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Aorta Valve Replacement Using Autologous Fascia Lata Transplants

Late Morphological Changes

An Electron Microscopic Study

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Summary. Ten transplants of autologous fascia lata used as human aortic valves were examined by light and electron microscopy. Failure of valvular function requiring resection occurred between 1 year 2 months and 7 years 10 months post transplantation. Gross examination showed thickening and retraction of the valvular cusps.

The significant light and electron microscopic findings are: absence of endothelialisation, late and sparse revascularisation, homogenous or organized superficial deposits, tissue necrosis, often accompanied by calcification, and proliferation of the connective tissue.

The pathogenesis of these lesions, which are probably metabolic and mechanical in origin, is discussed.

The changes in connective tissue adequately explain the grossly apparent thickening and retraction of the valves resulting in progressive aortic insufficiency.

Introduction

Various valvular prothesis are being used in cardiac surgery for the treatment of aortic incompetence. Patients receiving a valvular prothesis must be followed closely during the immediate and long term post-operative period because of the increased risk of hemolytic, infectious or thromboembolic complications. In the last decade, repeated attempts have been made to transplant hetero-, homo-, or autologous grafts of fascia lata to minimize these complications. The collagenous structure of a fascia lata graft is theoretically ideal for this purpose since it is easily incorporated by the surrounding tissue with no risk of immunologic rejection.

Most authors agree that the results of short term studies of transplanted fascia lata are excellent. The replaced valve remains fine and supple showing no signs of calcifications, even after 45 months. The transplant of fascia lata persists as viable collagen tissue retaining its morphological and especially functional characteristics. Recent studies have shown, however, that aortic insufficiency may arise even during the initial post operative months (Cox and Marti, 1972; Rothlin *et al.*, 1973; Rothlin *et al.*, 1974; Senning, 1971) or at a later date (Ionescu *et al.*, 1970; Senning, 1967; Silver and Trimble, 1972). Bacterial endocarditis is often noted as a late complication (Cox and Marti, 1972; Ionescu *et al.*, 1970; Ross, 1966).

The present study attempts to clarify some aspects of the pathogenesis of the late valvular lesions on an ultrastructural level.

Material and Methods

Ten autologous fascia lata grafts resected from patients showing symptoms of aortic insufficiency were provided by the Department A of Surgery, Kantonsspital University of Zurich (Prof. Dr. Å. Senning). In this Department more than 200 aortic valves were replaced by autologous fascia lata grafts from 1962 to 1971.

In the 10 cases studied, an average of $3^1/_2$ years elapsed before requiring replacement due to aortic insufficiency. The shortest interval was 1 year 2 months, the longest being 7 years 10 months (see Table 1).

The tissue for ultrastructural study was fixed in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide and embedded in Epon. Semi-thin sections, approximately 1.5 μ thick were stained with a mixture of methylene and azur blue II. Gold sections (600–800 Å) were sectioned using an LKB ultratome with a diamond knife, and stained with uranyl acetate and lead citrate. A Zeiss EM 9 electron microscope was used to examine the tissue.

Paraffin sections stained with hematoxylin and eosin, Alcian blue and van Gieson stain were used for light microscopy. For histological comparison, normal nongrafted fascia lata was studied in three of the same patients receiving a valvular graft. This tissue and the resected fascia lata transplant were prepared in identical manner for histological examination.

Results

Gross examination revealed no fundamental differences among the fascia lata grafts and their duration of transplantation. In most transplants, the form of the valvular pocket was preserved, although the valves exhibited various degrees of thickening and retraction. The free edge of the valve was sometimes normal, but more often indented. The commissures, when present in the resected material, were intact, free of adhesions or dehiscence of the sutures. The thickness of the resected valves often varied greatly, even within one valvular cusp, from 1 to 4 mm. (At time of transplantation, the fascia lata of the constructed valve is free of surrounding connective and adipose tissue, and is no thicker than 0.6–0.9 mm (Cox and Marti, 1972; Silver and Timble, 1972)). Pathological lesions were most pronounced on the ventricular side of the graft. The degree and extent of these lesions varied not only from case to case but also from cusp to cusp. The normal texture of the fascia lata was often easily recognisable, although it was sometimes concealed behind a white gelatinous coating similar to drops of candlewax.

In more than half of the cases, the graft contained focal calcifications. In three cases (case no. 7, 8, 10), small verrucosities were found. Perforation of a cusp was present in only one case (case no. 10, following bacterial endocarditis).

Light Microscopy

The valvular surface is scattered with deposits of varying thickness. The deposits are dense, either homogenous or pitted with small lacunae containing cellular fragments. In some areas, the surface is covered by young connective tissue with many fibroblasts. In the valvular pocket, there are intact areas, containing normal numbers of fibrocytes and isomorphic collagen fibrils lying parallel to one another. There are also large anucleate areas of tissue necrosis, often with calcifications.

At the periphery of these necrotic foci, the cells frequently exhibit vacuolation of the cytoplasm and pyknotic nuclei encircled by a clear halo. Between the cells,

Table 1

Case No.	Patient	Age/ Sex	Etiology of Endocarditis	Indication for valve grafting	Operation	Duration of transplant function (months)	Indication for graft replacement	Method of valve replacement
1	H.O.	62, ♂	?	Aortic insufficiency	Fascia lata graft	13.5	Insufficiency	Regraft of fascia lata
2	P.L.	47, ♀	Rheumatic fever	Aortic insufficiency + stenosis	Fascia lata graft	18	Insufficiency	Björk
3	M.A.	62, ♂	Rheum. fever	Aort. insuff.	Fascia lata graft	20	Insufficiency	Björk
4	G.G.	25, ♂	?	Aortic + mitral insufficiency	Fascia lata graft + annuloraphy	22	Insufficiency	Björk
5	S.A.	43, ♂	?	Aortic insuff. + stenosis	Fascia lata graft	27	Insufficiency	Björk
6	A.P.	31, ♂	Rheum. fever, Klippel-Trénauney-syndrome	Aortic insufficiency + stenosis	Fascia lata graft	30	Insufficiency	Ionescu
7	S.F.	35, ♀	Rheumatic fever	Aortic + mitral insufficiency	Fascia lata graft	35	Insufficiency	Regraft of fascia lata
8	H.K.	38, ♀	Rheumatic fever	Aortic insufficiency	Fascia lata graft	78	Insufficiency + calcification	Björk
9	P.A.	57, ♀	?	Aortic + mitral insufficiency	Fascia lata graft + mitral annuloraphy	84	Insufficiency + endocarditis	Björk
10	S.P.	42, ♀	Event. gonococcus	Aortic insufficiency + stenosis	Fascia lata graft	94	Insufficiency + endocarditis with small perforation	Björk

there is an abundance of a fine fibrillar, faintly eosinophilic substance which also stains faintly with Alcian blue. Some of these areas have the general appearance of primitive cartilage. These lesions have often been described as chondroid degeneration. The few remaining recognisable collagen fibrils in these areas are swollen, pale and of varying thickness. Blood vessels are found only at the base of those grafts transplanted for as long as 22 months (case no. 4–10). At a later stage, vessels are also visible in the valvular stroma, but always far from the free edge of the valve.

Although cases 9 and 10 (7 years and 7 years 10 months) had clinically diagnosed bacterial endocarditis, the graft revealed only very small foci of cellular infiltration. Lymphocytes and monocytes predominated, with only a rare polymorphonuclear leucocyte.

Electron Microscopy

Normal Human Fascia Lata. The middle third of normal human fascia lata appears as a tissue largely devoid of cells, formed essentially by collagen microfibrils. The microfibrils lie generally parallel to one another and are oriented either perpendicularly to the main axis of the fascia lata in the thin layer, or longitudinally in the deep layer. It is the much thicker deep layer which forms the important element of the valvular graft. The microfibrils show the periodicity of mature collagen (about 640 Å). In transverse sections, there is a great difference in size between the microfibrils in the same microscopic field, 800 Å to 2500 Å. It is, however, the microfibril of average thickness which predominates (Fig. 7). Elastic fibers are rare in normal fascia lata. The cellular elements are comprised of fibroblasts or fibrocytes. They are characterized by a well-developed cytoplasm lined with fine extensions and by an oval nucleus, rich in chromatin, often with a distinct nucleolus. The cytoplasm contains few mitochondria and a well developed ergastoplasm lined with many ribosomes. In addition to these typical elements, a few cells of histiocytic or monocytic type were present, as well as an occasional poorly differentiated mesenchymal cell. The vascular element is represented merely by an occasional fine capillary.

Fascia Lata from Resected Valvular Transplants. Semi-thin sections possess the following main features:

1. Predominantly homogenous deposits on the surface of the graft.
2. Extended areas of necrosis, with or without calcification.
3. Proliferation of collagen tissue.

The ultrastructure shows superficial deposits of a dense, finely granular substance (Fig. 1). This substance is pitted sporadically by irregularly edged lacunae, containing numerous cellular fragments (Fig. 2). Light microscopic study shows that these deposits are generally of hyaline nature. They are present in varying degrees of thickness and are situated sporadically on the surface of all the examined valves. It is particularly noteworthy that no real endothelium is present on the surface of these deposits (Fig. 1). Although a continuous cell layer was never observed, an occasional isolated cell of endothelial type was seen.

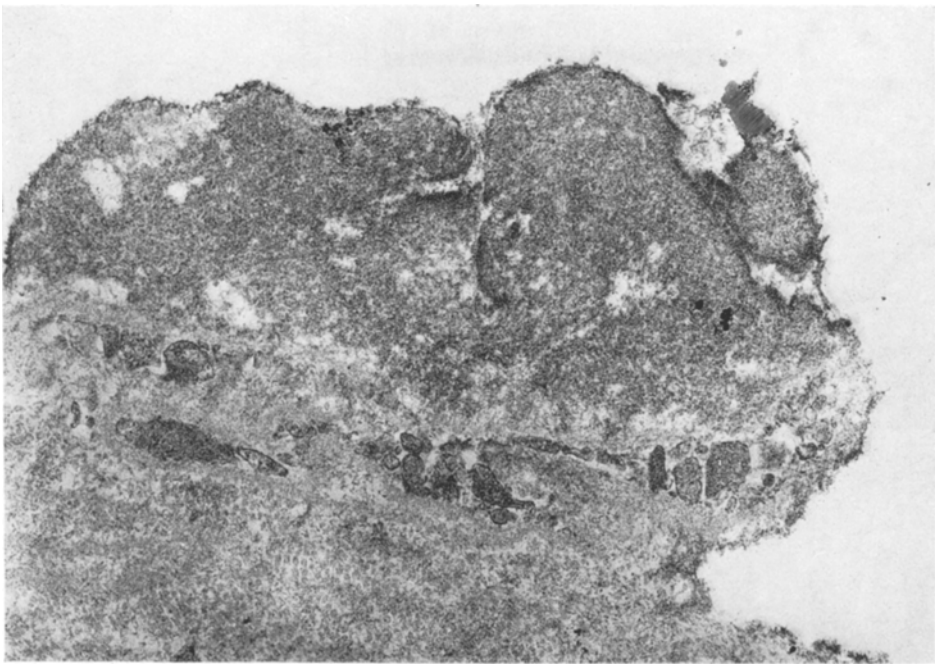

Fig. 1. Superficial deposit lacking endothelial layer. (× 21000. Case no. 8, HZ 4940/72)

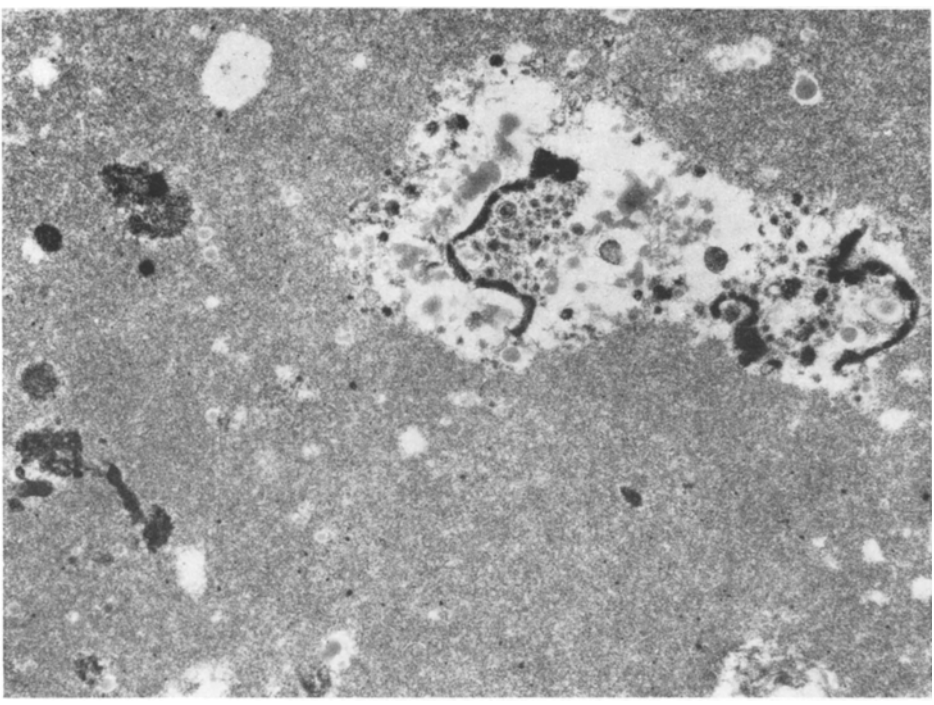

Fig. 2. Superficial deposit with lacunae and cellular remnants. (× 14000. Case no. 1, HZ 4470/72)

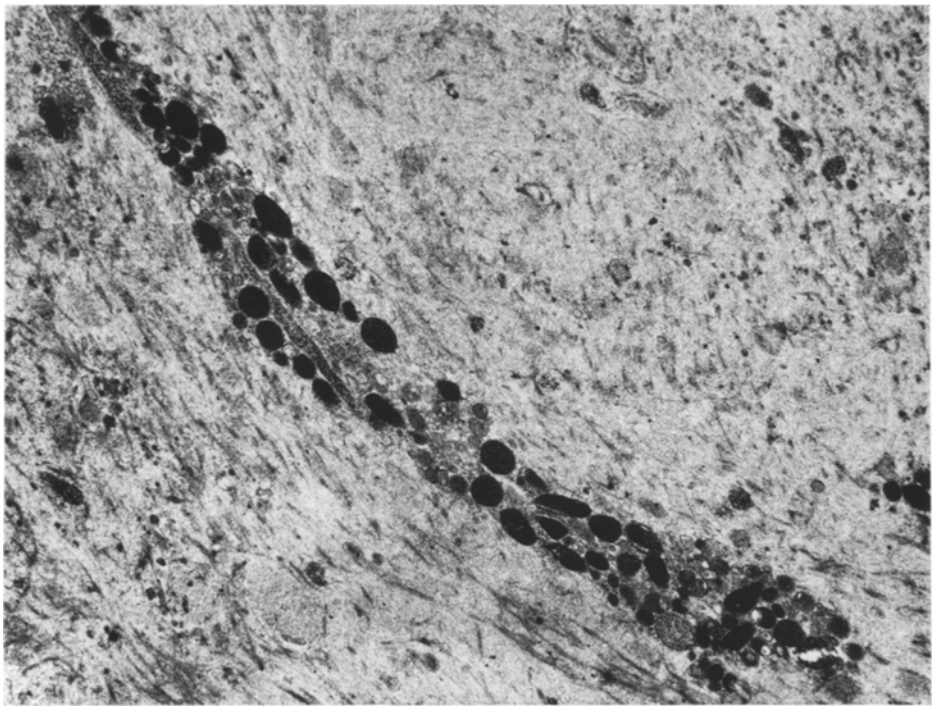

Fig. 3. Degenerating fibrocyte at the periphery of necrotic area. The cytoplasm is filled with lipid laden vacuoles. (× 20000. Case no. 5, HZ 5042/72)

Beneath the surface, areas of necrosis occupy a part or occasionally even the entire thickness of the graft. They consist of areas of fibrolysis within which the collagen microfibrils are swollen, seemingly disintegrating into microfilaments. They have lost their characteristic periodicity taking on a fine granulated appearance distributed evenly over the length of the microfibrils. The interfibrillar space is permeated with an osmiophilic, finely granular-fibrilar substance containing numerous cellular fragments. At the periphery of the necrotic areas, a few fibrocytes are intact. Their cytoplasm contains vacuoles filled with lipids or an electronlucent substance (Fig. 3). In many cases, these zones contain numerous hydroxyapatite crystals.

Areas of cellular proliferation are composed of numerous cells of fibroblastic type (Fig. 4) (as many as 15 per low power field compared to 3 per low power field seen in normal fascia lata). These cells exhibit a particularly welldeveloped rough endoplasmic reticulum, often expanding into large vacuoles which contain a flocculant material (Fig. 5). The microfibrils surrounding these cells are remarkably fine and regular in size (300 to 400 Å, normal fascia lata 800 to 2500 Å). They also have the typical periodicity of collagen. Instead of forming fibers in a parallel arrangement, they exhibit a totally random pattern (Figs. 4 and 6). In addition to these elements, there are also very thin microfibrils (approximately 70 Å thick), having a periodicity of approximately 200 Å.

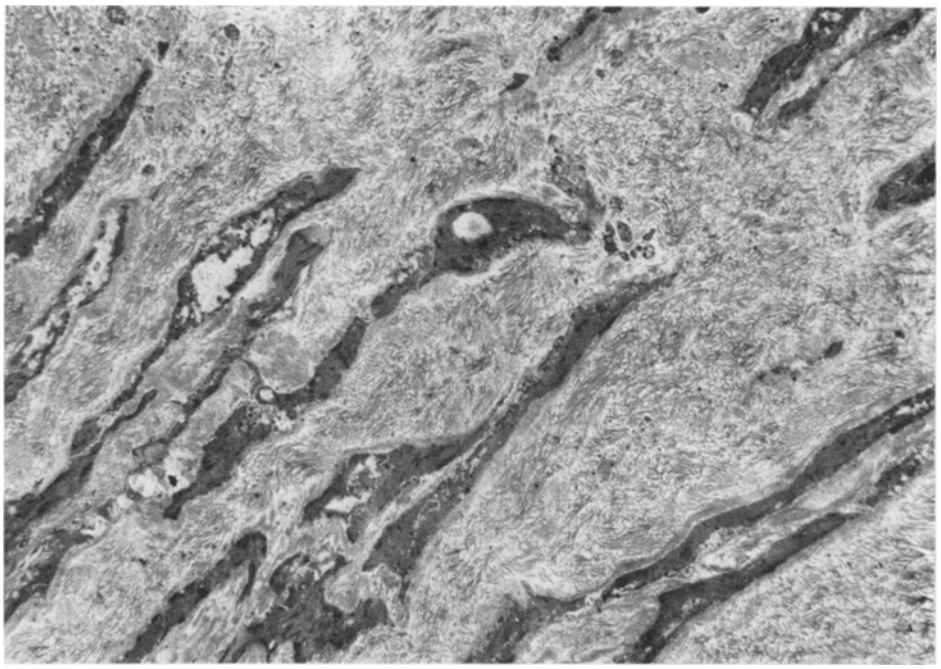

Fig. 4. Area of proliferation. Several fibroblasts and newly formed fibrils are shown. (× 50000. Case no. 7, HZ 4794/72)

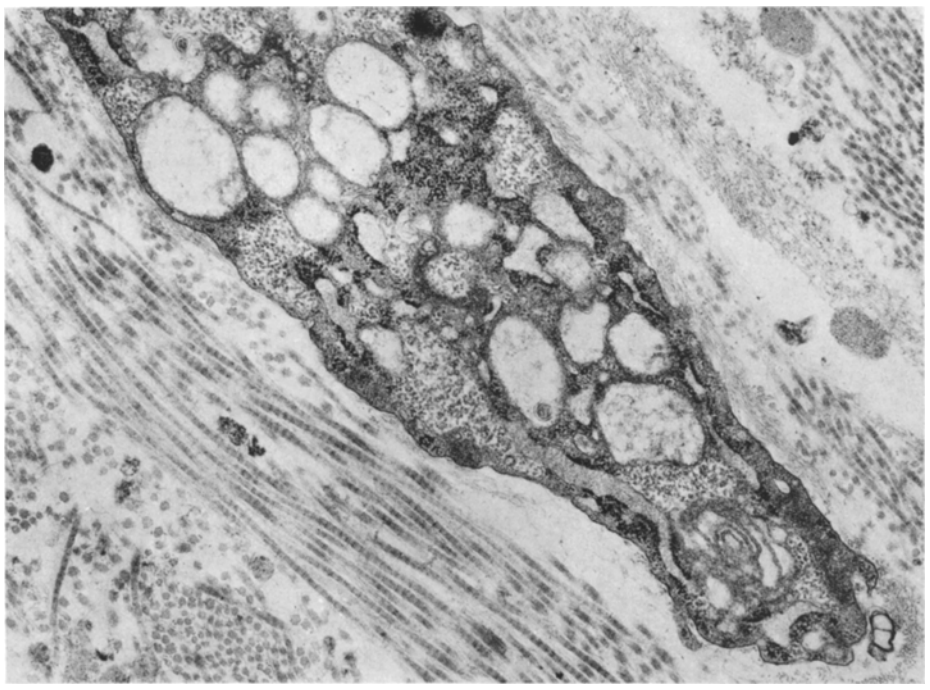

Fig. 5. Cytoplasmic extension of an activated fibroblast. The dilated rough endoplasmic reticulum contains a flocculent material. Around the cell, there are newly formed microfibrils. (× 19000. Case no. 10, HZ 5396/72)

Fig. 6. Randomly arranged, newly formed microfibrils in area of proliferation (×64000. Case no. 8, HZ 4883/72)

The structure of the central areas of the graft is variable. Normal appearing connective tissue, areas with intercellular finely granular osmiophilic substance, and areas of definite necrosis are present. Superficial deposits as well as necrotic foci and cellular proliferations are consistently more pronounced on the ventricular rather than on the outer (aortic) side of the valve.

In case no. 4 (22 months post transplantation), obliterated degenerating capillaries were seen in the valvular stroma. Valves removed after a longer period of transplantation occasionally exhibited newly formed, erythrocyte-filled capillaries of normal structure. They were situated deep within the stroma, always distant from the free edge of the valve.

Discussion

Approximately two hundred autologous fascia lata grafts have been used to replace diseased aortic valves from 1962 to 1972 at the University of Zürich surgical clinic. Conventional light and electron microscopic examination of ten transplants resected because of aortic insufficiency was undertaken. Duration of fascia lata function varied from $1^{2}/_{12}$ to $7^{10}/_{12}$ years, with a mean of $3^{1}/_{2}$ months. The most important findings are as follows:

1. The presence of predominantly homogenous deposits on the surface of the graft.
2. The absence of an endothelial layer.
3. Late, scant vascularization.
4. The presence of extended areas of necrosis, with or without calcification.
5. Proliferation of collagen tissue.

The pathogenesis of the superficial deposits is not clear. While some authors (Gavin *et al.*, 1972; Silver and Trimble, 1972; Wheeler *et al.*, 1972) contend the deposit found on the surface of homografts or heterografts to be of thrombotic origin, others (Barratt-Boyes *et al.*, 1969; Reichenbach *et al.*, 1969) mention only the presence of plasma proteins. Cox and Marti (1972) as well as Senning *et al.* (1967, 1973) believe the superficial deposits on recently transplanted fascia lata to be fibrin. Theoretically, it seems that the strong adhesiveness of thrombocytes to the elements of connective tissue would rather indicate a thrombotic origin. In the cases described, we were unable to find superficial deposits of thrombocytes or fresh fibrillar fibrin with its typical periodicity. The granular substance which we observed is typical of old fibrin. Parts of the superficial deposits are organized by proliferating fibroblasts, thereby forming a "pseudointimal" layer (Silver and Trimble, 1972).

The surface of valvular homo- and heterografts of fascia lata lack an endothelial layer. The absence of an endothelial coating is difficult to explain. The superficial deposits which obviously form very quickly, could by their very presence be capable of preventing the formation of an endothelium. It is also possible, that since the fascia lata graft must be carefully cleaned and scraped to avoid thrombosis, its surface is no longer suitable for the growth of endothelium. Local hemodynamic factors present at the aortic valve may also inhibit proliferation of endothelial cells. Thrombotic deposits in the small and medium arteries become quickly endothelialised, in contrast to those formed in the aorta. On the other hand, synthetic material (e.g. dacron and teflon prosthesis) quickly becomes endothelialised in the aortic position. These variable findings suggest the concommittant influence of tissue and local factors.

In a normal human fascia lata, the vessels are so fine and so sparse that one considers this tissue to be relatively avascular (Ionescu *et al.*, 1970), depending therefore on diffusion (Rasche and Koski, 1972). The few degenerating capillaries we noted represent the remains of the capillaries of the fascia lata excised from its normal connections. It is only after about 2 years that the stroma becomes vascularised, though very sparsely and never exceeding the proximal third of the valve cusp.

The ultrastructure of the areas of necrosis are similar in appearance to that of artificially altered collagen (e.g. heat) (Verzar and Zs-Nagy, 1970). Fatty degeneration of fibrocytes noted at the periphery of necrotic areas occurs frequently in unfavorable metabolic conditions (Schwarz *et al.*, 1962). During this time, these cells are no longer capable of protein synthesis, and therefore revert to lipid synthesis. The lesions noted within the fascia lata graft therefore might

be explained on a metabolic basis. The surface deposits retard or inhibit the diffusion of oxygen and essential nutrients into the depth of the tissue. In support of this theory, focal tissue necrosis was localized in those areas where the superficial deposits were most prominent. The bradytrophic nature of connective tissue would help explain the relatively long latent period before onset of necrosis (Fitton Jackson, 1957; Gross, 1961) even though fascia is less bradytrophic than normal cardiac valves (Schwarz).

This difference in metabolic behaviour suggests that connective tissue of fascia lata is unsuited for survival under the same metabolic conditions as a normal heart valve. Other factors must also be considered, since necrosis may be found in areas free of superficial deposits.

The grafts in six of ten cases exhibited focal calcifications. This finding is in contrast to most publications (Ionescu *et al.*, 1970; Reichenbach *et al.*, 1969; Senning, 1971). The difference may be explained by the fact, that in this study, the grafts had been transplanted for many years. Thus, the calcifications, like the necrosis, are a late event.

The proliferation of connective tissue represents an important qualitative rnd quantitative aspect. The fibroblasts of this connective tissue possess partiaularly well developed ergastoplasm indicating an intense cellular activity. This cesults in the formation of new connective tissue microfibrils. Some authors (Dahmen, 1966; Giesking, 1966) believe that the flocculent material seen in the cysternae of the rough endoplasmatic reticulum is molecular tropocollagen. The tangled order and the regular size (300–400 Å) of the newly formed microfibrils (Fig. 7) is also very typical of the proliferated connective tissue. In addition, microfibrils of an embryonic type are present. They are about 70 Å wide with a periodicity of no more than 200–250 Å (Fig. 7). If one compares these figures with those of the normal fascia lata using Dahmen's method (1966) (a method of graphing the number of fibers of equal size in one single microscopic field), a relatively rough Gaussian curve is formed with a peak between 1 200 and 1 400 Å. The smaller collagen microfibrils such as those observed in our grafts of fascia lata also occur in other pathological conditions, e.g. inflammatory granulation tissue, scar tissue, in various forms of fibromatosis, scleroderma, normal collagen tissue of the embryo (Fitton Jackson, 1957; Hehrlein *et al.*, 1969) and certain aging processes especially in the skin, where an important neoformation of particularly fine microfibrils is accompanied by an excessive formation of fundamental substance (Leroy, 1972).

In the absence of a normal endothelial filter, the balance between the blood and the fundamental substance of the collagen is modified. The extreme sensitivity of the collagen matrix to changes in temperature, pH, ionic concentration as well as the critical influence of such changes on collagen metabolism is well known (Delaunay and Bazin). Disturbed vascularisation in connective tissue rich in collagen stimulates the neoformation of numerous fine collagen microfibrils arranged in a totally random manner (Dahmen, 1966). These changes are even more pronounced if the tissue is exposed to repeated microtrauma. Fascia lata transplanted as an aortic valve functions in an analogous environment to that created experimentally by Dahmen (1966).

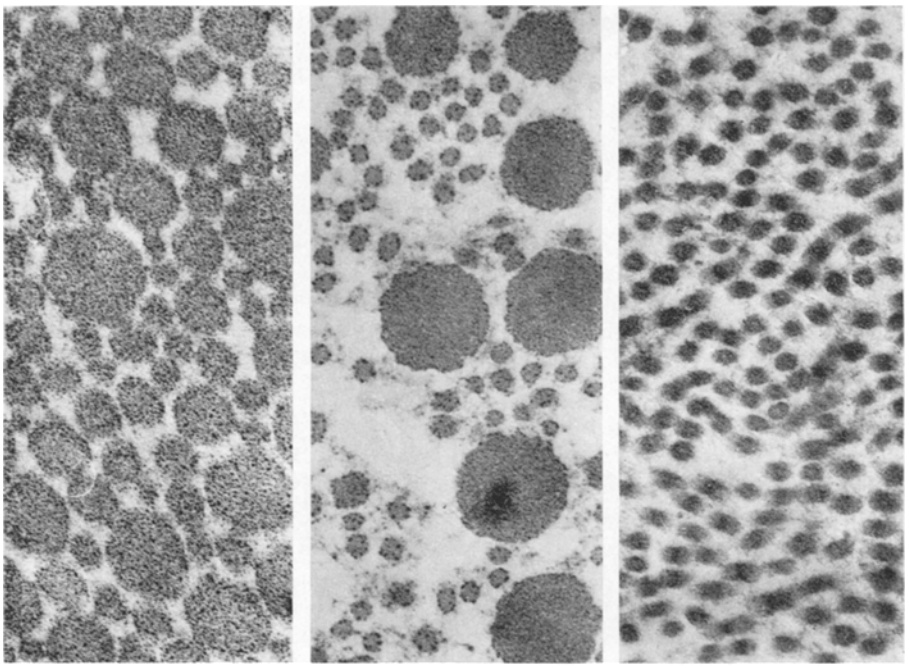

Fig. 7A—C. Comparison of collagen microfibrils size. (× 64000). (A) Normal non-grafted fascia lata. Microfibril diameter varies from 800 to 2500 Å. (HZ 441/73). (B) Grafted fascia lata at periphery of an area of proliferation. Microfibrils are normal size with very fine microfibrils (300 to 600 Å). Case no. 7 HZ 4787/72. (C) Grafted fascia lata at the center of an area of proliferation showing abundant collagen neoformation of embryonic type. The microfibrils exbibit a very regular diameter. Case no. 3, HZ 4107/72

There is no close correlation between the duration of fascia lata transplantation and the extent of the lesions. While the degree of proliferation and tissue necrosis are generally equal during the early stages of transplantation, connective tissue proliferation slowly become the more predominant morphological feature. When connective tissue proliferation exceeds tissue necrosis, the valves gradually become sclerosed and retracted, consequently resulting in a gradual yet progressive aortic insufficiency.

Concurrent necrosis and proliferation seen in grafted fascia lata probably represent reactions to different degrees of metabolic disturbance. It is noteworthy that the neoformation of collagen becomes truly significant only when the necrotic lesions begin to diminish, after about 2 years. It is also at this time that the first fine capillaries in the valvular stroma become apparent.

The lesions are comparable to those described in aortic valve homo- and heterografts (Barratt-Boyes *et al.*, 1969; Duvoisin *et al.*, 1968; Gavin *et al.*, 1972; Fitton Jackson, 1957; Rothlin *et al.*, 1973; Stephens and O'Brien, 1973) except that in this study tissue proliferation is much more pronounced.

The most frequently proposed pathogenetic explanation for the degenerative changes seen in homo- and heterograft transplants is that the endothelial lesions

are brought about by the methods used for tissue conservation or sterilisation (Barrat-Boyes *et al.*, 1969; Gavin *et al.*, 1972; Hehrlein *et al.*, 1969; Heimbecker, *et al.*, 1968; Innes *et al.*, 1971), e.g. chlorhexidine, ethylene dioxide, antibiotics, solution of Hankl, formaldehyde, B-propiolactone, lyophilisation, etc). In support of this view, *Heimbecker* (1968) reported better results with homografts which had not been pretreated. In addition, Barrat-Boyes (1969) described perforations only within those valvular grafts which had been pretreated. These findings further emphasize the importance of an intact endothelium which was lacking in our fascia lata transplants.

The immunological etiology of lesions observed in homografts and heterografts of the cardiac valves has also been debated (Fernando *et al.*, 1964). In autologous grafts of fascia lata, only formation of autoantibodies could be considered (Clark and Veist, 1972).

Several drugs are known to influence the metabolism of collagen (Trnavsky and Trnavsky, 1971). Chloroquine has a catabolic effect on newly formed collagen molecules, while cortisone is known to diminish the synthesis of the fundamental substance. Phenylbutazone totally prevents collagen synthesis. Presently there have been no published studies relating the effects of these drugs on fascia lata valve prothesis.

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