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Ultrastructural changes in atrial myocardium of the ageing rat

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INTRODUCTION

Although age-related changes in the contractile performance and other functional properties of heart muscle have been demonstrated (Heller & Whitehorn, 1972; Cavoto, Kelliher & Roberts, 1974; Goldberg, Baskin & Roberts, 1975; Goldberg, 1978), morphological studies have not established plausible structural correlation for such changes. Most ultrastructural studies on ageing mammalian myocardium have concentrated on the accumulation of dense bodies, including lipofuscin granules, and have discussed the possible origins of such structures (Glees, Hasan & Spoerri, 1974; Koobs, Schultz & Jutzy, 1978; Tomanek & Karlsson, 1973; Travis & Travis, 1972); in particular, the proposition that mitochondria or lysosomes may be converted into dense residual bodies has been examined. Sachs, Colgan & Lazarus (1977), in their investigation of the hamster heart, have gone further into the problem by considering changes in organelles such as the sarcoplasmic reticulum which they found was disproportionately reduced in ageing myocardial cells. However there is little or no comment in the literature on possible changes in the structure and organisation of myofilaments.

The present study on atrial muscle, taken from rats ranging from 2 months to 32 months in age, reveals that a wide variety of structural changes may occur in ageing myocardial cells. These include not only alterations in mitochondria and accumulation of dense bodies but also the appearance of unfamiliar arrays of strands alongside normally-aligned myofilaments.

MATERIALS AND METHODS

The animals used in this study were male albino rats drawn from an outbred Sprague–Dawley derived strain maintained as an ageing rat colony at the Charles River Breeding Laboratories (Wilmington, Massachusetts, U.S.A.); the 50% mortality point in the colony occurs at about 25 months and relatively few animals survive beyond 32 months. Colony animals beyond the age of 12 months were retired breeders and all animals were fed Purina laboratory chow and water *ad libitum*. The animals were perfused at the Boston University School of Medicine immediately upon their arrival from the supplier but those which exhibited grossly detectable pathology, such as respiratory infection, abscesses, tumours or paralysis, were excluded from the study.

Sixteen rats at the following ages were studied: 2–3 months (3 rats), 6 months

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(3 rats), 18 months (2 rats), 26 months (3 rats) and 31–32 months (5 rats). Although body weights did vary slightly within the age groups, they generally increased steadily with age and ranged from about 250 gm for the youngest animals to over 900 gm for the oldest animals.

The animals were anaesthetised with chloral hydrate injected intraperitoneally, artificially respired with a mixture of 95 % oxygen and 5 % carbon dioxide, and perfused through the left ventricle with a two-stage cacodylate buffered glutaraldehyde–paraformaldehyde fixative in accordance with the procedures published by Peters & Walsh (1972). Drainage of the perfusate was allowed by opening the margin of the right auricular appendage. Pre- or post-fixative buffer rinses were not used. Tissue blocks from both left and right atria, excluding the nodal regions, were removed on the day following perfusion and stored in cold fixative. Following osmication and dehydration, blocks were embedded in Araldite; ultrathin sections were stained with uranyl acetate and lead citrate and viewed under an AEI 6B electron microscope.

OBSERVATIONS

A wide range of abnormal morphological features appeared in the ageing myocardial cells examined in this study. Examples included increase in nucleolar material, degenerative foci in the cytoplasm, myofilament alterations, mitochondrial changes and the appearance of a variety of inclusions not characteristic of young adult animals. In the present report, however, we wish to confine our observations to the following three main features: (i) the appearance of helically aggregated strands alongside normal myofilaments; (ii) accumulation of dense bodies; (iii) changes in mitochondria.

Helically aggregated strands

Myocytes displaying abnormal filamentous arrays were encountered consistently in all the animals except those in the youngest age group (2–3 months). Typically the abnormal arrays lay in large clusters within the sarcoplasm (Fig. 1), occupying space that in other cells would be occupied by normal myofilaments, and often extended across the full thickness of the cell. In addition there were smaller foci of helically aggregated strands, situated between fascicles of normal myofilaments (Fig. 1), which were easy to overlook unless one specifically sought them. In the present study, careful examination of thin sections indicated that myocytes with helically arranged strands increased in frequency with age and in animals over 18 months examples could be found on every grid.

Each strand was composed of what appeared to be two or more elements or subunits wound round each other in helical fashion (Figs. 2, 3). Where the orientation

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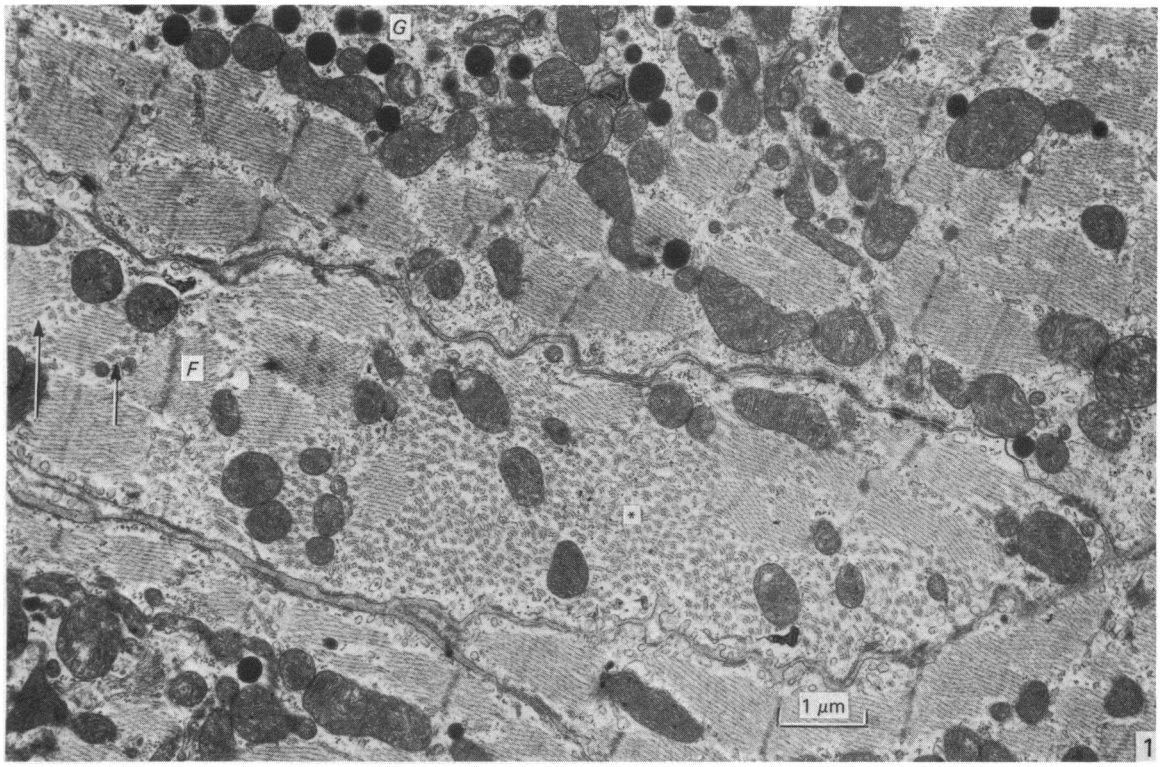
Fig. 1. Right atrial myocardium from a 26 months old rat showing helically aggregated strands. The strands occur both in the form of large masses (asterisk) and smaller foci (arrows) between fascicles of unaltered myofilaments (*F*). Near the top of the picture there are numerous specific atrial granules (*G*) among the mitochondria.

Fig. 2. Right atrial myocardial cell from a 31 months old rat showing helically aggregated strands. In favourable planes of section, the strands exhibit transversely oriented crossbridges (arrows) between the spiralling filaments. *F* indicates some myofilaments in normal configuration.

Fig. 3. Right atrial myocardial cell from a 18 months old rat. In longitudinal section, the strand composition varies from relatively simple twining of elements to more complex arrangements (double arrow). Crossbridges can also be seen in the region indicated by the double arrow. *Ob* indicates the crescentic appearance of strands viewed in oblique section.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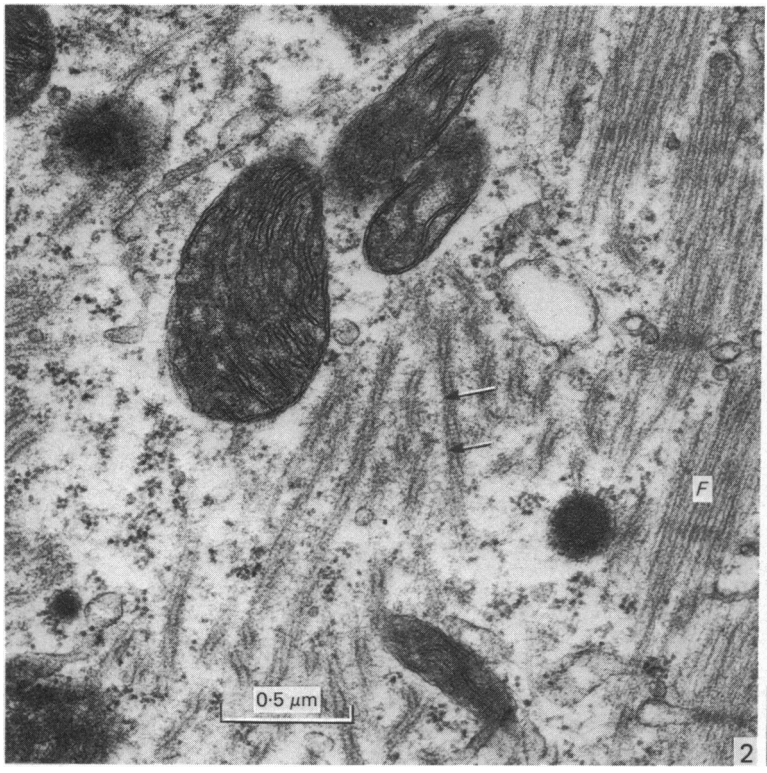
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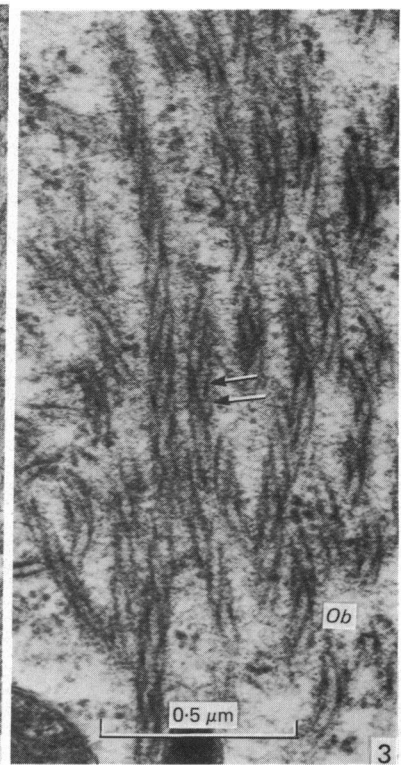
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3

of the strand was parallel to the plane of section (as in Fig. 3) a strand could be resolved into two elements at some points along its course but more than two elements at other points. Preliminary measurements indicated that each element was about 12 nm thick. In most, but not all, instances of helically aggregated strands cut longitudinally there was a fine transverse striation between the elements (Fig. 2) giving the appearance of crossbridges; the distance between successive striations was 15–20 nm. In oblique section (Fig. 3) typically short, crescentic profiles were observed. While sometimes such profiles appeared to be composed of only two filamentous elements (Figs. 5, 7), multiple stranding was more commonly suggested (Figs. 3, 4, 6).

Figure 4 shows some of these strands in cross section and it reveals quadratic lattices which contain several filaments (usually ranging between 6 and 16) interlinked by crossbridges. In several instances, apparent transitions between normal fascicles of myofilaments and the helically aggregated strands were observed. When such a transitional zone was viewed in transverse section (Fig. 5) it gave the impression that individual thick myofilaments separated from a normal fascicle to lie in rosette-like groups which frequently had 5 filaments. These aggregates then appeared to undergo transition to the quadratic lattice form. Evidence of similar transition was seen in the longitudinal plane (Figs. 6, 7) where it appeared that thick myofilaments became rearranged from their normal pattern to form helically aggregated strands.

As indicated earlier, preliminary measurements indicated that the filamentous elements in helically aggregated strands were approximately 12 nm in thickness. The centre-to-centre separation in a typical quadratic lattice was 25–30 nm (for comparison, the thick myofilaments at A band level in a normal myofibril have a thickness of up to 15 nm; they are hexagonally arranged and their centre-to-centre separation is about 35–45 nm).

Dense bodies

Dense bodies (Figs. 8, 9) were observed in all animals examined in this study but their frequency clearly increased with age. In the youngest animals (2–3 months) it was usual to find only one or two dense bodies in low magnification fields which included portions of up to 20 muscle cells. In old animals, on the other hand, as

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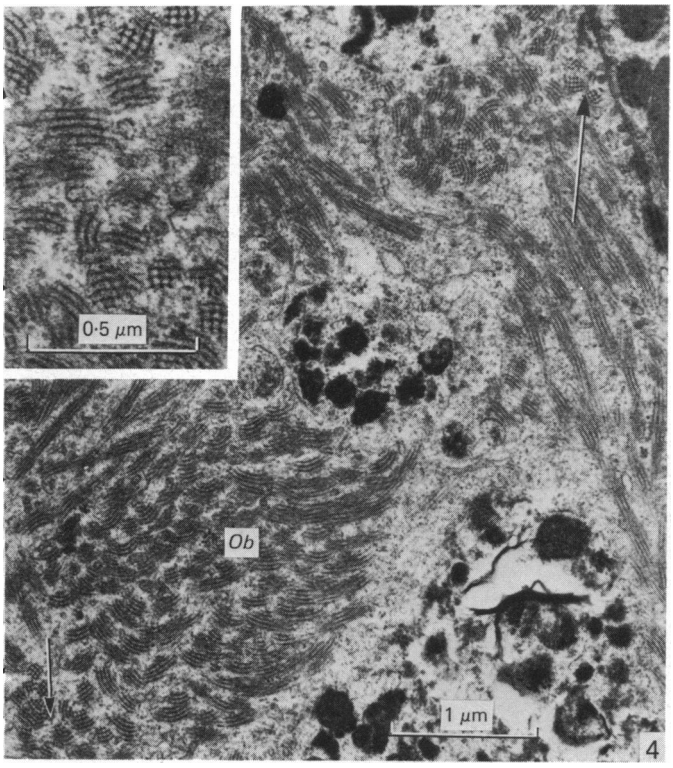
Fig. 4. Left atrial myocardium from a 6 months old rat. The myocyte illustrated contains large numbers of helically aggregated strands and very few normal myofilaments and in addition it contains masses of degenerating material. The arrows indicate the lattice-like arrangement seen when strands are cut transversely. *Ob* indicates obliquely cut strands. Inset: Enlargement of the transversely sectioned strands indicated by the arrow in the upper right portion of the picture.

Fig. 5. Right atrial myocardium from a 26 months old rat showing helically aggregated strands. The arrow indicates the rosette appearance found in a position intermediate between unaltered filaments (*F*), which have been cut transversely, and obliquely sectioned helical strands on the left.

Fig. 6. Left atrial myocardial cell from a 6 months old rat showing helically aggregated strands. Some of these strands are in apparent continuity (arrow) with normally arranged myofilaments (*F*). The cell shown here also contains patches of atypically expanded Z-line material (Z_1), also seen in a neighbouring cell (Z_2). Z_3 indicates the normal appearance of a Z-line.

Fig. 7. Right atrial myocardium from a 26 months old rat showing helically aggregated strands. The arrow indicates apparent continuity between a fascicle of unaltered myofilaments (*F*) and an array of helical strands one of which, to the left of the asterisk, is sectioned longitudinally.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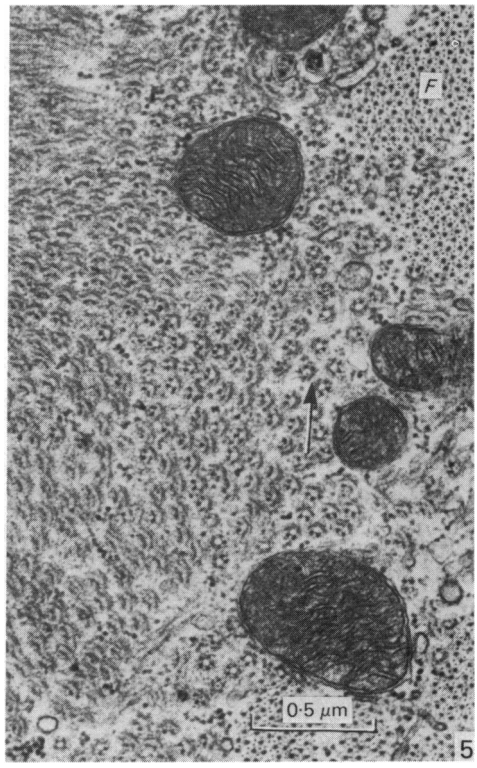
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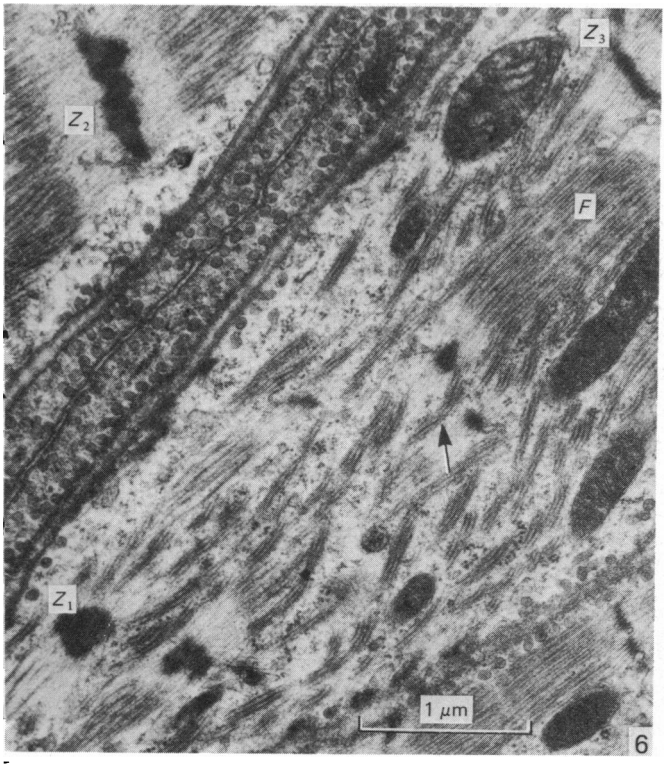
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Z2
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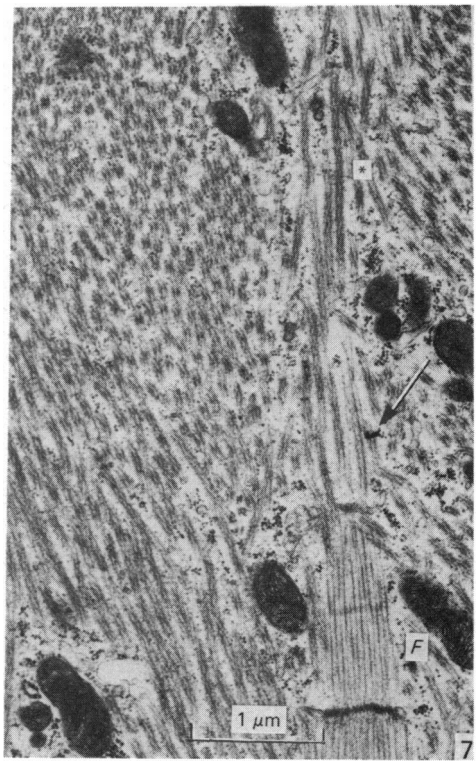
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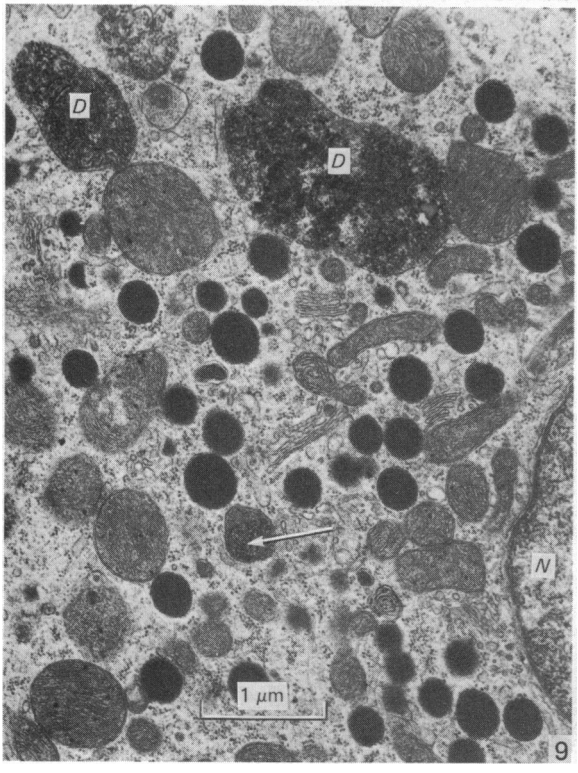

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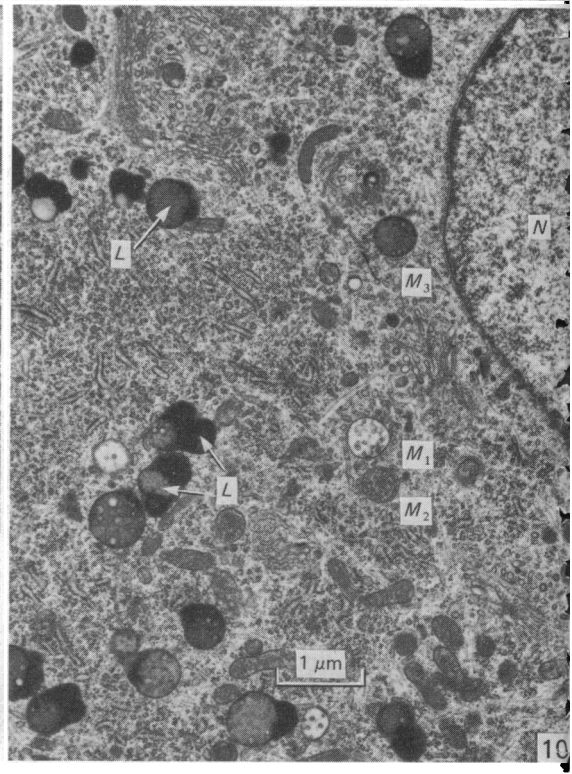

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many as 15 dense bodies could be observed in the sarcoplasmic cone related to one nuclear pole of a single muscle cell. Moreover, there was a distinct increase in size of individual dense bodies with advancing age. In the young animals dense bodies did not exceed average mitochondrial size (Fig. 8) but with increasing age they enlarged appreciably so that by 32 months of age they might be several times the size of the largest mitochondria (Fig. 9).

There were two preferential sites at which dense bodies were found. The first of these was along the columns of mitochondria which lay between myofibrillae (Fig. 8). The second preferential site was within the sarcoplasmic cone at each nuclear pole where the dense bodies were interspersed among mitochondria and specific atrial granules (Fig. 9); in many instances, when the two sarcoplasmic cones related to the same nucleus were examined, it was found that dense bodies were preferentially aggregated in one or other cone. In addition we had the distinct impression that the frequency of dense bodies was greater in cells exhibiting helically aggregated strands than in comparable sarcoplasmic areas associated with normal filaments.

In young animals, dense body profiles were round or oval and had smooth contours. With advancing age, however, irregularities of shape became progressively more frequent. The increases in size and irregularity of dense bodies in older animals suggested that dense body growth occurred by coalescence or accretion. All dense bodies were membrane-bound and, in high resolution images, the membrane was seen to be double, resembling that of a mitochondrion. Although an interior substructure was difficult to discern, the matrix of each dense body was of medium electron density superimposed on which were variable amounts of amorphous high density material, often in the form of coarse granules of varying sizes (Figs. 8, 9). The relative proportions of medium density matrix and high density material varied among dense bodies. However the smallest bodies, which had a relatively smooth outline, possessed little high density material (Fig. 9). Such forms were characteristic of the infrequent dense bodies in young animals and might represent an early stage in dense body formation. In a large number of instances, a laminar component could be discerned within the matrix of dense bodies (Fig. 9). At high magnification these components often appeared pentalaminar with dark margins and a lighter streak in which a central linear density was situated. In a few instances, dense bodies contained inclusions which resembled degenerated mitochondria (Fig. 13).

The majority of dense bodies observed in this study did not possess the large globular components characteristic of neuronal lipofuscin (Fig. 10) and, indeed, of lipofuscin generally found in human myocardial cells. For this reason we prefer

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Fig. 8. Right atrial myocardial cell from a 6 months old rat showing dense bodies (*D*) along the rows of mitochondria. The myofilaments appear normal.

Fig. 9. Right atrial myocardial cell from a 26 months old rat showing dense bodies (*D*) in the sarcoplasmic cone adjacent to the nucleus (*N*). The smaller dense body on the left shows a laminar substructure which is obscured in the larger dense body by highly electron-dense material. The profile indicated by the arrow may represent an earlier stage in dense body formation; a laminar substructure is present and the matrix density is similar to that of a mitochondrion.

Fig. 10. Lipofuscin granules (*L*) within a neuronal perikaryon on the left atrial wall of a 31 months old rat. Note the large vacuolar component of neuronal lipofuscin which is not a feature of myocyte dense bodies. The vacuolar component may be derived from multivesicular bodies such as M_1 in a sequence illustrated by $M_1 \rightarrow M_2 \rightarrow M_3$. *N*, nucleus.

to retain the term dense bodies. Occasional dense bodies in the oldest animals examined (31–32 m) might contain small globular inclusions (Fig. 13) but they never attained the relative size of those in neuronal or in human myocardial lipofuscin. Also they showed no morphological evidence of the relationship to multivesicular bodies that frequently exists in neurons (Fig. 10).

Mitochondrial changes

Several types of mitochondrial abnormalities have been observed in the present material. These included hypertrophy of mitochondria coupled with apparent disruption of the cristae or even dissolution, where the interior of the organelle consisted of homogeneous matrix. These changes occurred in individual scattered mitochondria, neighbouring organelles usually being unaffected. Although such disruption was more prevalent in older animals, it could be occasionally seen in animals of the 2–3 months old group.

A second type of abnormality, considerably more common, seemed to be confined to older animals, particularly to those over 2 years old. It consisted of ensheathment of a mitochondrion by lamellae closely applied to the mitochondrial surface (Figs. 11, 12). It has not proved possible to determine the source of the lamellae, or to trace individual lamellae to determine whether they were continuous or discrete. In some instances, however, the peripheral lamellae appeared to be closely related to cisternae of sarcoplasmic reticulum (Fig. 11). In some preparations ensheathed mitochondria appeared in the same region of a muscle cell as helically aggregated strands but the majority of organelles in these regions appeared to be normal in structure.

The mitochondria which were ensheathed might display a variety of intrinsic structural abnormalities (Fig. 12), particularly disruption or dissolution of cristae. In addition, what appeared to be remnants of sheaths, containing degenerated material, were occasionally encountered along mitochondrial rows between myofibrillae (Fig. 12). These observations suggested that ensheathment followed by mitochondrial degeneration is a possible sequence in age-related loss of mitochondria. Other sequences are also possible, such as mitochondrial hypertrophy mentioned earlier, or the incorporation or transformation of mitochondria into dense bodies (Fig. 13).

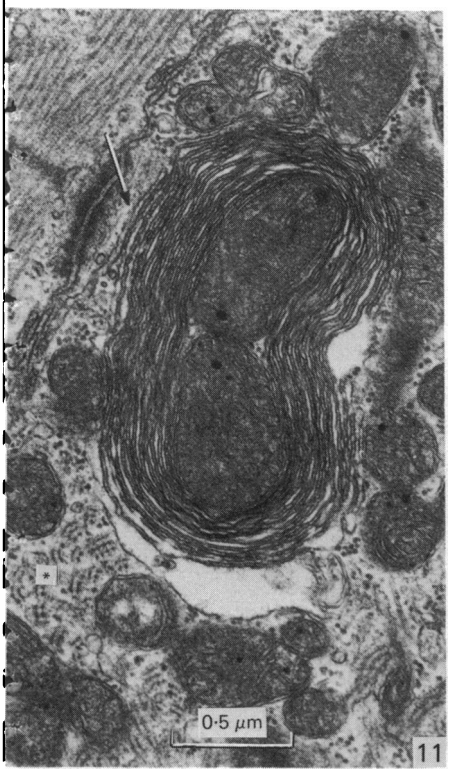
A further observation seen only in old animals comprised the accumulation of large numbers of small mitochondria (Fig. 14) in sarcoplasmic tracts between myofibrillae. When examined under high magnification, many of these mitochondria exhibited abnormalities such as cristae disruption or matrix vacuolisation.

Fig. 11. Two mitochondrial profiles ensheathed by lamellae of unknown origin in a 26 months old rat. The periphery of the sheath is closely apposed to a cistern of sarcoplasmic reticulum (see arrow). The matrix density of the affected mitochondria appears normal. Note the helically aggregated strands cut in transverse section near the asterisk.

Fig. 12. Ensheathed mitochondrial profiles in a 26 months old rat. In the example near the bottom of the figure, there is disruption of cristae and the density of mitochondrial matrix is reduced. In the two examples near the top of the picture, remnants of a lamellar sheath are evident but the mitochondrial content is lacking.

Fig. 13. Dense bodies in a 31 months old rat. The upper dense body contains a degenerating mitochondrion while the lower body contains a small vacuole-like inclusion which is very rarely seen in rat myocytes and then only in the oldest animals.

Fig. 14. Accumulations of mitochondria in an atrial myocyte from a 26 months old rat.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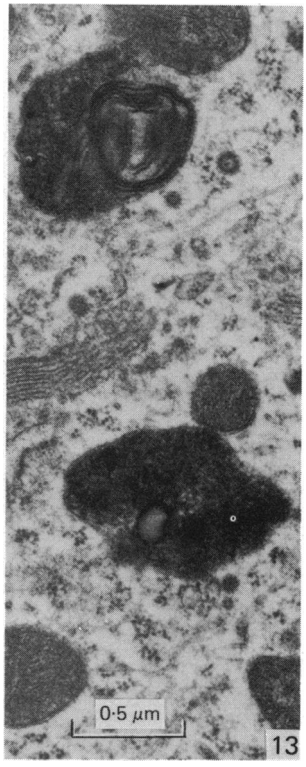
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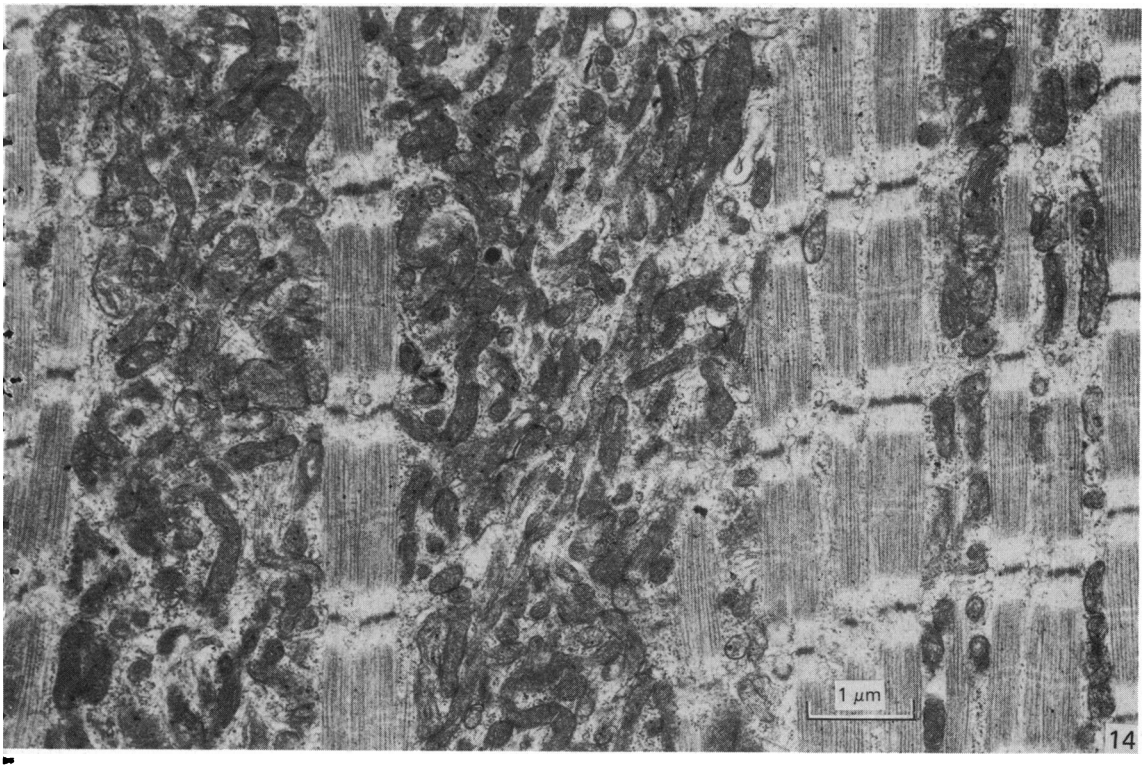
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DISCUSSION

The cellular changes encountered in this study are variable in their time course and there is no single stage at which the ageing process of atrial myocytes can be said to begin. Dense body accumulation starts early, some being present in the youngest animals examined, and continues throughout life. On the other hand, mitochondrial ensheathment is not observed till senescence while other changes, such as the appearance of helically aggregated strands, commence at intermediate stages.

The helically aggregated strands described in this communication seem to represent an alteration of thick filament arrangement. In many instances there is apparent continuity or transition between normally arranged thick filaments and the elements of the helical strands; indications of such transition can be observed both among filaments cut longitudinally and in those cut transversely. It is also worth noting that the crossbridges between the strand filaments display a 15–20 nm spacing as compared to a spacing of 14 nm reported by Huxley & Brown (1967) for crossbridges in skeletal muscle. The significance of helically aggregated strands is not known and it is not clear whether they represent the disruption of myofilaments which had previously been arranged in normal fashion or whether they arise *de novo* with advancing age. It does seem reasonable, however, to assume that their presence represents an impediment to the normal contractile behaviour of muscle cells; a normal mode of contraction would seem unlikely in view of their intertwining nature and the absence of thin filaments.

The progressive accumulation of dense bodies has been previously described in ventricular myocardial cells from rat (Travis & Travis, 1972; Tomanek & Karlsson, 1973) and hamster (Sachs *et al.* 1977). Travis & Travis, in particular, have drawn attention to similarities between these structures and mitochondria regarding their size and shape and the thickness of the double limiting membrane; they have concluded that the dense bodies originate from the degradation of mitochondria. Several later studies on lipofuscin have reached similar conclusions (Glees *et al.* 1974; Koobs *et al.* 1978), although the mitochondria–lipofuscin sequence was denied by Tomanek & Karlsson (1973). The present observations favour the interpretation of Travis & Travis for reasons including the almost invariable occurrence of dense bodies among mitochondria, the similarity of matrix density in the two structures and the not infrequently observed morphological association between them (Fig. 13).

The presumptive degradation of mitochondria into dense bodies is only one among several ways to account for the age-related deficits reported in mitochondrial number (Herbener, 1976) and function (Abu-Erreisch & Sanadi, 1978), assuming that these occur in atrial as well as in ventricular muscle. Other mitochondrial changes observed in the present study include hypertrophy with alteration of cristae, consistent with alterations observed in mouse myocardium by Tate & Herbener (1976), and lamellar ensheathment. The latter change was also encountered in the rat left ventricle by Travis & Travis (1972), who considered the lamellae to be derived from autophagic vacuoles (secondary lysosomes). The proximity of sarcoplasmic reticulum to the periphery of the sheath in some instances suggests that the reticulum may be involved in the synthesis of the sheath membrane.

SUMMARY

Ageing changes in the fine structure of atrial myocardial cells were studied in rats ranging from 2–32 months in age. The most striking change observed was the increasingly frequent appearance, from about 6 months onwards, of helically aggregated strands containing filaments which in respects other than arrangement bore resemblance to thick myofilaments. Other ageing changes included the accumulation of dense bodies and various types of mitochondrial degradation.

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