Distribution of type IV collagen, laminin, nidogen and fibronectin in the haemodynamically stressed vascular wall

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Summary. Changes in the extracellular matrix of haemodynamically stressed blood vessel walls were studied by immunofluorescence histochemistry in venous-pouch aneurysms fashioned on the site of the common carotid artery of nine sheep. Tissues from the thickened walls of the experimental aneurysms were examined from 11 to 98 months post-operatively for changes in the distribution of the basement membrane components type IV collagen, laminin, nidogen and fibronectin. In the younger aneurysms, there was an increase of the basement membrane components in the thickened area. Very little basement membrane was detected in older aneurysms. Diffuse staining for fibronectin was noted in aneurysms of all ages. Thick deposits of basement membrane material were observed in calcified tissues. The changes in the matrix proteins were similar to alterations occurring during the development of atherosclerosis in human vascular tissue.

Key words: Aneurysm, Basement membrane, Blood vessel, Fibronectin, Haemodynamics

Introduction

The role of haemodynamics is important in the localization and pathogenesis of atherosclerosis (Stehbens, 1979a, 1987; Texon, 1980; Caro and Parker, 1987). In experimental aneurysms in rabbits and in arteriovenous fistulae in sheep proliferative changes similar to those observed in human atherosclerosis developed in the haemodynamically stressed wall (Stehbens, 1981a,b, 1985a). Changes in the morphology of basement membranes have been observed in atherosclerosis of human arteries (Stehbens, 1975a) and in berry aneurysms (Stehbens, 1975b). These have included thickening of endothelial cell basement membranes and

the appearance of multilaminated, reticulated or dystrophic, smooth muscle cell basement membranes. Similar basement membrane alterations have been found in haemodynamically stressed blood vessel walls of experimental aneurysms (Stehbens, 1985b).

Basement membranes are continuous sheets of extracellular matrix material, found at the boundary between cells and the extracellular interstitium. They are composed of a number of components including the type IV and type V collagens, the glycoproteins laminin, nidogen and fibronectin and at least two heparan sulfate proteoglycans (Martínez-Hernández and Amenta, 1983; Paulsson, 1987). To study the effects of haemodynamic stress on basement membranes, the distribution of type IV collagen, laminin, nidogen and fibronectin, were studied in experimental saccular aneurysms. Nine sheep of postoperative ages ranging from 11 to 98 months, were examined by immunofluorescence histochemistry.

Materials and methods

Antibodies and antisera

The antiserum against nidogen was provided by Dr Marie Dziadek (the Murdoch Institute, Parkville, Australia). Monospecific antisera against collagen type IV was obtained from Bioscience Products AG, Emmenbruecke, Switzerland, against laminin from Gibco Laboratoires, New York, USA and against fibronectin from Bethesda Research Laboratories, Gaithersburg, USA.

Surgery

Saccular aneurysms were fashioned on the side of the common carotid artery using the venous pouch technique (Stehbens, 1973) in nine sheep less than 12 months old. Control arteriotomies and phlebotomies were performed on the left common carotid artery and external jugular vein, respectively.

Tissue sample preparation

The sheep were killed at 11, 15, 24, 25, 44, 50, 97 and 98 months postoperatively, by an overdose of intravenous Nembutal. The vessels were excised as rapidly as possible and samples were taken from the aneurysmal sac, the control artery and vein. Tissues were embedded in O.C.T. compound (Lab-Tek Products, Naperville, Illinois) and quickfrozen in liquid nitrogen. Sections 5-8 μm thick were cut at right angles to the direction of flow and mounted on glass slides, coated with poly-L-lysine (MW 350 000, Sigma, St Louis, USA) (Huang et al., 1983) and air dried.

Immunofluorescence staining

To prevent interference from elastic fibre autofluorescence, the improved technique for suppression of autofluorescence in vascular tissues as recently described by Kittelberger et al. (1989), was used. Briefly it involves:

– Incubation of the tissue sections with diluted first antibody (anti-type IV collagen 1:50, anti-laminin 1:100, anti-nidogen 1:200, anti-fibronectin 1:100) at 4° C overnight. The sections are washed three times (each of three minutes) with a solution of PBS.

– Incubation with FITC-conjugated goat anti-rabbit serum (Miles Lab Inc, Elkhart, USA) (diluted 1:40) at room temperature for 30 minutes. Then follows 2 x 3 minutes washes with PBS.

– Staining for 5 minutes at room temperature with 0.3% Eriochrome black T (C.I. 14645, Sigma, St Louis, USA) in PBS followed by washing twice (3 minutes each) with PBS.

– Mounting in glycerol/PBS, containing 0.1% oxidized p-phenylenediamine.

The sections were examined under a Zeiss fluorescence microscope equipped with an 09 filter set.

Controls were performed either by incubation of the tissue sections overnight at 4° C with PBS, 1% BSA or by incubation with antibodies against laminin or nidogen, preabsorbed with the purified antigen instead of the first incubation with diluted antibody as described above.

Results

Distribution of basement membrane components in control vessels

Type IV collagen, laminin and nidogen showed an identical distribution pattern in all blood vessel samples. This is demonstrated for three control vein sections in figure 1A-C. Strong staining was found along the luminal edge of the tissue sections, outlining the endothelial cell basement membranes, as well as in the media, showing clusters of smooth muscle cells. The adventitia, except for the vasa vasorum, was generally free of staining.

In figure 2 nidogen staining of control artery is shown. Staining of the endothelial cell basement membrane is visible between the lumen and internal elastic lamina. The media is completely covered with fluorescence staining, derived from the basement membranes of densely packed smooth muscle cells. The region of the adventitia is free of basement membrane staining, except for the nerve fibre bundles running parallel to the vessel axis.

Fibronectin antibodies were found to stain basement membranes in a manner similar to type IV collagen, laminin and nidogen antibodies. It was identified in the endothelial cell basement membrane and around smooth muscle cells. In addition, weaker fibrous staining was visible throughout the tissue (Fig. 1D).

No remarkable age-dependent changes were observed in control veins and arteries. In a few older veins the endothelial cell basement membrane seemed to be thicker or multi-layered in localized areas of the blood vessel (Figs. 1E, F). In the control arteries diffuse thickening was often found, with basement membrane staining around smooth muscle cells. These thickenings seemed to be independent of the age of the tissues.

Distribution of basement membrane components in the aneurysms

Considerable thickening of the walls of the experimental aneurysms as previously described (Stehbens, 1979b) was visible in all the examined specimens. One of the two oldest aneurysms (97 months) was extensively calcified while the other (98 months) showed calcification mainly proximally and distally and not in the central region of the dilatation. Areas of calcification were also observed in the 50 month old aneurysm.

The younger aneurysms (up to 25 months) exhibited strong, laminar staining patterns with type IV collagen, laminin and nidogen antibodies in the thickened intima and media, but virtually no staining in the adventitia (Fig. 3A). Differences in the distribution of these three components could not be observed. Fibronectin antibodies stained these laminar structures but also showed a more diffuse fluorescence in all the other parts of the tissue (Fig. 3B).

In the older aneurysms (44-98 months), basement membrane staining of laminin, nidogen and type IV collagen became weaker (Fig. 3C) or nearly disappeared (Fig. 3E), while diffusely scattered fibronectin staining still remained strong (Figs. 3D, F, H). In the two examples with calcification in the central part of the aneurysm sac, most of the basement membrane staining had disappeared, but local areas with large depositions of type IV collagen, laminin or nidogen were visible. These depositions often showed thick spindle-shaped structures (Figs. 3C, G), believed to be smooth muscle cells.

Major changes in the basement membrane components of the arterial region of the experimental aneurysms were observed in one case only, a two years old aneurysm. Pronounced thickening of the intima and extensive basement membrane staining in the intima and media were visible. The adventitia was free of basement membrane staining, except for the basement membrane surrounding nerve fibres. In all other arterial regions of aneurysms slight thickening similar to that found in the control arteries was often observed. The fibronectin
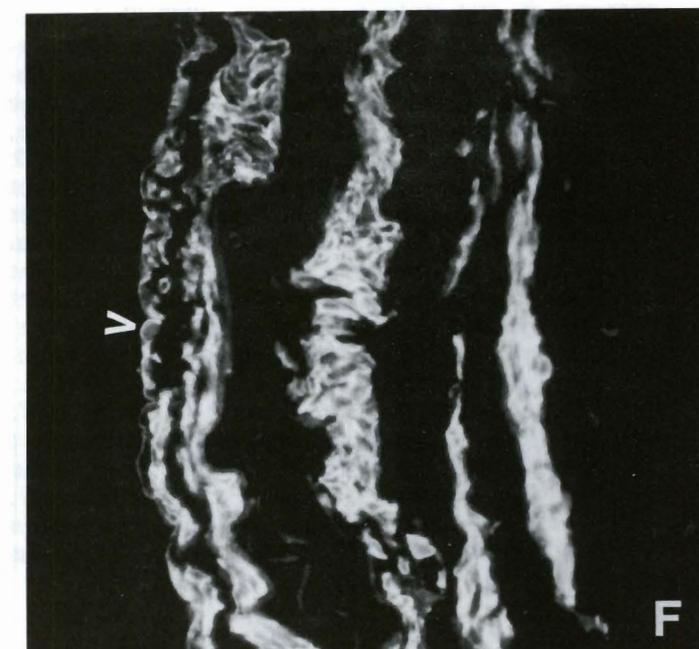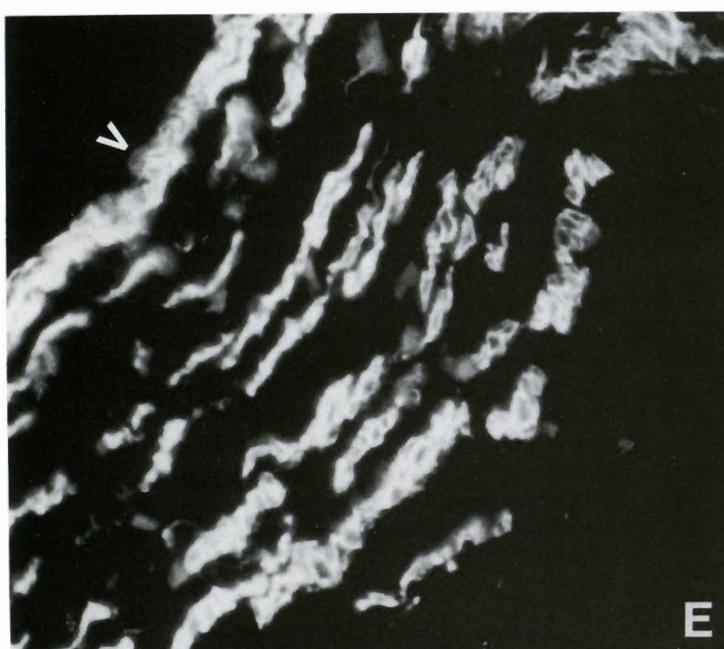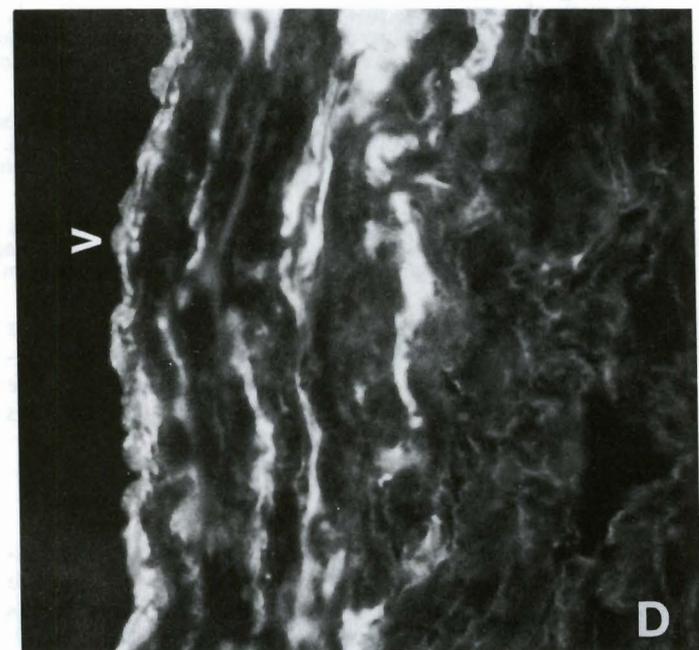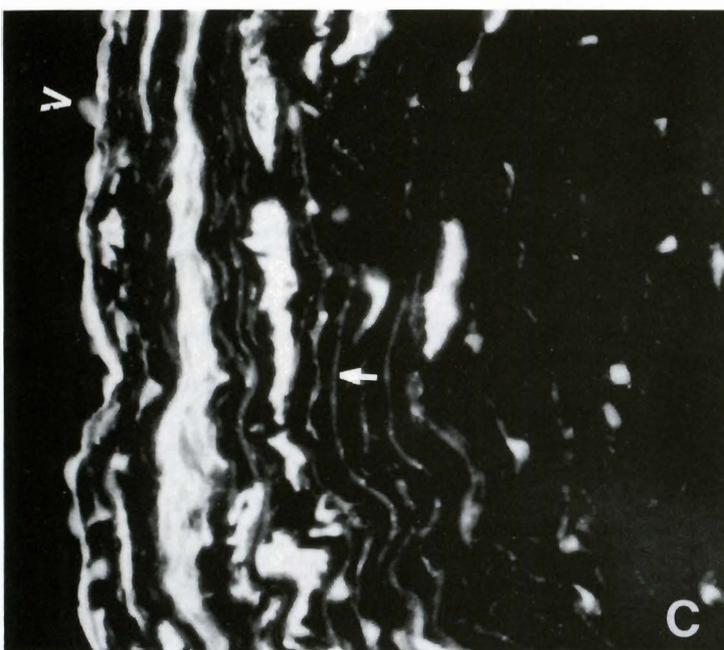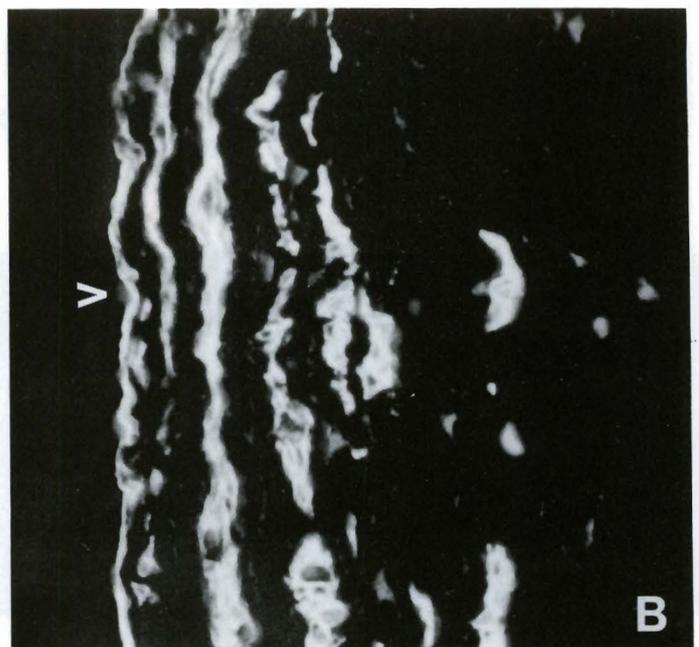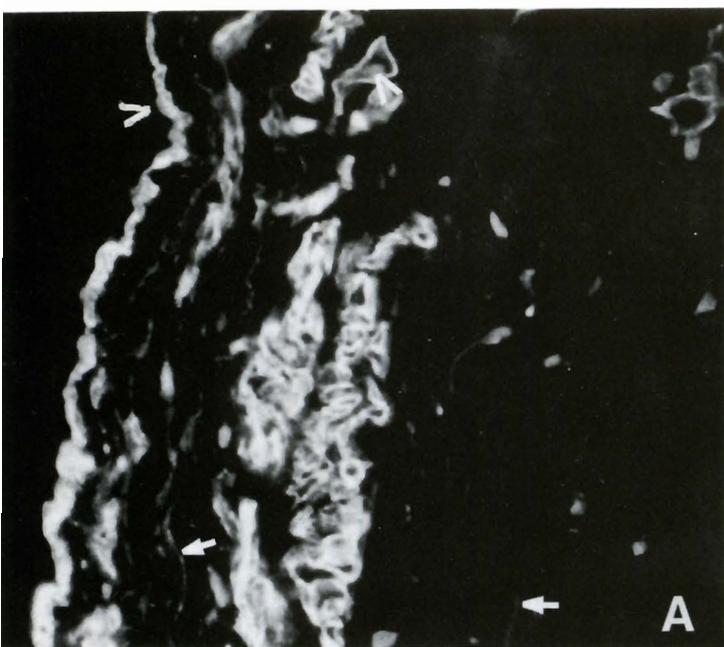
Fig. 1. Indirect immunofluorescence staining on jugular veins. A-D: vein sections from a 25 month old sheep (postpoeratively), stained with antibodies to A: type IV collagen, B: nidogen, C: laminin, D: fibronectin. E and F: staining of type IV collagen on a 44 months and on a 97 months old jugular vein respectively. The lumen is to the left in all photographs. Strong staining is derived from specific immunofluorescence (green on the original colour photograph), weak grey dots (especially along the intima) are cell nuclei (some of them are indicated by arrowheads; originally an amber staining) and weak grey bands are caused by autofluorescence of elastic fibres (some of them are indicated by arrows A and C; dark red on the original colour photograph). Original magnification. × 100

Fig. 2. Cross section of a control carotid artery of a sheep with a 24 month old aneurysm. Bright basement membrane staining (nidogen) is visible along the intima, around smooth muscle cells and nerve fibres (NF). The internal elastic lamina (IEL) appears as a grey meandering band. Cell nuclei (some are indicated by arrows) appear as either round dots in the endothelium or spindle-shaped inside smooth muscle cells. Original magnification × 100

Fig. 3. Sections of the saccular wall of aneurysms of different ages. A and B: 25 months, C and D: 50 months (partially calcified), E and F: 98 months, G and H: 97 months (calcified). A, C, E and G type IV collagen staining, B, D, F and H fibronectin staining. Note the thick basement membrane (type IV collagen) layers in C and G. Original magnification × 100

distribution was similar to the basement membrane staining of type IV collagen, laminin or nidogen respectively, but in addition, a diffuse staining throughout the vascular extracellular matrix was visible in all aneurysms examined.

Discussion

A considerable number of immunohistological examinations of the distribution of basement membrane components in healthy and thickened blood vessels has been published (Wick et al., 1979; Madri et al., 1980; Stenman et al., 1980; Natali et al., 1981; Fitch et al., 1982; Jensen et al., 1983; Palotie et al., 1983; Shekonin et al., 1985, 1987; Sariola et al., 1986; Voss and Rauterberg, 1986) reflecting the great interest in the mechanisms, leading to the changes in their distribution in the vascular wall. Blood vessel tissues, examined to date, were of human (Madri et al., 1980; Stenman et al., 1980; Jensen et al., 1983; Shekonin et al., 1985, 1987; Sariola et al., 1986; Voss and Rauterberg, 1986) bovine (Wick et al., 1979; Madri et al., 1980; Palotie et al., 1983) avian (Fitch et al., 1982) murine (Madri et al., 1980) rabbit (Natali et al., 1981; Palotie et al., 1983) and rat (Madri et al., 1980; Stenman et al., 1980; Natali et al., 1981) origin. The majority of studies have concentrated on the localization of type IV collagen (Madri et al., 1980; Fitch et al., 1982; Palotie et al., 1983; Shekonin et al., 1985, 1987; Voss and Rauterberg, 1986) and fibronectin (Stenman et al., 1980; Natali et al., 1981; Jensen et al., 1983; Palotie et al., 1983; Sariola et al., 1986; Shekonin et al., 1987; Voss and Rauterberg, 1986) and a smaller number on laminin (Palotie et al., 1983; Sariola et al., 1986; Voss

and Rauterberg, 1986) but virtually no data on nidogen are available.

There is general agreement on the distribution of type IV collagen and laminin in the unaffected aorta, artery and vein, viz. subjacent to the endothelium and around smooth muscle cells in the media. The adventitia is generally free of basement membrane components except for the vasa vasorum and nerve fibres (Wick et al., 1979; Madri et al., 1980; Fitch et al., 1982; Palotie et al., 1983; Shekonin et al., 1985, 1987; Sariola et al., 1986; Voss and Rauterberg, 1986). Our findings on the ovine carotid artery and jugular vein are consistent with these reports. However there are major differences between vein and artery, in the amount of basement staining, especially in the media. While the media of the artery was densely packed with bright fluorescence, reduced staining of basement membranes was visible in the vein, in the form of randomly distributed clusters of smooth muscle cells. Antibodies against nidogen exhibited staining patterns coincident with laminin and type IV collagen, reflecting its exclusive location in basement membranes (Timpl et al., 1983).

Compared with the immunostaining of the exclusive basement membrane components laminin, type IV collagen and nidogen (Martínez-Hernández and Amenta, 1983) antibodies against fibronectin exhibited strong fluorescence of endothelial and smooth muscle cell basement membranes and weaker, more diffuse staining in the interstitial connective tissue in the control blood vessels. This is in accord with several earlier reports on its distribution in the unaffected vascular wall (Jensen et al., 1983; Martínez-Hernández and Amenta, 1983;
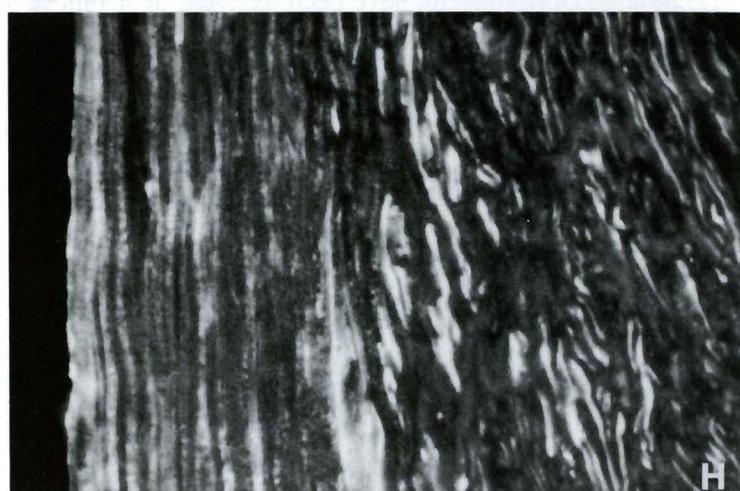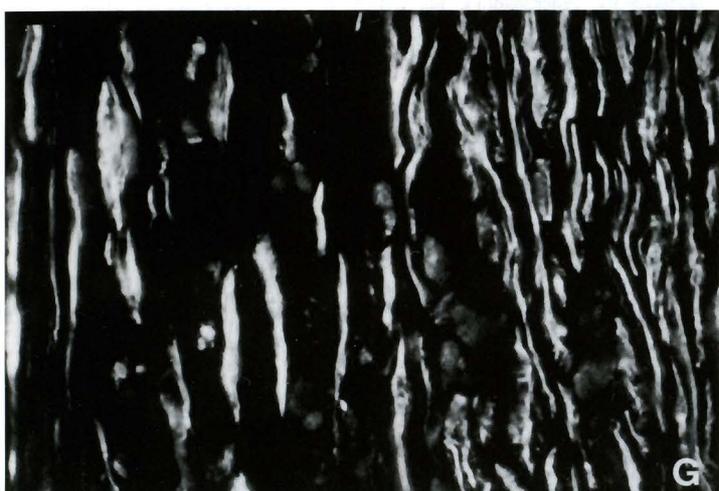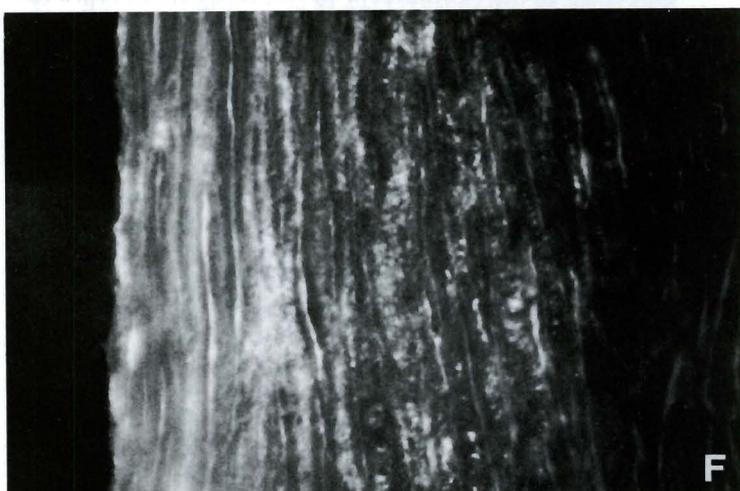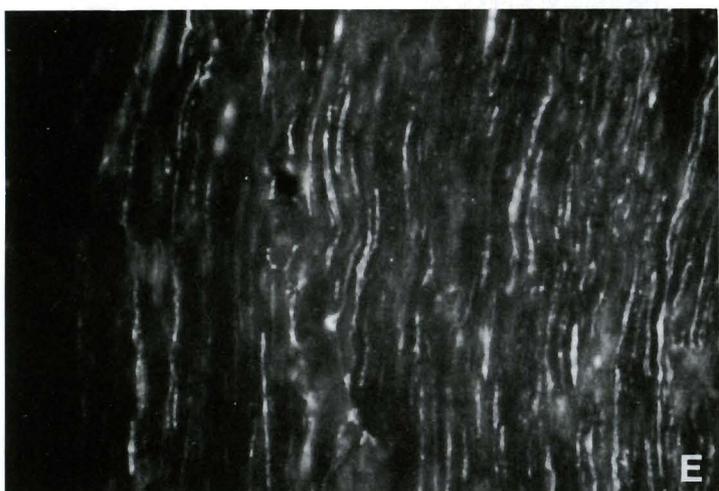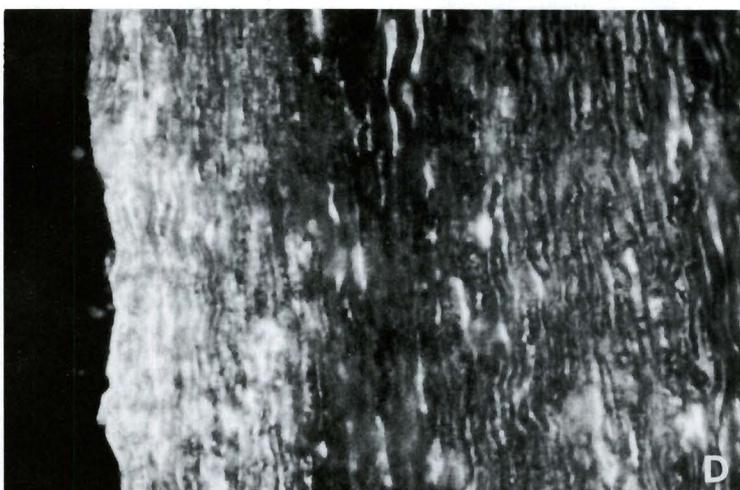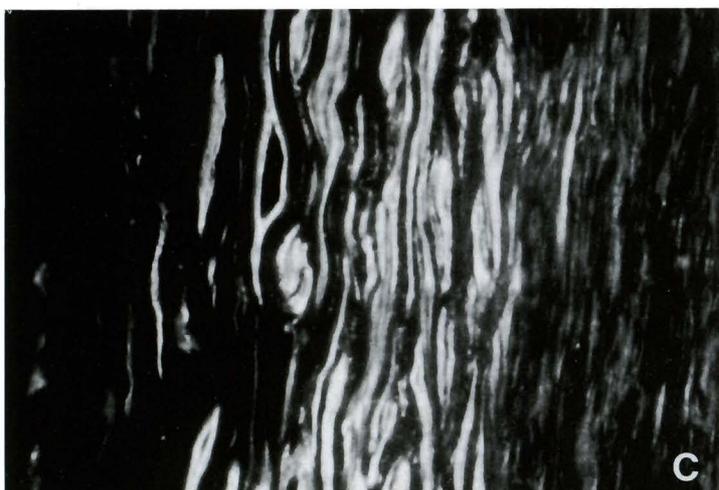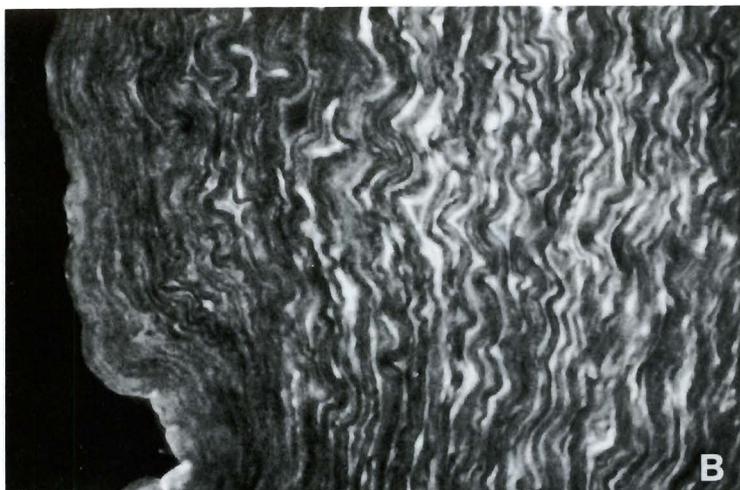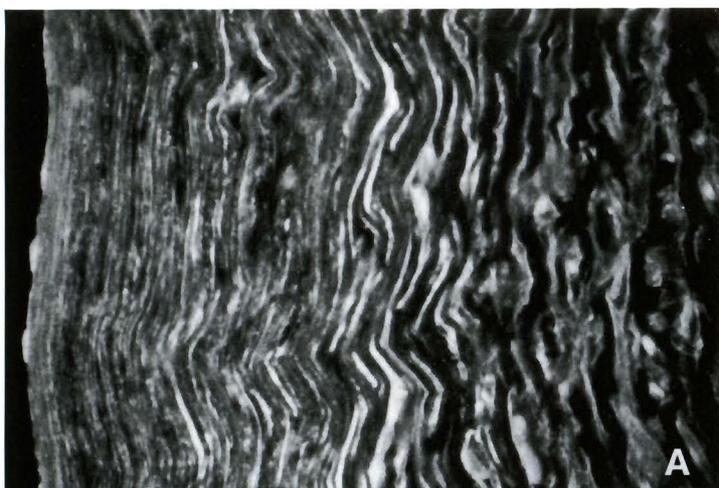
Palotie et al., 1983; Sariola et al., 1986; Shekonin et al., 1987).

Thickening of the aneurysm sac wall in younger aneurysms (up to 3-4 years) was always accompanied by widespread basement membrane staining, resembling the process of migration and proliferation of smooth muscle cells in the thickening intima during atherosclerosis (Campbell and Campbell, 1987). With advanced age of the aneurysms (4-9 years), most of the basement membrane staining had disappeared, while diffuse fibronectin staining was still present. Regions either devoid of or alternatively with low amounts of type IV collagen and laminin have been reported in advanced atherosclerotic plaques in humans (Shekonin et al., 1985, 1987; Sariola et al., 1986; Voss and Rauterberg, 1986). Degeneration of smooth muscle cells and degradation of their basement membranes may occur in the advanced blood vessel thickening, due to the development of a more so-called interstitial type of vascular extracellular matrix (Sariola et al., 1986).

An interesting finding was the observation of «spindle shaped» fluorescent structures (Fig. 3C, G) in some areas of the thickened vascular wall. They often appeared to be surrounded by thick, multiple layers of basement membranes. Similar appearance of smooth muscle cells has been noted in atherosclerotic fibrous plaques (Ross et al., 1984; Shekonin et al., 1985) especially in the fibrous cap. In this study these types of thickened basement membranes were observed only in calcified tissues which indicates a possible correlation with the process of calcification. Tanimura et al. (1986a, b) recently reported calcium deposits in association with smooth muscle cell basement membranes in humans and experimental animals.

In the haemodynamically thickened vascular wall of younger aneurysms fibronectin was clearly distributed identically to the exclusive basement membrane components, and distributed diffusely in the interstitium. Most of the basement membrane staining of fibronectin disappeared in the older aneurysms, but the diffuse staining was still prominent. Diffuse and prominent distribution of this glycoprotein in early atherosclerotic lesions (Stenman et al., 1980) and small fibrous plaques (Shekonin et al., 1987) has been reported. Distorted expression, reduced amounts or absence of fibronectin have been reported in advanced atherosclerotic lesions (Sariola et al., 1986; Shekonin, 1987; Stenman et al., 1980). Such observations are, in general, in agreement with our findings. Differences in the staining methods may be responsible for differences in basement membrane associated localization. We have applied an improved staining method in order to reduce autofluorescence of elastic fibres and have thus been able to use high antisera dilutions. No precautions to avoid autofluorescence were taken by other authors who therefore probably used higher antibody concentrations. Basement membrane fluorescence as well as diffuse interstitial fluorescence might then appear to be strong and might not be readily distinguished.

The profound changes in the distribution of basement membrane material in the haemodynamically stressed walls of experimental saccular aneurysms, as visualized by immunofluorescence staining of laminin, type IV collagen, nidogen and fibronectin, show a high degree of similarity with changes in the atherosclerotic blood vessel wall. The saccular aneurysm therefore is suitable for further studies of the distribution of extracellular matrix components at an ultrastructural level.

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