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Cardiac cell damage: a primary myocardial disease in streptozotocin-induced chronic diabetes

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Summary. Ultrastructural changes in heart muscle due to chronic diabetes subsequent to a single injection of streptozotocin (65 mg/kg body wt, i.v.) were studied in rats. Presence of diabetes was indicated by hyperglycaemia (plasma glucose, control, 120 ± 7; diabetic, 448 ± 21 mg/dl) as well as hypo-insulinaemia (plasma insulin, control, 25.6 ± 5.2; diabetic, 11.2 ± 0.5 μU/ml). After 8 weeks of diabetes, the hearts were processed for electron microscopic examination. Cardiac muscle cells in diabetic hearts showed condensation of nuclear chromatin and folding of nuclear membranes. Swelling of mitochondria, clearing of mitochondrial matrix and incorporation of lysosomal membranes into mitochondrial matrix was also noted. A marked increase in both lysosomes and lipid droplets was apparent. Focal areas in diabetic hearts showed contracted sarcomeres, myofibrillar degeneration and separation of the intercalated disc. Atherosclerotic plaque formation as well as structural changes in the smooth muscle or endothelial cells in the small arteries, arterioles or capillaries were not seen to accompany the structural changes in the cardiac muscle cells of the diabetic hearts. This study provides strong evidence for the occurrence of primary myocardial disease in streptozotocin-induced chronic diabetes.

Keywords: cardiac cell damage, myocardial disease, streptozotocin, diabetes

Death due to heart failure is abnormally high among diabetic patients (Partamian & Bradley 1965; Kannel 1978; Tunbridge 1981). Although atherosclerotic plaque formation in the coronary arteries is considered to be the major cause of depressed myocardial function (Kessler 1971), its exact role in the causation of heart failure has not been unequivocally established (Blumenthal *et al.* 1960; Ledet 1968; Vihert *et al.* 1969; Hamby *et al.* 1974; Regan *et al.* 1977). It is possible that cardiac death in diabetics is due to primary myocardial disease which may or may not be accompanied by coronary artery disease. Defects in contractile function (Fein *et al.* 1980; Heyliger *et al.* 1982; Ganguly *et al.* 1983), adrenergic receptors (Heyliger *et al.* 1982) and various subcellular mechanisms (Puckett & Reddy 1979; Pierce & Dhalla 1981, 1983; Ganguly *et al.* 1983; Pierce *et al.* 1983) have been well documented in the myocardium of chronic diabetic animals indicating the presence of myocardial cell disease. However, it remains to be established whether these changes are primary or secondary to the obstructive

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lesions caused by atherosclerosis. In a study of genetically diabetic mice of 5–24 weeks of age, it has been reported that cardiomegaly and pathological alterations in cardiac muscle cells are accompanied by degenerative changes in perivascular nerve endings as well as changes in intramural coronary arteries including arterioles and capillaries (Giacomelli & Wiener 1979). Since these lesions in genetically diabetic mice were found in the absence of structural changes in the large coronary arteries, this was considered to be a demonstration of primary myocardial disease. However, the study did not rule out the possibility of myocardial cell changes being secondary to intramural coronary artery pathology.

In the present ultrastructural study, we wish to describe lesions of the myocardial cell in rat hearts due to streptozotocin-induced chronic diabetes. In the absence of any demonstrable changes in intramural arteries, arterioles and capillaries, these changes can be seen to represent a primary cardiomyopathy in this animal model.

Materials and methods

Ten male Sprague–Dawley rats weighing 175–200 g were made diabetic with a single intravenous injection of streptozotocin (65 mg/kg body wt) delivered in a citrate-buffered vehicle (pH 4.5) into the femoral vein. Ten control rats were injected with citrate buffer alone. All animals were maintained on normal rat chow and water *ad libitum* for 8 weeks. For haemodynamic studies animals were anaesthetized with a single i.p. injection of Nembutal (50 mg/kg). After intubation of the trachea for maintaining adequate ventilation, the right carotid artery was cannulated for recording the blood pressure. Heart rate and blood pressure were monitored for 1 h. At the termination of these studies, a blood sample was collected in a heparinized syringe and plasma was prepared. These plasma samples were then employed for quantitative analyses of glucose (Worthington Statzyme glucose reagent kit) and for insulin (Amersham) radioimmunoassay procedures. The data were analysed by Students *t*-test.

The hearts were removed quickly from the anaesthetized animals, the atria and connective tissue was dissected free, and the wet ventricular weight was obtained. For ultrastructural examination, five hearts each from the diabetic and the control group were fixed in 0.1 M phosphate buffer (pH 7.4) containing 2% glutaraldehyde at 4°C. Small tissue pieces 4–6 mm in size were taken from four different areas of mid-myocardial layer of the free left ventricular wall between middle of the chamber and the apex of the heart. These tissue samples were immersed for 15 min in the aldehyde fixation solution, cut into pieces

Table 1. Effects of diabetes on different parameters in rats

	Control	Diabetic
Body weight (g)	448±8 (10)	268±8* (10)
Ventricular weight (g)	1.16±0.04 (10)	0.82±0.05* (10)
Ventricular/body weight ratio (mg/g)	2.58±0.04 (10)	3.06±0.05* (10)
Plasma glucose (mg/dl)	120±7 (6)	448±21* (6)
Plasma insulin (μU/ml)	25.6±5.2 (6)	11.2±0.5* (6)
Blood pressure (mmHg)	140.0±1.2 (5)	137.2±2.4 (5)
Heart rate (beats/min)	409.3±13.7 (5)	331.9±9.2* (5)

Values represent mean ± S.E. Numbers of animals are given in parentheses.
* Significantly different from control ($P<0.05$).

of approximately 1 mm^3 and allowed to stand in the solution for a total of 2 h for further fixation. In the experimental as well as in the control group, heart muscle samples were also fixed by the procedure of perfusion-fixation described previously (Singal *et al.* 1979). The tissue pieces were washed overnight in cold phosphate buffer containing sucrose, post-fixed for 1 h with 1% osmium tetroxide, dehydrated in a graded alcohol series and embedded in Epon 812 according to the method of Luft (1961). Sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined by a Zeiss EM9 electron microscope.

Results

The presence of diabetes in the streptozotocin injected animals was confirmed by elevated glucose and depressed insulin levels in plasma in comparison with control rats (Table 1). Diabetic animals exhibited significantly lower body and ventricular weights, and an increase in heart-to-body weight ratio (Table 1). Although a significant depression in the heart rate of diabetic animals was apparent, there was no difference noted in the blood pressure of diabetic and control rats (Table 1).

Since animals were given anaesthesia in this study, ultrastructure of the hearts from rats anaesthetized for 60 min with Nembutal (65 mg/kg) was compared with the hearts from control animals. Ultrastructural details were quite similar and comparable to those seen in the perfusion-fixed hearts (Stenger & Spiro 1961; Singal *et al.* 1979). A moderate

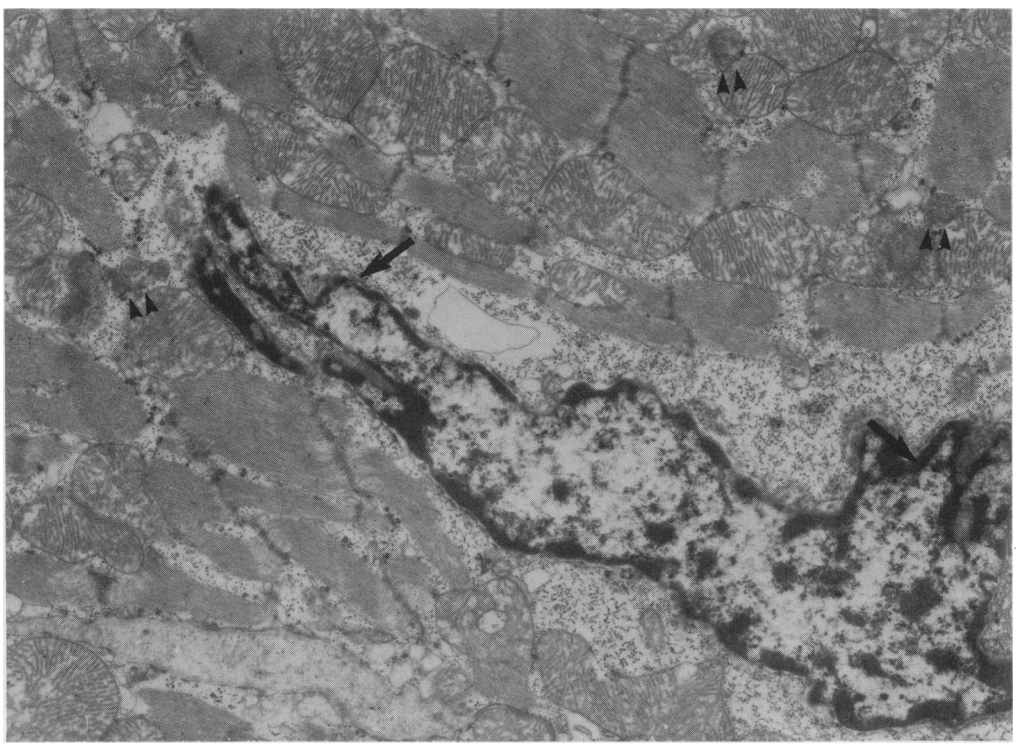

Fig. 1. This and all other electron micrographs are from the diabetic hearts. Condensation of nucleic chromatin and marked folding of the nuclear membrane (arrows) as well as swelling of mitochondria and clearing of their matrix are apparent. Double arrow-heads indicate lysosomes. × 14 000.

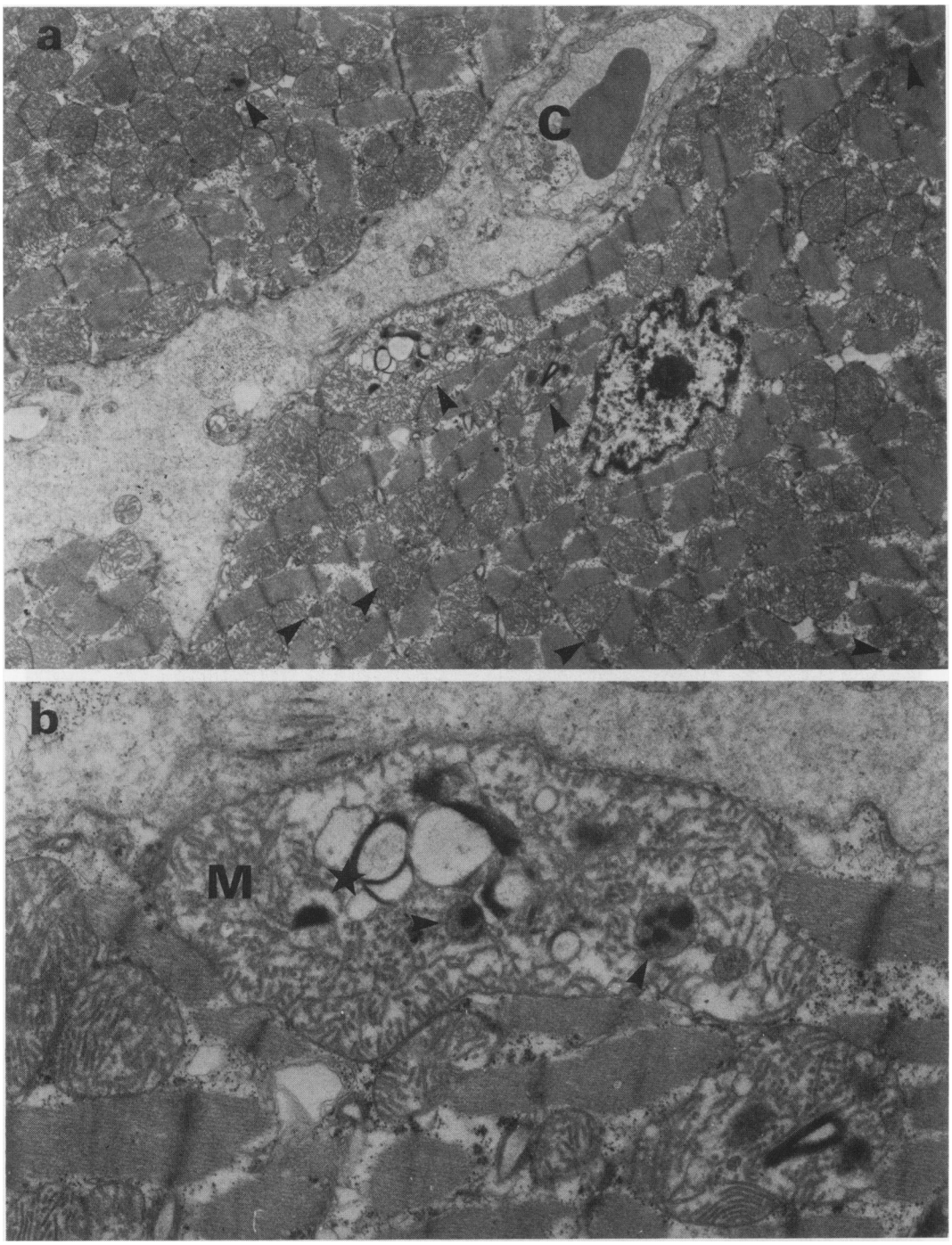

Fig. 2. (*a*) Increased number of lysosomes either in close contact with or inside the mitochondria (arrow-heads). Wavy contours of the nuclear membrane and heterogenous appearance of chromatin are apparent. A capillary (C) with normal-looking endothelial cells can also be seen. × 10 000. (*b*) Enlarged view of mitochondria (M) to show intramitochondrial lysosomes (arrow) and membrane whorls (*). × 32 000.

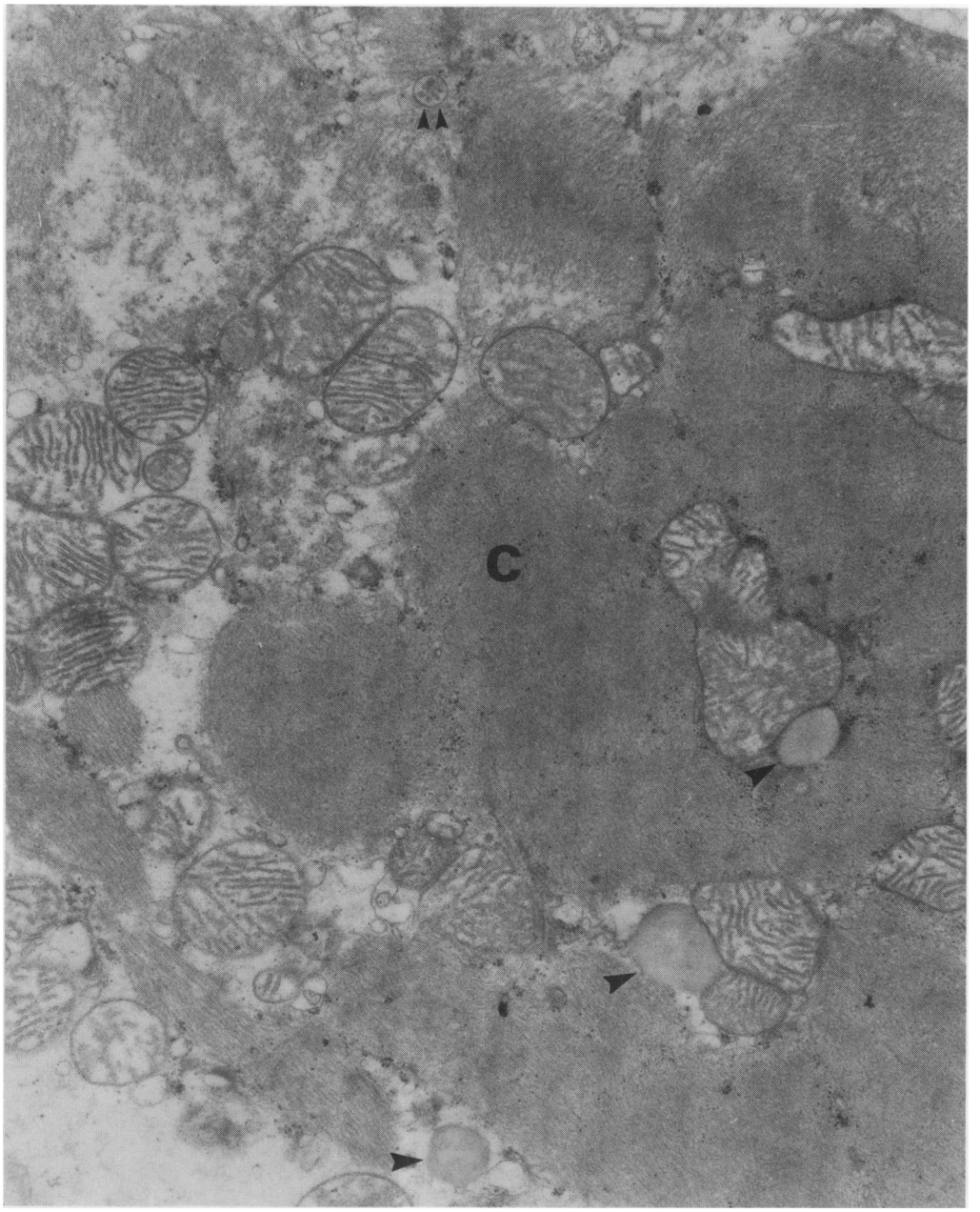

Fig. 3. Disruption of myofibrils and formation of contraction bands (C). Arrow-heads point to lipid droplets and a double arrow-head indicates lysosomes. × 16 000.

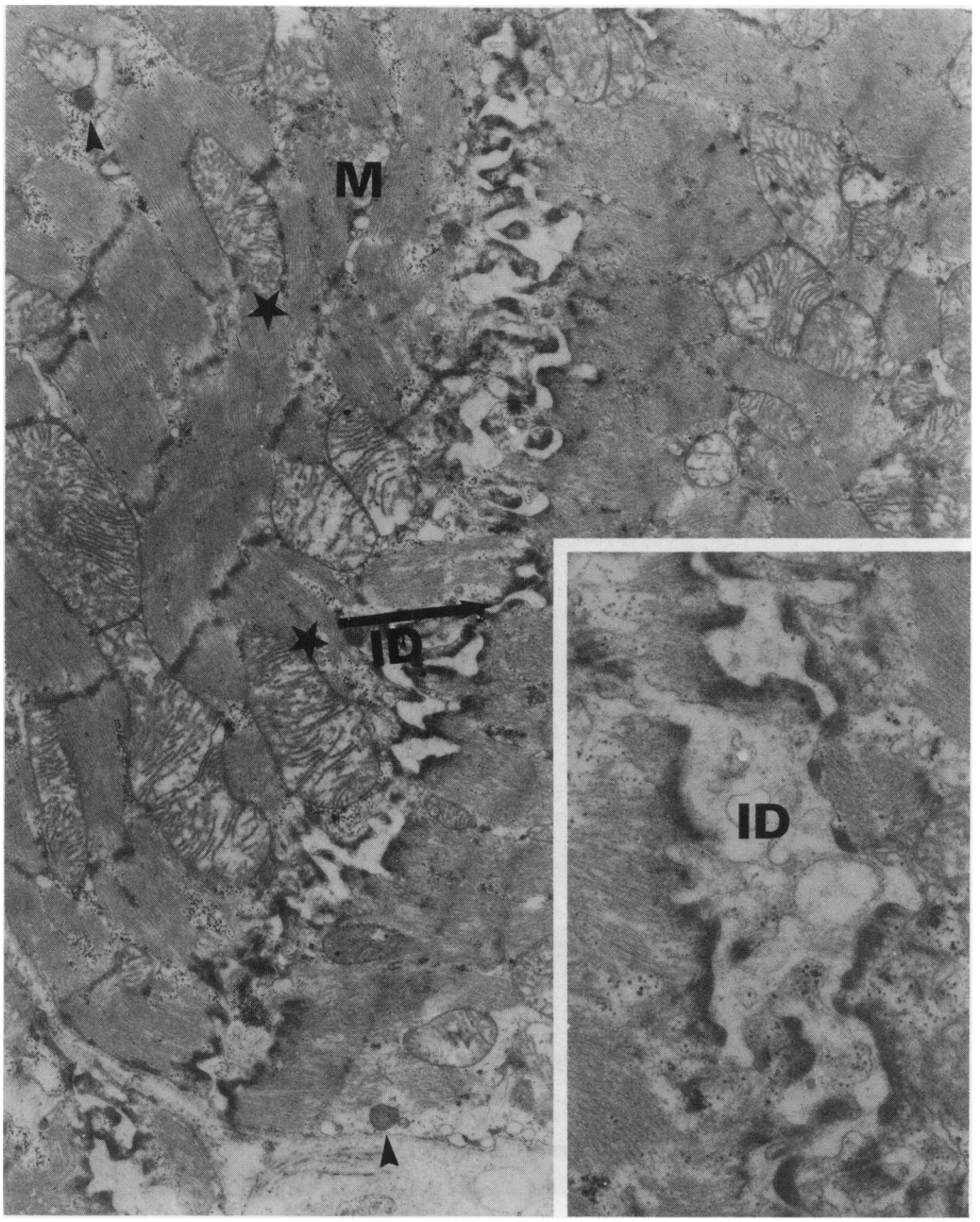

Fig. 4. Cell-from-cell separation at the intercalated disc (ID) in the diabetic hearts. Typical arrangement of a sarcomere is disrupted (*). Arrow-heads point to lysosomes. M, mitochondria. × 16 000. Inset shows another area with a wider gap in the intercalated disc (ID). Appearance of membrane vesicles in the gap can be noticed. × 17 000.

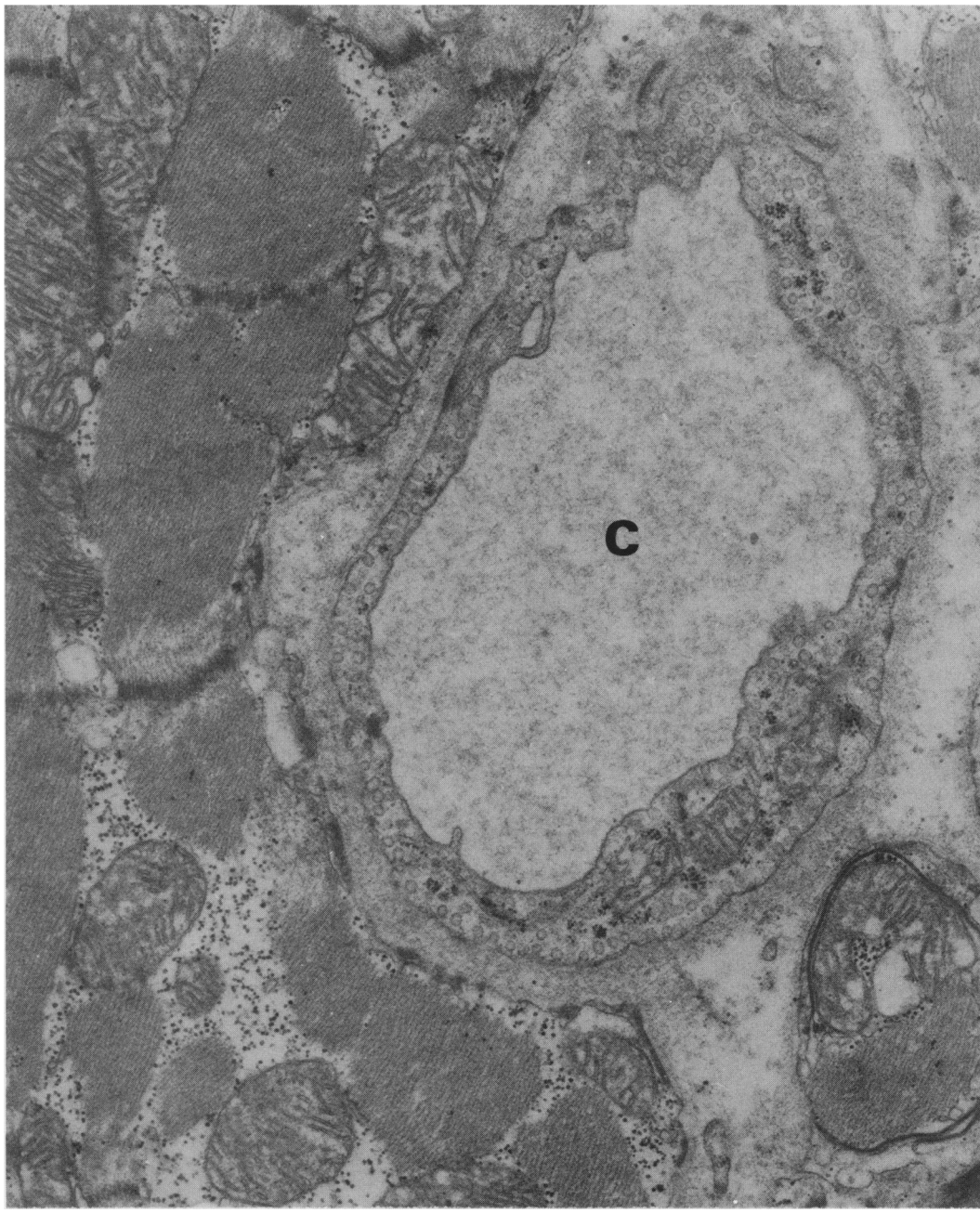

Fig. 5. Normal appearance in the capillary (C) endothelial cells in a diabetic heart. Mitochondria in the cardiac cell are swollen and sarcomeres show wavy Z line. × 30 000.

expansion of the intercellular space in those portions of the intercalated disc running parallel to the myofibrils as well as the appearance of small vesicles in the transverse tubular spaces appear to be normal features in these electron microscope preparations of the heart (Singal *et al.* 1979).

Varying degrees of ultrastructural change were apparent in the ventricular myocardium of the diabetic animals. The nucleus of myocytes was lobulated due to marked indentations of the nuclear membrane and nuclear chromatin was condensed and marginated (Figs 1 and 2). Mitochondria in the streptozotocin-induced diabetic hearts appeared swollen and their matrix was relatively clear (Figs 1–5). Occasional enlargement of mitochondria was also noted. Focal degeneration of mitochondria was indicated by the incorporation of lysosomal as well as lipoid bodies in these organelles (Fig. 2). There was a marked increase in the lysosomes and lipid droplets seen in the cytoplasm of cardiac myocytes in the diabetic animals (Figs 2 and 3). In addition to these changes, some cardiac muscle cells exhibited the formation of contraction bands and the structural details of a typical sarcomere were lost in these cells (Fig. 3). Disruption of sarcomere arrangements expressed as a wavy appearance of the Z line was seen in almost all of the cardiac muscle cells of the diabetic hearts. Cell-to-cell relationships at the intercalated disc level were altered by the appearance of gaps between the cells (Fig. 4). In some areas this intercellular gap also contained membrane vesicles and small granules (Fig. 4).

The ultrastructural changes in the cardiac muscle cells were not accompanied by any changes in the endothelial cells of the tunica intima or in the smooth muscle cells of the tunica media in the intramural small arteries, arterioles and capillaries (Figs 1 and 2). The appearance of the lumen as well as of other cellular details of the coronary arteries in the streptozotocin-induced diabetic hearts was normal (Fig. 5) and quite comparable to that in the control hearts.

Discussion

The streptozotocin-induced animal model of experimental diabetes is considered to be better than other experimental models because of its closer resemblance to insulin-dependent diabetes mellitus in humans and a relative lack of any non-specific necrotic effects of this drug in rats (Agarwal 1980; Srivastava *et al.* 1982). The hyperglycaemic condition concomitant with the hypo-insulinaemic state seen in the present study due to a single intravenous injection of streptozotocin is consistent with other studies employing similar experimental protocols (Penpargkul *et al.* 1981; Ganguly *et al.* 1983; Pierce *et al.* 1983). The diabetic animals in the present study showed a very small gain in the body or ventricular weights after streptozotocin injection as compared to the vehicle-treated controls in spite of the fact that diabetic animals consume more food and water (Hofteizer & Carpenter 1973). The reduced weight gain may have been due to the dehydration and abnormally high rate of protein and fat catabolism which have been reported during diabetes (Oakley 1968). The elevated ventricular-to-body weight ratio observed in this study has also been reported by others (Penpargkul *et al.* 1980; Vadlamudi *et al.* 1982) and could be due either to a true heart hypertrophy or to a decrease in body weight. The occurrence of cardiac cell hypertrophy in diabetic rats has been suggested before (Onishi *et al.* 1981).

The present ultrastructural observations on the myocardium of streptozotocin-induced diabetic rats clearly demonstrate changes in the cardiac muscle cells. In the absence of any accompanying lesion in the intramural coronary arteries, arterioles and capillaries, this study provides a strong evidence for the occurrence of a primary myocardial disease in the streptozotocin-induced experimental model of diabetes. Based on their histological studies, Giacomelli & Wiener (1979) reported the presence of a primary cardiomyopathy in genetically diabetic mice even though degenerative

changes in the intramural coronary vasculature as well as in the perivascular nerve endings were seen to accompany the pathological changes in the cardiac muscle cells. Their conclusion in favour of a primary cardiomyopathy was mainly based on the absence of large (epicardial) coronary artery pathology in the genetically diabetic mice (C 57 BL/KsJ db^+/db^+) which together with other myocardial lesions seem to fulfil the criteria for a primary myocardial disease (Hamby *et al.* 1974; Regan *et al.* 1975). However, our study documents the possibility that cardiac cell damage in diabetics may precede any changes in the coronary, epicardial or intramural vasculature.

It should be noted that ultrastructural changes in the cardiac muscle cell recorded by us in this study do not appear to be unique to the streptozotocin-induced diabetics. Alloxan-induced diabetes has been shown to be accompanied by similar changes in the mitochondria and widening of the intercalated disc has also been seen (Regan *et al.* 1975; Tarach 1976; Onishi *et al.* 1981). Formation of contraction bands, appearance of mitochondrial inclusions and an increase in lysosomes and lipid droplets have been seen in genetically diabetic mice (Giacomelli & Wiener 1979). It is possible that irrespective of the inciting stimulus for chronic insulin deficiency, the terminal pathological changes due to this condition may follow a common pathway. An increased number of lysosomes as well as the incorporation of these organelles into mitochondria noted in this study may also represent a non-specific response to a yet undefined cardiac injury as similar lysosomal changes are known to accompany a variety of human and experimental pathological conditions (Sulkin & Sulkin 1965; Wheat 1965; Hug & Schubert 1970; Ferrans *et al.* 1972; Singal *et al.* 1979).

Although the present study does not allow us to define the subcellular defect/s leading to the changes noted, formation of contractile bands coupled with cell-from-cell separation and swelling of mitochondria may indicate an intracellular increase in calcium. It may be noted that presence of general cardiac sarcolemmal lesions (Pierce *et al.* 1983; Pierce & Dhalla 1983) as well as increases in sodium and calcium contents have been reported to occur during diabetes (Nagase *et al.* 1981; Regan *et al.* 1981). Intracellular Ca^{2+} overload and mitochondrial changes can be seen to result in reduced consumption of substrates such as cardiac lipid as well as impaired energy production (Dhalla *et al.* 1980). Accumulation of lipid droplets was apparent in the myocardium of streptozotocin-induced animals in this study. High intracellular levels of calcium can also stimulate proteases, phospholipases and lysosomal enzymes (Dhalla *et al.* 1982; Singal *et al.* 1984). A lower energy state as well as activation of lysosomal hydrolases have been implicated in the pathogenesis of heart disease (Dhalla *et al.* 1980, 1982; Katz & Reuter 1979; Singal *et al.* 1984). Further investigation in terms of time-related changes in streptozotocin-induced diabetic hearts will not only provide the sequence of early subcellular changes taking place primarily in the cardiac myocytes but will also reveal that stage of the disease where coronary vasculature may become involved.

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