Virchows Archiv A Pathol Anat (1992) 420:499–506

Virchows Archiv A
Pathological Anatomy and Histopathology

Effects of combined renovascular hypertension and diabetes mellitus on myocardial cells, non-vascular interstitium and capillaries: a stereological study on rat hearts

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Received June 30, 1991 / Received after revision September 4, 1991 / Accepted September 5, 1991

Summary. The effects of combined renovascular hypertension and diabetes mellitus on the rat heart were investigated in order to detect possible synergistic effects of the two conditions. Hypertensive diabetic and hypertensive non-diabetic animals were compared to diabetic and non-diabetic controls. Hypertension was established for 12 weeks by a surgical stenosis of the left renal artery; diabetes mellitus was maintained for 8 weeks by a single intraperitoneal injection of 60 mg/kg streptozotocin. Light microscopic stereology did not reveal significant divergences between diabetic hypertensives and non-diabetic hypertensives. Hypertension induced a focal perivascular and interstitial fibrosis with increased volume densities of non-vascular interstitium and fibrosis ($P<0.001$). Capillary density (Q_A) was decreased in transverse sections ($P<0.01$) and increased in longitudinal sections ($P<0.01$). This indicates a three-dimensional remodelling of the capillary bed with an increased number of obliquely running capillaries. At least the length density (L_V) of capillaries (mm/mm^3) tends to be normalized in long-term renovascular hypertension. At the ultrastructural level, a synergism of hypertension and diabetes mellitus was observed: the volume ratio of mitochondria to myofibrils was significantly decreased in hypertensive diabetics, but not in non-diabetic hypertensives or in diabetics. This may enhance the risk of cardiac deterioration. We conclude that the primary target of the synergistic damage in hypertensive diabetic heart muscle disease is the myocardial cell and not the cardiac interstitium.

Key words: Diabetes mellitus – Renovascular hypertension – Myocardium – Stereology – Ultrastructure

Introduction

Hypertension and diabetes mellitus are frequent disorders in modern industrial countries. Epidemiological studies have established both conditions as risk factors of congestive failure even in the absence of coronary atherosclerosis (McKee et al. 1971; Kannel et al. 1974; Factor et al. 1980).

Recently, co-existence of diabetes mellitus has been suggested to potentiate myocardial alterations and a specific hypertensive diabetic heart muscle disease has been described (Fein et al. 1989) on the basis of autopsy studies (Factor et al. 1980) and experimental investigations (Factor et al. 1981; Fein et al. 1984). It has been reported that one histological hallmark of the synergistic effects on the heart is the enhancement of cardiac fibrosis.

However, this has never been established with objective quantitative morphological methods. Furthermore, a recent experiment on short-term combination of hypertension and diabetes with quantitative methods did not reveal synergistic effects on the cardiac interstitium. At the ultrastructural level, however, synergistic damage of myocardial cells was observed (Mall et al. 1987a). The present experiment was designed in order to establish possible long-term synergistic effects on cardiac structure, including myocardial cells and interstitial space.

Materials and methods

For this experiment 93 young male Wistar rats were primarily divided into two groups by the use of random numbers (see below). Sixty-six animals were treated with a surgical stenosis of the left renal artery with a silver clip of 0.20–0.22 mm internal diameter. Twenty-seven animals served as sham-operated control. After induction of hypertension, a 2% NaCl solution was offered in order to prevent malignant hypertension (Möhring et al. 1976).

* Preliminary results of this study have been published in: Mall G (1991) Morphometric study on the rat heart in combined renovascular hypertension and diabetes mellitus. In: Nagano N, Dhalla NS (eds) The diabetic heart. Raven Press, New York, pp 115–124

** Dedicated to Prof. Dr. med. G. Seifert on the occasion of his 70th birthday

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All animals were caged individually and received chow pellets and tap-water ad libitum. In total, 34 animals died before the end of the experiment and had therefore to be excluded (see Results for details). Within 4 weeks, 21 of the operated animals died. After 8 weeks, the surviving animals were divided into four groups: a *hypertensive group* where 20 animals served as non-diabetic hypertensives for further 12 weeks; a *diabetic group* where 16 sham-operated animals were treated with 60 mg/kg body weight i.p. streptozotocin, thus inducing diabetes mellitus for 12 weeks; a *hypertensive diabetic group* in which 25 operated animals were treated with 60 mg/kg body weight streptozotocin i.p. in order to generate a hypertensive-diabetic group for 12 weeks; and a *control group* of 15 sham-operated rats.

Systolic blood pressure was determined by means of a tail-plethysmographic method in slight ether anaesthesia. Operated animals with a systolic blood pressure lower than 140 mm Hg at any time were excluded from the study. Streptozotocin-injected animals were investigated with glucose oxidase kits (Boehringer, Mannheim, FRG).

At the end of the experiments the viscera were fixed by retrograde vascular perfusion at a pressure of 110 mm Hg after catheterization of the abdominal aorta, as described elsewhere (Mall et al. 1987b). Left ventricular papillary muscles were randomly cut either longitudinally or transversely with a tissue sectioner as described elsewhere (Mall et al. 1978; Mattfeldt and Mall 1984). Seven transversely cut 200-μm slices of two longitudinally cut 200-μm slices were randomly selected for stereology and embedded in Epon-Araldite as described elsewhere (Mall et al. 1986, 1987b). Semithin sections (1 μm) were stained with methylene blue and basic fuchsin (Di'Sant'Agnese and De Mesy Jensen 1984) and examined light microscopically using oil immersion and phase contrast. Ultrathin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss EM 10 electron microscope.

In addition, six transversely cut slices of the total heart were embedded in paraffin, sectioned at 3–4 μm, and stained with haematoxylin and eosin and a specific collagen stain (Sirius red).

Details of the quantitative stereology (morphometry) method used have been described elsewhere (Mall et al. 1978, 1986, 1987a and b, 1988; Weibel 1979; Mattfeldt and Mall 1984). Briefly, volume densities (V_V : volume of a structure per unit reference volume) can be determined by point counting (Weibel 1979). This is true in transverse, oblique as well as longitudinal sections of the myocardium. In contrast, estimation of length densities of capillaries (L_V : length per unit reference volume) depends on the distribution of the length elements in space and the angle α between the section plane and the preferred orientation (axis of anisotropy in stereological terms). In practice, L_V of capillaries is derived from the number of transsects per unit transverse sectional area $[Q_A(\alpha=0)]$ and the number of capillary transects per unit longitudinal sectional area $[Q_A(\alpha=\pi/2)]$ according to the equation:

$$L_V = c_1(K_L, \alpha=0) \cdot Q_A(\alpha=0)$$

The coefficient of correction $c_1(K_L, \alpha=0)$ is determined by the ratio $Q_A(\alpha=0)/Q_A(\alpha=\pi/2)$ as described elsewhere (Mattfeldt and Mall 1984).

Capillary transects were counted on semithin sections, and a Zeiss eyepiece containing 100 test points was used to count points on capillaries, non-vascular interstitium and myocardial cells, as described elsewhere in detail (Mall et al. 1986, 1987a and b). Myocardial cell organelles were evaluated on electron microscopic images which were visualized on-line with a television monitor and a point grid (Mall et al. 1986, 1987a and b, 1988). V_V of fibrosis was determined in left ventricles (including ventricular septum) on six transverse sections of the heart with an automatic image analysing system IBAS II (Kontron, Eching, FRG). Sirius-red-stained paraffin sections were digitized under continuous visual control at a primary magnification of 40:1 using a grey value threshold. The area fraction of the sirius-red-stained myocardium corresponds to the volume density of fibrosis in the left ventricle (Jalil et al. 1989).

Dunnett's test was used to detect divergences between the arithmetic mean of the hypertensive diabetic group and the means of the others. A result was considered to be significant if the probability of error, P, was lower than 0.05 (Sachs 1974).

Results

Thirty-four animals out of 93 died before the end of the experiment. Clipping of the renal artery was followed by death of 21 animals within 4 weeks. Later, 6 rats died in the hypertensive group, 1 animal in the diabetic group, and 6 in the hypertensive diabetic group. Congestion from cardiac failure was not observed. Autopsy studies excluded the presence of infective disease such as pneumonia or myocarditis. In the hypertensive group, 6 animals were normotensive at the end of the experiment as a result of complete atrophy of the clipped kidney. All rats included in the diabetic group presented with diabetes mellitus. In the hypertensive diabetic group, 7 animals developed both diseases, whereas 8 animals presented only with hyper-

Table 1. Heart weights and body weights

Parameter	Control group $n=15$	Hypertensive group $n=8$	Diabetic group $n=15$	Hypertensive diabetic group $n=7$
Body weight (g)	543* ± 44	533* ± 39	378 ± 42	386 ± 71
LVW/body weight (mg/g)	2.13* ±0.31	2.75* ±0.33	2.58* ±0.19	3.60 ±0.75
LVW (mg)	1158 ± 124	1467 ± 284	976* ± 80	1389 ± 180

Means ± standard deviations
LVW, Left ventricular weight
* Significant divergences (Dunnett's test) from the hypertensive diabetic group ($P<0.05$)

Table 2. Biological variables

	Control group $n=15$	Hypertensive group $n=8$	Diabetic group $n=15$	Hypertensive diabetic $n=7$
Systolic pressure (mm Hg)	126* ± 9	200 ± 16	136* ±14	196 ± 23
Serum glucose (mg/dl)	107* ± 12	104* ± 15	376 ± 28	384 ± 72
Creatinine (mmol/l)	0.64 ±0.08	0.70 ±0.09	0.59 ±0.06	0.65 ±0.07
Urea (mg/dl)	42 ± 6	50 ± 7	64 ±16	60 ± 8

Means ± standard deviations

* Significant divergences (Dunnett's test) from the hypertensive diabetic group ($P<0.05$)

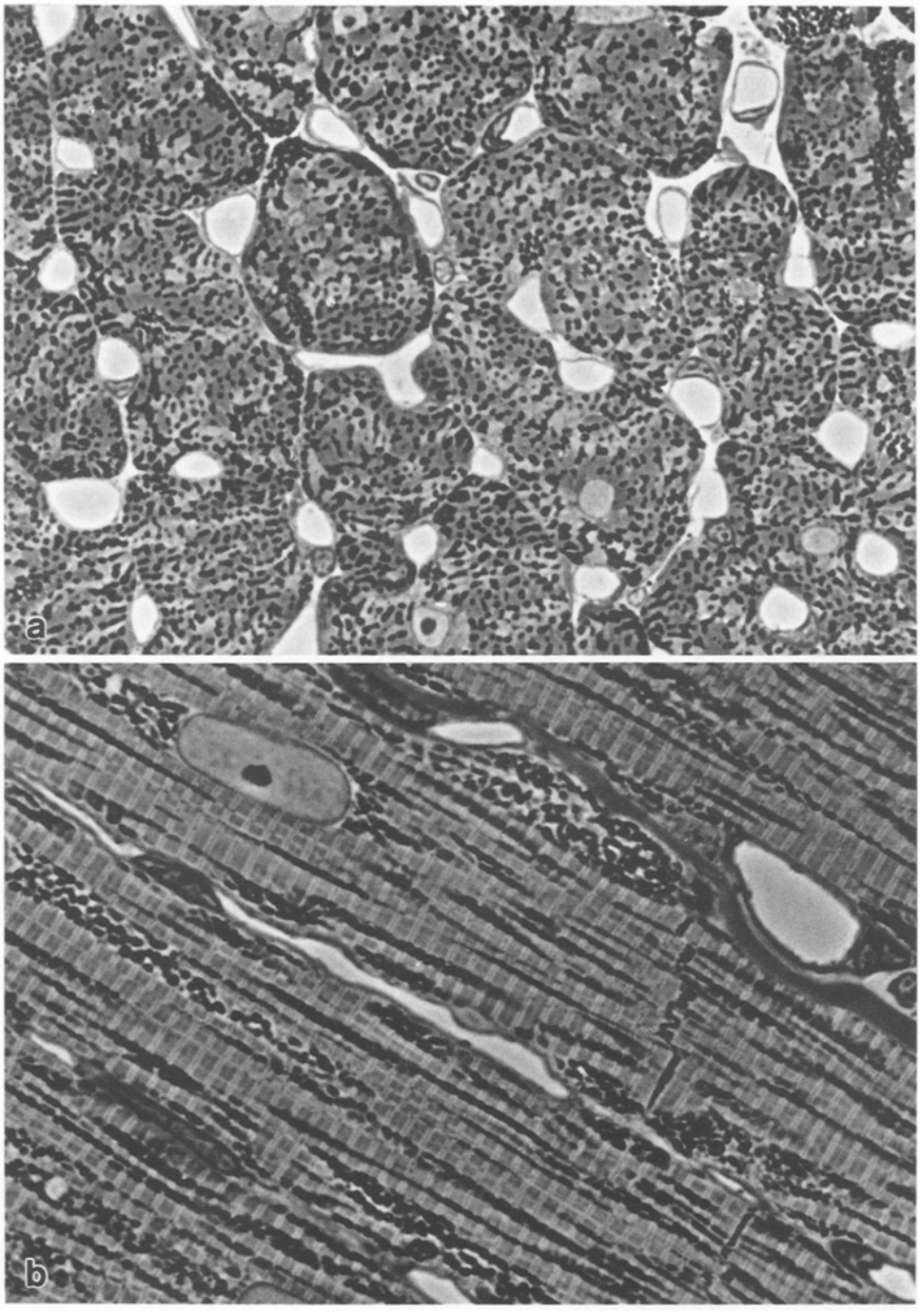

Fig. 1a, b. Transverse and longitudinal section of a papillary muscle of a control rat. Since capillaries are orientated nearly in parallel in normal hearts, a high number of capillary profiles can be seen in transverse sections and only a few profiles in longitudinal sections. Semithin section, ×2096

tension, and 2 with neither the one nor the other. Out of the surviving 15 diabetic rats and 15 controls, a random sample of 11 rats was selected in each group for stereological analysis.

Mean body weights were higher in the non-diabetic groups (Table 1). Systolic blood pressure was similar in diabetic and non-diabetic hypertensives (Table 1). Serum glucose levels were increased 3.5 times in both diabetic groups. Renovascular hypertension increased the left ventricular weight and the left ventricular weight to body weight ratios to similar degrees in diabetic and non-diabetic rats, by 28% and 31% respectively) (Table 1).

Serum creatinine and urea levels were normal in all groups (Table 2).

Qualitative histological investigations revealed a focal perivascular and interstitial fibrosis in diabetic and non-diabetic hypertensives (Figs. 3, 4). Occasionally, focal scarring could be detected in both groups. The normotensive diabetics did not show any histological abnormalities. Light microscospical stereology indicated the absence of specific cardiac effects of diabetes on the hypertensive rats (Fig. 4). In hypertensives, non-vascular interstitium and fibrosis were increased (Table 2). In hypertensive diabetics and even in non-diabetic hyperten-

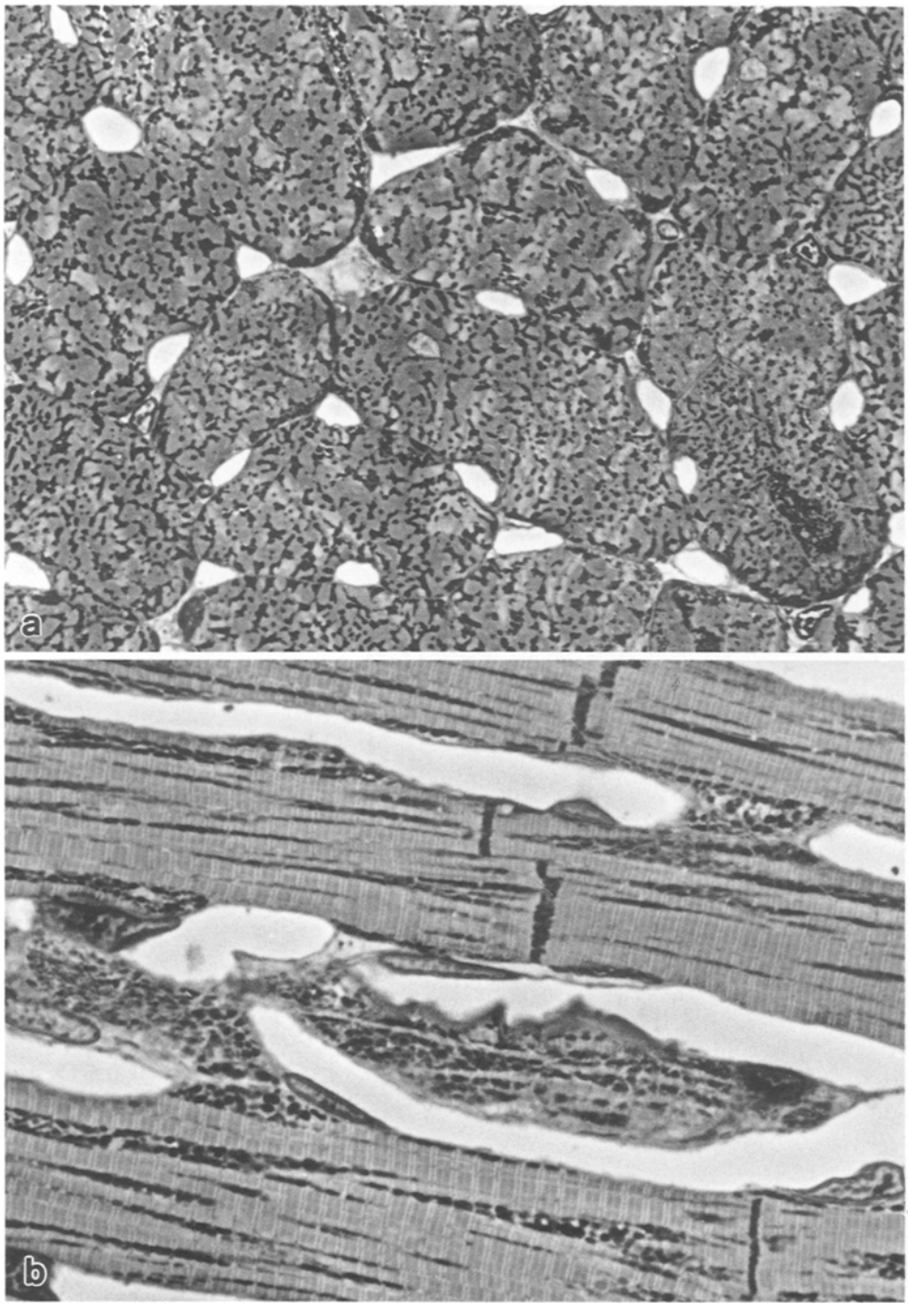

Fig. 2a, b. Transverse and longitudinal section of a papillary muscle of a non-diabetic hypertensive rat. The density of capillary profiles in transverse sections is reduced and the density of profiles in longitudinal sections is increased. This is due to neoformation of capillary branches in oblique and transverse direction refered to the longitudinal axis of the muscle. Semithin section, ×2096

sives, capillary density $\{Q_A(\alpha=0)\}$ was decreased in transverse sections (Figs. 1a, 2a), increased in longitudinal sections $\{Q_A(\alpha=\pi/2)\}$ (Figs. 1b, 2b) and the three-dimensional estimator of capillarization, V of capillaries, showed a slight decrease only. This indicates that in long-term renovascular hypertension an adaption of the capillary network occurs which compensates almost completely for the increased width of the hypertrophic myocytes. Adaption seems to be realized by neoformation of obliquely and transversely running capillaries [cf. increased c_1 values, and $Q_A(\alpha=\pi/2)$ (Fig. 2a, b)].

At the ultrastructural level, the hypertensive diabetic group showed an alteration of the quantitative structure of myocardial cells which was not found in non-diabetic hypertensives or in normotensive diabetics: the volume ratio of mitochondria to myofibrils was significantly decreased. In the diabetic group, the volume density of sarcoplasm was significantly increased.

Discussion

The majority of experimental animals died early after clipping the renal artery. Between 12 and 20 weeks, when

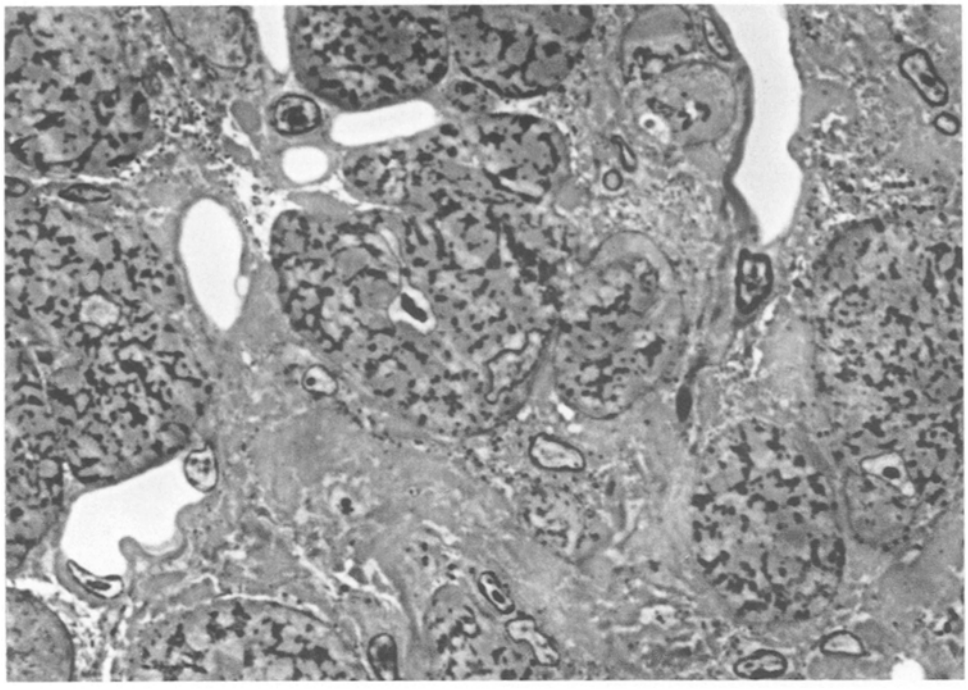

Fig. 3. Myocardium of a non-diabetic hypertensive rat. Pronounced interstitial fibrosis can be seen which leads to broad sheets of fibrous tissue between the myocytes. Semithin section, ×2096

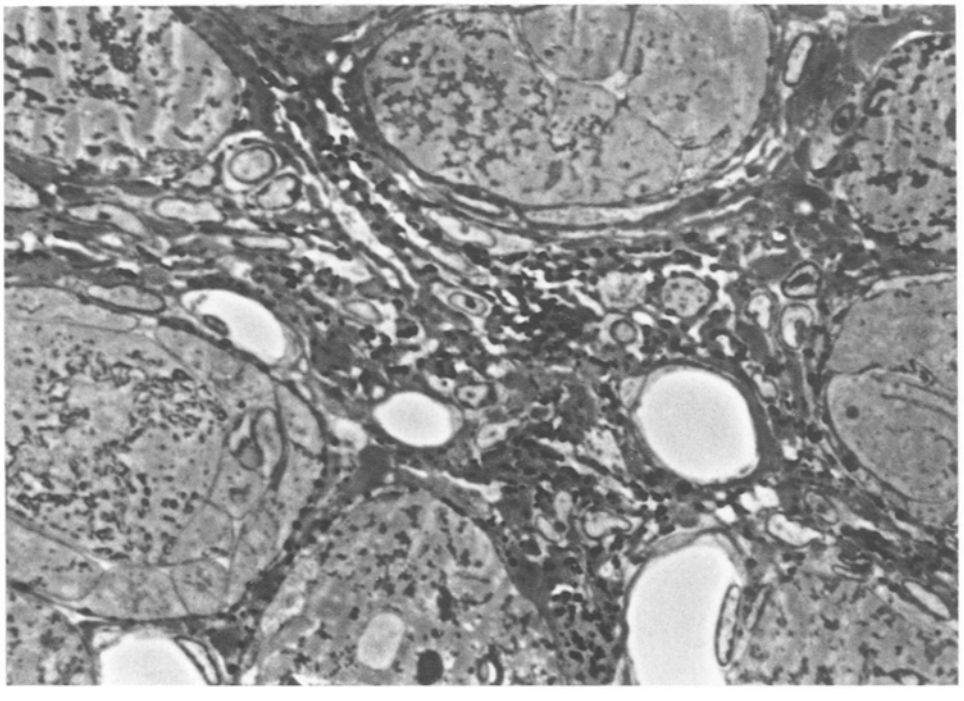

Fig. 4. Myocardium of a diabetic hypertensive animal. Marked increase in collagen which surrounds the myocytes. As far as the fibrosis is concerned, there is no significant difference in comparison with non-diabetic hypertensive rats. Semithin section, ×2096

diabetes mellitus had been induced, lethality was 45% in the non-diabetic hypertensives as well as in the diabetic hypertensives. Histologically, these animals showed a pronounced cardiac fibrosis without divergences between both hypertensive groups. All investigations of non-diabetic animals despite injection of streptozotocin were not different from animals of the non-diabetic groups (data not given). This precludes a direct toxic effects for streptozotocin.

Systolic pressure and left ventricular weights were higher in diabetic and non-diabetic hypertensives when compared to normotensives. Stereological investigations of the non-vascular interstitium, especially of fibrosis, provided evidence that the interstitial changes in hypertension were not potentiated by diabetes mellitus. Qualitatively, the typical pattern of cardiac fibrosis in hypertension was observed: perivascular fibrosis, interstitial fibrosis and reparative fibrosis (Thiedemann et al. 1983; Weber et al. 1987). Reparative fibrosis was not found more frequently in hypertensive diabetics when compared with hypertensives. The absence of effects of the diabetes on the interstitium agrees with findings of our

Table 3. Stereological parameters of myocytes, interstitium and capillaries

Parameter	Control group $n=11$	Hypertensive group $n=8$	Diabetic group $n=11$	Hypertensive diabetic group $n=7$
V_V myocytes (cm^3/cm^3)	0.90 ±0.01	0.89 ±0.01	0.89 ±0.01	0.89 ±0.02
V_V interst (cm^3/cm^3)	0.020* ±0.002	0.046 ±0.010	0.027* ±0.007	0.057 ±0.020
V_V capillaries (cm^3/cm^3)	0.060 ±0.010	0.061 ±0.010	0.071 ±0.020	0.053 ±0.010
L_V capillaries (mm/mm^2)	3804 ± 231	3667 ± 232	4082 ± 458	3699 ± 390
$Q_A(\alpha=0)$ (cap/mm^2)	3679* ± 239	3212 ± 311	3914* ± 431	3311 ± 462
$Q_A(\alpha=\pi/2)$ (cap/mm^2)	542* ± 56	913 ±176	643* ±131	857 ±135
$c_1(\alpha=0, K_L)$	1.02* ±0.008	1.13 ±0.07	1.03* ±0.01	1.12 ±0.06
V_V fibrosis (cm^3/cm^3)	0.009* ±0.003	0.020 ±0.004	0.011* ±0.002	0.019 ±0.004

Means ± standard deviations

* Significant divergences (Dunnett's test) from the hypertensive diabetic group ($P<0.05$)
V_V interst, Volume density of non-vascular interstitial space; L_V capillaries, length density of capillaries; Q_A ($\alpha=0$), number of capillary profiles per mm^2 in cross-sections; Q_A ($\alpha=\pi/2$), number of capillary profiles per mm^2 in longitudinal sections; K_L, constant of anisotropy; α, sectioning angle; c_1, coefficient of correction

group in short-term experiments [12 weeks of renovascular hypertension and 4 weeks diabetes mellitus (Mall et al. 1991) and 8 weeks of renovascular hypertension and 4 weeks of diabetes mellitus (Mall et al. 1987a, 1991)]. In contrast, Factor and coworkers (1981) found a more pronounced fibrosis (based on a semi-quantitative score) in a short-term model (8 weeks of renovascular hypertension, 4 weeks of diabetes mellitus) when compared with hypertension alone. It is difficult to explain the discrepant results convincingly. Quantitative evaluations of histological images lead to objective data, in contrast to the more subjective histological scores. However, it cannot completely be ruled out that a subjective score is more sensitive when focal changes are investigated. Furthermore, in the experiment of Factor et al. (1981), streptozotocin was injected intravenously before increased blood pressure had been established in contrast to the present experiment. Recently, chronic uraemia has been detected as an independent determinant of cardiac interstitial or perivascular fibrosis (Mall et al. 1988, 1990a). Whereas renal function was normal in our experiment, Factor et al. did not give any information on this point.

Earlier investigations (Mall et al. 1987a, 1991) agree with the present findings that cardiac fibrosis was associated with renovascular hypertension, but not with diabetes mellitus. It should be emphasized, however, that after long-standing diabetes melltitus, interstitial cardiac fibrosis develops (Baandrup et al. 1981). Thus, one may suggest that at advanced stages of the disease renovascular hypertension and diabetes act additively on cardiac fibroblasts.

At the ultrastructural level, the volume ratio of mitochondria to myofibrils was significantly lower in the hypertensive diabetic group when compared with the other experimental groups (Table 3). This agrees with our previously published results in a short-term combination model (Mall et al. 1987a), where the ratio was decreased after 12 weeks of renovascular hypertension even without diabetes (Mall et al. 1991). It is generally accepted that a reduced ratio of mitochondria to myofibrils corre-

Table 4. Stereological parameters of myocardial cell organelles

Parameters	Control group $n=11$	Hypertensive group $n=8$	Diabetic group $n=11$	Hypertensive Diabetic group $n=7$
V_V mitochondria (cm^3/cm^3)	0.28 ±0.01	0.30* ±0.02	0.28 ±0.01	0.26 ±0.01
V_V sarcoplasm (cm^3/cm^3)	0.05 ±0.01	0.04 ±0.01	0.10* ±0.02	0.06 ±0.01
V_V myofibrils (cm^3/cm^3)	0.66 ±0.02	0.65 ±0.01	0.61* ±0.02	0.67 ±0.01
V_V ratio mitochondria to myofibrils (cm^3/cm^3)	0.43* ±0.03	0.46* ±0.04	0.47* ±0.04	0.39 ±0.03

Means ± standard deviations

* Significant divergences (Dunnett's test) from the hypertensive diabetic group ($P<0.05$)

sponds to cardiac deterioration in hypertension (Wollenberger and Schulze 1962; Breisch et al. 1980). Thus, diabetes mellitus seems to enhance synergistically the pathological effects of hypertension on myocytes. The molecular basis of this maladaption is not yet known. A decrease in myofibrillar ATPase activity and increase in slow isomyosins occur in the rat heart after pressure overload (cf. Jacob 1983) as well as in diabetes mellitus (Dillmann 1980; Fein et al. 1984; Pierce and Dhalla 1981; Bimji et al. 1986). It cannot be precluded that the decrease in myofibrillar ATPase activity decreases not only oxygen consumption but also the mitochondrial mass.

The increase of the volume densities sarcoplasmic matrix in diabetic animals is caused by a slight sarcoplasmic oedema and by accumulation of β-glycogen granules (Frenzel et al. 1985; Jackson et al. 1985; Hsiao et al. 1987; Mall et al. 1987a). The less pronounced increase of V_V sarcoplasm in hypertensive diabetics is probably related to an over-proportionate increase of myofibrils in the combination group, which masks the increase in sarcoplasmic matrix.

An interesting finding of this study is the peculiar capillary reaction pattern in long-term pressure overload which is independent of the presence of diabetes. Hitherto, numerous quantitative morphological studies have demonstrated a decrease of capillary supply in hypertrophy induced by pressure overload (Anversa et al. 1980; Breisch et al. 1984). All these observations were based on the common parameter of capillarization, the (two-dimensional) capillary density [Q_A ($\alpha=0$) in stereological terms]. Even the three-dimensional parameter of capillarization, the L_V of capillaries, was considerably decreased in short-term experiments of renovascular hypertension (Mall et al. 1990b). In the present investigation, three-dimensional analysis of the capillary network provided evidence that the capillary supply tended to become normalized after long-term renovascular hypertension. However, the pattern of adaptive growth was different from other models of hypertrophy. After physical exercise and chronic thyroxine application capillary growth occurred without changes of the spatial distribution of capillary axes (Mall et al. 1990b). In long-term renovascular hypertension, capillary growth was realized predominantly by an increase of obliquely and transversely running capillary branches [increase in $c_1(\alpha=0)$ and Q_A ($\alpha=\pi/2$]. Measurements of sarcomere lengths in all groups precluded artificial effects of variable states of contraction on capillary tortuosity (Mall et al. 1990b).

Acknowledgements. The skilful technical assistance of J. Scheurer, Z. Antoni, G. Gorsberg, W. Nottmeyer and P. Rieger is gratefully acknowledged. The study was supported by grants from the Deutsche Forschungsgemeinschaft (Ma 912/1-3).

References

Anversa P, Olivetti G, Melissari M, Loud AV (1980) Stereological measurement of cellular and subcellular hypertrophy and hyperplasia in the papillary muscle of adult rat. J Mol Cell Cardiol 12:781–795

Baandrup U, Ledet T, Rasch R (1981) Experimental diabetic cardiopathy preventable by insulin treatment. Lab Invest 45:169–173

Bhimji S, Godin DV, McNeill JH (1986) Insulin reversal of biochemical changes in hearts from diabetic rats. Am J Physiol 251:H670–675

Breisch EA, Houser SR, Carey RA, Spann JF, Bove AA (1980) Myocardial blood flow and capillary density in chronic pressure overload of the feline left ventricle. Cardiovas Res 14:469–475

Breisch EA, White C, Bloor CM (1984) Myocardial characteristics of pressure overload hypertrophy. Lab Invest 51:333–341

Dillmann WH (1980) Diabetes mellitus induces changes in cardiac myosin of the rat. Diabetes 29:579–582

Di Sant'Agnese PA, De Mesy Jensen KL (1984) Dibasic staining of large epoxy sections and applications to surgical pathology. Am J Clin Pathol 80:25–29

Factor SM, Minase T, Sonnenblick EH (1980) Clinical and morphological features of human hypertensive-diabetic cardiomyopathy. Am Heart J 99:446–458

Factor SM, Minase T, Bhan R, Wolinski H, Sonnenblick EH (1981) Hypertensive-diabetic cardiomyopathy in the rat. An experimental model of human disease. Am J Pathol 102:219–317

Fein FS, Capasso JM, Aronson RS, Cho S, Nordin C, Miller-Green B, Sonnenblick EH, Factor SM (1984) Combined renovascular hypertension and diabetes in rats: a new preparation of congestive cardiomyopathy. Circulation 70:318–330

Fein FS, Cho S, Zola BE, Miller B, Factor SM (1989) Biventricular damage with right ventricular predominance. Am J Pathol 134:1159–1166

Frenzel H, Buerrig KF, Kasper M, Schwartzkopff B, Roesen P (1985) Funktionelle und morphometrische Untersuchungen an Kapillaren und Herzmuskelzellen im Herzen diabetischer Ratten. Verh Dtsch Ges Path 69:657

Hsiao YC, Suzuki KI, Abe H, Toyoda T (1987) Ultrastructural alterations in cardiac muscle of diabetic BB Wistar rats. Virchows Arch A 411:45–52

Jackson CV, McGrath GM, Tahiliani AG, Vadlamudi RVSV, McNeill JH (1985) A functional and ultrastructural analysis of experimental diabetic rat myocardium. Diabetes 34:873–876

Jacob R (1983) Chronic reactions of myocardium at the myofibrillar level. Reflections on "adaption" and "disease" based on the biology of long-term cardiac overload. In: Jacob R, Gülch RW, Kissling G (eds) Cardiac adaptation to hemodynamic overload, training and stress. Steinkopff, Darmstadt: pp 89–106

Jalil JE, Doering CW, Janicki JS, Pick R, Shroff SG, Weber KT (1989) Fibrillar collagen and myocardial stiffness in the intact hypertrophied rat left ventricle. Circ Res 64:1041–1050

Kannel WB, Hjortland M, Castelli WP (1974) Role of diabetes in congestive heart failure: the Framingham Study. Am J Cardiol 34:29–34

Mall G, Reinhard H, Kayser K, Rossner JA (1978) An effective morphometric method for electron microscopic studies on papillary muscles. Virchows Arch [A] 379:219–228

Mall G, Mattfeldt T, Möbius HJ, Leonhard R (1986) Stereological study on the rat heart in chronic alimentary thiamine deficiency – absence of myocardial changes despite starvation. J Mol Gen Cardiol 18:635–643

Mall G, Klingel K, Hasslacher GH, Mann J, Mattfeldt T, Baust H, Waldherr R (1987a) Synergistic effects of diabetes mellitus and renovascular hypertension on the rat heart – stereological investigations on papillary muscles. Virchows Arch [A] 411:531–542

Mall G, Schikora I, Mattfeldt T, Bodle R (1987b) Dipyridamole-induced neoformation of capillaries in the rat heart. Lab Invest 57:86–93

Mall G, Rambausek M, Neumeister A, Kollmar S, Vetterlein F, Ritz E (1988) Myocardial interstitial fibrosis in experimental uraemia – implications for cardiac compliance. Kidney Int 33:804–811

Mall G, Huther W, Schneider J, Lundin P, Ritz E (1990a) Diffuse intramyocardiocytic fibrosis in uraemic patients. Nephrol Dial Transplant 5:39–44

Mall G, Zimmer G, Baden S, Mattfeldt T (1990b) Capillary neoformation in the rat heart – a stereological study on papillary muscles in hypertrophy and physiologic growth. Basic Res Cardiol 85:531–540

Mall G (1991) Morphometric study on the rat heart in combined renovascular hypertension and diabetes mellitus. In: Nagano N, Dhalla NS (eds) The diabetic heart. Raven Press, New York, pp 115–124

Mattfeldt T, Mall G (1984) Estimation of length and surface of anisotropic capillaries. J Microsc 135:181–190

McKee PA, Castelli WP, McNamara PM, Kannel WB (1971) The natural history of congestive heart failure: the Framingham study. N Engl J Med 285:1441–1446

Möhring J, Petri M, Szokol M, Haack D, Möhring B (1976) Effects of saline drinking on malignant course of renal hypertension in rats. Am J Physiol 230:849–857

Pierce GN, Dhalla NS (1981) Cardiac myofibrillar ATPase activity in diabetic rats. J Mol Cell Cardiol 13:1063–1069

Rodrigues G, McNeill JH (1986) Cardiac function in spontaneously hypertensive diabetic rats. Am J Physiol 521:H571–H586

Sachs L (1974) Angewandte Statistik. Springer-Verlag Berlin Heidelberg New York, p 386

Thiedemann KU, Holubarsch C, Medugorac I, Jacob R (1983) Connective tissue content and myocardial stiffness in pressure overload hypertrophy. A combined study of morphologic, morphometric, biochemical, and mechanical parameters. Basic Res Cardiol 78:140–155

Weber KT, Janicki JS, Pick R, Abrahams C, Shroff SG, Bashey RI, Chen RM (1987) Collagen in the hypertrophied, pressure-overloaded myocardium. Circulation 75 [Suppl I]:1–40

Weibel ER (1979) Stereological methods, vol 1. Academic Press, London, pp 26–36

Wollenberger A, Schulze W (1962) Über das Volumenverhältnis von Mitochondrien zu Myofibrillen im chronisch überlasteten, hypertrophierten Herzen. Naturwissenschaften 49:161–162