role in other cardiomyopathies as well.[34] Since we have now demonstrated that many HD rats die with severe congestive heart failure or experience sudden death (Fein et al, unpublished observations, 1983), like cardiomyopathic hamsters and humans, we believe that the HD rat model has great relevance for the understanding of the pathogenesis of this particular cardiomyopathy. In the future, if therapy can be devised to prevent microvascular lesions in hypertension and diabetes, with concurrent prevention of morphologic cardiac lesions, then we may have new insight into the means for preventing clinical cardiomyopathy in this combined disease.

References

1. Factor SM, Minase T, Sonnenblick EH: Clinical and morphological features of human hypertensive-diabetic cardiomyopathy. Am Heart J 1980, 99:446-458
2. Factor SM, Bhan R, Minase T, Wolinsky H, Sonnenblick EH: Hypertensive-diabetic cardiomyopathy in the rat: An experimental model of human disease. Am J Pathol 1981, 102:219-228
3. Factor SM, Minase T, Bhan R, Wolinsky H, Sonnenblick EH: Hypertensive diabetic cardiomyopathy in the rat: Ultrastructural features. Virchows Arch [Pathol Anat] 1983, 398:305-317
4. Factor SM, Minase T, Cho S, Dominitz R, Sonnenblick EH: Microvascular spasm in the cardiomyopathic Syrian hamster: A preventable cause of focal myocardial necrosis. Circulation 1982, 66:342-354
5. Factor SM, Cho S: Alpha adrenergic blockade of the cardiomyopathic Syrian hamster: Further evidence for the microvascular etiology of micronecrosis. Fed Proc 1983, 42:920
6. McMillan DE: Deterioration of the microcirculation in diabetes. Diabetes 1975, 24:944-957
7. McMillan DE: Diabetic angiopathy: Its lessons in vascular physiology. Am Heart J 1978, 96:401-406
8. Williamson JR, Kilo C: Basement-membrane thickening and diabetic microangiopathy. Diabetes 1976, 25: 925-927
9. Jordan SW, Perley MJ: Microangiopathy in diabetes mellitus and aging. Arch Pathol 1972, 93:261-265
10. Silver MD, Huckell VF, Lorber M: Basement membranes of small cardiac vessels in patients with diabetes and myxoedema: Preliminary observations. Pathology 1977, 9:213-220
11. Vracko R, Pecoraro RE, Carter WB: Overview article:

Basal lamina of epidermis, muscle fibers, muscle capillaries, and renal tubules: Changes with aging and in diabetes mellitus. Ultrastruct Pathol 1980, 1:559–574
12. Williamson JR, Kilo C: Vascular complications in diabetes mellitus. N Engl J Med 1980, 302:399–400
13. Cogan DG, Toussaint D, Kuwabara T: Retinal vascular patterns: IV. Diabetic retinopathy. Arch Opthalmol 1961, 66:366–378
14. Rand LI: Recent advances in diabetic retinopathy. Am J Med 1981, 70:595–602
15. Bloodworth JMB Jr: A re-evaluation of diabetic glomerulosclerosis 50 years after the discovery of insulin. Hum Pathol 1978, 9:439–453
16. Nakamoto Y, Takazakura E, Hayakawa H, Kawai K, Dohi K, Fijoka M, Kida H, Hattori N, Takeuchi J: Intrarenal microaneurysms in diabetic nephropathy. Lab Invest 1980, 42:433–439
17. Factor SM, Okun EM, Minase T: Capillary microaneurysms in the human diabetic heart: N Engl J Med 1980, 302:384–388
18. Parving HH, Rasmussen SM: Transcapillary escape rate of albumin and plasma volume in short- and long-term juvenile diabetes. Scand J Clin Lab Invest 1973, 32:81–87
19. Fischer VW, Barner HB, LaRose LS: Quadriceps and myocardial capillary basal laminae: Their comparison in diabetic patients. Arch Pathol Lab Med 1982, 106: 336–341
20. Joyner WL, Mayhan WG, Johnson RL, Phares CK: Microvascular alterations develop in Syrian hamsters after induction of diabetes mellitus by streptozotocin. Diabetes 1981, 30:93–100
21. Bohlen HG, Hankins KD: Early arteriolar and capillary changes in streptozotocin-induced diabetic rats and intraperitoneal hyperglycaemic rats. Diabetologia 1982, 22:344–348
22. Mueller SM, Mueller TM, Ertel PJ: Sympathetic and vascular dysfunction in early experimental juvenile diabetes mellitus. Am J Physiol 1982, 243:H139–H144
23. Giese J: Acute hypertensive vascular disease: Two studies on vascular reaction patterns and permeability changes by means of vital microscopy and colloidal tracer technique. Acta Pathol Microbiol Scand 1964, 62:497–515
24. Byrom FB: The pathogenesis of hypertensive encephalopathy and its relation to the malignant phase of hypertension: Experimental evidence from the hypertensive rat. Lancet 1954, 2:201–211
25. Prewitt RL, Chen IIH, Dowell R: Development of microvascular rarefaction in the spontaneously hypertensive rat. Am J Physiol 1982, 243:H243–H251
26. Christlieb AR: Diabetes and hypertensive vascular disease: Mechanisms and treatment. Am J Cardiol 1973, 32:592–606
27. Knowler WC, Bennett PH, Ballintine EJ: Increased incidence of retinopathy in diabetics with elevated blood pressure: A six-year follow-up study in Pima Indians. N Engl J Med 1980, 302:645–650
28. Parving HH. Andersen AR, Smidt UM, Oxenboll B, Edsberg B, Sandahl Christiansen J: Diabetic nephropathy and arteriol hypertension. Diabetologia 1983, 24: 10–12
29. Mogensen CE: Long-term antihypertensive treatment (over six years) inhibiting the progression of diabetic nephropathy. Acta Endocrinol 1981, Suppl 242:31–32
30. Wolinsky H: Effects of hypertension and its reversal on the thoracic aorta of male and female rats. Circ Res 1971, 28:633–637
31. Schaper W: The collateral circulation of the heart. Amsterdam, North Holland, 1971, p 5
32. Okun EM, Factor SM, Kirk ES: End-capillary loops in the heart: An explanation for discrete myocardial infarctions without border zones. Science 1979, 206: 565–567
33. Factor SM, Okun EM, Minase T, Kirk ES: The microcirculation of the human heart: End-capillary loops with discrete perfusion fields. Circulation 1982, 66: 1241–1248
34. Factor SM, Sonnenblick EH: Hypothesis: Is congestive cardiomyopathy caused by a hyperreactive myocardial microcirculation (microvascular spasm)? Am J Cardiol 1982, 50:1149–1152
35. Fischer VW, Leskiw ML, Barner HB: Myocardial structure and capillary basal lamina thickness in experimentally diabetic rats. Exp Mol Pathol 1981, 35: 244–256
36. Regan TJ, Ettinger PO, Khan MI, Jesrani MU, Lyons MM, Oldewurtel HA, Weber M: Altered myocardial function and metabolism in chronic diabetes mellitus without ischemia in dogs. Circ Res 1974, 35:222–237
37. Neetans A: Microcirculation in the diabetic eye, The Microcirculation in Diabetes. Edited by E Davis, Basel, S. Karger, 1979, pp 55–93
38. Altura BM, Halevy S, Turlapaty PDMV: Vascular smooth muscle in diabetes and its influence on the reactivity of blood vessels,[37] pp 118–150
39. Bohlen HG, Niggl BA: Adult microvascular disturbances as a result of juvenile onset diabetes in Db/Db mice. Blood Vess 1979, 16:269–276
40. Bohlen HG, Niggl BA: Arteriolar anatomical and functional abnormalities in juvenile mice with genetic or streptozotocin-induced diabetes mellitus. Circ Res 1979, 45:390–396
41. Pfaffman MA, Hilman R, Darby A: Contractile and relaxing activity of arterial smooth muscle from streptozotocin-diabetic rats. Res Commun Chem Pathol Pharmacol 1980, 30:283–299
42. Click RL, Gilmore JP, Joyner WL: Direct demonstration of alterations in the microcirculation of the hamster during and following renal hypertension. Circ Res 1977, 41:461–467
43. Bhan RD, Giacomelli F, Wiener J: Adrenoreceptor blockade in angiotensin-induced hypertension. Effect on rat coronary arteries and myocardium. Am J Pathol 1982, 108:60–71
44. Hill GS, Heptinstall RH: Steroid-induced hypertension in the rat: A microangiographic and histologic study on the pathogenesis of hypertensive vascular and glomerular lesions. Am J Pathol 1968, 52:1–39
45. Eng C, Cho S, Factor SM, Sonnenblick EH, Kirk ES: Myocardial micronecrosis produced by microsphere embolization: Role of an alpha adrenergic tonic influence on the coronary microcirculation. Circ Res 1984, 54:74–82

Acknowledgments

We thank Ms. Renee Dominitz for her outstanding technical support on this project and Mrs. Marilyn Sasso for her excellent work in the preparation of the manuscript.