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Ultrastructure of the chronically vagotomised atrial myocardium in the monkey *(Macaca fascicularis)*

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Summary. The ultrastructure of the atrial myocardium in the monkey *(Macaca fascicularis)* was studied after bilateral cervical vagotomy and survival times of 100, 175 and 367 days. Changes were observed in the nucleus and the cytoplasm of the myocyte. Sequestered within the nuclei of the affected myocytes were cytoplasmic organelles and inclusions. In the late stages, there was a tendency towards condensation and margination of the heterochromatin. Changes in the cytoplasm included increased glycogen, mitochondrial degeneration and myofibrillar disorganisation and degeneration. There was increased collagen and mononuclear cell infiltration in the extracellular space in the later stages. This study has shown that the long term structural integrity of the atrial myocyte depends on an intact vagal innervation. The survival of the monkey after chronic bilateral vagotomy suggests that this nonhuman primate is a suitable model for functional studies of the parasympathetically denervated heart.

Key words: Ultrastructure, Vagotomised, Myocardium, Monkey

Introduction

Although the normal ultrastructure of the mammalian atrial myocardium has been well described (Canale et al., 1986; Sommer and Jennings, 1986) accounts of the effects of vagotomy on its structural integrity are relatively few. Tchervova (1960) has described, histologically, leucocytic infiltration, dedifferentiation of connective tissue cells and myocardial fibers following vagotomy, while Chernukh et al. (1974) mentioned mitochondrial changes in their ultrastructural study. Imataka et al. (1982) observed cellular enlargement, nuclear degeneration and fibrosis in the interstitial spaces in the rabbit ventricle 7 to 8 days after a unilateral vagotomy. More recently, Wong et al. (1987) followed the sequential ultrastructural changes in the monkey's atrial myocardium up to 4 weeks after a bilateral vagotomy. In that study, it was uncertain if the observed atrophic changes were transient and reversible or prolonged and irreversible. The present study was therefore undertaken to answer that question and to describe the ultrastructural changes in the chronically vagotomised atrial myocardium in the monkey. A brief account of the results has been published (Wong et al., 1988).

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Materials and methods

Six healthy adult monkeys *(Macaca fascicularis)* of either sex and weighing 2.0-4.2 kg were used. Their ages could not be ascertained as they were trapped in the wild by animal suppliers. In 3 animals, under intraperitoneal Sagatal (sodium pentobarbital 30 mg/kg) anesthesia and aseptic conditions, a ventral midline incision was made in the neck to expose both vagi. The nerves were sectioned above the omohyoid muscle and to avoid reinnervation a 10 mm length of each nerve was removed. Survival times were 100, 175 and 367 days. One monkey was sham operated in which the vagi were exposed but not sectioned and was allowed to survive 175 days. The 2 remaining monkeys were used as unoperated controls. At the time of sacrifice each animal was reanesthetized and received artificial respiration through a tracheostomy by a Palmer animal ventilator. The mediastinum was exposed and the pericardium slit to allow the routine intraventricular injection of 1000 units of heparin (1 ml) and 1 ml of 1% sodium nitrite per kg body weight. The animal was rapidly perfused through the left cardiac ventricle with 500 ml of Ringer's solution at pH 7.3-7.4 followed by 1000 ml of dilute fixative (1% paraformaldehyde + 1.25% glutaraldehyde) and then 2000 ml of concentrated fixative (4% paraformaldehyde + 5% glutaraldehyde) in 0.1M cacodylate buffer at pH 7.4.

After perfusion, the heart was removed and placed in

fresh concentrated fixative overnight at 4°C. The interatrial septum was carefully dissected out and trimmed into approximately 1 mm cubes which were placed in ice-cold 0.1M cacodylate buffer (pH 7.4) containing 5% sucrose. Following 2 changes of buffer at intervals of 10 minutes, the tissues were post-fixed in ice-cold 1% osmium tetroxide containing 1.5% potassium ferrocyanide (Hayat, 1981) for 2 hours. The tissue blocks were then dehydrated in an ascending series of ethanol and embedded in Araldite.

Semithin 1μm thick sections were cut with Porter-Blum MT, or Reichert OMU4 ultramicrotomes and stained with 1% methylene blue and azure II. Selected areas of the blocks containing the atrial myocardium sectioned longitudinally or transversely were trimmed for ultrathin sectioning. The ultrathin sections were mounted on coated grids, doubly stained with uranyl acetate and lead citrate and viewed in a Philips 400T or Jeol 1200 EX electron microscope.

Results

Control animals

The ultrastructure of the atrial cells in the sham operated and unoperated controls (Figs. 1, 2) resembled that described previously in the monkey (Wong et al., 1987) and in other mammals. The nucleus was usually central but may occasionally be subsarcolemmal in position. The nuclear outline, though relatively smooth in most cells may be indented in some. The nucleoplasm contained evenly dispersed chromatin with small amounts of heterochromatin on the inner aspect of the nuclear envelope. Mitochondria were abundant but giant forms were rarely encountered. Glycogen was present as beta particles in the sarcoplasm and occasionally within mitochondria. Atrial granules, sarcoplasmic reticulum and typical intercalated discs were readily identified.

Legends

Abbreviations

Ag	Atrial granule
Co	Collagen
Dg	Dense granule
Gd	Glycogen deposit
Gg	Glycogen granule
Gp	Alpha glycogen particle
H	Heterochromatin
Id	Intercalated disc
Iv	Cytoplasmic invagination
L	Lymphocytic cell
Lp	Lipid droplet
M	Mitochondrion
Mc	Macrophage
Mf	Myofilaments
My	Myofibril
N	Nucleus
Rb	Residual body
S	Sarcoplasm
Sr	Sarcoplasmic reticulum
Tv	Tubulo-vesicular profiles
Z	Z disc

Fig. 1. Transverse section of control atrial myocyte. The nucleus is central and its outline even. Cytoplasmic contents include myofilaments, atrial granules and mitochondria. Note the occasional intramitochondrial glycogen granule (arrows). × 20,000

Fig. 2. Longitudinal section of control atrial myocytes. The regular arrangement of the sarcomeres, intercalated disc, glycogen granules, sarcoplasmic reticulum and mitochondria are readily identified. × 20,000

Fig. 3. Located within a deep nuclear invagination are: a large residual body; dense granules; a mitochondrion; myofilaments and portions of sarcoplasm. 100 days post-vagotomy. × 12,000

Fig. 4. A nucleus with a deeply indented outline and mitochondria and a lipid droplet lying freely in the nucleoplasm. One of the mitochondria, sectioned tangentially (M), contains many glycogen granules. 100 days post-vagotomy. × 18,000

Fig. 5. A nucleus with tubulo-vesicular profiles lying freely in the nucleoplasm. 100 days post-vagotomy. × 20,000

Fig. 6. A nucleus with a bizarre outline; its chromatin is patchily distributed and there is condensation of the heterochromatin. Note also the cytoplasmic invaginations. 100 days post-vagotomy. × 12,000

Fig. 7. An eccentrically located nucleus within a sarcoplasmic projection which also contains a giant mitochondrion. 175 days post-vagotomy. × 12,000

Fig. 8. A large membrane-bounded glycogen deposit, probably a degenerated giant mitochondrion. 100 days post-vagotomy. × 26,000

Fig. 9. Mitochondria showing disintegration of cristae and massive accumulation of glycogen particles. 100 days post-vagotomy. × 30,000

Fig. 10. The profile is filled with mitochondria, sarcoplasmic reticulum and numerous alpha glycogen particles. Some remnants of myofilaments are barely discernible. 175 days post-vagotomy. × 20,000

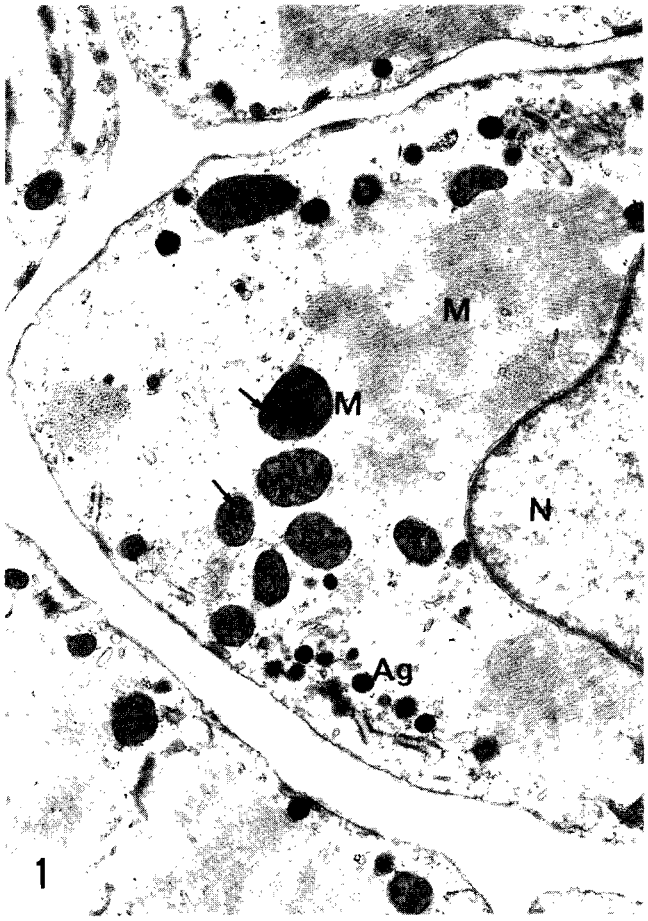
Fig. 11. Several mitochondria entrapped within a large glycogen deposit and undergoing dissolution. 100 days post-vagotomy. × 20,000

Fig. 12. Part of a longitudinal section of an edematous myocyte with fragmented myofibrils and vacuolated mitochondria. Note the undefined granular substance in the cytosol. 367 days post-vagotomy.
× 10,000

Fig. 13. Part of a longitudinal section of an edematous myocyte with remnants of myofilaments. Note the distorted Z discs. 175 days post-vagotomy. × 10,000

Fig. 14. A low power view to show infiltration of mononuclear cells and increased collagen in the extracellular space. Macrophages and lymphocytic cells are readily identified. 175 days post-vagotomy. × 3,900

Fig. 15. A detached myocyte (bottom of figure) in close relationship with an active macrophage. 175 days post-vagotomy. × 12,000



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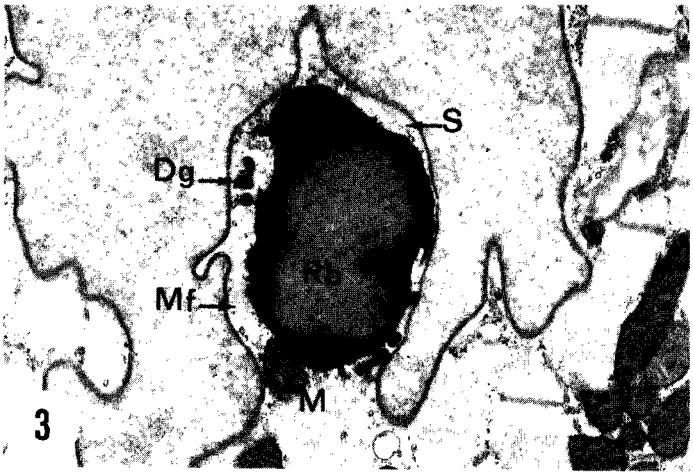

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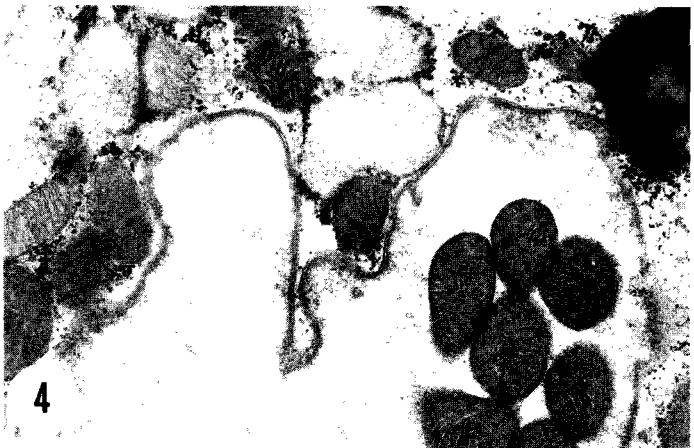

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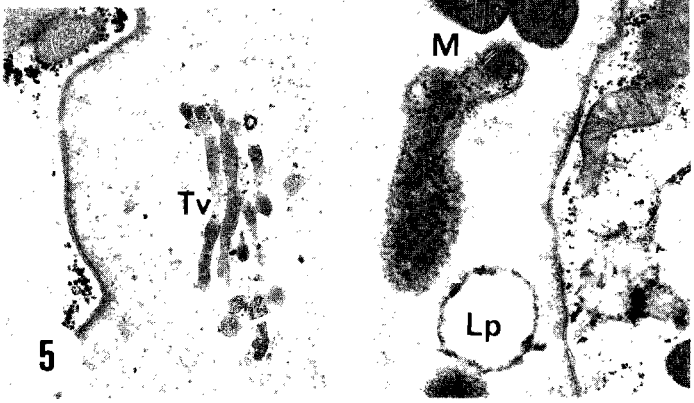

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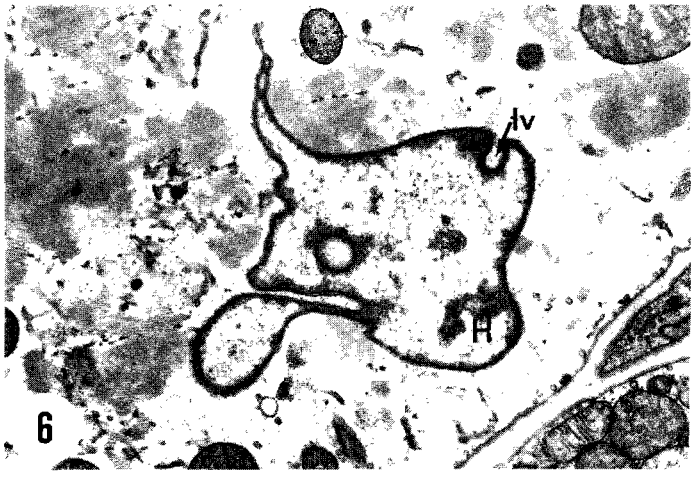

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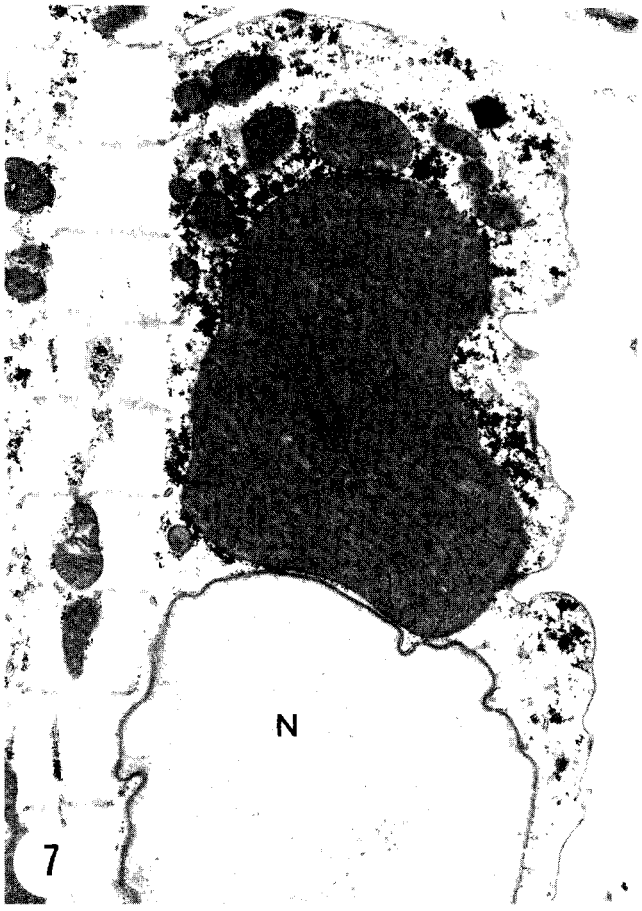

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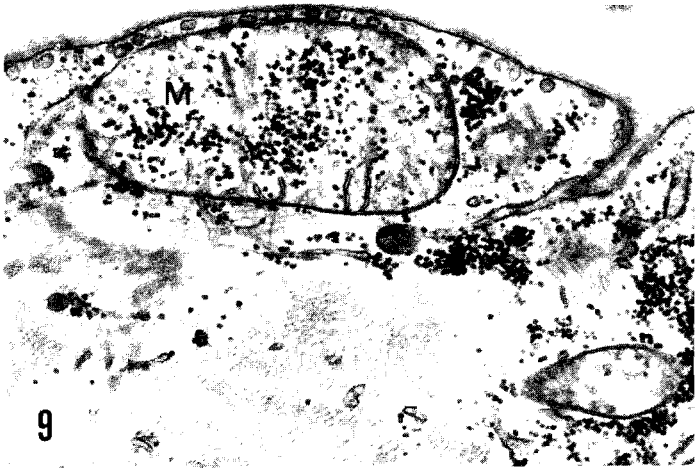

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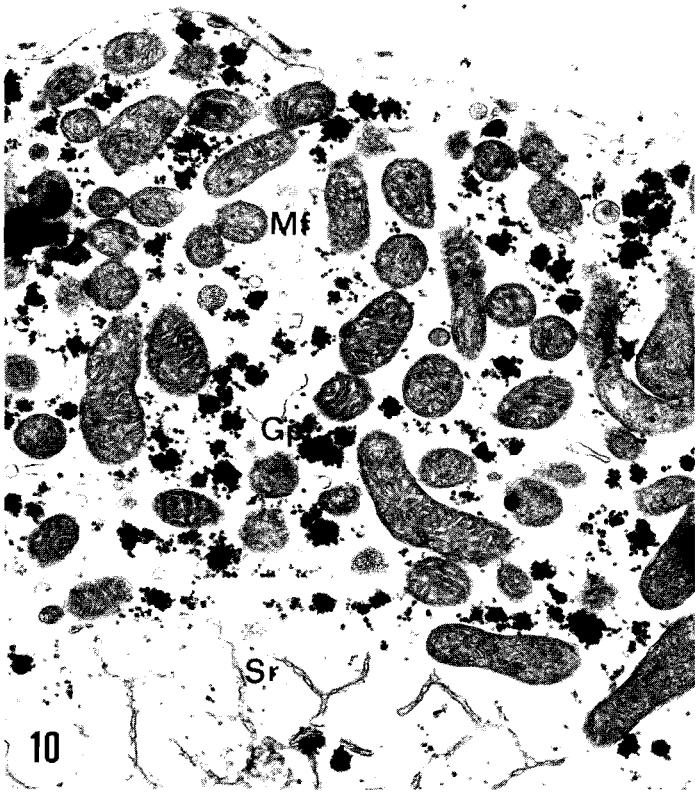

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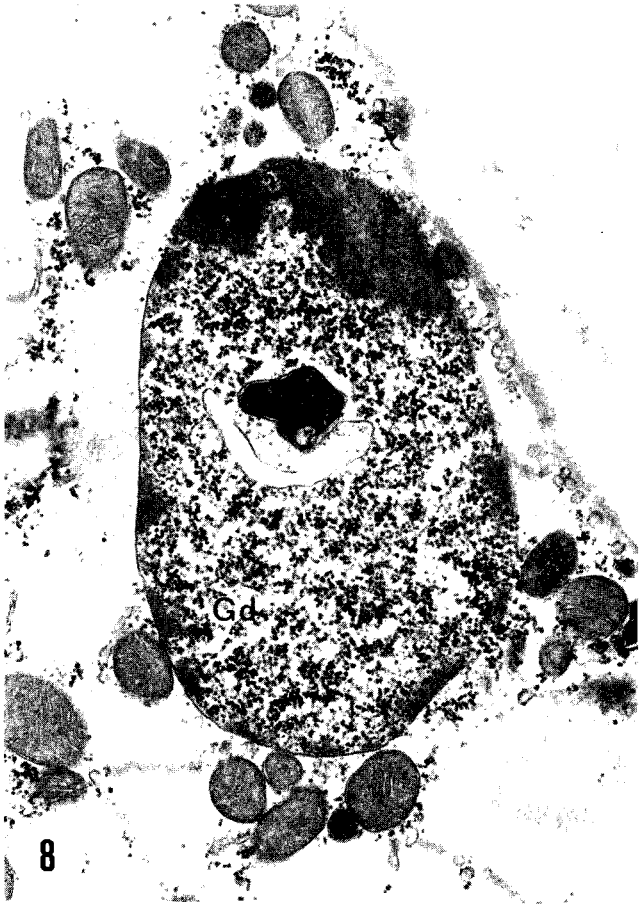

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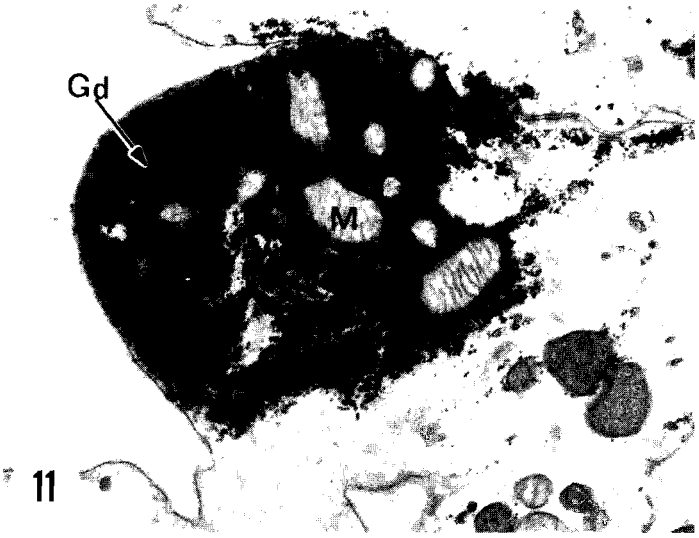

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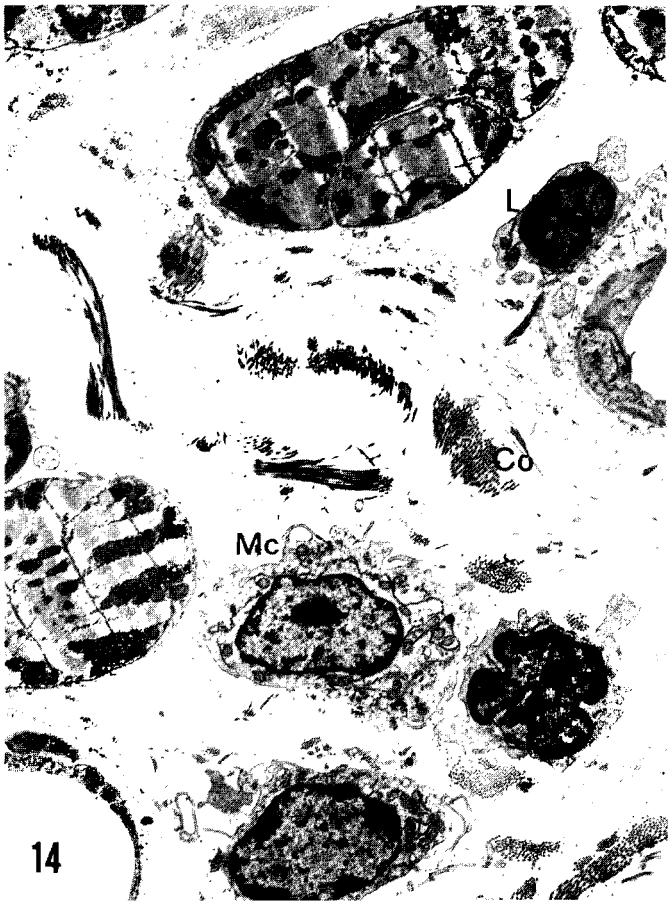

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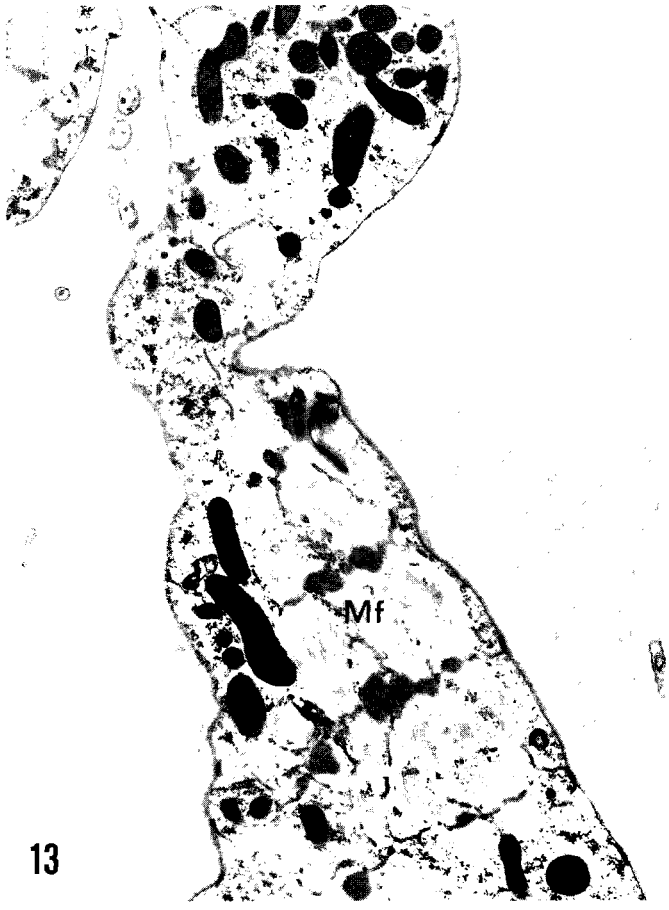

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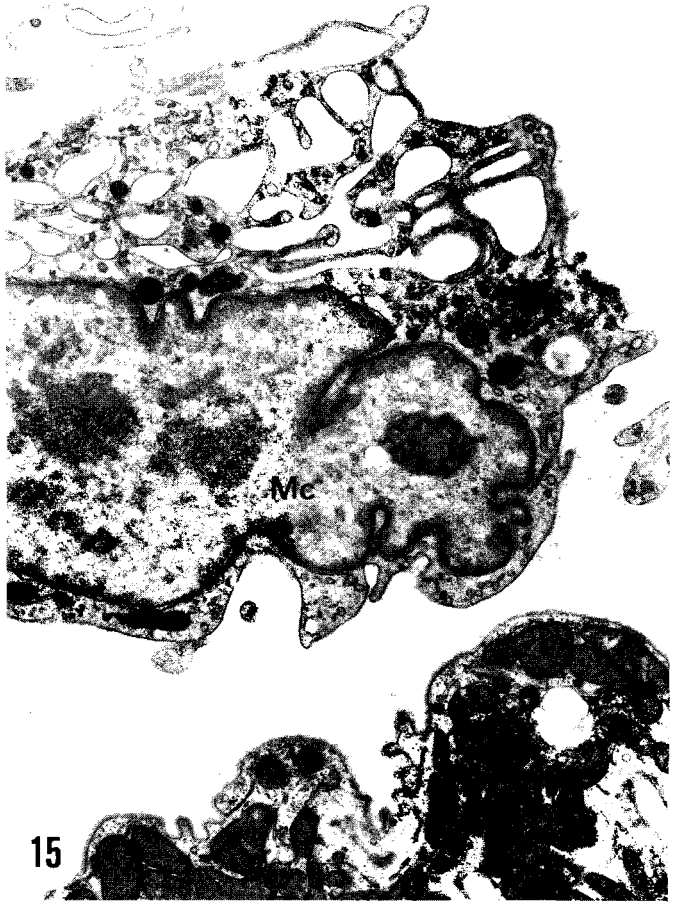

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Experimental animals

A spectrum of abnormal myocytic profiles was seen in the chronically vagotomised myocardium. Changes were observed in the nucleus and cytoplasm.

Cytoplasmic organelles and inclusions such as mitochondria, atrial granules, sarcoplasmic reticulum, tubulovesicular structures, myofilaments, lipid droplets and electron-dense bodies were located within deep nuclear invaginations which communicated with the cytosol (Figs. 3, 4). In other instances, the cytoplasmic organelles were apparently lying freely in the nucleoplasm (Figs. 4, 5). The outlines of other nuclei were deeply indented assuming polylobular forms (Fig. 6). The chromatin in these and other nuclei was patchily distributed. There was a tendency towards condensation and margination of the heterochromatin, especially in the later stages.

The sarcolemma of some atrial cells was deeply folded with outpockets or cell projections resulting in peripheral displacement of the nucleus (Fig. 7). Giant mitochondria (Figs. 7, 8) were more commonly seen than in control animals. Many mitochondria showed disintegration of cristae, vacuolation and sequestered glycogen granules (Figs. 4, 8, 9). Glycogen granules were increased in many myocytes with the formation of alpha particles, hardly encountered in control animals (Fig. 10). Large glycogen deposits may entrap within themselves mitochondria which undergo dissolution (Fig. 11).

Myofibrillar disorganisation was reflected in a general edema of the myocyte, loss of myofilaments and distortion, fragmentation or disappearance of Z discs (Figs. 12, 13). The loss of myofilaments in the myocytes may be replaced by a proliferation of sarcoplasmic reticulum, mitochondria and accumulation of alpha glycogen particles (Fig. 10) or an undefined granular substance in the cytosol (Fig. 12).

There was increased collagen in the extracellular space, especially in the 175- and 367-day animals with infiltration of mononuclear cells (Fig. 14), some of which were in close relation with myocytes (Fig. 15).

Discussion

The present study provides the first direct evidence that the long term structural integrity of the atrial myocardial cell is dependent upon an intact vagal innervation. As an extension of our earlier observations (Wong et al., 1987), the present results also suggest that the ultrastructural changes in the chronically vagotomised myocardium are progressive and irreversible. These changes may be attributed to the bilateral vagotomy as none were observed in the sham operated or unoperated control animals. Moreover, experimental and control animals were subjected to a similar procedure before and during perfusion.

The intranuclear inclusion of cytoplasmic organelles and inclusions observed in the present study compare with those described in a variety of normal, degenerating and diseased mammalian tissues (Leduc and Wilson, 1959; Brandes et al., 1965; Bloom, 1967; Sobel et al., 1969; De Lellis et al., 1980; Sanford et al., 1987), including human myocardial cells in coronary heart disease (Schaper and Schaper, 1986). Interestingly, the nuclei of smooth muscles in the cerebral artery wall also assumed polylobular forms after long-term sympathectomy (Dimitriadou et al., 1988).

Various explanations have been proposed to account for the phenomenon. They include undulation of the nuclear envelope and lobulation of the nucleus (Brandes et al., 1965; De Lellis et al., 1980), abnormal or unusual mechanisms during cell growth (Brandes et al., 1965; Bloom, 1967) and the maintenance of a constant ratio of nuclear surface to nuclear volume (Leduc and Wilson, 1959; Sanford et al., 1987). In the present study, the second alternative is not likely since such nuclear profiles were not encountered in the control animals; nor indeed were they in the short-term survival studies (Wong et al., 1987). The invagination of the nuclear membrane resulting in an increase of the nuclear surface may reflect functional demands, since in the atrophic myocytes there was degradation and degeneration of cytoplasmic organelles.

A lowered pH has been associated with ultrastructural changes in the myocardium, including aggregation of nuclear chromatin (Armiger et al., 1977). In the present results the changes observed in the nuclear chromatin could be a reflection of the accumulation of metabolites or breakdown products in the atrophied myocytes. Such changes may be indicative of cell injury.

In the experimentally-induced ischemic myocyte there is a rapid depletion of glycogen due to the acceleration of glycogenolysis and anaerobic glycolysis (see Reimer and Jennings, 1986). In contrast, in the chronically vagotomised myocyte there is increased glycogen, possibly due to decreased demand or accelerated synthesis. The deformation and disintegration of the mitochondria entrapped within the large glycogen deposits could be a mixed physical and chemical phenomenon. Another feature is the increased glycogen within the mitochondria. Intramitochondrial glycogen has been reported in normal rat retinal receptors (Ishikawa and Pei, 1965), frog spinal ganglia (Berthold, 1966) and dystrophic axons (Lampert, 1967) among others. An occasional glycogen granule has been observed by us in the normal myocyte. The abnormal accumulation may be due to a decreased demand and metabolism.

Tomanek and Cooper (1981) have demonstrated the role of stretch and/or tension in maintaining the structural integrity of the myocardial cell by severing the chordae tendineae of a ventricular papillary muscle. They described rapid and marked cellular degeneration in the myocytes. In the present study, the bilateral section of the vagus nerves would have interrupted not only their efferent but also their afferent components which are concerned with the cardiac reflexes (Higgins et al., 1973; Corr et al., 1986). Sectioning of the vagus nerves would remove the parasympathetic innervation to the atrium and abolish these reflexes. This could be a contributing factor to the changes in the myocytes which

included myofibrillar degeneration and extensive loss of contractile elements.

While bilateral vagotomy in the present study has been shown to be correlated with degenerative changes in the atrial myocardium, whether the operation also affected its functional integrity and to what degree is not known. Previous functional studies have emphassied the poor survival of animals after a bilateral cervical vagotomy (Sharpey-Schafer, 1920; MacCanon and Horvath, 1957; Schmitt and Meyers, 1957; Sloan et al., 1959; Shephard and Whitty, 1964). Consequently, it has been difficult to obtain a chronically parasympathectomised heart (Kaye, 1984). Some observations on the isolated cat papillary heart muscle one week after a bilateral cervical vagotomy (Vergara and Penna, 1984) suggest a possible modulatory role of the vagus nerve in the synthesis and/or release of the adrenergic neurotransmitter in the ventricular myocardium. The present successful survival of our animals following chronic bilateral cervical vagotomy, coupled with our earlier shorter term study (Wong et al., 1987) suggests that the monkey could serve as a suitable experimental model for functional studies of an extended timeframe of the parasympathetically denervated heart.

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References

Armiger L.C., Seelye R.N., Phil D., Elswijk J.G., Carnell V.M., Garvin J.B. and Herdson P.B. (1977). Fine structural changes in dog myocardium exposed to lowered pH *in vivo*. Lab. Invest. 37, 237-242.

Berthold C.-H. (1966). Ultrastructural appearance of glycogen in the B-neurons of the lumbar spinal ganglia of the frog. J. Ultrastruct. Res. 14, 254-267.

Bloom G.D. (1967). A nucleus with cytoplasmic features. J. Cell. Biol. 35, 266-267.

Brandes D., Schofield B. and Anton E. (1965). Nuclear mitochondria? Science 149, 1373-1374.

Carnale E.D., Campbell G.R., Smolich J.J. and Campbell J.N. (1986). Cardiac Muscle. Springer-Verlag. Berlin. Heidelberg. New York. Tokyo.

Chernukh A.M., Chernysheva G.V. and Kopteva L.A. (1974). Certain biochemical and ultrastructural features of the ventricular myocardium following cardiac denervation. Circ. Res. 34-35 (Suppl. III) 99-107.

Corr P.B., Yamada K.A. and Witkowski F.Y. (1986). Mechanisms controlling cardiac autonomic function and their relation to arrhythmogenesis. In: The Heart and Cardiovascular System, Scientific Foundations. Fozzard H.A., Haber E., Jennings R.B., Katz A.M. and Morgan H.E. (eds). Raven Press. New York. Vol. 2. pp 1343-1403.

DeLellis R.A., Suchow E. and Wilfe H.J. (1980). Ultrastructure of nuclear «inclusions» in pheochromocytoma and paraganglioma. Human Pathol. 11, 205-207.

Dimitriadou V., Aubineau P., Taxi J. and Seylaz J. (1988). Ultrastructural changes in the cerebral artery wall induced by long-term sympathetic denervation. Blood Vessels 25, 122-143.

Hayat M.A. (1981). Fixation for Electron Microscopy. Academic Press. New York.

Higgins C.B., Vatner S.F. and Braunwald E. (1973). Parasympathetic control of the heart. Pharmacol. Rev. 25, 119-155.

Imataka K., Takahashi H., Seki A. and Fujii J. (1982). Experimental production of papillary muscle and mitral valve lesions by vagal manipulations in rabbits. Jpn. Circ. J. 46, 362-368.

Ishikawa T. and Pei Y.F. (1965). Intramitochondrial glycogen particles in rat retinal receptor cells. J. Cell. Biol. 25, 402-407.

Kaye M.P. (1984). Dennervation and reinnervation of the heart. In: Nervous Control of Cardiovascular Function. Randall W.C. (ed). Oxford University Press. New York. Oxford. pp 278-306.

Lampert P.W. (1967). A comparative electron microscopic study of reactive, degenerating, regenerating and dystrophic axons. J. Neuropathol. Exp. Neurol. 26, 345-368.

Leduc E.H. and Wilson J.W. (1959). An electron microscopic study of intranuclear inclusions in mouse liver and hepatomas. J. Biophysic. Biochem. Cytol. 6, 427-430.

MacCanon D.M. and Horvath S.M. (1957). Effect of bilateral cervical vagotomy in the dog. Am. J. Physiol. 189, 569-572.

Reimer K.A. and Jennings R.B. (1986). Myocardial ischemia, hypoxia and infarction. In: The Heart and Cardiovascular System, Scientific foundations. Fozzard H.A., Haber E., Jennings R.B., Katz A.M. and Morgan H.E., (eds). Raven Press. New York. Vol. 2. pp 1133-1201.

Sanford S.E., Miller R.B. and Hook D.M. (1987). A light and electron microscopical study of intranuclear cytoplasmic invaginations in interstitial cell tumours of dogs. J. Comp. Pathol. 97, 629-636.

Schaper J. and Schaper W. (1986). Morphological changes in myocardium from patients with coronary heart disease and cardiac hypertrophy. Adv. Cardiol. 34, 16-24.

Schmitt G.H. and Meyers F.H. (1957). Characterization of the acute pulmonary edema that follows vagal section in the guinea pig. Am. J. Physiol. 190, 89-92.

Sharpey-Schafer E. (1920). Experiments on the cervical vagus and sympathetic. Q.J. Exp. Physiol. 12, 231-301.

Shepard R.S. and Whitty A.J. (1964). Bilateral cervical vagotomy: a long-term study on the unanesthetized dog. Am. J. Physiol. 200, 265-269.

Sloan R.D., Webb W.R, Meena A.L. and Lee S.S. (1969). The cause of fatalities in bilateral vagotomies. Surg. Forum, 9, 427-429.

Sobel H.J., Schwarz R. and Marquet E. (1969). Nonviral nuclear inclusions. 1. Cytoplasmic invaginations. Arch. Pathol. 87, 179-192.

Sommer J.R. and Jennings R.B. (1986). Ultrasctructure of cardiac muscle. In: The Heart and Cardiovascular System, Scientific Foundations. Fozzard H.A., Haber E., Jennings R.B., Katz A.M. and Morgan H.E. (eds). Raven Press. New York. Vol 1. pp 61-100.

Tchervova I.A. (1960). On structural and functional alterations in heart caused by section of vagal nerves. Arch. Anat. 13-22. (In Russian).

Tomanek R.J. and Cooper G. (1981). Morphological changes in the mechanically unloaded myocardial cell. Anat. Rec. 200, 271-800.

Vergara V. and Penn M. (1984). Influence of chronic vagotomy on inotropic responses in isolated cat heart papillary muscle. Res. Commun. Chem. Pathol. Pharmacol. 46, 93-102.

Wong W.C., Yick T.Y. and Ling E.A. (1987). Effects of vagotomy on the ultrastructure of the atrial myocardium in the monkey *(Macaca fascicularis).* Anat. Embryol. 177, 147-152.

Wong W.C., Yick T.Y. and Ling E.A. (1988). Ultrastructure of chronically vagotomised monkey atrial myocardium. Anat. Rec. 200, 106 (Abstract).

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