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Electron microscopic observations of elastic fibres in the lung and aorta of tight-skin and beta-aminopropionitrile-fed mice

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Summary. The lung of the tight-skin (TSK) mouse was characterized by enlargement of the air spaces. Elastin in the alveolar walls of the TSK mouse exhibited fragmentation. The aorta of the TSK mouse was characterized by marked hyperplasia of loose connective tissue in the adventitia. Collagen fibres and ruthenium red-positive materials were markedly increased. Microfibrils surrounding elastin in the adventitia of the aorta were not clear in the TSK mouse.

In the lung of the beta-aminopropionitrile (BAPN)-fed mouse, enlargement of the alveolar air spaces was not prominent compared with the TSK mouse. Elastic fibres in the alveolar walls did not show the fragmentation observed in the TSK mouse, and microfibrils surrounding elastin were clearly observed. However, elastic laminae in the media of the BAPN-fed mouse aorta were swollen and fragmented. Elastic fibres in the adventitia exhibited a normal appearance and microfibrils surrounding elastin in the adventitia were clearly observed.

The results suggest that the mechanism of the connective tissue abnormality in the TSK mouse is different from that of BAPN, which inhibits the activity of lysyl oxidase. The abnormality of elastin and microfibrils surrounding elastin in the TSK mouse probably plays a role in the deformity or degradation of elastic fibres and the structural changes of the lung.

Key words: Elastic fibre, Lung, Aorta, Tight-skin mouse, Beta-aminopropionitrile

Introduction

The tight-skin (TSK) mouse is a dominant mutation in which heterozygote animals (TSK/+) have tight skins with marked hyperplasia of subcutaneous connective tissue, increased growth of cartilage and bone, small tendons with hyperplasia of tendon sheaths, increased thoracic size, and large distended lungs (Green et al., 1976). It was reasonable to hypothesize that the TSK mouse might be a hereditary model of scleroderma (Jiménez et al., 1984) and emphysema (Szapiel et al., 1981; Rossi et al., 1984). In the subcutaneous connective tissue of the TSK mouse there are large accumulations of microfibrils in the intercellular space (Green et al., 1976). Jiménez et al. (1984) reported that abnormalities in collagen metabolism similar to those occurring in the skin of patients with scleroderma have been demonstrated in the TSK mouse. On the other hand, degradation of elastin was studied in terms of the pathogenesis and development of pulmonary emphysema in the TSK mouse (Houya, 1986). He reported that the urinary desmosine excretion increased every week after birth (five to ten weeks). It was suggested that connective tissue metabolism including elastin might be abnormal in the TSK mouse.

It is well known that beta-aminopropionitrile (BAPN) causes serious abnormalities in the connective tissue. BAPN has proved very useful in studying the biosynthesis of elastin as well as that of collagen since it inhibits the copper-dependent enzyme lysyl oxidase, which catalyzes the oxidative deamination of lysine and hydroxylysine residues of the soluble precursors of elastin and collagen (Miller et al., 1965; O'Dell et al., 1966; Narayanan et al., 1972; Harris et al., 1974; Sykes and Partridge, 1974).

This study describes the morphological changes of elastic fibres in the lung and aorta of the TSK mouse compared with those in the BAPN mouse.

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Materials and methods

Animals

TSK mice: Five heterozygous tight-skin (TSK/+)

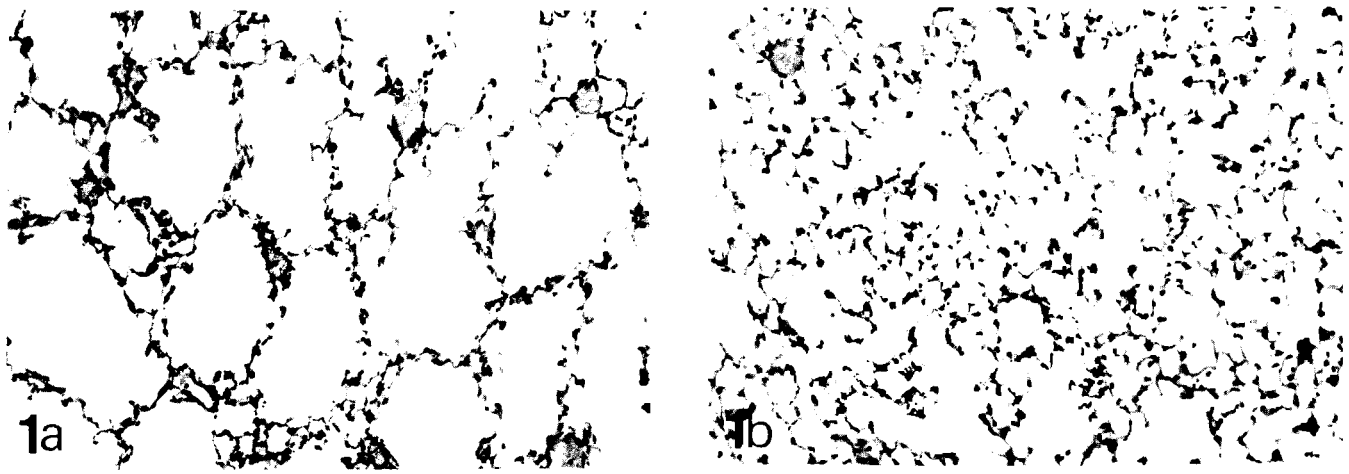

Fig. 1. a. TSK mouse lung. Enlargement of the alveolar air spaces are observed. **b.** Normal mouse lung. The alveolar air spaces are small and uniform in size. × 150

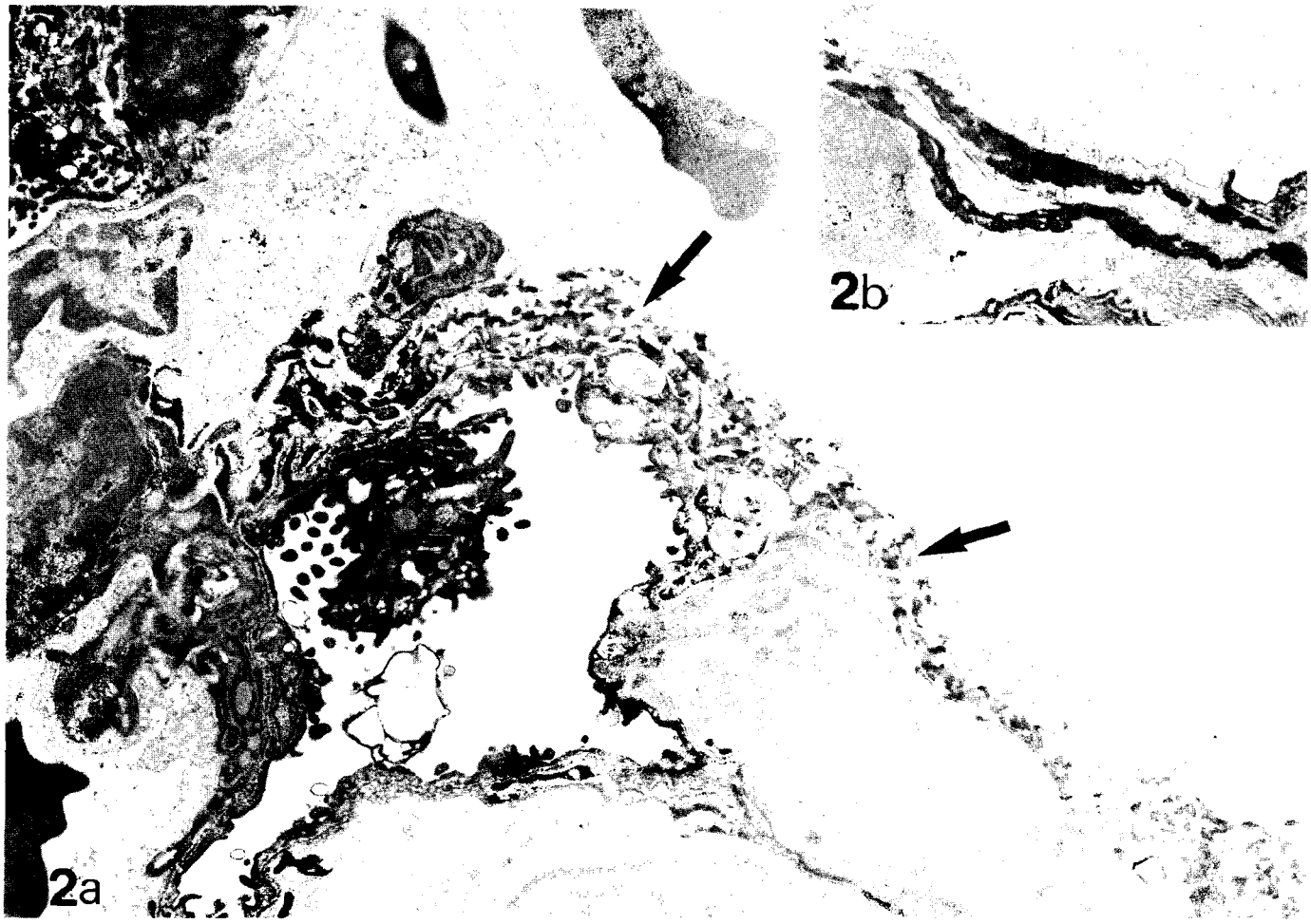

Fig. 2. a. TSK mouse lung. Elastic fibres (arrows) in the lung are fragmented. **b.** Normal mouse lung. × 10.000

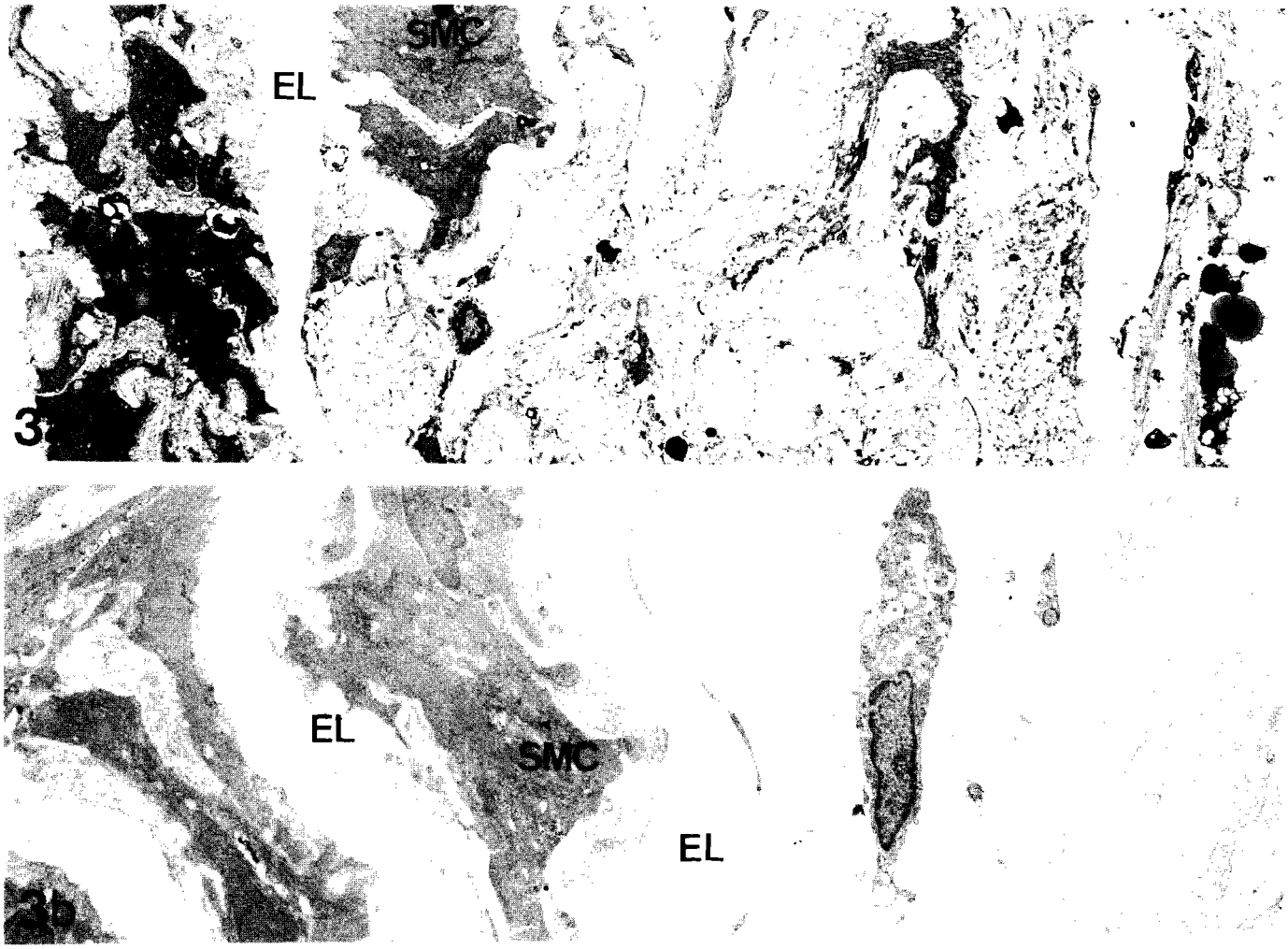

Fig. 3. a. TSK mouse aorta. Hyperplasia of loose connective tissues in the adventitia are observed. **b.** Normal mouse aorta. Ruthenium red fixation. El = elastic lamina; SMC = smooth muscle cell. × 6,000

mice (five weeks old) were obtained from the Jackson Laboratory (animals were given by courtesy of Dr. Akira Yoshida, Tokyo Kyosai Hospital). Three normal homozygous (+/+) mice were used as controls.

BAPN-fed mice: Five pregnant mice of the DDY strain were used. After delivery the mothers were fed a diet to which was added 0.4% (by weight) BAPN (Sigma). Forty young mice were weaned at 3 weeks. These post-weaned mice were fed the same diet and sacrificed when 3, 4, and 5 weeks old. Regular diet-fed mice were used as controls.

Morphological methods

Animals were sacrificed by intraperitoneal injection of sodium pentobarbital. The trachea was cannulated with a teflon catheter (Szapiel et al., 1981). The lung and trachea were excised en block and fixed inflated at 25 cm H_2O for 3 h with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). The lung and aorta fixed with glutaraldehyde were cut into small pieces, and post-fixed with 1% OsO_4 for electron microscopy. Tannic acid and ruthenium red-glutaraldehyde fixations (Luft, 1971; Mizuhira et al., 1972) were also performed. Fixed materials were dehydrated through graded alcohols and embedded in epoxy resin. Ultrathin sections were observed with electron microscopy after staining with uranyl acetate and lead citrate.

Results

TSK mice

The lung of TSK mice was characterized by enlargement of the alveolar air spaces to several times their normal size (Fig. 1). The lung of normal mice had a fine fishnet pattern of alveoli, and the alveolar air spaces were small and uniform in size. The enlargement of the alveolar air spaces in TSK mice was associated with destruction of the alveolar walls

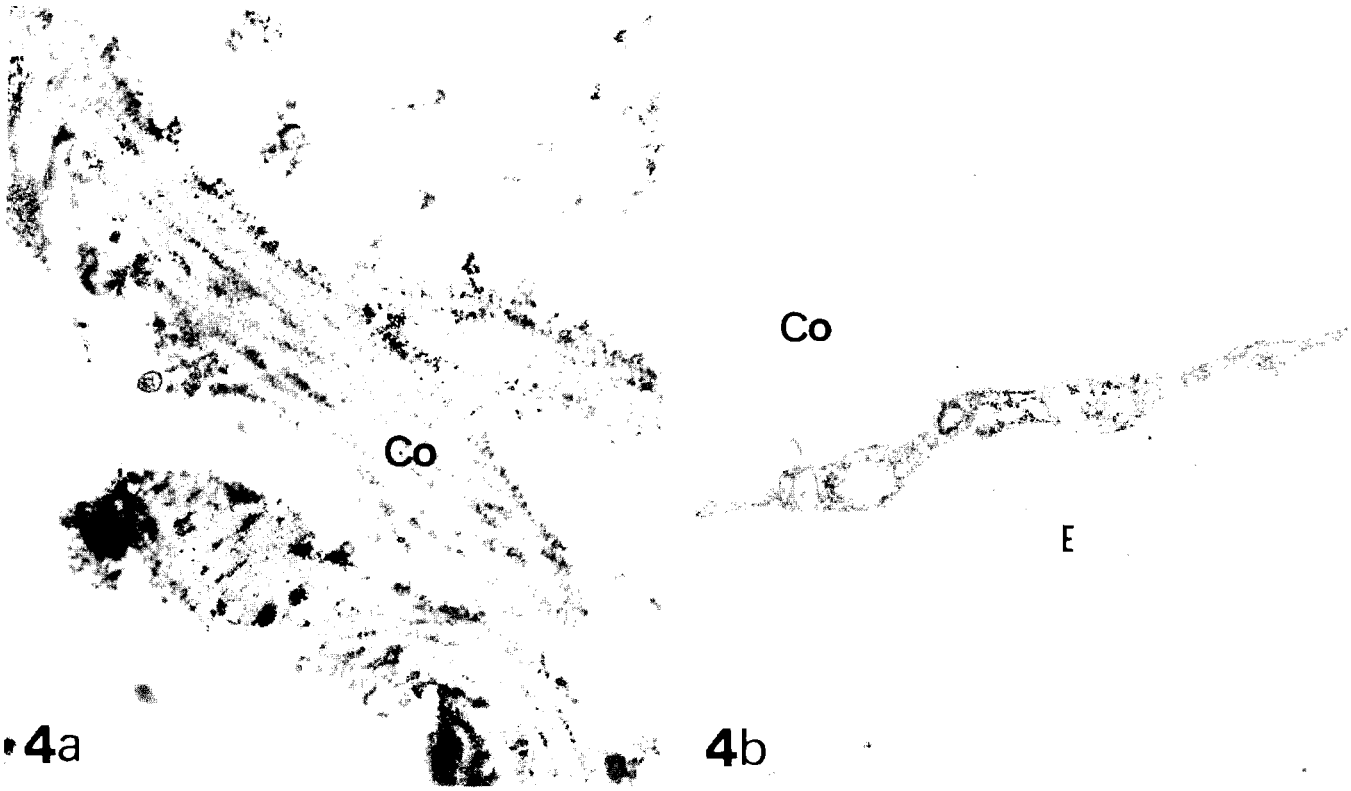

Fig. 4. a. TSK mouse aorta. Ruthenium red positive materials surrounding collagen (Co) in the adventitia are markedly increased. **b.** Normal mouse aorta. E = elastin. × 24,000

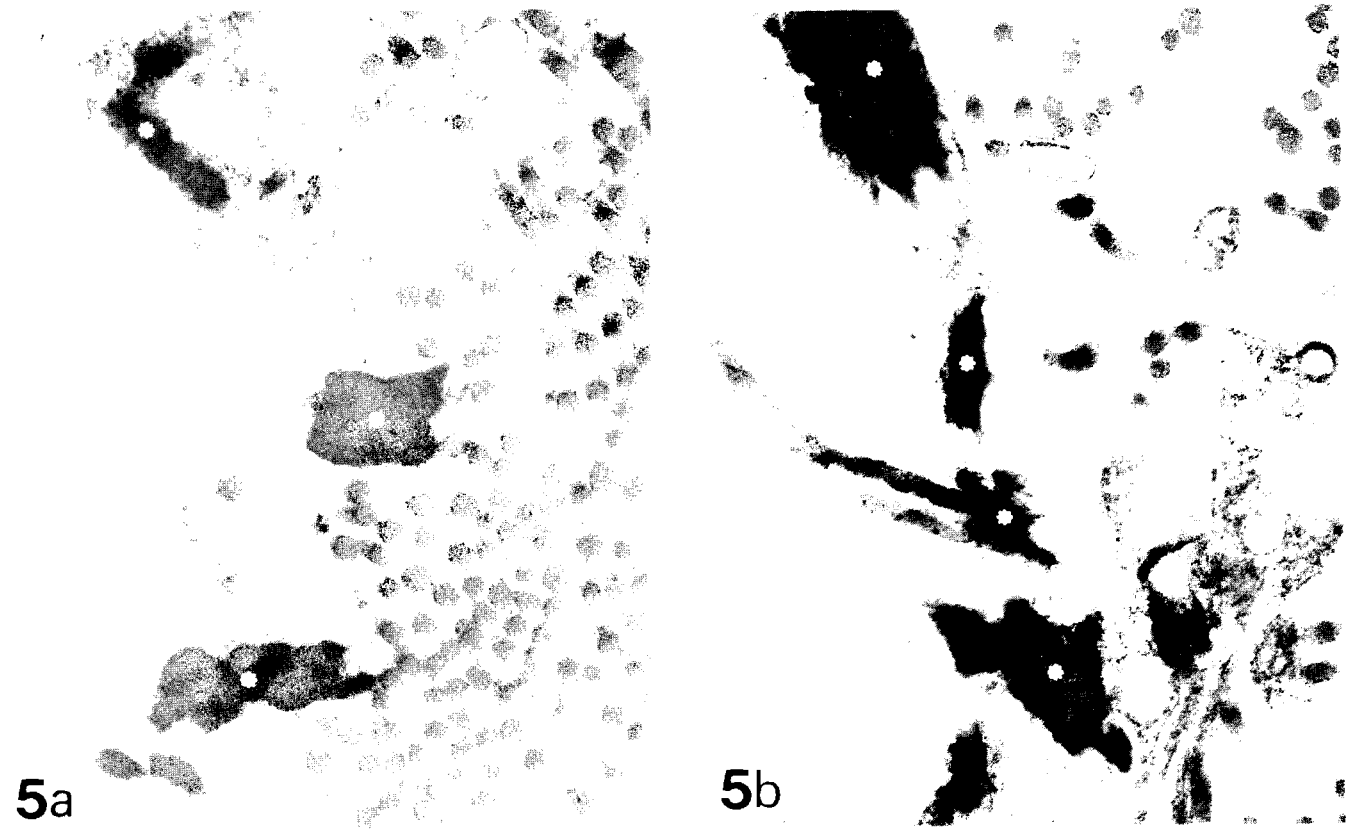

Fig. 5. a. TSK mouse aorta. Microfibrils surrounding elastin (asterisks) are not clear compared with normal mouse aorta **(b).** × 40,000

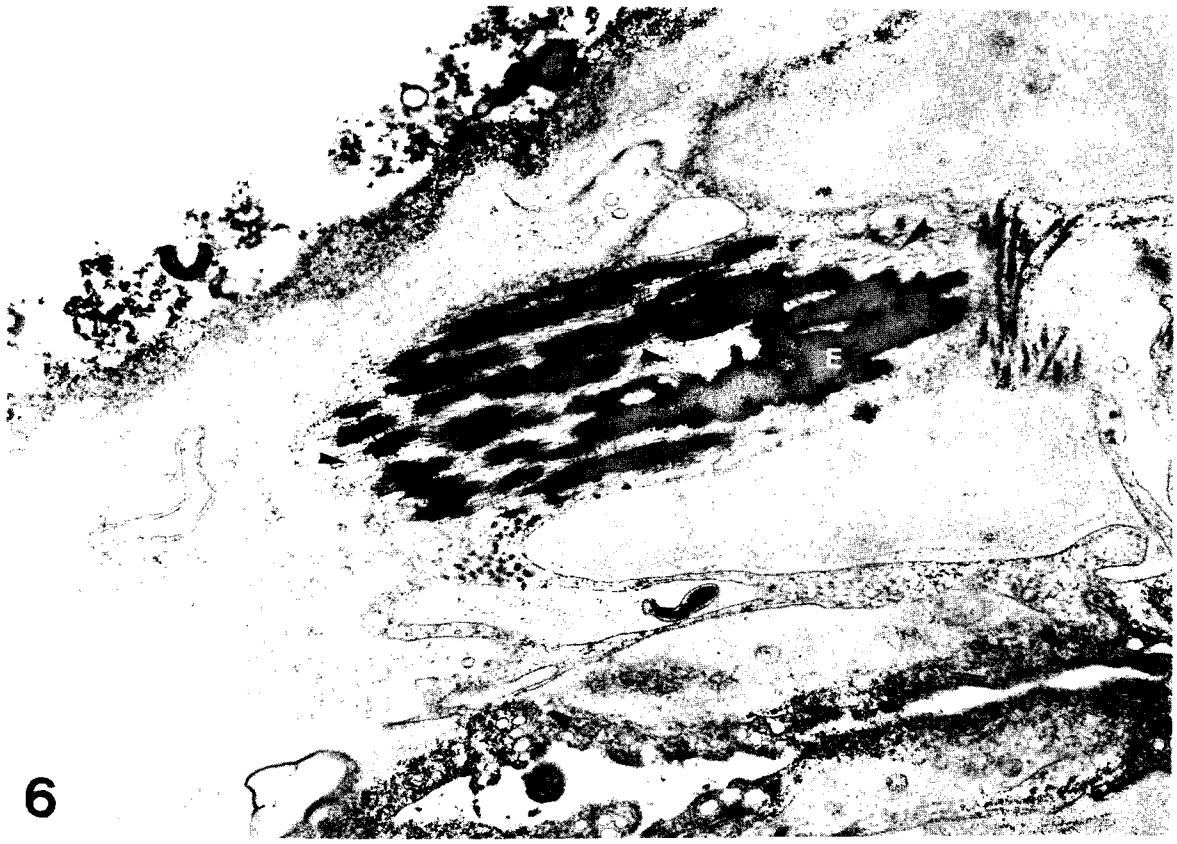

Fig. 6. BAPN fed mouse lung. Microfibrils (arrow heads) surrounding elastin (E) are clearly observed. × 36,000

Fig. 7. BAPN fed mouse aorta. Elastic lamina (EL) is swollen and fragmented. × 12,000

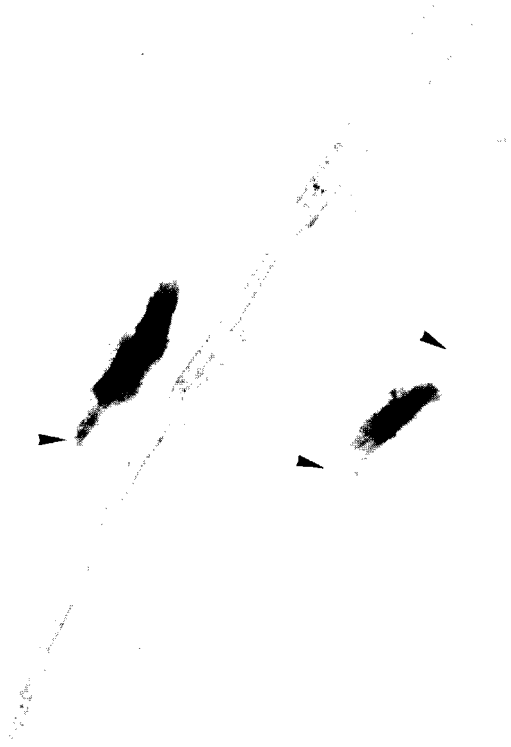

Fig. 8. BAPN fed mouse aorta. Microfibrils (arrow heads) surrounding elastin are clearly observed in the adventitia. × 24,000

and with formation of bullae and subpleural cysts. The lung of TSK mice on electron microscopy exhibited fragmentation of elastin in the alveolar walls (Fig. 2).

The aorta of TSK mice was characterized by marked hyperplasia of loose connective tissue in the adventitia. Collagen fibres and ruthenium red-positive materials were markedly increased (Figs. 3, 4). In the aorta of normal mice, elastic fibres consisted of amorphous components (elastin) and surrounding microfibrils, and microfibrils of elastic fibres in the adventitia were more apparent than those in the media. However, micro fibrils surrounding elastin in the adventitia were not clear in TSK mice (Fig. 5).

BAPN-fed mice

The lung of BAPN-fed mice had a fine fishnet pattern of alveoli, although the alveolar walls were slightly thickened. Enlargement of the alveolar air spaces were not apparent as observed in the lung of TSK mice. Elastic fibres in the alveolar walls did not exhibit fragmentation, and microfibrils surrounding elastin were clearly observed (Fig. 6).

The media of the aorta of BAPN-fed mice contained swollen and fragmented elastic laminae (Fig. 7). However, elastic fibres in the adventitia of the aorta exhibited a normal appearance, and microfibrils surrounding elastin in the adventitia were clearly observed (Fig. 8).

Discussion

Green et al. (1976) described that the skin of TSK mice revealed a marked hyperplasia of the subcutaneous loose connective tissue. Menton et al. (1978) and Menton and Hess (1980) reported that the dermis of TSK mice was both stiffer and thicker than normal and lacked the regular weave of normal dermal collagen fibres. Jiménez et al. (1984) also reported that the connective tissue hypertrophy was predominantly due to a marked increase in tissue collagen. Biochemical data revealed that total ^{14}C-hydroxy-proline synthesized in TSK mice skin organ culture, a measure of collagen production, was almost twice as much when compared with that of control skin. Soluble collagen was significantly greater in TSK mice than in controls (Jiménez et al., 1984). Ross et al. (1983) and Shikata et al. (1986) reported that a highly significant increase in glycosamino-glycan (GAG) content was found in TSK mice skin compared with normal mice skin. In this study, we observed that the aorta of TSK mice showed a hyperplasia of the adventitial connective tissue. There was a marked increase in the number of collagen bundles. In addition to this abnormality, ruthenium red-positive materials (GAG) were more numerous than in normal mice. These findings were similar to that observed in the TSK mouse skin.

In the Blotchy mouse, the lungs are abnormally compliant and have structural changes resembling emphysema (Fisk and Kuhn, 1976). Mice including Blotchy mice with alleles at the x-chromosomal, Mottled, have connective tissue abnormalities. These mice show abnormalities similar to those found in BAPN-fed and copper-deficient animals, including aortic aneurysms and reduced skin tensile strength, and bone abnormalities (Searle, 1968; Rowe et al., 1974). There are some similarities between the TSK mouse and the Blotchy mouse. In the TSK mouse, however, aortic aneurysms were not observed. It is well known that BAPN causes aortic aneurysms. BAPN inhibits the activity of lysyl oxidase. Starcher et al. (1977) reported that the Blotchy mouse has a genetic deficiency of lysyl oxidase, and other Mottled mice have been shown to have markedly reduced copper levels (Hunt, 1974). In this study, abnormality of elastic lamina in the media, as was observed in the BAPN-fed mouse, was not observed in the TSK mouse. It is suggested that the mechanism of connective tissue abnormality in the TSK mouse differs from that in the BAPN-fed mouse and the Blotchy mouse.

Elastin in the alveolar walls and the adventitia of the aorta associates with numerous microfibrils in normal mice. However, elastin in TSK mice had few associated microfibrils. Microfibrils play a primary role in development and determination of the shape of elastic fibres (Ross and Bornstein, 1969). It is suggested that the abnormality of microfibrils has a close relationship to the formation of elastic fibres and structural abnormalities in the TSK mouse lung. Biochemical data showed that there was no significant difference in total GAG and GAG concentration between TSK and normal mice lungs (Rosenberg et al., 1984). Increase of ruthenium red-positive materials was not seen in the alveolar walls. Therefore, structural abnormalities in the TSK mouse lung were not demonstrated to be due to qualitative nor quantitative changes in GAG. Defect of elastin in the emphysematous lung was observed (Wright, 1961; Thurlbeck, 1963). In this study, difference in amount of elastin between TSK and normal mice was not examined. However, fragmentation of elastin was observed in the alveolar walls in TSK mice. In TSK mice the levels of the urinary desmosine was significantly high, as compared with controls (Houya, 1986). It was suggested that abnormality of microfibrils and deformity or degradation of elastin probably play an important role in the pathogenesis and development of pulmonary emphysema.

Ultrastructural studies on the early stages of elastogenesis have shown that the bundles of microfibrils first appear in the juxtacellular space, and later the amorphous materials of elastin are deposited in the centre of surrounding microfibrils (Fahrenbach et al., 1966; Greenlee et al., 1966). In the media of the aorta, however, the early elastogenesis shows some different patterns (Albert, 1972; Nakamura, 1988). We have pointed out that the formation of elastic fibres in the aortic medial cells was different from that in the

adventitial cells in vitro (Akita et al., 1988). The results obtained in this study support the idea that the formation of elastic fibres and the relationship between elastic and microfibrils in the media may differ from those of the adventitia.

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