through the micropores of the membrane to reach the mitochondria of the sperm. Direct evidence for the suggested functions must await future experiments.


* To whom correspondence should be addressed.

1 Baccetti, B., Bigliardi, E., and Floriana, R., J. Ultrastr. Res. *35* (1971) 582.
2 Mann, Th., Spermatophores, pp. 217. Springer, Berlin 1984.
3 Osanai, M., Aigaki, T., Kasuga, H., and Yonezawa, Y., Insect Biochem. *16* (1986) 879.
4 Osanai, M., Aigaki, T., and Kasuga, H., Insect Biochem. *17* (1987) 71.
5 Kasuga, H., Aigaki, T., and Osanai, M., Insect Biochem. *17* (1987) 317.
6 Aigaki, T., Osanai, M., and Kasuga, H., Insect Biochem. *17* (1987) 323.
7 Osanai, M., Kasuga, H., and Aigaki, T., J. Morph. *193* (1987) 1.
8 Osanai, M., Aigaki, T., and Kasuga, H., in: New Horizons in Sperm Cell Research, p. 185. Ed. H. Mohri. Jap. Sci. Press, Tokyo 1987.
9 Katsuno, S., Appl. Ent. Zool. *13* (1978) 47.
10 Osanai, M., Kasuga, H., and Aigaki, T., Experientia *43* (1987) 593.
11 Osanai, M., Kasuga, H., and Aigaki, T., J. Morph. *198* (1988) 231.
12 McLeen, I. W., and Nakane, P. K., J. Histochem. Cytochem. *22* (1974) 1077.
13 Osanai, M., Kasuga, H., and Aigaki, T., Intern. J. Invert. Reprod. Devl. *13* (1989) 97.
14 Fawcett, D. W., The Cell, 2nd edn, p. 862. Saunders Company, Philadelphia 1981.
15 Brown, C. R., von Glos, K. I., and Jones, R., J. Cell Biol. *96* (1983) 256.
16 Burger, M. M., Nature *227* (1970) 170.
17 Fawcett, D. W., Devl. Biol. *44* (1975) 394.
18 Feldherr, C. M., Exp. Cell Res. *93* (1975) 411.
19 Bonner, A., in: The Cell Nucleus, Part C, p. 97. Ed. H. Busch. Academic Press, New York 1978.
20 Dworetzky, S. I., and Feldherr, C. M., J. Cell Biol. *106* (1988) 575.
21 Dworetzky, S. I., Lanford, R. E., and Feldherr, C. M., J. Cell Biol. *107* (1988) 1279.
22 Osanai, M., Kasuga, H., and Aigaki, T., J. Insect Physiol. *35* (1989) 401.
23 Unpublished data.
24 Gray, J. P., Drummond, G. I., Luk, D. W. T., Hardman, J. G., and Sutherland, E. W., Archs Biochem. Biophys. *172* (1976) 20.
25 Lindemann, C. B., Cell *13* (1978) 9.
26 Morisawa, M., and Okuno, M., Nature *295* (1982) 703.
27 Øyen, O., Myklebust, F., Scott, J. D., Hansson, V., and Jahnsen, T., FEBS Lett. *246* (1989) 57.
28 De Lamirande, E., and Gagnone, C., J. Cell Biol. *102* (1986) 1378.


0014-4754/90/030261-04$1.50 + 0.20/0


## Type VI collagen in experimental atherosclerosis

R. Kittelberger, P. F. Davis and W. E. Stehbens

*The Malaghan Institute of Medical Research, Wellington School of Medicine, Private Box 7060, Wellington South, Wellington (New Zealand)*
*Received 9 June 1989; accepted 3 August 1989*

*Summary.* Diffuse intercellular immunofluorescence staining of type VI collagen was found in the experimentally thickened vascular wall and in control blood vessel tissues as well, superimposed by more intense staining around basement membranes. While the basement membrane staining disappeared in advanced mural thickenings, the diffusely distributed network of type VI collagen remained.
*Key words.* Aneurysm; atherosclerosis; haemodynamics; immunofluorescence; type VI collagen.

Type VI collagen was originally isolated from human aortic intima and named intima collagen [1]. Subsequently it has been localized in all three layers of the vascular wall and in a large number of other tissues (for recent review see Timpl and Engel [2]). The ubiquitous occurrence of this collagen variant indicates an important biological role for this protein. Despite its original detection in vascular tissue, virtually no data on its involvement in atherosclerosis are available. In this study, experimental saccular aneurysms have been examined by immunofluorescence histochemistry in thirteen sheep of post-operative ages, ranging from 11 to 98 months. It has been shown that histological changes in these vessels resemble human atherosclerosis [3, 4]. Comparison of the distribution of type VI collagen in haemodynamically stressed vessels with that in sham-operated control vessels, is reported here.

*Materials and methods*

The antiserum to type VI collagen was provided by Dr Mark Gibson (Univ. Adelaide, Adelaide, Australia). Monospecific antiserum to collagen type IV was obtained from Bioscience Products AG, Emmenbrucke, Switzerland. Saccular aneurysms were produced by anastomosing the right jugular vein to the right common carotid artery and then ligating the vein, proximally and distally to the anastomotic site, in sheep less than 12 months of age [3]. Control arteriotomies and phlebotomies were performed on the left common carotid artery and external jugular vein. Tissue sample preparation and immunofluorescence staining for type IV and type VI collagen were performed as described elsewhere [5].

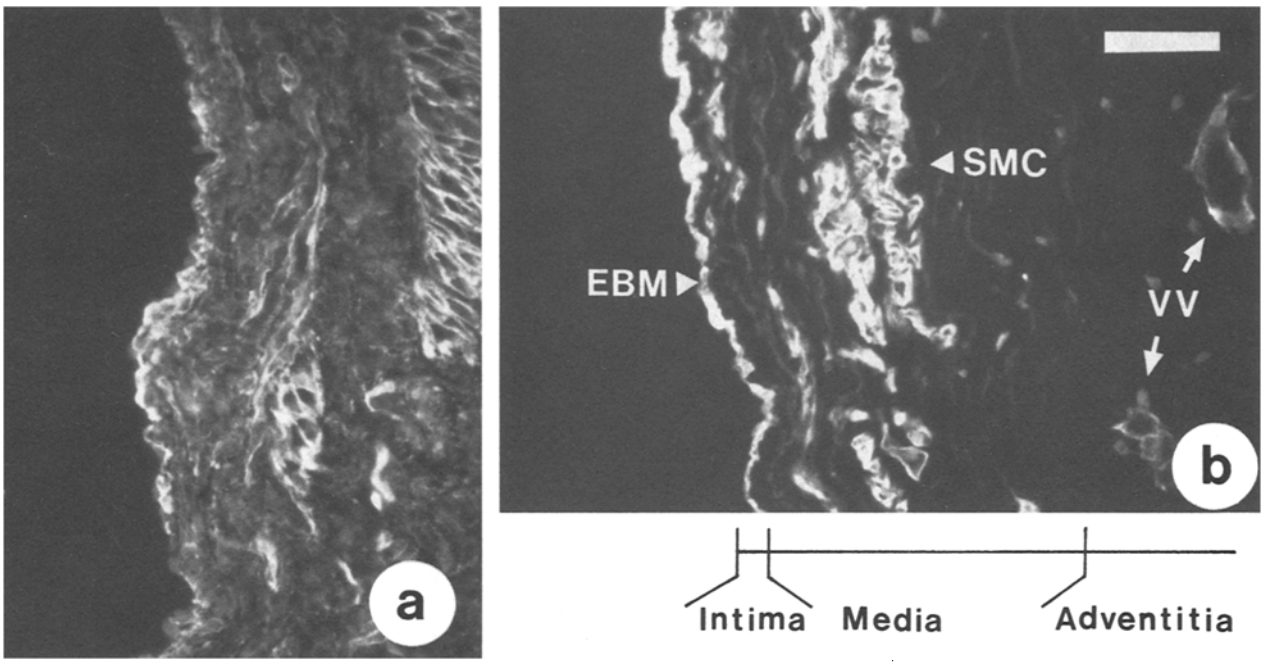


Figure 1. Cryosections of jugular veins stained for collagen type VI in (*a*) and collagen type IV in (b). Staining is strongest around smooth muscle cells for both collagen variants. Additionally, diffuse staining throughout the vascular wall is visible for type VI collagen. Lumen is to the left. The three layers of the vein, namely intima, media and adventitia, are indicated in (*b*). EBM, endothelial basement membrane; SMC, smooth muscle cell clusters; VV, vasa vasorum. Original magnification ×100. Scale bar 50 µm.

*Results and discussion*

The distribution of type VI collagen in a control vein and in thickened aneurysm sac walls is compared with the distribution of type IV collagen on identical tissue samples in figures 1 and 2. In all control veins and also in the control arteries diffuse staining for type VI collagen was visible throughout the blood vessel wall. More pronounced staining was found around smooth muscle cells, around vasa vasorum and nerve fibres. This pronounced fluorescence was very similar to the staining found with antibodies to type IV collagen (compare fig. 1, a and b). These findings are in agreement with previous studies [6, 7] on human and bovine aortas. Both groups reported diffuse distribution of type VI collagen throughout the aorta with increased density on the smooth muscle cell surface.

In the thickened vascular wall of younger aneurysms (up to 35 months post-operatively) type VI collagen staining was also diffusely distributed throughout the blood vessel but seemed to become gradually stronger towards the adventitia. Strong laminar staining, identical to the basement membrane staining of type IV collagen, was superimposed on the diffuse staining in both the thickened intima and the media (fig. 2, a, b). This laminar staining nearly disappeared in the older aneurysms (over 3 years) but the diffuse fluorescence of type VI collagen was still present (fig. 2, c, d). Some of the older aneurysm sac walls showed varying degrees of calcification. In some parts of these tissues thickened laminar basement membrane structures could be visualized with type IV collagen and type VI collagen antibodies (fig. 2, e, f). Diffuse distribution of type VI collagen still remained prominent.

Little is known about the changes of type VI collagen under pathological conditions. An increase was demonstrated in cirrhotic liver [8, 9] neurofibroma [10], sun-damaged skin [11], cutis laxa [12] and in brain vessels of chronic hypertensive patients [13]. In the latter study, small focal intimal proliferation of smooth muscle cells on basal arteries was observed, resembling minimal arteriosclerotic changes. In these lesions a fine network of type VI collagen could be demonstrated. One report on quantitative changes of collagen type VI in human atherosclerosis showed that the amount of this collagen variant seemed to increase with the severity of the disease [14].

Immunoelectron microscopic localization has shown that type VI collagen forms a network of microfibrils which appears to be independent in orientation from the major fibrillar elements [6]. This second network possibly functions to support or provide an organizational network for the larger collagen fibrils [15]. Our results show that type VI collagen exhibits a diffuse distribution throughout the blood vessel wall of healthy and of atherosclerotic tissue. This diffuse distribution is still present even when the type VI collagen, that codistributed with basement membrane structures, had disappeared. Thus this collagen variant also seems to form a second network during the process of vascular mural thickening and this appears to be maintained throughout the disease process.

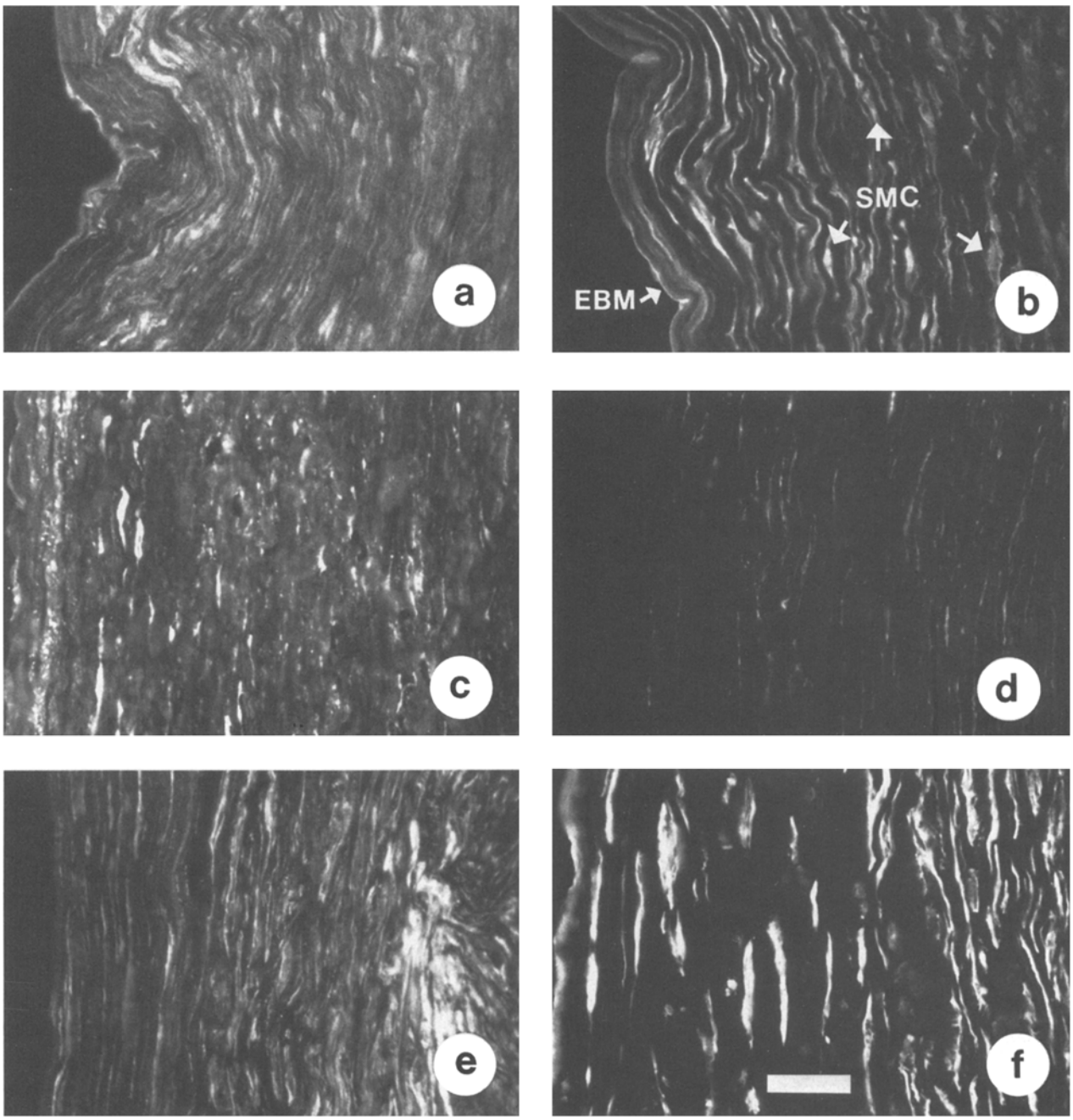


Figure 2. Thickened intimal areas of aneurysm sac walls of a two-year-old (*a* and *b*), an eight-year-old (*c* and *d*) and an eight-year-old calcified aneurysm (*e* and *f*). Media and adventitia are not visible in these figures. Staining is for type VI collagen in *a*, *c* and *e* and for type IV collagen in *b*, *d* and *f*. Some smooth muscle cells (SMC) and the endothelial basement membrane (EBM) are indicated in (*b*). Lumen in all figures is to the left. Original magnification ×100. Scale bar 50 µm.

Acknowledgments. The authors would like to thank Dr Mark Gibson, University of Adelaide, South Australia, for supplying us with type VI collagen antiserum. This work was supported by the National Heart Foundation of New Zealand and the Medical Research Council of New Zealand.

1 Chung, E., Rhodes, R. K., and Miller, E. J., Biochem. biophys. Res. Commun. *71* (1976) 1167.
2 Timpl R., and Engel, J., in: Structure and Function of Collagen Types, p. 105. Eds R. Mayne and R. E. Burgeson. Academic Press, Orlando 1987.
3 Stehbens, W. E., Surg. Gynec. Obstet. *149* (1979) 43.
4 Stehbens, W. E., Proc. R. Soc. Lond. B *185* (1985) 357.
5 Kittelberger, R., Davis, P. F., and Stehbens, W. E., Acta histochem. (1989) in press.
6 von der Mark, H., Aumailly, M., Wick, G., et al., Eur. J. Biochem. *142* (1984) 493.
7 Gibson, M. A., and Cleary, E. G., Coll. Rel. Res. *3* (1983) 469.
8 Biempica, L., Mokrecki, R., Wu, C. H., et al., Gastroent. *77* (1977) 1213 (abstr.).
9 Schuppan, D., Ruhlmann, T., and Hahn, E. G., Analyt. Biochem. *149* (1985) 238.

10 Fleischmajer, R., Timpl, R., Dziadek M., et al., J. Invest. Dermat. *85* (1985) 54.
11 Chen, V. L., Fleischmajer, R., Schwartz, E., et al., J. Invest. Dermat. *87* (1986) 334.
12 Crawford, S. W., Featherstone, J. A., Holbrook, K., et al., Biochem. J. *227* (1985) 491.
13 Roggendorf, W., Opitz, H., and Schuppan, D., Acta neuropath. *77* (1988) 55.
14 Murata, K., Kotake, C., and Moroyama, T., Artery *14* (1987) 229.
15 Mayne, R., Arteriosclerosis *6* (1986) 585.

0014-4754/90/030264-04$1.50 + 0.20/0


## Metabolism of 3β-hydroxycholest-5-en-26-oic acid in hamsters

Y. Ayaki*, E. Kok and N. B. Javitt

**Department of Biochemistry, Medical School of Tottori University, Yonago, Tottori 683 (Japan), and Division of Hepatic Diseases, New York University Medical Center, New York (New York 10016, USA)*
*Received 26 June 1989; accepted 9 August 1989*

*Summary.* Metabolism of 26-hydroxycholesterol to 3β-hydroxychol-5-en-24-oic acid and other C24-bile acids has been expected to occur by way of 3β-hydroxycholest-5-en-26-oic acid in studies in vitro. 3β-Hydroxycholest-5-en-26-oic acid was infused intravenously into bile fistula hamsters and the following C24-bile acids were identified: 3β-hydroxychol-5-en-24-oic acid, lithocholic acid, chenodeoxycholic acid, and a small amount of cholic acid.
*Key words.* 3β-Hydroxycholest-5-en-26-oic acid; bile acids; hamsters; metabolism.

Previous studies in hamsters reported that 26-hydroxycholesterol is metabolized to 3β-hydroxychol-5-en-24-oic acid, lithocholic acid, chenodeoxycholic acid, and cholic acid[1] and that 3β-hydroxychol-5-en-24-oic acid is also metabolized to lithocholic acid and chenodeoxycholic acid[2,3]. In vitro studies of rat liver by Mitropoulos and Myant indicated that 3β-hydroxycholest-5-en-26-oic acid was the intermediate in the conversion of the C24 bile acids from cholesterol[4]. We synthesized [16,22-$^3$H]-3β-hydroxycholest-5-en-26-oic acid from [16,22-$^3$H]-26-hydroxycholesterol[5] and determined its metabolism in the hamster in vivo.

*Materials and methods*

[16,22-$^3$H]-3β-hydroxycholest-5-en-26-oic acid (0.28 μCi/μmol) was previously prepared from [16,22-$^3$H]-26-hydroxycholesterol which was synthesized from diosgenin[1,5]. 24-$^{14}$C-labeled lithocholic, chenodeoxycholic, and cholic acids were purchased from Amersham/Searle (Arlington Heights, IL) and/or New England Nuclear (Boston, MA). [24-$^{14}$C]-3β-hydroxychol-5-en-24-oic acid was the compound described previously[3].

The steps used to identify metabolites were (a) solvolysis, (b) hydrolysis, (c) excretion, (d) methylation, (e) column chromatography using glycophase G on controlled-pore glass 80–100 mesh (Pierce Chemical Co., Rockford, IL), (f) HPLC using μPorasil (10 μM silica, Waters Associates, Milford, MA) or thinlayer chromatography, and (g) reverse isotope dilution. All these steps have previously been described in detail, including the quantification of radioactivity by liquid scintillation spectrometry using a Beckmann CPM200 instrument[2,3,6].

Male Syrian hamsters weighing about 100 g each were prepared during pentobarbital anesthesia as described previously[6]. The animal's bile acid pool was depleted by overnight drainage. Then an infusion, made by dissolving the sodium salt of [16,22-$^3$H]-3β-hydroxycholest-5-en-26-oic acid in 0.2 ml of propylene glycol and adding it, with vortexing, to a sterile solution of 5% dextrose in 0.45% NaCl containing 25% of human serum albumin, was administered intravenously over a 10-min period.

*Results*

Bile samples were collected separately at 30-min intervals over a period of 4 h and were then pooled. Eighty percent of the radioactivity from the infused material was recovered in the bile. An aliquot of bile was solvolyzed, hydrolyzed, extracted with ethyl acetate, and methylated. The methyl esters of the radioactive metabolites obtained from hamster bile were fractionated on glycophase G (fig.).

After addition of a [24-$^{14}$C] labeled standard (3β-hydroxychol-5-en-24-oic acid or lithocholic acid) to the radioactive samples obtained from the monohydroxy region, HPLC analysis as both the methyl ester and the methyl ester acetate detected both 3β-hydroxychol-5-en-24-oic acid and lithocholic acid. A small amount (4–10%) of the infused 3β-hydroxycholest-5-en-26-oic acid was still present in this fraction.

On HPLC analysis, the radioactive material obtained from the dihydroxy region of the glycophase column had the retention time of chenodeoxycholic acid methyl ester. Addition of authentic [$^{14}$C]-chenodeoxycholic acid methyl ester and HPLC analysis as both the methyl ester