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Age-related changes of aorta in Syrian hamsters of APA strain

J. Yamanouchi[1], Y. Sugawara[2], S. Itagaki[1] and K. Doi[1]

[1]Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Bunkyo-ku, Tokyo and

[2]Toxicology Laboratory, Basic Research Laboratories, Toray Industries, Inc., Sonoyama, Otsu-shi, Shiga, Japan

Summary. Age related changes in thoracic aorta (TA) and abdominal aorta (AA) of male APA hamsters from 3 to 12 months of age were examined morphometrically and ultrastructurally. The nuclear density of smooth muscle cells (SMCs) was larger in AA than in TA, and it decreased with advancing age. In contrast, the collagen fibre density was larger in TA than in AA, and it increased correlatively with aging, especially in TA. Electron microscopic examinations revealed that subendothelial cystic spaces and aggregations of fragments of elastic and collagen fibres were found at 3 months of age and progressed with advancing age in TA, while they were not evident in AA even at 12 months of age. Irregularity of medial SMC contours and an amount of SMC-associated collagen fibres were more prominent in TA than in AA throughout the experimental period. Degenerative changes of endothelial cells and medial SMCs progressed with aging in both TA and AA, and degenerated SMCs were characterized by aggregations of swollen mitochondria.

Key words: Aging, Aorta, APA hamster, Morphometry, Ultrastructure

Introduction

Syrian hamsters of APA strain (APA hamsters) have been developed in Japan (Tajima, 1968) and maintained as a closed colony by random breeding in our laboratory since 1988. APA hamsters are known to develop spontaneous mesangial thickening in the renal glomeruli from a young age (Han et al., 1992a). On and after 6 months of age, they also develop focal and segmental glomerulosclerosis (FSG) (Doi et al., 1987), of which ultrastructural progressions had been reported by Yamanouchi et al. (1994).

As a second step of a series of pathological surveys of age-related changes in APA hamsters carried out in our laboratory, this paper describes the morphometrical and ultrastructural progressions of spontaneous changes in thoracic and abdominal aorta of male APA hamsters from 3 to 12 months of age.

Offprint requests to: Dr. J. Yamanouchi, Department of Biomedical Science, Faculty of Agriculture, The University of Tokyo, Yayoi 1-1-1, Bunkyo-ku, Tokyo 113, Japan

Materials and methods

Fifteen male APA hamsters obtained from our breeding colony were used. They were maintained under controlled conditions (temperature, 24±1 °C; humidity, 55±5%) in plastic cages with sterilized wood shavings for bedding, and fed a commercial diet, CMF (Oriental Yeast Co. Ltd., Tokyo) and tap water *ad libitum*.

Five animals each were weighed and killed by exanguination under ether anaesthesia at 3, 6 and 12 months of age, respectively. Proximal thoracic aorta (TA) and abdominal aorta (AA), just below the bifurcation of the left renal artery, were taken from each animal and fixed in 10% neutral buffered formalin. Paraffin sections (4 μm) were made and stained with haematoxylin and eosin (HE). In addition, they were also stained with resorcinol and fuchsin (RF) for elastic fibres, and picrofuchsin (PF) for collagen fibres.

Morphometrical analysis was performed on these sections using an image-analyzer SP500 (Olympus Industry Co. Ltd., Tokyo). Namely, the thickness of aortic wall (intima+media) and the nuclear density of smooth muscle cells (SMCs) (number/wall) were measured on HE-stained sections, the density (%) of elastic fibres (area/wall) on PF-stained ones. These parameters were calculated on the whole area of one coronary aortic section of each animal and values were expressed as mean±SD of 5 animals of each age. Statistical analysis was performed using Student's t-test.

For electron microscopic examination, small pieces of TA and AA were fixed in 2.5% glutaraldehyde and 2.0% paraformaldehyde in 0.1M phosphate buffer (pH 7.4), postfixed in 1.0% osmium tetroxide in the same buffer, and embedded in epoxy resin, Quetol 812 (Nissin EM Co. Ltd., Tokyo). Ultrathin sections were double-stained with uranyl acetate and lead citrate.

Results

Body weight

Body weights increased correlatively with aging (Fig. 1).

Light microscopic and morphometric findings

At 3 months of age, a partial discontinuity of the internal elastic lamina (IEL) was sometimes observed in both TA and AA. In addition, slight vacuolization was found in the cytoplasm of a few medial SMCs. On and after 6 months of age, partial disintegration of elastic lamella (Fig. 2) was sometimes observed. At 12 months of age, very mild calcification in the medial interstice was sometimes observed.

The wall thickness of TA was significantly larger than that of AA at any age of animals examined (Fig. 3). The former reached the plateau at 6 months of age, while

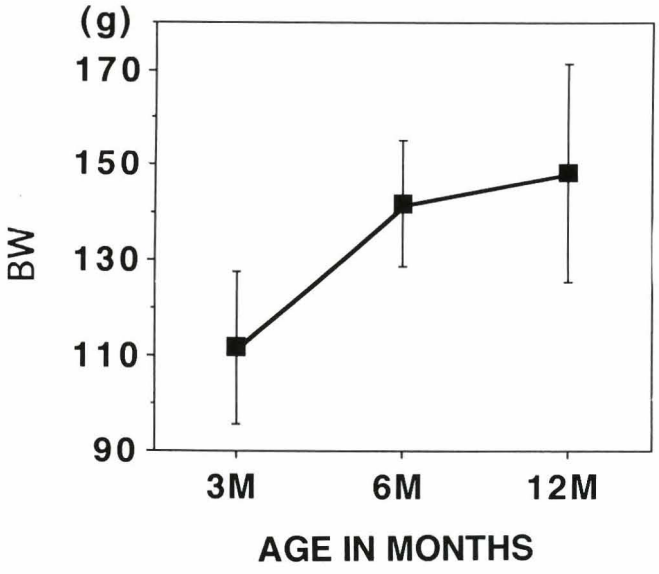

Fig. 1. Changes in body weight of APA hamsters.

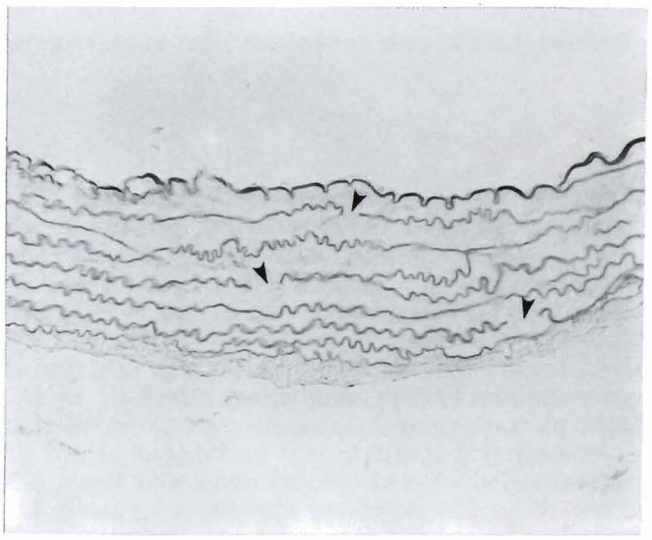

Fig. 2. Thoracic aorta of an APA hamster at 12 months of age. Partial disintegration of elastic lamella (arrowheads) is observed. RF. x 690

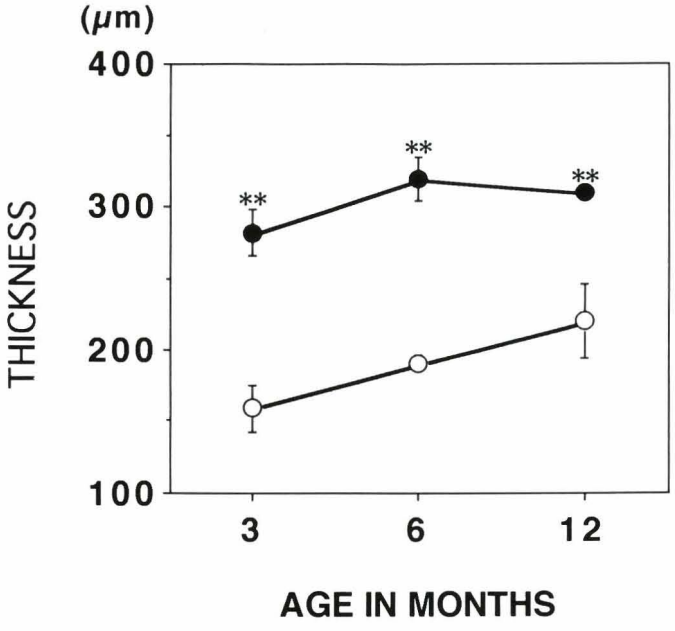

Fig. 3. Changes in aortic wall thickness of APA hamsters. Black circles: thoracic aorta (TA); white circles: abdominal aorta (AA); **: significantly different from AA ($p<0.01$).

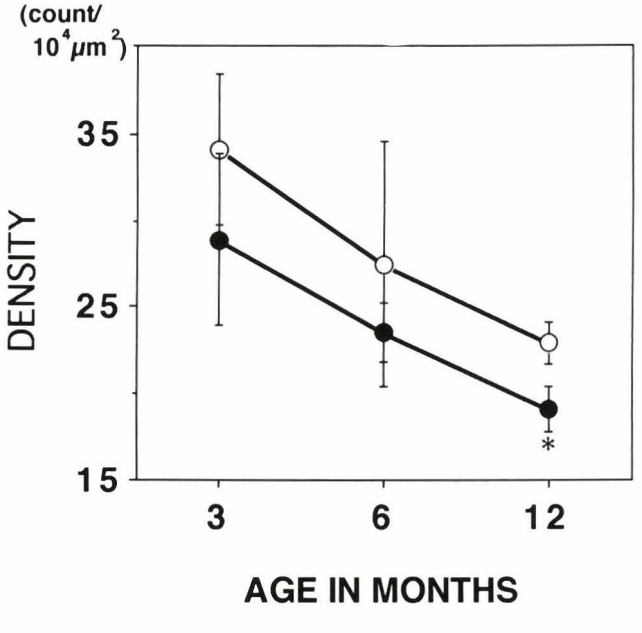

Fig. 4. Changes in the nuclear density of smooth muscle cells in the aorta of APA hamsters. Black circles: TA; white circles: AA; *: significantly different from AA ($p<0.05$).

the latter showed a tendency to increase up to 12 months of age.

On the other hand, the nuclear density of SMCs was always larger in AA than in TA, and it showed a tendency to decrease correlatively with aging in both TA and AA (Fig. 4).

The elastic fibre density showed no significant age-dependent changes either in TA or AA (Fig. 5). The collagen-fibre density of TA showed a tendency to increase correlatively with aging, while that of AA reached its peak at 6 months of age (Fig. 6). At 12 months of age, it was significantly larger in TA than in AA.

Electron microscopic findings

In the intima of TA at 3 months of age, cystic spaces and small aggregations of fragments of elastic and

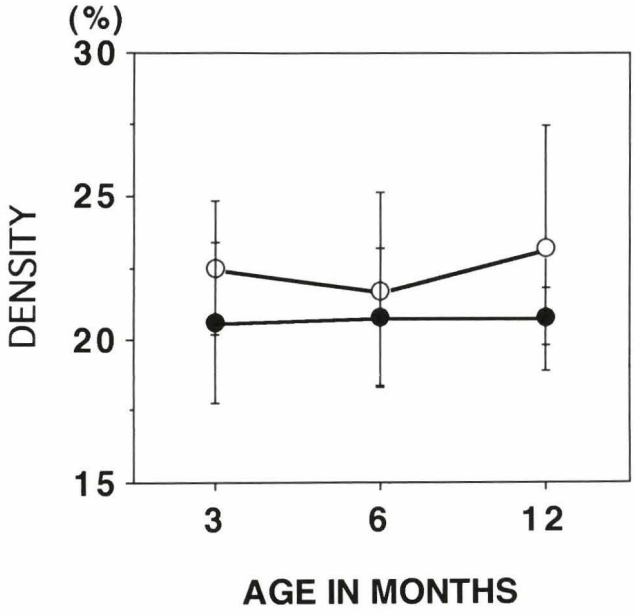

Fig. 5. Changes in the elastic fibre density in the aorta of APA hamsters. Black circles: TA; white circles: AA.

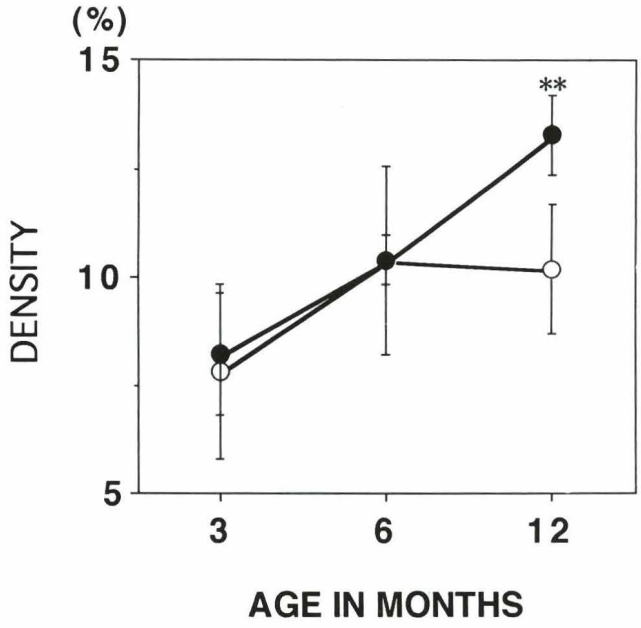

Fig. 6. Changes in the collagen fibre density in the aorta of APA hamsters. Black circles: TA; white circles: AA. **: significantly different from AA ($p<0.01$).

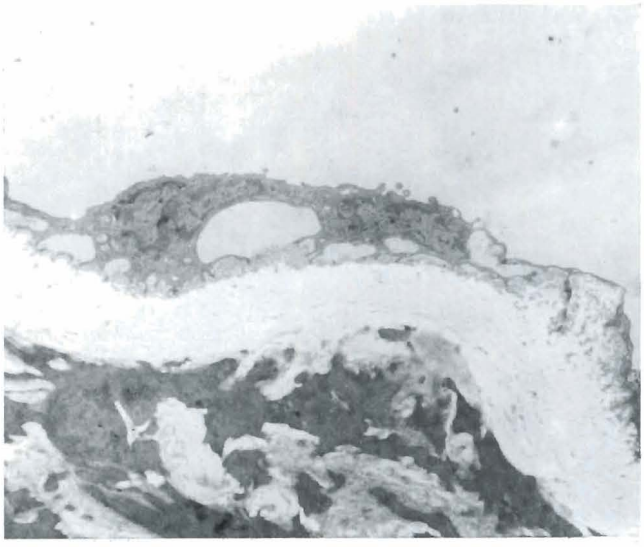

Fig. 7. Thoracic aorta of an APA hamster at 3 months of age. Cystic spaces and small aggregations of fragments of elastic and collagen fibres in the subendothelium. Collagen fibres around smooth muscle cells with irregular cytoplasmic projections in the media. x 3,700

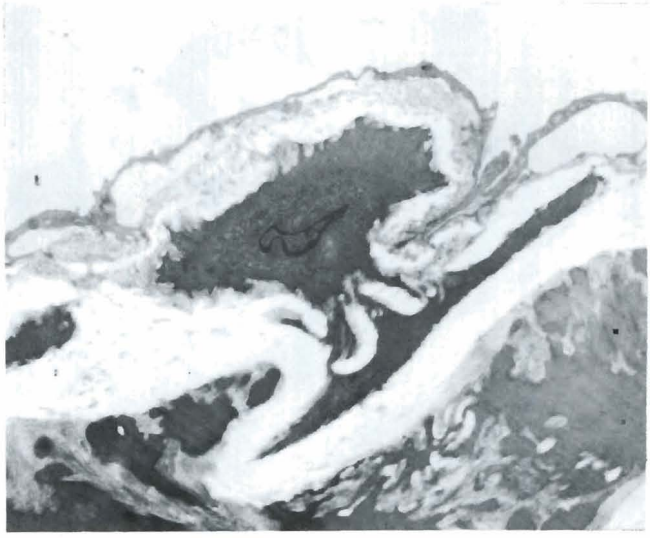

Fig. 8. Thoracic aorta of an APA hamster at 3 months of age. Split internal elastic lamina with an invasion of a smooth muscle cell. x 3,700

collagen fibres were frequently observed in the subendothelium (Fig. 7). The endothelial cells (ECs) over these aggregations were usually flat. In addition, ECs sometimes contained slightly swollen mitochondria. Thickened IEL often became split accompanied by invasion of SMCs with irregular cytoplasmic projections (Fig. 7), and an accumulation of swollen mitochondria were found in some SMCs.

On the other hand, in the intima of AA, endothelial cell changes such as mitochondrial swelling and dilation of endoplasmic reticula were more prominent when compared with those in TA (Fig. 9). Subendothelial cystic spaces and aggregations of fragments of elastic and collagen fibres were, however, not evident. In the media, SMCs were arranged vertical to the IEL (Fig. 9).

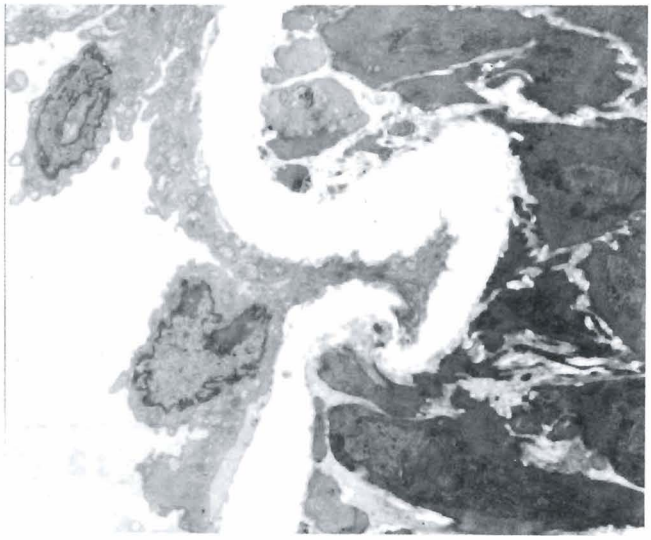

Fig. 9. Abdominal aorta of an APA hamster at 3 months of age. Swelling of mitochondria and dilation of endoplasmic reticula in endothelial cells. In the media, smooth muscle cells arrange vertically to the internal elastic lamina. x 3,700

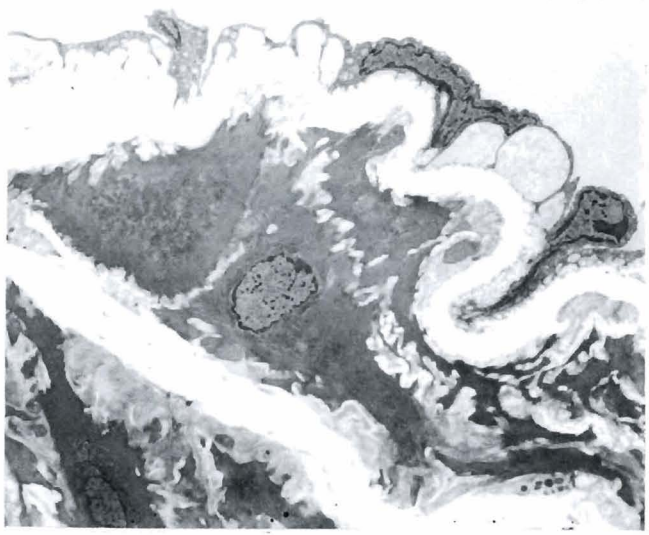

Fig. 10. Thoracic aorta of an APA hamster at 6 months of age. Prominent cystic spaces and aggregations of fragments of elastic and collagen fibres in the subendothelium. x 2,700

At 6 months of age, in both TA and AA, the above-mentioned changes observed at 3 months of age generally became more prominent (Fig. 10), but their severity varied between individuals. In addition, a large SMC with a syncytial cell-like appearance was sometimes found beneath the IEL in TA (Fig. 11).

In the intima of TA at 12 months of age, degeneration of ECs was frequently observed, and the intima was thickened with large amounts of fragments of elastic and collagen fibres (Fig. 12). In the media, between wavy elastic lamella, irregular-shaped SMCs sometimes showed degeneration with an aggregation of swollen mitochondria (Fig. 12).

In the intima of AA, except for subendothelial aggregations of fragments of elastic and collagen fibres, similar changes to those in TA were observed (Fig. 13). SMCs in the media showed degeneration characterized by a large aggregation of swollen mitochondria (Fig. 13). In addition, cellular debris was scattered in the widened intercellular spaces (Fig. 14).

Discussion

There are few reports on the details of age-related changes in the aorta of Syrian hamsters. In this study, we examined age-related changes in the aorta of APA hamsters morphometrically and ultrastructurally.

As generally said in other animal species, the wall thickness in APA hamsters was also larger in TA than in AA and its age-related increase was more obvious in AA than in TA. In the aortic wall of APA hamsters, as reported in rats (Toda, 1978), the collagen fibre density, which increased correlatively with aging, especially in TA, was larger in TA than in AA. In addition, the nuclear

Fig. 11. Thoracic aorta of an APA hamster at 6 months of age. A large smooth muscle cell with a syncytial cell-like appearance. x 2,000

Fig. 12. Thoracic aorta of an APA hamster at 12 months of age. The thickened intima with a large amount of fragments of elastic and collagen fibres. Endothelial cells and smooth muscle cells show degeneration. x 4,300

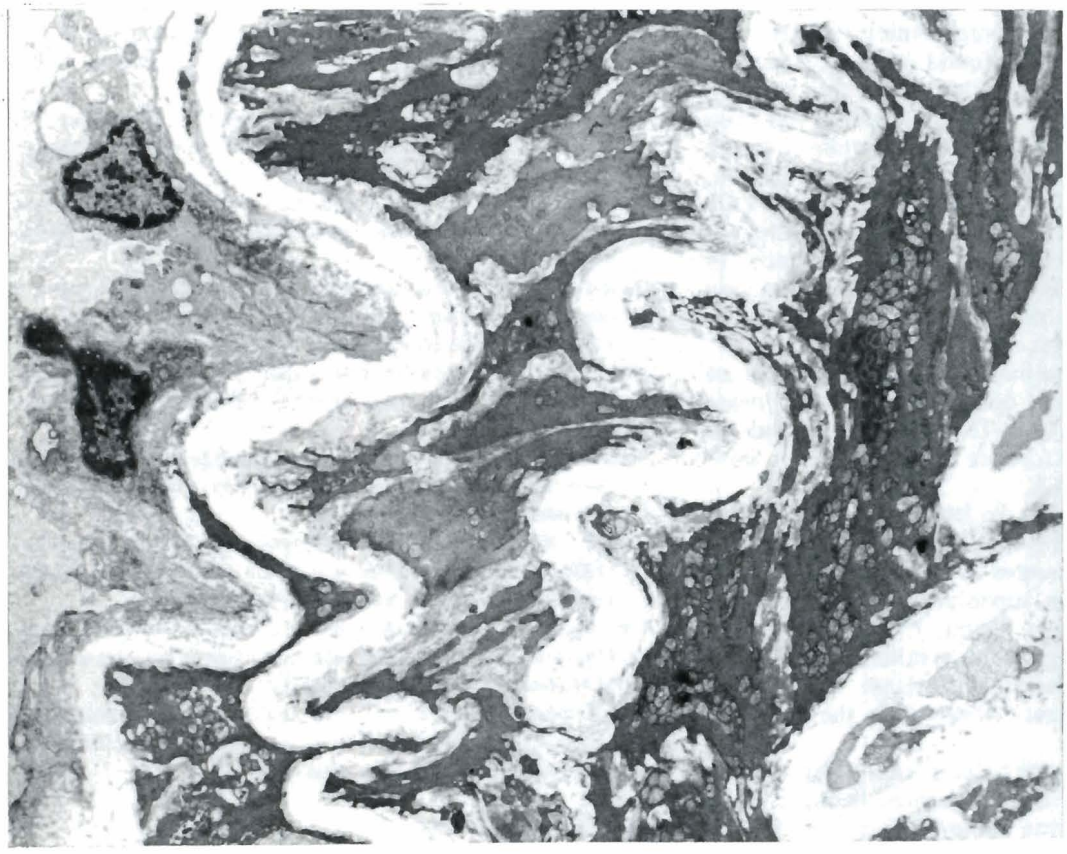

Fig. 13. Abdominal aorta of an APA hamster at 12 months of age. Degeneration of endothelial cells and smooth muscle cells. A large aggregation of swollen mitochondria is prominent in degenerated smooth muscle cells. x 4,300

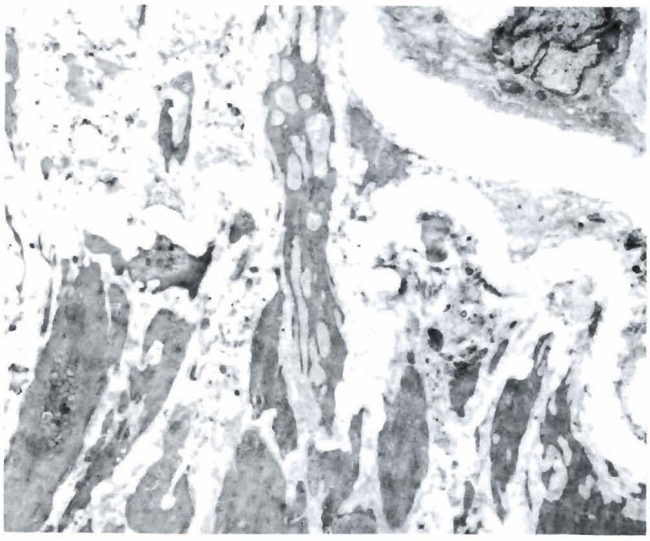

Fig. 14. Abdominal aorta of an APA hamster at 12 months of age. Cellular debris in the widened medial interstice. x 3,700

density of SMCs was larger in AA than in TA, though it decreased with advancing age in both TA and AA. From these morphometrical findings, the age-related increase in the aortic wall thickness in APA hamsters seems to largely depend on the increase in stromal materials, especially collagen fibres, as reported in human aorta (Toda et al., 1980).

Ultrastructural examinations revealed that subendothelial aggregations of fragments of elastic and collagen fibres were generally found in TA at 3 months of age and they progressed with advancing age. In contrast, such aggregations were not evident in AA even at 12 months of age. In addition, a formation of cystic spaces in the intima, indicating an intimal edema, probably due to an increase in permeability of ECs (Yasoshima, 1982), was more conspicuous in TA than in AA. One possible explanation for these differences in the intimal morphology between TA and AA is that they might reflect the fact that the changes in blood pressure are more frequent and larger in TA than in AA.

An irregularity of medial SMC contours and an amount of SMC-associated collagen fibres were more prominent in TA than in AA. This well corresponds to the above-mentioned difference in the collagen fibre density between TA and AA.

Degenerative changes in both ECs and SMCs progressed with advancing age, following no reactive changes. Degenerative changes of aortic SMCs are said to be characterized by an appearance of dense bodies and myelin figures in rats (Toda, 1978). They were characterized by an aggregation of swollen mitochondria in APA hamsters. The meaning of a large SMC with a syncytial cell-like appearance observed in the media of TA is obscure.

Recently, Syrian hamsters have been used for the induction of diet-induced atherosclerosis, because, as in humans, their major plasma cholesterol carrier is LDL (Nistor et al., 1987). However, it is also reported that it takes too many months to induce atherosclerotic changes in Syrian hamsters (Nistor et al., 1987). In this connection, APA hamsters are known to develop marked focal and segmental glomerulosclerosis with glomerular lipidosis in 3 months after a single injection of streptozotocin (40 mg/kg) (Han et al., 1992b), which had been suggested to be analogous to atherosclerosis by many researchers (Peric-Golia and Peric-Golia, 1983; Grond et al., 1986; Diamond and Karnovsky, 1988; Keane et al., 1988). This and the present results indicate a possibility that APA hamsters may develop atherosclerotic changes in a somewhat shorter period after streptozotocin-treatment.

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