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Composition of Surface Layers in Unfixed Autologous Fascia Lata Heart Valve Grafts

A transmission electron microscopical study

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ABSTRACT

Sixty-nine patients underwent aortic valve replacement with frame mounted non-fixated fascia lata grafts. Nine patients still have their fascia lata valve in place after a period of between 9 to 11 years. Fifty-two grafts were available for histo-pathologic examination. The mechanism of valve thickening was studied by transmission electron microscopy (TEM). The major factor contribution to the thickening seems to be a surface accumulation of complex proteins, fibrin and platelets. Within the valvular tissue itself, sub-cellular degenerative products in cell cytoplasm, the interstitium and in the sub-surface layer also contributed. The thickening never exceeded one fifth of the original thickness of the valve.

INTRODUCTION

Unfixed autologous fascia lata heart valve graft material is fairly rough on one surface, with slightly protruding smooth collagen bundles at the other (14). The rough side is initially covered by fat, connective tissue and vessels. After surgical tailoring, some remnants of small damaged fibers of fascial tissue remain. Implanted in the blood stream, the fascial tissue is rapidly covered by a fine, granular material, probably protein (2, 14). Small fragments of fibrin and platelets can be visualized (14). This covering makes the surface smoother and may explain the frequent lack of clinical thrombus-formation with these prostheses (6, 10, 11). The blood born elements which adhere to the surface later become organized mainly on the rough surface. Eventually the valvular tissue itself undergoes focal degenerative and hyper-plastic changes (3, 4, 15), probably as a result of mechanical stress (5). Different stages of tissue breakdown can be illustrated (14, 15). Finally, shrinkage calcification and valve thickening appear in some of the valves with subsequent failure due to rupture or dysfunction (4). The aim of this paper is to discuss the possible contribution to valve thickening by the endogenous breakdown of fascial tissue itself which becomes enveloped by the

surface pseudomembrane.

PATIENTS

Between November 13, 1969, and June 7, 1972, 69 patients underwent isolated aortic valve replacement with a frame-mounted fascia lata graft. Of the 55 patients released from the hospital, 30 died after between 5 months and 8½ years. In 3 of these cases the cause of death was graft failure and, in 27, myocardial failure sometimes in combination with septic infection (5 patients). Twenty-two patients were reoperated due to graft failure with aortic regurgitation and 6 of these died during or soon after the reoperation. Nine patients still have their original fascia lata valve graft in place after a period of between 9 and 11 years and most of them are doing well (fig. 1).

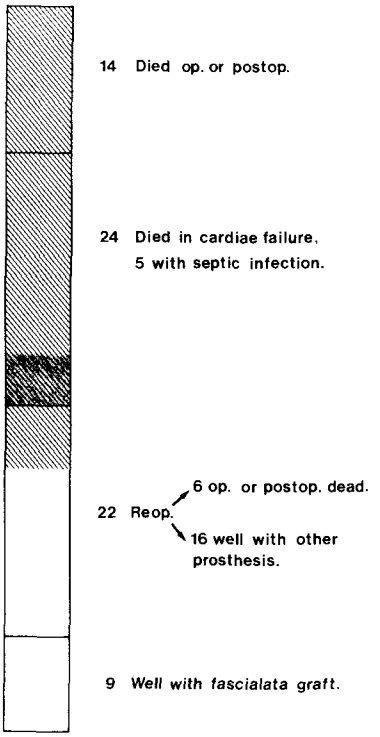

Fig. 1. Clinical material. Ultimate fate of 69 fascia lata heart aortic valve operated patients.

METHODS

The tissue was inbedded for scanning electron microscopy (SEM) as previously described (14, 15). In order to carry out transmission electron microscopy (TEM) the scanning electron microscopy tissue was trimmed into small blocks 3 x 3 mm and put into a mixture of propylenoxide/epon 1:1 over night. The following morning the tissue was left in epon for 4 to 6 hours. It was then put in Beem^{R}-capsules filled with freshly made resin mixture and hardened at 60^{o}C for 3 to 4 days. The blocks were cut in the usual way on a

LKB ultratome^{R}. The sections were mounted on formvar coated grids, stained with lead-citrate for 4 minutes, rinsed and dried, counterstained with uranyl-acetate for 30 minutes at 37^{o}C, and finally rinsed and dried. The gold layer put on the valvular surface in the preparation for the SEM investigation served as a marker for identification of fascial surface.

RESULTS

Central parts of the normal valvular tissue showed areas with well preserved fibrocytes and fibroblasts surrounded by numerous collagen fibers (14, 15). Sometimes markedly degenerated areas were observed, showing fibroblasts with endocytic activity, and interstitial tissue which contained amorphous material and only a very few collagen fibrils (fig. 2). Some of the cells contained partly degenerated rough and smooth endoplasmic reticulum, primary and secondary lysosomes and other cell debris (figs. 3, 4). Some fibroblasts

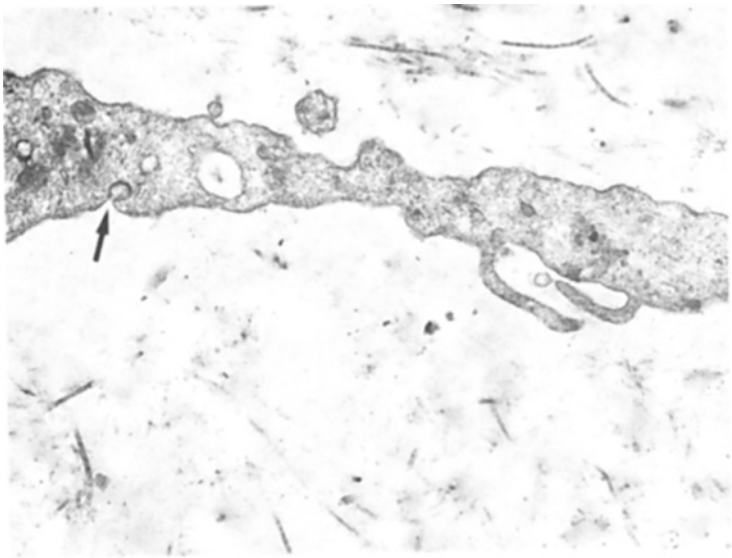

Fig. 2. Electron micrograph of fairly well preserved fibroblast with endocytic activity (arrow) and slender protrusions. Degeneration is evident in the intercellular space where mostly amorphous material and only scant collagen bundles are seen (orig. mag. x 5 000).

showed slight bulging of the plasma membrane in association with lysosomes and outside the cell expelled residual bodies were visualized (fig. 4). Sometimes the interstitium was filled with well preserved collagen bundles showing cross striation (fig. 5). Studies of areas closer to the surface showed abundant collagen fibrils together with light ground substance, cell debris and degenerated platelets (fig. 6). It was thus possible to find sub-cellular breakdown products within all parts of the valvular tissue; in cell cytoplasm as well as in the interstitium. An accumulation of these products was seen at

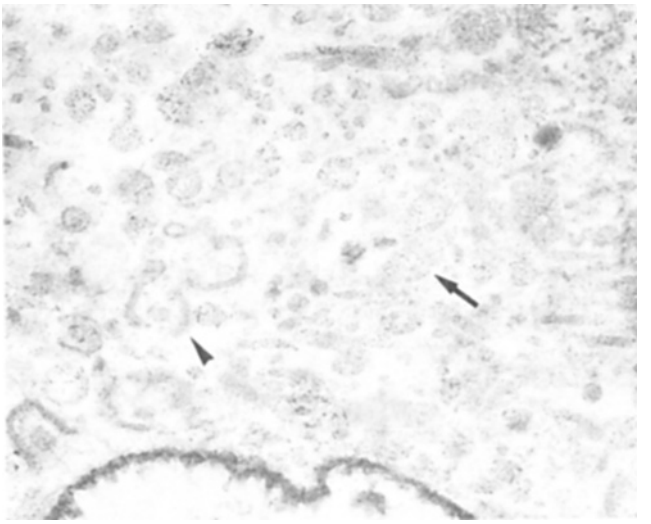

Fig. 3. Electron micrograph of fibroblast. Part of nucleus (bottom) and cytoplasm filled with rough endoplasmic reticulum autophagocytic vacuoles (arrow-head), primary lysosomes (arrow) and cell debris (orig. mag. x 16 000).

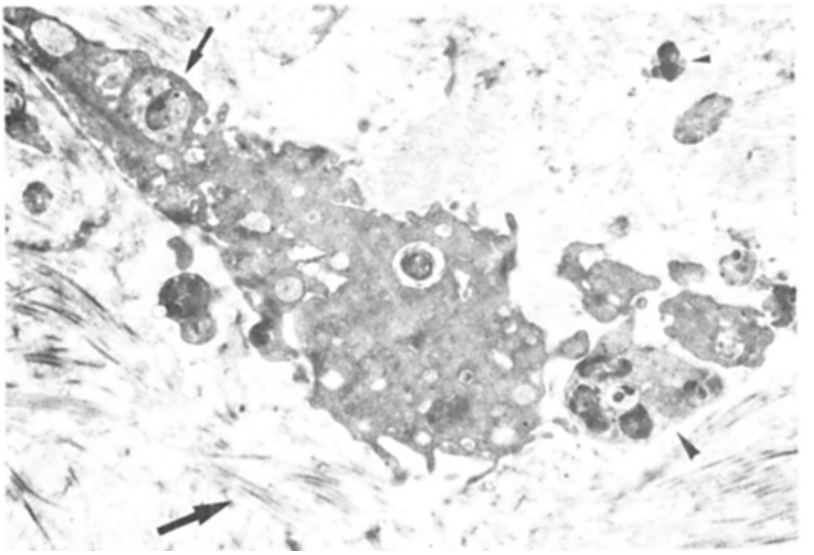

Fig. 4. Electron micrograph of partially degenerated fibroblast containing primary and secondary lysosomes (large arrow-head), slight bulging of plasma membrane in association with a lysosome (small arrow), and expelled lysosomal material (small arrow-head). In the interface abundant collagen fibers, showing cross striation (large arrow), (orig. mag. x 5 000).

the surface, but the thickness of this accumulation never exceeded one fifth of the original valvular thickness.

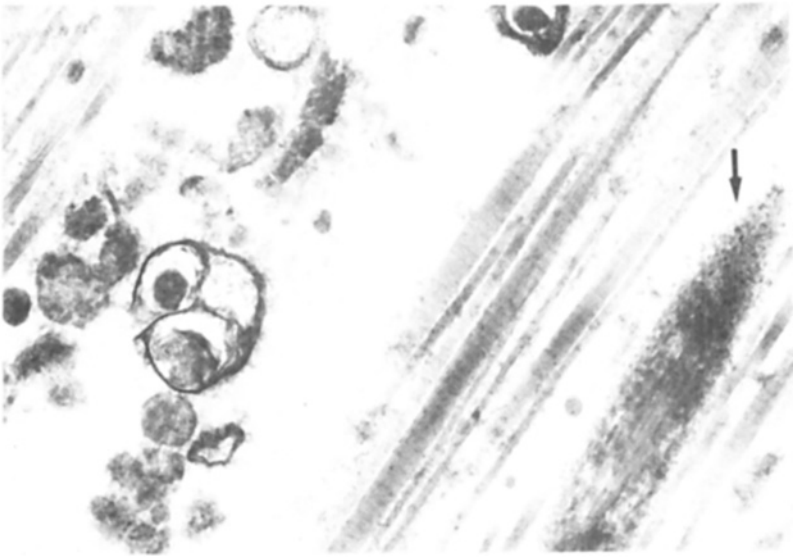

Fig. 5. Electron micrograph of collagen bundles, partly degenerated (arrow), partly showing evident cross striation. In the interface secondary lysosomes of different sizes and shapes (orig. mag. x 20 000).

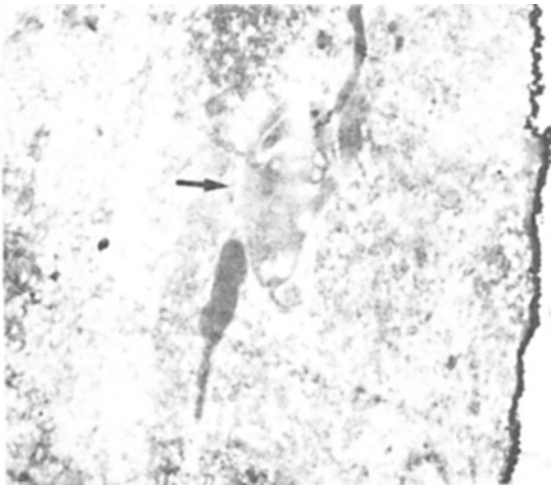

Fig. 6. Surface area containing degenerated platelets (arrow), amorphous material and cell debris. Outer surface is covered by electron opaque material.

DISCUSSION

From this and earlier studies (2, 4, 6, 7, 9, 10, 14, 15) it is evident that the events taking place at the surface of and centrally in bio-prosthetic valvular tissue are very complex and that the causes of thickening, shrinkage and dysfunction in these prostheses are not fully understood. In this study it has been shown that transitory adhesion of complex proteins and platelets

takes place on the surface of the tissue valve, building up a surface membrane. Within this membrane and in the center of the cusp, breakdown products from viable fascial cells could be visualized both within the cell cytoplasm as well as within the interstitial tissue. It was thought that these two processes in combination contributed to the thickening of the valve as adhesion and degradation products occupied so much space as visualized in the TEM investigation.

The formation of a complex protein layer at the surface was thought to prevent further platelet adhesion and that a tissue antithrombogenic inducing factor exists in the proteoglycan fraction (2). However, continuous adhesions of fibrin fragments or thrombous material seem to take place on the cusp surface. These organize to form a connective tissue pseudointima (3, 10, 11, 12, 13). In order to prevent this process, the use of anticoagulants in the initial phase after valve implantation e.g. 6 weeks to 10 months, has been advocated (1, 5, 9). When using non-fixed bio-prosthetic valvular tissue it was evident from this study that breakdown products from viable cells within the fascial tissue itself, contribute to the thickening of the cusp. This factor can only be reduced by fixation of the valvular tissue before insertion into the host by e.g. formaldehyd or glutaraldehyd. The latter is now in common practice.

ACKNOWLEDGEMENT

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REFERENCES

1. Bernhard, A., Thiede, A., Müller-Hermelink, H-K., Krug, A., Fischer, K. & Yankah, A.C.: Mitral valve replacement with autologous fascia lata. Result of morphologic examinations. J. Thorac. Cardiovasc. Surg. 64:94, 1973.
2. Carpentier, A., Relland, J., Carpentier, S., Lessana, A. & Gory, G.: Tissue antithrombogenic inducing factor: Experimental evidence and practical applications in the construction of carciac valves and the artificial heart. Thromb. Diates. Haemorrag. 34:557, 1975.
3. Dubiel, W.T., Johansson, L. & Willén, R.: Postoperative changes in autologous fascia lata heart valve grafts. Ann. Thorac. Surg. 15:140, 1973.
4. Dubiel, W.T. & Willén, R.: Viability Properties in Autologous Fascia Lata Heart Valve Grafts, late observations in: Shiley European Carciovascular Conference, Chamonix, France, 1979, p. 161.
5. Hartig, F.: Uberlagungen zur funktionellen Struktur der Fascie. Zbl Vet-Med. (Reie A) 13:85, 1966.
6. Jonescu, M.I., Pakrashi, B.C., Mary, D.A.S., Bartek, I.T. & Wooler, G.H.: Long term evaluation of tissue valves. J. Thorac. Cardiovasc. Surg. 68: 361, 1974.
7. Senning, Å.: Fascia lata replacement of aortic valves. J. Thorac. Cardiovasc. Surg. 54:465, 1967.

8. Senning, A.: Discussion of paper by Jonescu et al: J. Thorc. Cardiovasc. Surg. 68:379, 1974.
9. Shiley European Cardiovascular Conference, Chamonix, France, 1979, p. 108.
10. Silver, M.D., Hudson, R.E.B. & Trimble, A.S.: Morphologic observations on heart valve prostheses made of fascia lata. J. Thorac. Cardiovasc. Surg. 70:360, 1975.
11. Silver, M.D. & Trimble, A.S.: Structure of autologous fascia lata heart valve prostheses. Arch. Path. 93:109, 1972.
12. Trimble, A.S., Gunstensen, J., Silver, M.D., Aldridge, H.E., Schwartz, L. & Morch, J.E.: Aortic valve replacement with fascia lata: an encouraging late study. J. Thorac. Cardiovasc. Surg. 68:219, 1974.
13. Welch, W., Povliege, P. & Promo, G.: Autologous fascia lata cardiac valve replacement. Thorax 26:271, 1971.
14. Willén, R., Dubiel, W.T. & Johansson, L.: Viability and surface properties of autologous fascia lata heart valve grafts. Ann. Thorac. Surg. 18:597, 1974.
15. Willén, R.m Dubiel, W.T. & Johansson, L.: Viability properties in autologous fascia lata heart valve grafts. Ann. Thorac. Surg. 19:494, 1975.

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