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The presence of fine elastin fibrils within the elastin fibre observed by scanning electron microscopy

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INTRODUCTION

Fetal bovine elastic fibres consist of two major components; a microfibrillar protein externally and elastin internally (Ross & Bornstein, 1969). In adult elastic ligaments only traces of microfibrillar protein are present; this is associated with collagen to form a 'knitted sheath' structure surrounding the elastin (Finlay & Steven, 1973). The adult elastin fibre has been shown by transmission electron microscopy to consist of fine beaded filaments of elastin, 3–4 nm diameter (Quintarelli, Bellocci & Zito, 1973; Gotte, Giro, Volpin & Horne, 1974). The filamentous organization was thought to express the conformation of elastin molecules within the fibre. Earlier studies (Karrer, 1961; Fahrenbach, Sandberg & Cleary, 1966) also reported the presence of fine filamentous units. The gross organization of elastin fibres in fetal ligamentum nuchae has been observed after enzymic removal of the non-elastin components (Minns & Steven, 1974). The fetal elastin fibres were found to be much thinner than those obtained from the adult.

In the present paper we wish to present evidence for the existence of fine elastin fibrils (approximately 120 nm diameter) in the fetal elastin fibre, and to illustrate the way they associate in the adult ligament to form the thicker, bifurcated elastin fibres. This structural organization within the elastin fibre has been revealed by sequential enzymic and chemical removal of collagen and microfibrillar protein from the elastin fibres of bovine ligamentum nuchae, followed by critical point drying and scanning electron microscopy.

MATERIALS AND METHODS

The nuchal ligament was dissected from a 7½ month old fetal calf within 2 hours of the death of the mother – the age of the fetus being determined from its forehead to rump length (Bogart, 1959). Adult bovine nuchal ligament was obtained from a two year old cow.

Strips of tissue 2–3 mm thick and about 20–30 mm in length were cut parallel to the long axis of the ligament. Sequential chemical and enzymic treatments were applied to the tissue (Ross & Bornstein, 1969), with exhaustive washing with

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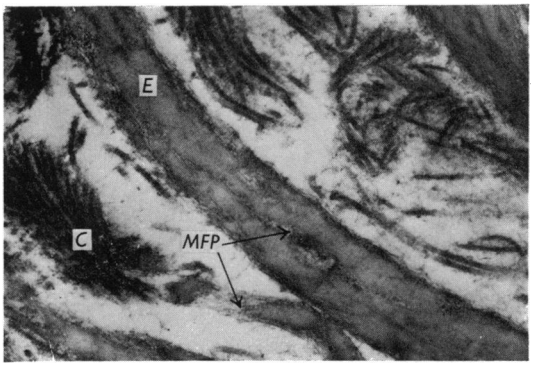

Fig. 1

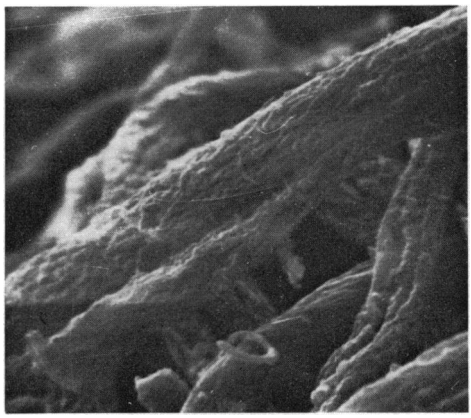

Fig. 2

G, C, M symbols refer to specific extraction proceedings – see text for explanation.

Fig. 1. Transmission electron micrograph of the fibrous elements of fetal ligamentum nuchae. The elastic fibre consists of an elastin core (*E*) surrounded by a coating of microfibrillar protein (*MFP*). This protein is also situated between the fibrils of elastin which make up the elastin core. Collagen fibrils (*C*) lie between elastic fibres. Stained with uranyl acetate–lead citrate. ×27000.

Fig. 2. Scanning electron microscopy of fetal ligament elastic fibres (GCGCG). The microfibrillar protein can be seen on the surface of the fibre – compare this with Fig. 5 in which the microfibrillar protein has been removed by a further treatment with (M). ×11500.

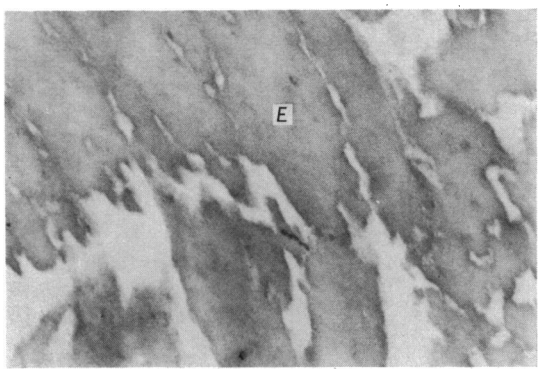

Fig. 3

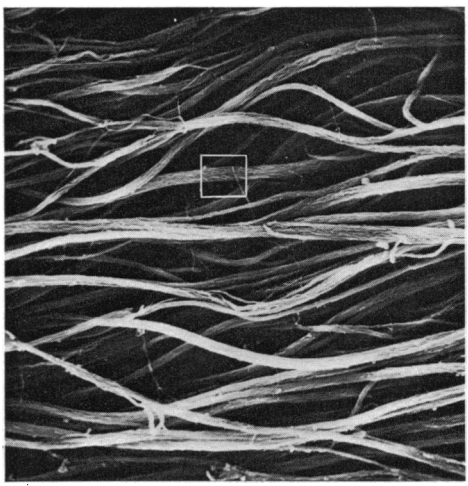

Fig. 4

Fig. 3. Transmission electron micrograph of a section of fetal elastin fibres (GCGCGM). The elastin fibres (E), stripped of their coating of electron-dense microfibrillar protein, are poorly stained and amorphous in cross sectional appearance. Stained with uranyl acetate–lead citrate. ×105000.

Fig. 4. Low power scanning electron microscopy of fetal elastin fibres (GCGCGM), showing unbranched thin elastin fibres, some of which can be seen to be composed of fine fibrillar elastin. ×2500.

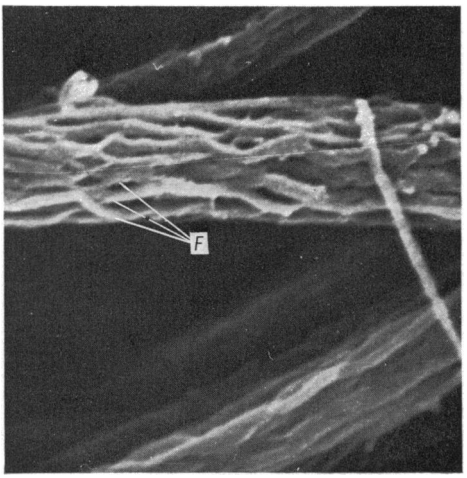

Fig. 5

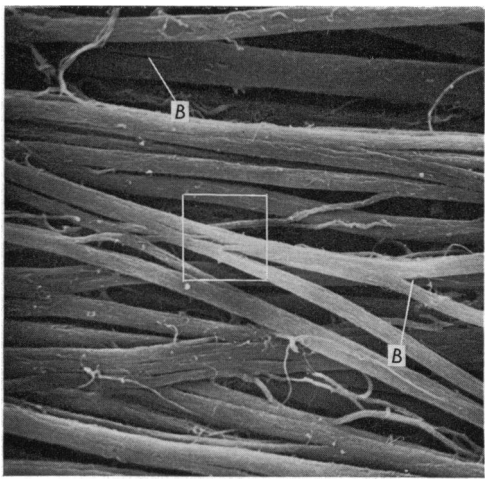

Fig. 6

Fig. 5. Higher power scanning electron micrograph of a fetal elastin fibre shown in the enclosed area of Fig. 4. Note the removal of microfibrillar protein (Fig. 2) with the exposure of the fine fibrillar elastin (*F*) which has an approximate diameter of 115–125 nm (including the thickness of the metallic coating required for scanning electron microscopy). × 23000.

Fig. 6. Low power scanning electron micrograph of adult ligament elastin fibres (GCGCGM). Note the variation in elastin fibre thickness and the bifurcations (*B*) typical of adult elastin. The insert is shown in Fig. 9. × 560.

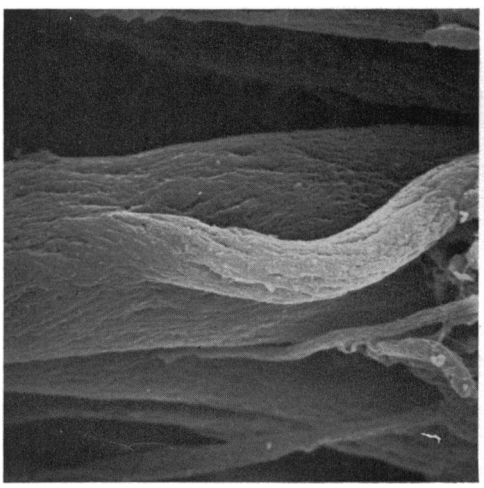

Fig. 7

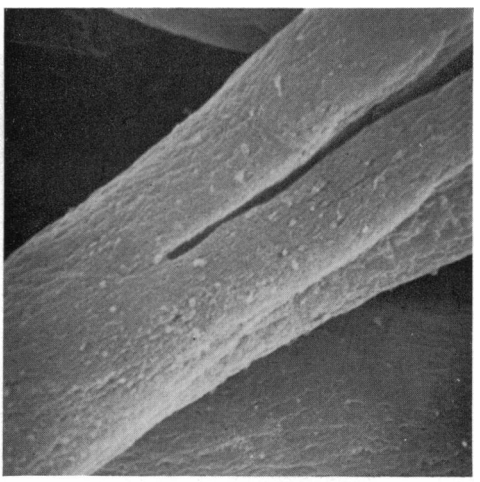

Fig. 8

Figs. 7, 8. Two examples of bifurcating elastin fibres (GCGCGM) from adult ligament. At this magnification the ridged appearance on the surface of the elastin fibres can be seen. × 2300.

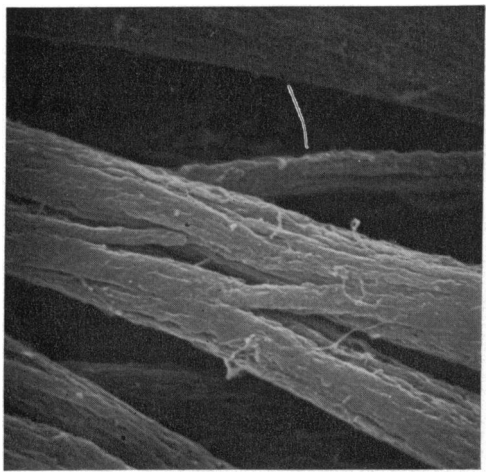

Fig. 9

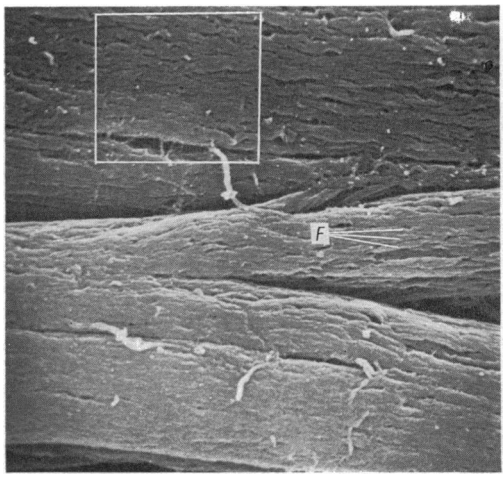

Fig. 10

Fig. 9. Detail from Fig. 6 illustrating the bridging of two adult elastin fibres (GCGCGM) at the site of a bifurcation. Note that two bridges shown in the picture both form a continuum with the major elements of the elastin fibre. × 2300.

Fig. 10. Detail of a bridge between two elastin fibres (GCGCGM). The ridged appearance of the elastin fibre surface can be seen to originate from aligned fine fibrils of elastin (*F*) of the same diameter as observed in unbranched fetal elastin fibres (Fig. 5). The enclosed area is shown in Fig. 11. × 5700.

distilled water between each treatment. The strips were treated with 6 M guanidinium chloride (buffered to pH 7·0 with 1 N NaOH) for 18 hours at 4 °C (coded G), followed by digestion with purified bacterial collagenase (Worthington CLSPA, coded C) in 0·05 M Tris-HCl buffer, pH 7·5, containing 0·005 M $CaCl_2$, at 37 °C for 18 hours (enzyme-substrate ratio approximately 1:100). The tissue (GC) was subjected to further guanidine and collagenase treatments (GCGCG). Finally, the strips were suspended in 6 M guanidinium chloride (pH 8·5), containing 0·1 M 2-mercapto-ethanol (coded M), and kept for 72 hours at 4 °C to solubilize the microfibrillar protein.

Scanning electron microscopy

Samples were fixed in 2·5 % glutaraldehyde in sodium cacodylate buffer (pH 7·4) + 3 mM calcium chloride, followed by washing in distilled water and dehydration with increasing concentrations of acetone over a period of 2 hours. The tissue was dried using a Polaron E 3000 critical point drying apparatus. The dried material was glued to marked metallic stubs and vacuum coated with approximately 25 nm gold using the scanning electron microscope coating unit E 5000 (Polaron Equipment Ltd). The coated specimens were examined in a Cambridge Instrument Co. Stereoscan S_4, operating at an accelerating voltage of 30 Kv. Results were recorded with an Exacta Vx 500 camera on Ilford FP4 35 mm film.

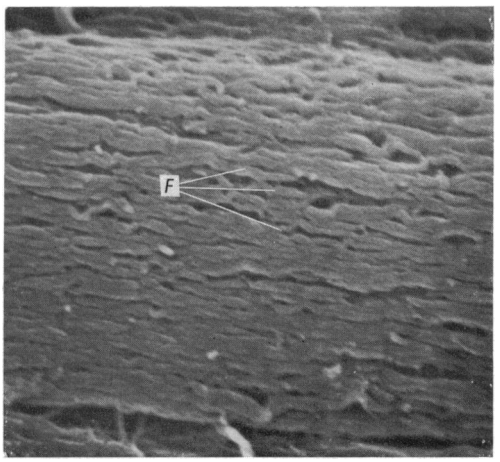

Fig. 11. Higher magnification of the area shown in Fig. 10. The adult elastin fibre is shown to be composed entirely of fine fibrils (*F*), aligned parallel to the main axis of the fibre. ×11 500.

Transmission electron microscopy

Part of the glutaraldehyde-fixed tissue was post-fixed with 1 % osmium tetroxide and embedded in Araldite. Sections were stained with uranyl acetate followed by lead citrate, and examined in a Philips EM 301 electron microscope.

RESULTS AND DISCUSSION

Transmission electron microscopy of the fibrous elements of ligamentum nuchae showed the tissue to consist of collagen fibrils, and elastin fibres, the latter surrounded by microfibrillar protein (Fig. 1). The collagen was removed by guanidine-collagenase (GCGCG) treatments, leaving the elastin fibres and associated microfibrillar protein (Fig. 2).

Further extraction with 0·1 M 2-mercaptoethanol in 6 M guanidium chloride (GCGCGM) removed the microfibrillar protein, leaving the purified elastin fibres (Fig. 3). Amino acid analysis of the elastin prepared by these techniques confirmed its purity.

The elastin fibres from GCGCGM-treated fetal and adult ligament were compared and two main differences were apparent.

1. The fetal fibres (0·5–2·5 μm diameter) were much more uniform than those of the adult ligament (2–14 μm diameter).

2. Fetal fibres showed no branching (Fig. 4) as opposed to the typical bifurcated adult fibres (Figs. 6–8).

The scanning electron microscope study of the fetal elastin showed each fibre to be composed of a number of thinner fibrils (approximately 120 nm diameter), wound together to form the composite elastin fibre (Fig. 5). It is probable that the microfibrillar protein normally occupies the spaces between these fibrils (supported by transmission electron microscopy observations, Ross & Bornstein, 1969), and that the reduction in the quantity of microfibrillar protein, which accompanies fibre

maturation, renders the fibrils more densely packed, and thus less easily visualized in scanning electron microscopy preparations.

Maturation is accompanied by thickening and bifurcation of the elastin fibres (Figs. 6–8). In addition, individual adult elastin fibres showed evidence of longitudinal splitting (Fig. 6). At high magnification (Figs. 9–11) the sites of splitting revealed a mass of fibrillar elements with a wavy appearance, aligned approximately parallel to the major axis of the fibre. These fibrils had a diameter range of 110–130 nm, very similar to that of the fine fibrils observed in fetal tissue. Bifurcation of adult elastin fibres was seen to be the result of a large number of these fibrillar elements crossing over from one fibre to another.

It may be concluded that the fine elastin fibrils are a fundamental building unit, leaving and rejoining fibres to produce bifurcated fibres of variable size in a three dimensional network.

The 'filamentous organization' within the adult elastin fibre reported by Quintarelli *et al.* (1973) is beyond the resolution of the scanning electron microscope and could not therefore have been seen in this study. It is very probable, however, that numerous 'filamentous organizations' make up each of the elastin fibril units observed in the present study.

SUMMARY

Scanning electron microscopy of critical point dried, enzymically and chemically purified fetal and adult elastin fibres from bovine ligamentum nuchae has shown that the fibres are composed of fine elastin fibrils (110–130 nm diameter). In the fetal tissue the elastin fibres were of relatively uniform thickness and did not bifurcate, but in the adult, much thicker, branching fibres were present. It would appear that, during maturation of the elastin fibre, thickening is the result of the aggregation of many fine fibrils, and bifurcations result from bundles of such fibrils crossing over from one fibre to another.

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