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Ultrastructural alterations in cardiac muscle of diabetic BB Wistar rats

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Summary. The cardiac muscle of BB Wistar rats suffering from diabetes for 8 and 16 weeks (8-Wk and 16-Wk of DM) were examined by light and electron microscopy. The diabetic rats were kept alive by injections of small doses of insulin and exhibited severe hyperglycaemia, glycosuria and weight loss. The heart/body weight ratio of all diabetic groups was greater than that of age matched controls. Over the experimental period, the left ventricular myocardium of the diabetic BB rats sustained damage that was progressively more serious with the duration of the diabetic state. In BB rats after 8-wk of diabetes the myocardium contained large numbers of lipid droplets and glycogen granules around mitochondria which showed patchy swelling, and slight loss of myofilaments. Disruption of mitochondrial membranes and extensive loss of myofilaments were seen in rats diabetic for 16 wk. In addition, dilatation of the sarcoplasmic reticulum-transverse tubular system, formation of a contraction band and myelin bodies and widening of the intercellular space at the fasciae adherens of the intercalated disc were characteristically observed in BB rats after 16-wk of diabetes. However, there were no evident alterations in the capillaries of any diabetic BB rats. Morphometric analyses showed the volume percentage of myofibrils in diabetic rats to be significantly decreased when compared with controls. The loss of myofibrillar elements may be a primary damage induced by insulin deficiency. The formation of contraction bands suggests Ca^{2+} overload caused by diabetic metabolic disturbances.

Key words: Diabetes – BB rat – Myocardium – Loss of myofilament – Contraction band

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Diabetic cardiomyopathy is characterized by cardiomegaly, ventricular dysfunction and congestive heart failure (Ahmed et al. 1975; Friedman et al. 1982; Hamby et al. 1973; Regan et al. 1975; Regan et al. 1977; Rubler et al. 1972). Some studies have suggested that these abnormalities in the diabetic myocardium are induced secondarily by microangiopathy (Blumenthal et al. 1960; Hamby et al. 1974; Ledet 1968 and 1976; Rubler et al. 1972; Sohar et al. 1970), but others consider the changes to result from metabolic disturbances rather than from impaired coronary circulation (Ahmed et al. 1975; Haider et al. 1977; Regan et al. 1974; Seneviratine 1977). Recent biochemical reports have demonstrated that the abnormalities in contraction and relaxation observed in diabetic hearts are associated with disturbance in calcium kinetics (Penpargkul et al. 1981; Pierce et al. 1983) and alteration in cardiac myosin (Dillmann 1980), also.

Several reports have dealt with the ultrastructure of the heart of diabetic animal models (Fischer et al. 1981; Giacomelli and Wiener 1979; Orth and Morgan 1962; Reinilä and Åkarblom 1984; Tarach 1976). These investigations have yielded varying results according to the severity of the diabetic state and the different animal models. Almost all models in these investigations were produced by injections of streptozotocin and alloxan. Recently, spontaneously diabetic BB Wistar rats have been used as an animal model for insulin dependent diabetes mellitus (Brismar and Sima 1981; Marliss et al. 1981; Nakhooda et al. 1977; Sima 1980). In these rats, the diabetic syndrome, consisting of hypoinsulinaemia, hyperglycaemia and glycosuria occurs concomitantly with autoimmune insulitis characterized by the destruction of beta cells. The changes occur at approximately the time of sexual maturation, in the absence of obesity.

The purpose of the present study was to clarify the morphologic changes in the myocardium in BB Wistar diabetic rats.

Materials and methods

Two pairs of BB rats were given kindly by P. Thibert from the Health Protection Branch, Health and Welfare, Canada, Ottawa in 1982. Age- and sex-matched non-diabetic BB Wistar rats served as controls. All animals were maintained by T. Nobunaga at the Institute of Experimental Animals, Tohoku University School of Medicine. Food and water were given ad libitum. The onset of diabetes (about at 10 weeks of age) was determined by 2+ urine glucose (Dextrostix; Ames), at which time insulin treatment was initiated. A small daily dose of Lente insulin (2–4 U/day) was administered for the animals to survive and to maintain them at mild hyperglycaemic levels. Blood glucose levels were monitored on a biweekly basis from onset to death. Blood was withdrawn from the tail, and blood glucose values were determined by the glucose oxidase method. Body weight and heart weight were measured at death, and the heart/body weight ratio was calculated. Groups of 5 diabetic rats and an equal number of control rats were killed after the duration of diabetes for 8 and 16 weeks for light microscopic study; groups of another 5 diabetic BB rats and the corresponding control were prepared for electron microscopic study.

Under sodium pentobarbital anesthesia (30 mg/kg body weight) the chest was opened. Thereafter, for light microscopic observation, the heart was quickly removed, weighed and trimmed. The specimens were fixed in 10% buffered formalin (pH 7.2), dehydrated, embedded in paraffin, cut into 2.5 μm thick sections and stained with haematoxylin and eosin. For electron microscopic observation a tiny cut was given to the cardiac apex. Ringer solution was perfused (37° C) for several minutes via a tube inserted into the ascending aorta through the left ventricle, followed by 2.5% glutaraldehyde buffered at pH 7.3 with 0.1 M sodium cacodylate. After perfusion, the free walls of the left ventricles were removed. They were divided into several pieces and further fixed in the same fixative for another 2 h to 3 h followed by postfixation in 1% osmium tetroxide buffered at pH 7.3 with 0.1 M sodium cacodylate for 1 hour at 4° C. After being rinsed several times in distilled water, the specimens were block stained in 2% uranyl acetate for 60 min, dehydrated in graded ethanols and embedded in Epon 812.

Semi-thin sections were cut from each block and stained with toluidine blue. Ultrathin sections were cut and stained with uranyl acetate and lead citrate. Ultrastructural observations were performed on a Hitachi H-600.

For the measurement of the volume percentage of sarcoplasmic organelles of cardiac myocytes, ten random fields were micrographed from four blocks obtained from each rat at ×10000 and printed at a final magnification of ×24000. A square grid of 576 sampling points with a area of 11.3 cm × 11.3 cm was used for determining the volume fraction of myofibrils, mitochondria, lipid droplets, T-system and sarcoplasmic reticulum.

The volume percentage (Vi/Vcell) of any sarcoplasmic organelle (i) was obtained from the equation:

$$Vi/Vcell = Pi/Pcell$$

where Pi is the number of points falling on all profiles of the organelles on the print, and Pcell is the total number of prints falling on all of the myocardial cells in the print (Page et al. 1971).

The results are expressed as means ± SEM. The statistical differences between mean values were calculated by Student's t-test, and were considered to be significant if the probability of error (p) was found to be less than 0.05.

Results

Blood sugar levels of the diabetic rats ranged from 330 mg/dl to 400 mg/dl, whereas those of the control rats were 105 mg/dl to 115 mg/dl. The body weights of rats diabetic for 8 wk and for 16 wk and the heart weights of rats diabetic for 16 wk were significantly reduced when compared with the control rats, but the heart weights of rats diabetic for 8 wk showed no significant difference from those of the controls. The heart/body weight ratios of rats diabetic for 8 wk and for 16 wk of DM were significantly greater than those of the controls (Table 1).

On light microscopy cardiac muscle cells in control rats were arranged in parallel array. Each cell might be partially divided into two or more branches. They showed regular cross striations (Fig. 1). The most marked morphological alteration in rats diabetic for 16 wk was coagulative myocytolysis. This lesion consisted of a single

Table 1. General observations of control and diabetic BB rats

Duration of diabetes	Body wt (g)	Heart wt (g)	HW/BW (mg/g)	FBG (mg/dl)
8-Wk				
Control ($n=5$)	349 ± 7*	0.86 ± 0.03	2.45 ± 0.05*	110 ± 5**
Diabetic ($n=5$)	278 ± 10*	0.89 ± 0.02	3.20 ± 0.12**	350 ± 20**
16-Wk				
Control ($n=5$)	460 ± 10*	1.30 ± 0.03**	2.82 ± 0.05**	112 ± 3**
Diabetic ($n=5$)	228 ± 13**	0.75 ± 0.05**	3.32 ± 0.12**	380 ± 20**

* $p<0.05$

** $p<0.01$ comparing each diabetic group with its age-matched control group

Results are mean ± SEM; HW/BW = heart weight to body weight ratio; FBG = Fasting blood glucose

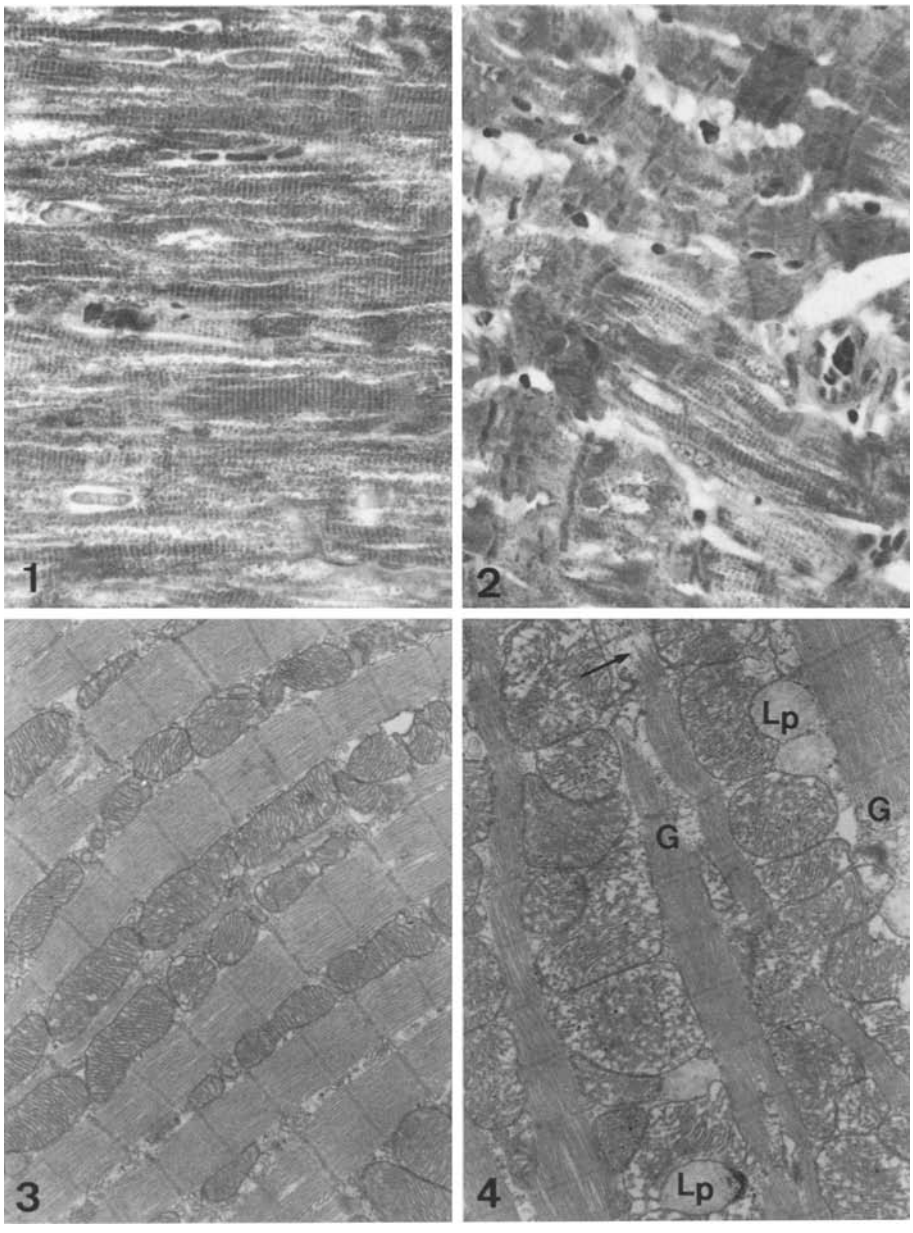

Fig. 1. Left ventricle from control rat. ×425

Fig. 2. Left ventricle from rats diabetic for 16 wk showing contraction bands. ×425

Fig. 3. Left ventricle from 8-Wk control. ×10700

Fig. 4. 8-Wk diabetic left ventricle showing numerous lipid droplets (*Lp*) and glycogen (*G*), swelling mitochondria and slight loss of contractile protein (*arrow*). ×10700

transverse band formed by the hypercontraction of 5 to 10 sarcomeres at one end of the cell adjacent to the disc (Fig. 2). There were no hypercontraction bands in rats diabetic for 8 wk. On electron microscopy the cardiac muscle cells of the left ventricle contained parallel arrays of myofilaments and electron dense mitochondria. All I and A bands were distinctly observed. The sarcomere was segmented between two successive prominent Z lines. The M line was observed as a faint striation. The mitochondria were cylindrical in form and usually almost one sarcomere long with their ends near the Z lines. They also contained normal cristae. A well developed sarcoplasmic reticulum was interspersed among myofilament bundles, as were transverse tubules. Normal amounts of glycogen granules were noted, but lipid droplets were scanty (Figs. 3 and 5). The intercalated disc exhibited three distinct types of junctional specializations corresponding to the macula adherens (MA), fascia adherens (FA), and nexus (N) (Fig. 11). Blood capillaries had a widely patent lumena and a thin basal lamina (Fig. 5).

In rats diabetic for 8 wk numerous lipid droplets and glycogen granules were observed around mitochondria. Patchy swellings of the matrix was found in mitochondria, as well as a slight loss of myofilament bundles (Fig. 4).

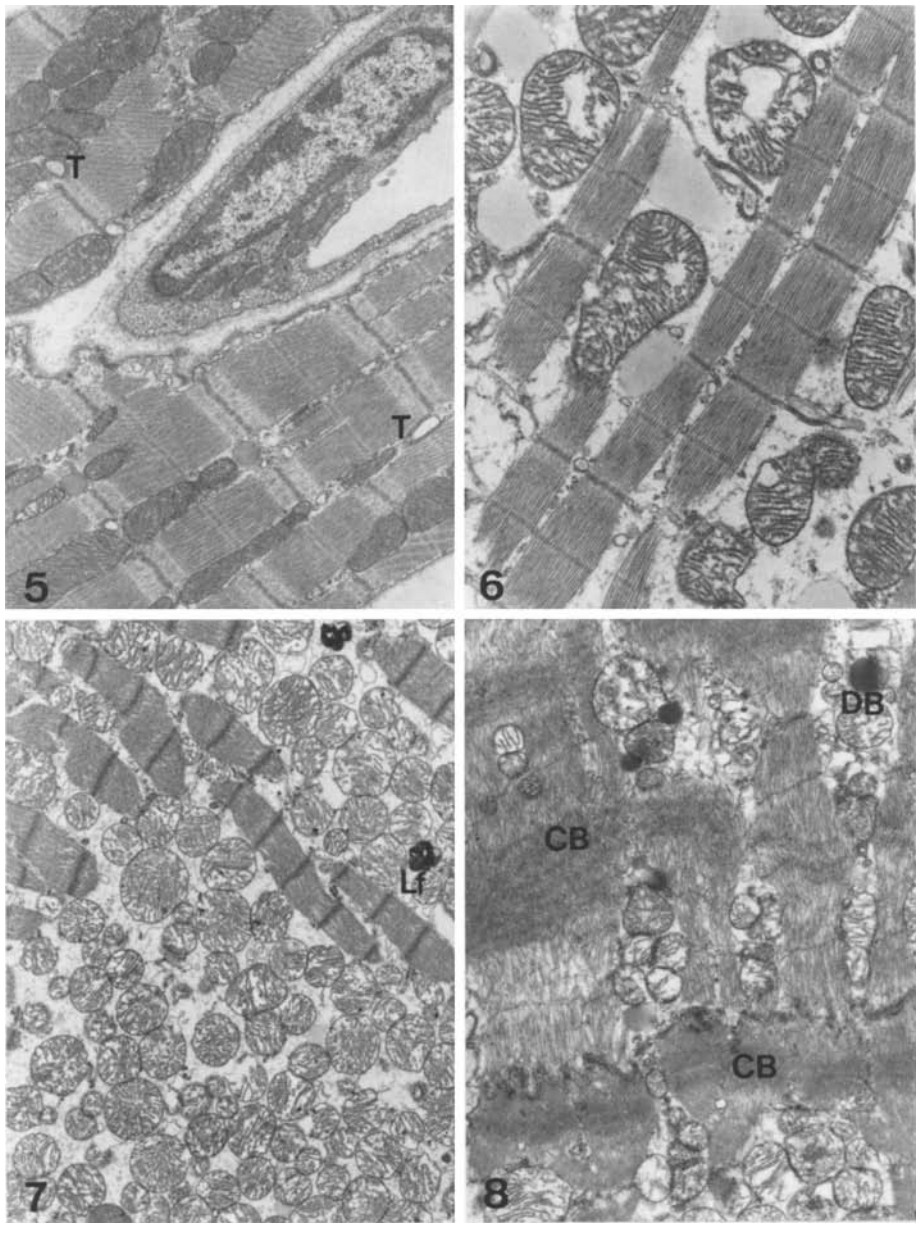

Fig. 5. 16-Wk control left ventricle. The A and I bands are distinct. An adjacent capillary and normal transverse tubule are seen (*T*). ×10200

Fig. 6. 16-Wk diabetic left ventricle showing focal loss of myofilaments, disrupted mitochondria and destruction of sarcoplasmic reticulum-transverse tubular system. ×12000

Fig. 7. 16-Wk diabetic left ventricle showing extensive loss of contractile protein with accumulation of mitochondria. Some disrupted mitochondria and a few of lipofuscins (*Lf*) are seen. ×6000

Fig. 8. 16-Wk diabetic left ventricle showing paradiscal contraction bands (*CB*) and dense bodies (*DB*). ×7200

After 16 weeks of diabetic state the cardiac muscle cells had sustained more severe damage. Generally, a focal loss of myofilament bundles and destruction of the sarcoplasmic reticulum-transverse tubular system were evident (Fig. 6). Disrupted mitochondria with separated cristae and electron lucent matrix were observed and numerous lipid droplets were situated near the abnormal mitochondria (Fig. 6). Occasionally, there were wide areas of cytoplasm where contractile protein disappeared and many mitochondria and a few lipofuscins clustered (Fig. 7). Other lesions were represented by the formation of contraction bands (Fig. 8). They were mostly adjacent to the intercalated disc. In the contraction band, the A band and the I band could not be clearly recognized. Z lines were seen as electron-dense remnants (Fig. 8). Many dense bodies were noted near the contraction band. The contraction bands were never observed in rats diabetic for 8 wk or in any of the control preparations.

The transverse tubles in the diabetic cardiac muscle cells were dilated (Fig. 9). The high number of large vacuoles located between the dilated transverse tubules might be represent the swelling of the sarcoplasmic reticulum (Fig. 9). A number of myelin figures were seen between myofilaments (Fig. 10). Intercellular spaces in fascia adherentes

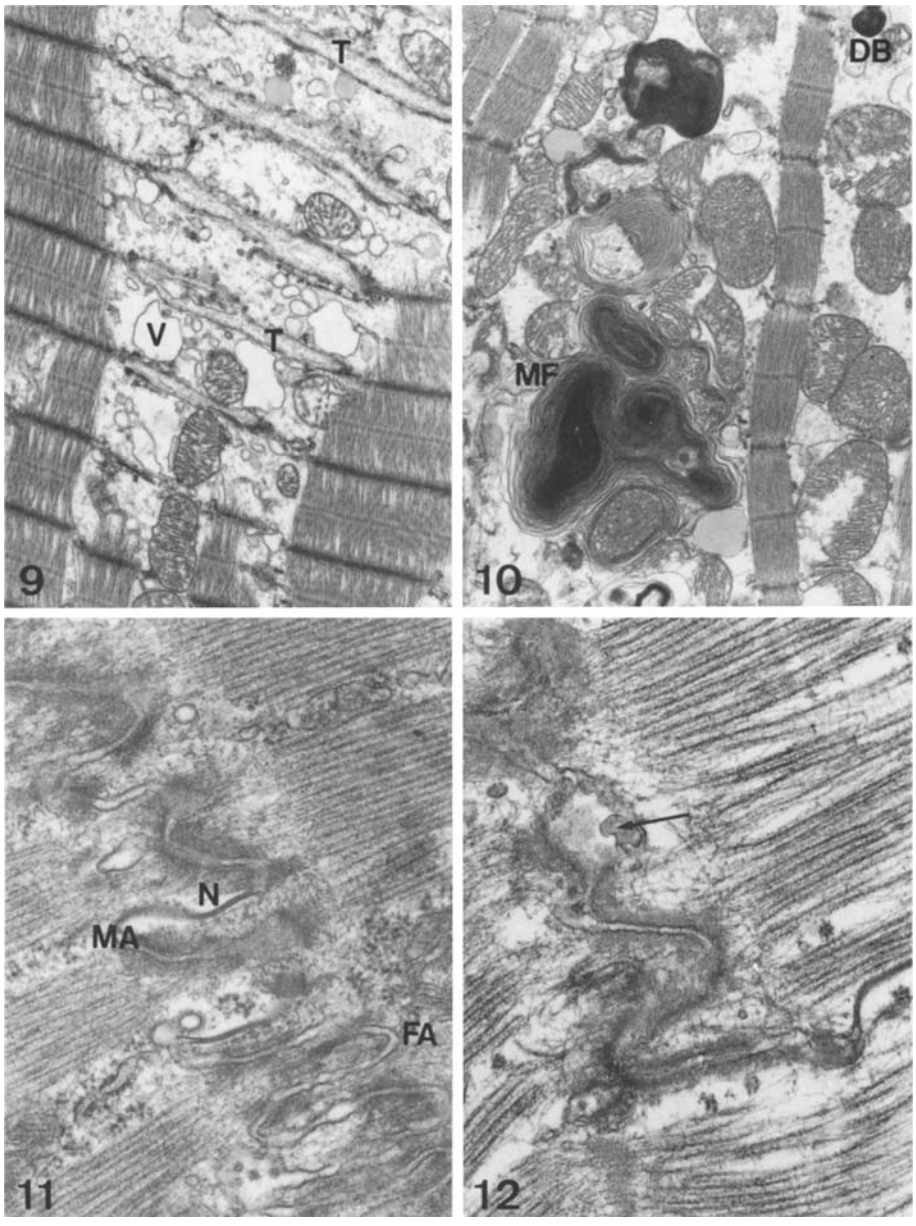

Fig. 9. 16-Wk diabetic left ventricle showing dilatation of transverse tubules (*T*) and vacuoles (*V*). ×10000

Fig. 10. 16-Wk diabetic left ventricle showing numerous dense bodies (*DB*) and myelin figures (*MF*). ×11200

Fig. 11. An intercalated disc from 16-Wk control left ventricle contained three types of specialized regions; fascia adherens (*FA*), macula adherens (*MA*), and nexus (*N*). ×33900

Fig. 12. An intercalated disc from 16-Wk diabetic left ventricle showed that the fascia adherens is markedly dilated and contains fragments (*arrow*). ×34200

Table 2. Volume percentage of intra-myocardium organelles in control and diabetic BB rats

Duration of diabetes	Myofibrils	Mitochondria	Lipid droplets	T-tube	Sarcoplasmic reticulum
8-Wk					
Control ($n=5$)	56.2±2.5*	31.3±1.0**	0.82±0.27**	1.24±0.24	3.02±0.20
Diabetic ($n=5$)	51.4±2.1*	34.1±1.2**	2.43±0.52**	1.23±0.26	2.98±0.25
16-Wk					
Control ($n=5$)	55.4±2.0**	32.7±1.7	0.88±0.35**	1.31±0.23*	3.23±0.34*
Diabetic ($n=5$)	39.5±4.2**	32.2±2.6	2.64±0.43**	1.82±0.30*	4.04±0.48*

* $p<0.05$

** $p<0.01$ comparing each diabetic group with its age-matched control group

Results are mean±SEM

were markedly dilated and often contained fragmented substance (Fig. 12), whereas maculae adherentes and nexuses remained intact (Fig. 12). There were no structural alterations in the blood capillaries in rats diabetic for 8 wk and 16 wk.

Table 2 summarizes our morphometric results for the volume percentage of myofibrils, mitochondria, lipid droplets, T-system and sarcoplasmic reticulum from control and diabetic hearts. A significant decrease in the volume percentage of myofibrils was seen in all diabetic rats. A significantly increase in the volume percentage of lipid droplets in all diabetic rats was demonstrated when compared with controls. The volume percentage of mitochondria in rats diabetic for 8 wk and the T-system and sarcoplasmic reticulum in rats diabetic for 16 wk were also significantly increased when compared with controls.

Discussion

In this study, distinct damage to the myocardium in diabetic rats was identified as loss of myofilaments, disruption of mitochondria, dilatation of sarcoplasmic reticulum-T system, formation of contraction bands and myelin bodies, swelling of the intercalated disc and an increase in lipid droplets and glycogen granules. Morphometric analysis showed the volume of myofibrils to be significantly decreased. In the absence of microangiopathy, these lesions might be ascribed to a primary myocardial disease.

The growth in body weight was significantly retarded in rats diabetic for 8 wk and for 16 wk. In rats diabetic for 8 wk, heart weight showed no evident decrease, although slight myocytolytic necrosis and numerous lipid droplets and glycogen granules were noted in the myocardium. Subsequently, in rats diabetic for 16 wk heart weight was significantly diminished and myofilaments were disrupted. The significant increase in heart/body weight ratio of rats diabetic for 8 wk and for 16 wk suggested that the hearts from diabetic rats were in a state of hypertrophy. This result was compatible with previous reports demonstrating cardiomegaly in diabetic rats (Giacomelli and Wiener 1979; Pierce et al. 1983). Cardiac muscle cells from rats diabetic for 8 wk showed numerous lipid droplets and glycogen granules, swelling of mitochondria, and focal myofibrillolysis. These findings indicate an early stage of diabetic cardiomyopathy as described by Factor et al. (1983). In accordance with a previous study on the myocardium of streptozotocin-induced diabetic rats (Jackson et al. 1985), the diabetic myocardium progressively deteriorated with the duration of illness. Our data revealed an extensive loss of contractile protein, disruption of mitochondrial membranes, myelin formations and alteration of the sarcoplasmic reticulum-transverse tubular system in the myocardium of rats diabetic for 16 wk. More severe alterations took place in the rats diabetic for 16 wk than for 8 wk.

Dillmann (1980) has demonstrated that a close correlation exists between contractility and the activity of Ca^{2+} ATPase of purified actinomyosin and myosin. Penpargkul et al. (1981) have also shown that calcium control by the sarcoplasmic reticulum plays an important role in modulating cardiac relaxation. Thus, the loss of contractile protein and the alteration of the sarcoplasmic reticulum-transverse tubular system observed in diabetic BB myocardium may explain abnormal cardiac contraction and relaxation.

Another possible explanation for impaired myocardial function in diabetes is based on an abnormal energy producing system. Miller (1979) perfused hearts of acute alloxan diabetic rats and found that impaired myocardial function was associated with low ATP stores. Mitochondria have always been considered as the system for harvesting the energy liberated during the operation of the respiratory chain (synthesis of ATP) (Carafoli and Roman 1980). The present study revealed alterations of mitochondria in the diabetic myocardium which may be responsible for low ATP stores and decreased ventricular function.

Another type of myocardial lesion in rats diabetic for 16 wk was represented by the formation of contraction bands. Decreases in sarcolemmal sialic acid and phosphatidyl-ethanolamine contents in concert with an increase in lysophosphatidylcholine level in the hearts of diabetic rats have been shown to result in augmented permeability and enhanced Ca^{2+} entry into the cardiac muscle cells (Pierce et al. 1983). In addition, alteration in the cardiac sarcolemmal membrane in diabetic rats also seems to influence Ca^{2+} entry into the myocardial cells (Dhalla et al. 1982; Pierce et al. 1983). Therefore, Ca^{2+} overload may result from these abnormal myocardial calcium kinetics and this may induce contraction bands. Contraction bands were demonstrated not only in the diabetic myocardium (Jackson et al. 1985; Giacomelli and Wiener 1979) but also in catecholamine cardiomyopathy (Rona 1985; Todd et al. 1985). The same pathogenesis of contraction bands was suggested by Fleckenstein (1971) in catecholamine-induced cardiomyopathy.

Insulin is necessary for proper protein synthe-

sis. A decrease in myofibrillar protein was found in rats diabetic for 8 wk in which the formation of contraction bands was not yet found. Thus, it is postulated that the initial loss of myofibrillar protein was associated with hypoinsulinaemia. Furthermore, hypoinsulinaemia overlapping with Ca^{2+} overload results in more distinct loss of contractile protein later on.

In rats diabetic for 16 wk intercellular space of the fascia adherens is widened as has been described in alloxan diabetic hearts (Tarach 1976). Furthermore, membrane fragments were found in the widened fascia adherens. This may have a negative influence on the synchrony of heart contraction. Groniowski (1974), and Grzycki and Tochmann (1975) demonstrated that intercalated disks make a direct communication between muscle cells which contributes to ion continuity and by this help to the synchrony of contraction.

The presence of numerous lipid droplets and glycogen granules is probably due to the disturbance of carbohydrate and lipid metabolisms in the diabetic myocardium (Chen et al. 1984; Murthy and Shipp 1977; Neely and Morgan 1974; Nikkila et al. 1963; Opie 1968). In rats diabetic for 16 wk mitochondria are damaged so severely that they no longer metabolize cardiac lipid properly. This may then result in the breakdown of mitochondria, accumulation of lipid, and finally the formation of myelin bodies.

Our results failed to show valid differences in laminar thickening around cardiac blood capillaries between diabetic and control rats. This result was compatible with previous studies demonstrating microangiopathy in diabetic animals (Cameron et al. 1973; Salazar et al. 1973) since Fischer et al. (1981) have reported that a period of at least 6 months following induction of the diabetic state is required to produce laminar thickening around capillaries. In the present study, since the duration of diabetic states was shorter than 6 months, we were unable to demonstrate any marked alteration in capillaries in diabetic rats. Therefore the impaired cardiac structures observed in the present study seem to be due to diabetic metabolic disturbances rather than to microangiopathy.

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References

Ahmed SS, Jaferi GA, Narang RM, Regan TJ (1975) Preclinical abnormality of left ventricular function in diabetes mellitus. Am Heart J 89:153–158

Blumenthal HT, Alex M, Goldenberg S (1960) A study of lesions of the intramural coronary artery branches in diabetes mellitus. Arch Pathol 70:27–42

Brismar T, Sima AAF (1981) Changes in nodal function in nerve fibres of the spontaneously diabetic BB-Wistar rat: Potential clamp analysis. Acta Physiol Scand 113:499–506

Cameron DP, Amherdt M, Leuenberger P, Orci L, Stauffacher W (1973) Microvascular alterations in chronically streptozotocin-diabetic rats. In: Camarini-Davalos RA, Cole HS (eds) Vascular and neurological changes in early diabetes. Academic Press, New York, p 257

Carafoli E, Roman I (1980) Mitochondria and disease. Molec Aspects Med 3:295–429

Chen V, Ianuzzo CD, Fong BC, Spitzer JJ (1984) The effects of acute and chronic diabetes on myocardial metabolism in rats. Diabetes 33:1078–1084

Dhalla NS, Pierce GN, Panagia V, Singal PK, Beamish RE (1982) Calcium movements in relation to heart function. Basic Res Cardiol 77:117–139

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

Factor SM, Minase T, Bhan R, Wolinsky H, Sonnenblick EH (1983) Hypertensive diabetic cardiomyopathy in the rat: Ultrastructural features. Virchows Arch (Pathol Anat) 398:305–317

Fischer VW, Leskiw ML, Barner HB (1981) Myocardial structure and capillary basal laminar thickness in experimentally diabetic rats. Exp Mol Pathol 35:244–256

Fleckenstein A (1971) Specific inhibitors and promotors of calcium action in the excitation-contraction coupling of heart muscle and their role in the prevention of production of myocardial lesions. In: Harris P, Poie LH (eds) Calcium and the heart. Academic Press, London, p 135

Friedman NE, Levitsky LL, Edidin DV, Vitullo DA, Lacina SJ, Chiemmongkoltip P (1982) Echocardiographic evidence for impaired myocardial performance in children with type I diabetes mellitus. Am J Med 73:846–850

Giacomelli F, Wiener J (1979) Primary myocardial disease in the diabetic mouse. Lab Invest 40:460–473

Groniowski J (1974) Patologia komórkowa mieśnia serca. Kardiol Pol 17:437–446

Grzycki S, Tochmann A (1975) Some observations on the intercalated disc of a rat cardiac muscle. Z Mikrosk Anat Forsch 89:219–230

Haider B, Ahmed SS, Moschos CB, Oldewurtel HA, Regan TJ (1977) Myocardial function and coronary blood flow response to acute ischemia in chronic canine diabetes. Cir Res 40:577–583

Hamby RI, Zoneraich S, Sherman L (1973) Primary myocardial disease and diabetic mellitus (abstr). Circulation 41–42 (Suppl 3):44

Hamby RI, Zoneraich S, Sherman L (1974) Diabetic cardiomyopathy. JAMA 229:1749–1754

Jackson CV, McGrath GM, Tahiliani AG, Vadlamudi RVSV, McNeill JH (1985) A functional and ultrastructural analyis of experimental diabetic rat myocardium. Diabetes 34:876–883

Ledet T (1968) Histological and histochemical changes in the coronary arteries of old diabetic patients. Diabetologia 4:268–272

Ledet T (1976) Diabetic cardiopathy. Quantitative histological studies of the heart from young juvenile diabetics. Arch Pathol Microbiol Scand 84:421–428

Marliss EB, Sima AAF, Nakhooda AF (1981) Spontaneous diabetes in the "BB"-wistar rat. Proc Int Symposium on the Etiology of Diabetes, Toronto (1980). Raven Press, New York

Miller TB (1979) Cardiac performance of isolated perfused

hearts from alloxan diabetic rats. Am J Physiol 236:H808–H812

Murthy VK, Shipp JC (1977) Accumulation of myocardial triglycerides in ketotic diabetes: evidence for increased biosynthesis. Diabetes 26:222–229

Nakhooda AF, Like AA, Chappel CI, Murrya FT, Marliss EB (1977) The spontaneously diabetic Wistar rat. Metabolic and porphologic studies. Diabetes 26:100–112

Neely JR, Morgan HE (1974) Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. Annu Rev Physiol 36:413–457

Nikkila EA, Torsti P, Penttila O (1963) The effect of exercise on lipoprotein lipase activity of rat heart, adipose tissue and skeletal muscle. Metabolism 12:863–865

Opie LH (1968) Metabolism of the heart in health and disease. Part I*. Am Heart J 76:685–698

Orth DN, Morgan HE (1962) The effect of insulin, alloxan diabetes and anoxia on the ultrastructure of the rat heart. J Cell Biol 15:509–523

Page E, McCallister LP, Power B (1971) Stereological measurement of cardiac ultrastructures implicated in excitation-contraction coupling. Proc Natl Acad Sci USA 68:1465–1466

Penpargkul S, Fein F, Sonnenblick EH, Scheuer J (1981) Depressed cardiac sarcoplasmic reticular function from diabetic rats. J Mol Cell Cardiol 13:303–309

Pierce GN, Nutryk MJB, Dhalla NS (1983) Alteration in Ca^{2+} binding by and composition of the cardiac sarcolemmal membrane in chronic diabetes. Proc Natl Acad Sci USA 80:5412–5416

Regan TJ, Ettinger PO, Khan MI, Jesrani MU, Lyons MM, Oldewurtel HA, Weber M (1974) Altered myocardial function and metabolism in chronic diabetes mellitus without ischemia in dogs. Circ Res 35:222–237

Regan TJ, Ahmed SS, Levinson GE, Oldewurtel HA, Ahmad MR, Haider B, Lyons MM (1975) Cardiomyopathy and regional scar in diabetes mellitus. Trans Assoc Am Phys 88:217–223

Regan TJ, Lyons MM, Ahmed SS, Levinson GE, Oldewurtel HA, Ahmad MR, Haider B (1977) Evidence for cardiomyopathy in familial diabetes mellitus. J Clin Invest 60:885–899

Reinilä A, Åkerblom HK (1984) Ultrastructure of heart muscle in short-term diabetic rats: influence of insulin treatment. Diabetologia 27:397–402

Rona G (1985) Editorial review: catecholamine cardiotoxicity. J Mol Cell Cardiol 17:291–306

Rubler S, Dlugash J, Yuceoglu YZ, Kumral T, Branwood AW, Grishman A (1972) New type of cardiomyopathy associated with diabetic glomerusclerosis. Am J Cardiol 30:595–602

Salazar H, Chez RA, Pardo M (1973) Absence of ultrastructural changes in the basement membrane of muscle capillaries in streptozotocin-induced carbohydrate intolerance in Rhesus monkeys. Amer J Pathol 71:437–446

Seneviratine BIB (1977) Diabetic cardiomyopathy: the preclinical phase. Br Med J 1:1444–1446

Sima AAF (1980) Peripheral neuropathy in the spontaneously diabetic "BB"-Wistar rat. Acta Neuropathol (Berl) 51:223–227

Sohar EH, Ravid M, Ben-Shaul Y, Reshef T, Gafni J (1970) Diabetic fibrillosis. A report of three cases. Am J Med 49:64–69

Tarach JS (1976) Histomorphological and ultrastructural studies of a rat myocardium in experimental conditions. Z Mikrosk Anat Forsch 90:1145–1157

Todd GL, Baroldi G, Pieper GM, Clayton FC, Eliot RS (1985) Experimental catecholamine-induced myocardial necrosis I. Morphology, quantification and regional distribution of acute contraction band lesions. J Mol Cell Cardiol 17:317–338

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