Amyloid Deposits in Bioprosthetic Cardiac Valves After Long-Term Implantation in Man

A New Localization of Amyloidosis

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Congo red staining with microscopic examination under polarized light was performed in 30 porcine bioprosthetic cardiac valves and one autologous fascia lata valve explanted from 31 patients in order to detect the presence of amyloid. Microdeposits of amyloid were present in the sewing ring of the fascia lata valve and in 10 porcine bioprostheses, and this finding was confirmed by transmission electron microscopy in 3 porcine bioprostheses. All amyloid-laden porcine valves had been implanted for at least 33 months before removal, and all except two showed dysfunction and/or severe degeneration of cuspal tissue. Statistical analyses failed to establish any correlation between the presence of amyloid and patient-related factors. In a majority of porcine bioprostheses amyloid was permanganate-sensitive and tryptophan-positive. The pathogenesis of this new form of heart valve amyloidosis might consist in penetration of human macrophages in deteriorated bioprosthetic cusps and their interaction with blood-borne amyloid precursors. (Am J Pathol 1984, 114:431–442)

SEVERAL HISTOCHEMICAL CHANGES have been described in porcine bioprosthetic cardiac valves after long-term implantation.[1,2] In many cases of valvular dysfunction the changes occur together with severe alterations of the cusp surface and collagen fibers. According to all ultrastructural studies, erosions and microscopic slits appear in the cusp surface, mainly at the outflow side, while the underlying bundles of collagen fibers degenerate, break down in many places, and become infiltrated by small lakes of granular proteins, presumably derived from human plasma. These alterations are associated with polymorphonuclear leukocytes and macrophages. In most cases calcium salts, lipids, platelets, and fibrin are found in the deep layers.

No Congo-red-positive deposition with typical amyloid fibrils at ultrastructural level has been reported thus far, neither in porcine bioprosthetic valves of the Hancock or Carpentier-Edwards types nor in any other type of biologic valve. In one instance, finely fibrillar electron-dense material, blending with granular proteins and degenerated collagen fibers, has been displayed in an electron micrograph, published by Platt et al in 1978, but the pattern of the fibrils was not characteristic or suggestive of amyloid. The discovery by one of us of Congo-red-positive material with characteristic apple-green birefringence in the cuspal tissue of a porcine bioprosthetic valve explanted from a patient with Q fever endocarditis[5] led us to speculate that the deposition was amyloid of human origin. In addition, it was postulated that amyloid had been formed in the degenerated heterologous bioprosthesis after its penetration by amyloid precursors present in the circulating plasma of the host. In some respects, this unexpected and peculiar congophilic deposition shows morphologic similarities with the micro-deposits of so-called dystrophic

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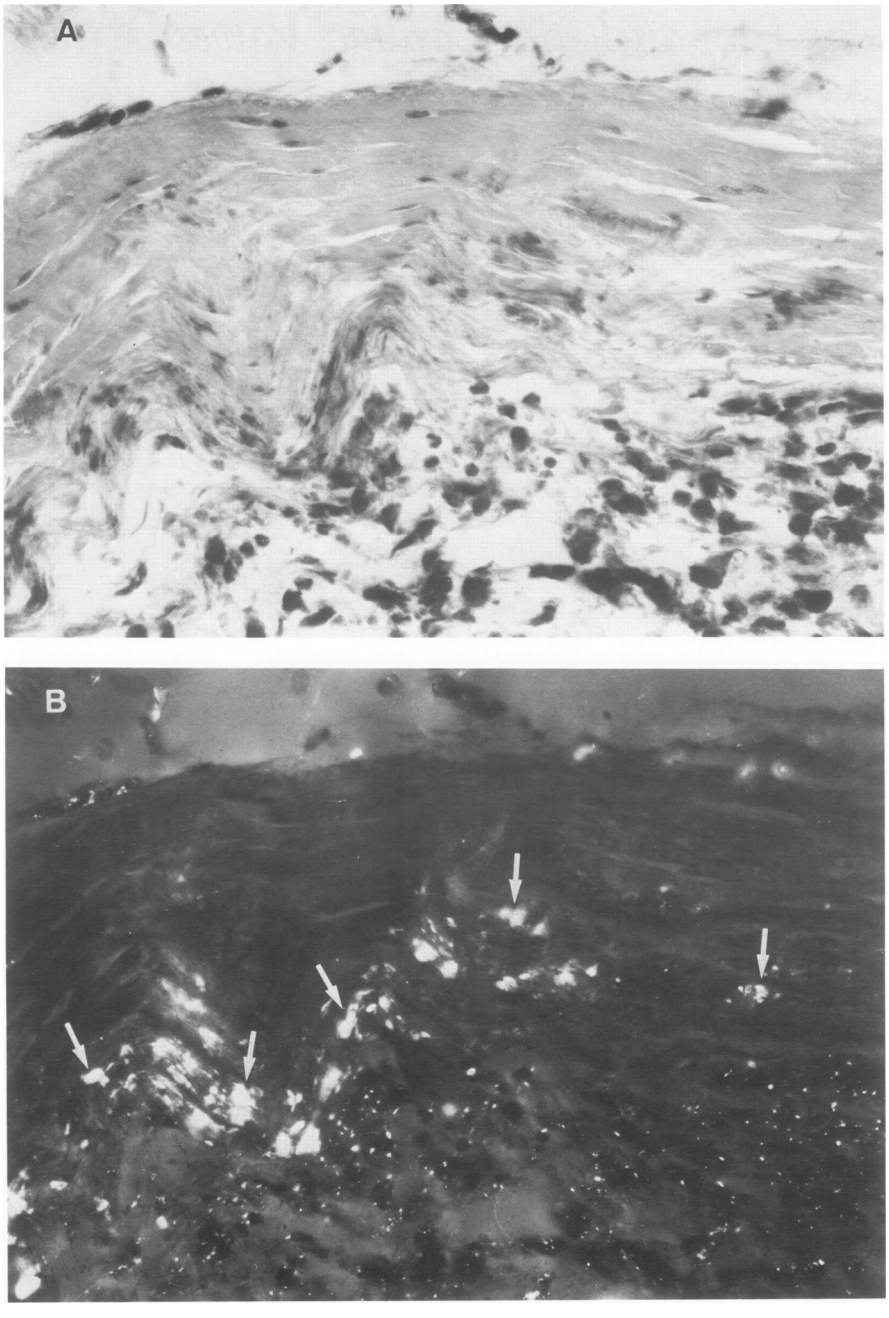


Figure 1 — Photomicrographs of a Hancock porcine bioprosthesis implanted for 64 months in the mitral position in a 34-year-old man (Case 9). **A** — Small Congo-red-positive deposits in the fibrous layer of the annulus. **B** — Typical strong birefringence of the deposits under polarized light. (Common landmarks are indicated by *arrows*). Collagen fibers display moderate unspecific birefringence. (Alkaline Congo red, ×156)

amyloidosis originating in sclerocalcific human cardiac valves.[6]

In order to confirm the presence of amyloid in bioprosthetic cardiac valves implanted in man and to study its various aspects, we performed Congo red staining in a series of porcine bioprosthetic valves recovered from patients. An autologous fascia lata recovered from a patient after 132 months of implantation was studied as well. Valves that contained amyloid deposits were further studied for tryptophan content, permanganate sensitivity, and in a few cases ultrastructural features.

Materials and Methods

A total of 31 bioprosthetic cardiac valves were examined from 31 different patients: 30 porcine aortic bioprostheses, 15 of the Hancock and 15 of the Carpentier–Edwards type, and 1 autologous fascia lata valve. Twenty-four specimens were explanted during surgery, and 7 porcine bioprostheses were recovered at postmortem examination. The age of the patients varied from 9 to 69 years. Fifteen were males and 16, females. The clinical–pathologic diagnosis of the native valvular pathology was "chronic or recurrent

rheumatic endocarditis" in 19 cases and "congenital or degenerative valvulopathy" in 12. Controls consisted of 3 nonimplanted glutaraldehyde-fixed porcine bioprosthetic valves: 1 Hancock and 2 Carpentier-Edwards (courtesy of Edwards Laboratories, Santa Ana, Calif). Of the porcine bioprostheses, 23 were implanted at the mitral and 7 at the aortic site. The postimplantation interval varied from 41 days to 82 months; 21 of 30 showed severe cusp dysfunction due to collagen breakdown, calcifications and/or leaflet rupture; 1 case showed a moderate pressure gradient, and the 8 others showed normal valvular function. The fascia lata valve was explanted from the aortic site of a 53-year-old man after 132 months' (11 years') implantation because of severe fibrotic cuspal shrinking. The patient died a few days after the operation from heart failure. None of the 31 patients presented with clinical evidence of systemic amyloidosis, nor were there any amyloid deposits at postmortem examination in the 7 fatal cases. All specimens were fixed in 4% formaldehyde or, when calcified, in a solution containing 4% formaldehyde and 7.5% nitric acid. Paraffin sections, 5 μ thick, were stained with hematoxylin–erythrosin–saffron, alkaline Congo red, orcein–Van Gieson, phosphotungstic acid hematoxylin, and alcian blue–periodic acid–Schiff (PAS) at pH 2.5 (Mowry method). Congo-red–stained sections were mounted in Kaizer's gelatin–glycerin or gum arabic–sucrose for prevention of nonspecific birefringence.[7] Material that was Congo-red–positive and showed typical green birefringence under polarized light was identified as amyloid. In all positive cases the sections were treated with potassium permanganate prior to Congo red staining[8] and with the DMAB method for tryptophan.[9] Control tissues for these two methods consisted in renal tissue containing permanganate-sensitive amyloid and normal pancreas, respectively. Three porcine bioprostheses containing amyloid deposits, as defined above, were processed for transmission electron microscopy. Blocks were postfixed in 1% buffered osmium tetroxide, contrasted with uranyl acetate, and embedded in Epon. Thin sections were contrasted with lead acetate and analyzed on a Philips 201 electron microscope. Chi-square and Student t tests were applied for statistical analysis of the results.

Results

Morphologic Aspects in Light and Electron Microscopy

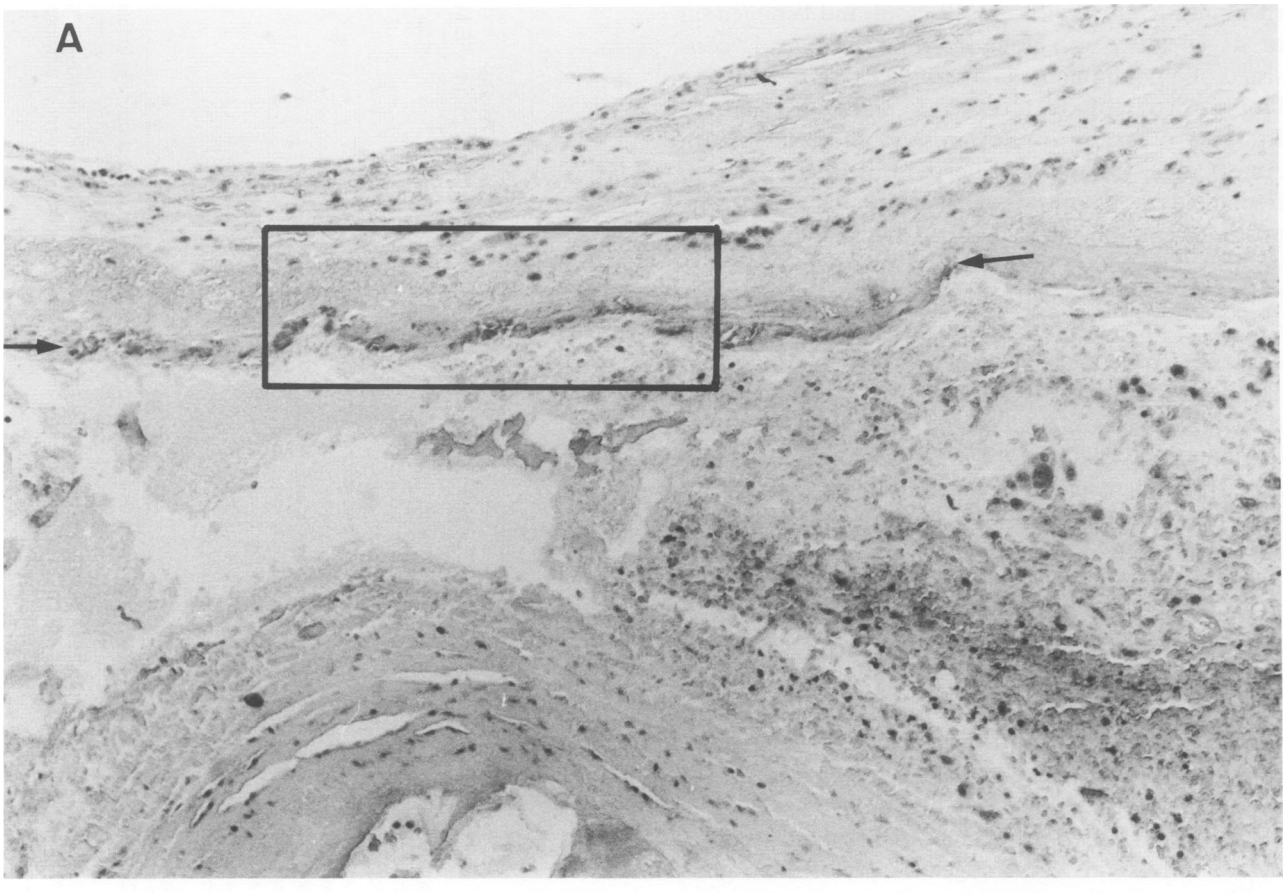
Deposition of Congo-red–positive material with characteristic apple-green dichroism under polarized

Table 1—Eleven Bioprosthetic Cardiac Valves With Amyloid Deposition

Case	Age (yr) and sex	Site of implant	Diagnosis of natural valve	Type of prosthesis	Duration of implementation (mo)	Form of cusp alteration	Site of amyloid deposits	Permanganate reaction	Tryptophan	EM
1	19 M	Ao	Congenital	C E	48	Calcification	Paraannular fibrosa	S (90%)	+	+
2	61 M	Ao	Calcific stenosis	C E	33	Endocarditis	3 layers	R	+	–
3	53 F	M	C R E	H	48	Moderate pressure gradient	Muscular annulus	S (100%)	+ +	
4	13 F	M	Unknown	C E	50	Calcification	Fibrosa and adipocytes; spongiosa	R	+	+
5	9 M	M	C R E	C E	33	Rupture	Spongiosa	S (100%)	+ +	
6	16 M	Ao	Aortoventricular canal	C E	40	Calcification and ulceration	Spongiotic annulus	± S (50%)	+	
7	67 F	M	C R E	C E	57	Normal function	Muscular annulus	R	–	
8	21 F	M	Mucoid degeneration	H	40	Calcification	3 layers	S (80%)	+	
9	34 M	M	C R E	H	64	Calcification and perforation	Fibrous annulus	S (80–90%)	+	
10	63 F	M	C R E	H	82	Normal function	Fibrosa	R	+	+
11	53 M	Ao	C R E	F L	132	Retractile fibrosis	Annulus	R	+	

+, positive; + +, strongly positive; ±, moderately +; –, absent or negative; Ao, aortic; CE, Carpentier-Edwards; CRE, chronic rheumatic endocarditis; EM, electron microscopy; FL, fascia lata; H, Hancock; M, mitral; R, resistant; S, sensitive.

The alkaline Congo red method used on the potassium-permanganate-treated sections was the Highman method. Recently, in a subsequent study on remaining tissues of this series, using the Puchtler and Sweat method, Case 1 and 6 amyloids turned resistant. Thus, the choice of the Congo red method is critical when one is performing permanganate pretreatment of amyloid-positive bioprostheses.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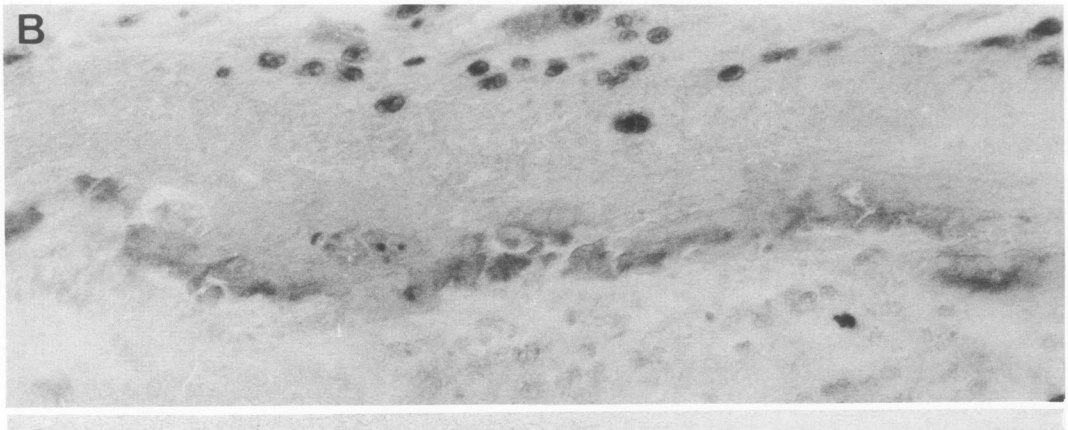
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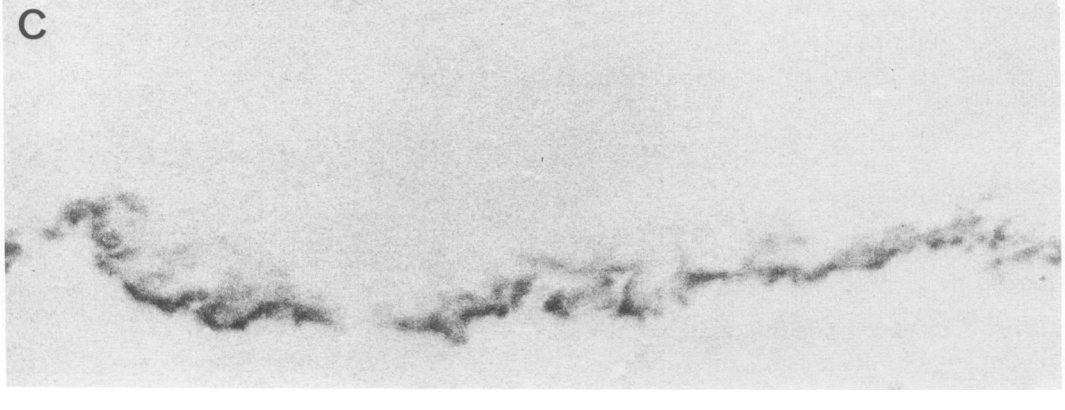
C

Figure 3 – Photomicrograph of a Hancock porcine bioprosthesis implanted for 40 months in the mitral position in a 21-year-old woman (Case 8). Linear deposits of amyloid (*arrows*) are located close to calcifications (*asterisks*) in the fibrosa of the basal part of a cusp. (Alkaline Congo red. ×63)

light (Figure 1) was identified in 10 porcine bioprosthetic valves and in the fascia lata valve, all implanted for more than 32 months (Table 1). The amyloid deposits were generally scanty and of small size and appeared in some particular locations: the annulus and parabasal part of the cusps in 4 porcine bioprostheses; close to the sewing ring in the fascia lata valve; in the spongiotic tissue in 5 bioprostheses. In 3 cases, groups of macrophages were seen in the vicinity of the deposits (Figure 2). In 2 others, linear spots of amyloid were located close to calcifications (Figure 3). The most peculiar aspects of Congophilic deposition with green birefringence occurred 1) in the sewing ring of the fascia lata valve, presenting as small rings around Dacron fibers (Case 10, Figure 4), and 2) in the muscle shelf of the right coronary cusp of a porcine bioprosthesis within several devitalized porcine capillaries and a few partly calcified myocardial cells (Case 7). The three control unimplanted porcine bioprosthetic valves contained no amyloid but displayed some loosening in the spongiotic layer with moderate decrease of proteoglycans, with respect to unprocessed pig valves.

Permanganate Sensitivity and Tryptophan Staining

The results of potassium permanganate treatment and Adams method for tryptophan staining are shown in Table 1: 1) Amyloid deposits in the porcine bioprostheses were moderately to highly permanganate-sensitive in 6 cases, with a 50–100% decrease in Congophilia after treatment, and permanganate-resistant in the others. Tryptophan was present in all amyloids (Figure 2C) except in the permanganate-re-

Figure 2 – Photomicrographs of a Carpentier-Edwards porcine bioprosthesis implanted for 33 months in the mitral position in a 9-year-old boy (Case 5). **A** – Linear deposits of amyloid (*arrows*) along the ventricularis side of the spongiosa. **B** and **C** – Higher magnification of area included in the box. **B** – Numerous macrophages in the vicinity of the amyloid deposition (macrophages on *top*). **C** – Tryptophan stain positive at the level of the amyloid; *F*, fibrosa; *S*, spongiosa; *V*, ventricularis. (**A** and **B**, alkaline Congo red; **C**, DMAB method; **A**, ×63; **B** and **C**, ×156)

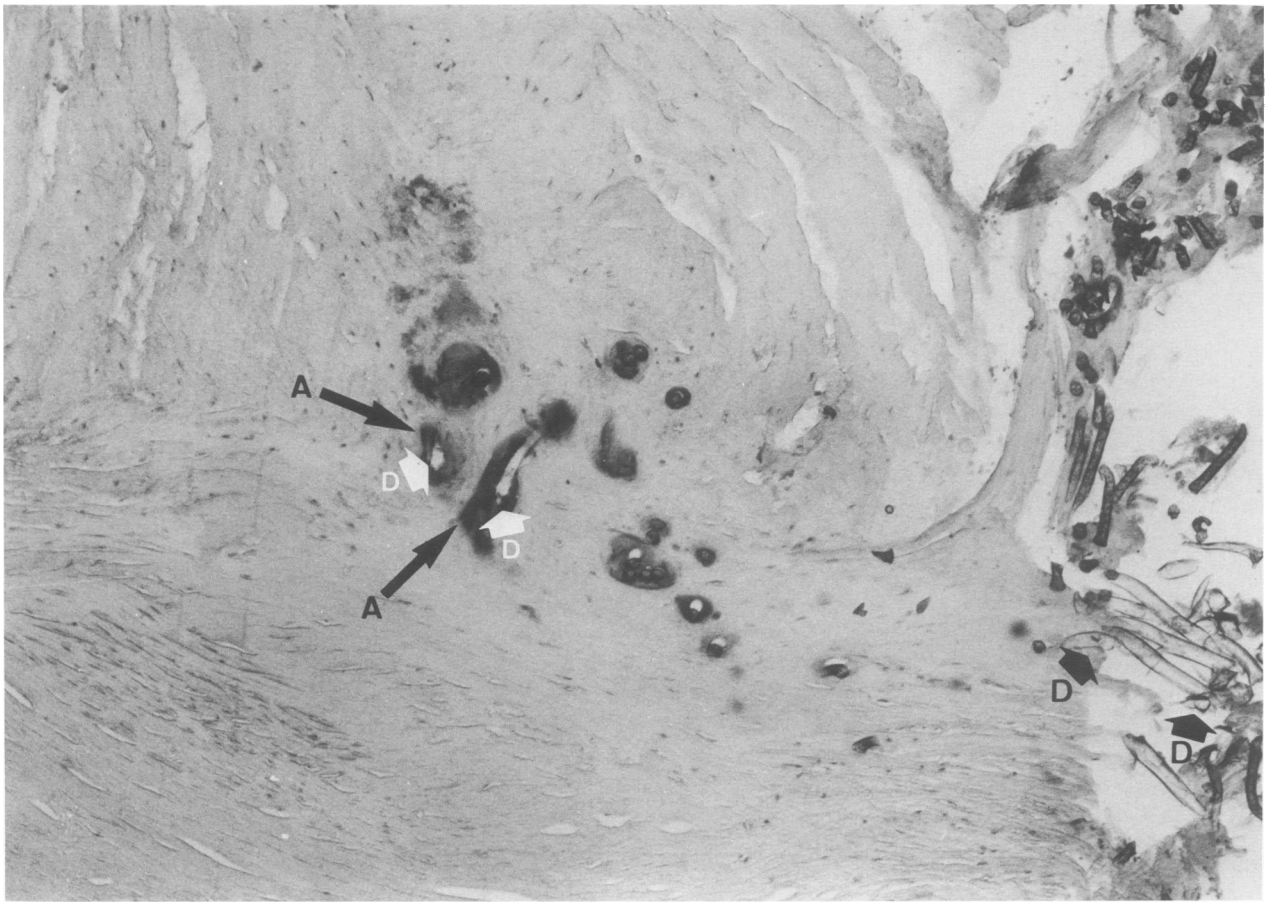

Figure 4 — Photomicrograph of the autologous fascia lata valve implanted for 132 months in the aortic position (Case 10). Amyloid presenting as dark circular deposits (*long arrows*) around the Dacron fibers (*short arrows*) close to the sewing ring of the bioprosthesis. A bundle of Dacron fibers is marked in the *right lower corner.* (Alkaline Congo red, ×25)

sistant deposits which occurred in Case 7 within devitalized porcine capillaries and myocardial cells. 2) Amyloid of the fascia lata valve was permanganate-resistant and tryptophan-positive.

Electron Microscopy

In the case of a 13-year-old girl, electron microscopic study of a Carpentier–Edwards porcine bioprosthesis, removed after 50 months (Case 4, Table 1), confirmed the presence of fibrils with a characteristic ultrastructure of amyloid: fibrils were nonbranching, showed typical random orientation with a fibril width of about 9 nm, and were disseminated in a matrix of slightly altered or partly disrupted collagen fibers (Figure 5). The paraffin embedded block of another porcine bioprosthesis (Case 1, Table 1) after reembedding in epon also showed microfibrillar material at ultrastructural level.

In the third case of degenerated porcine bioprosthesis, electron-microscopic study was made of a permanganate-resistant amyloid deposit, which was observed in the fresh-frozen section of the fibrosa layer (Figure 6A & B). Ultrastructurally, the porcine cusp appeared to consist of well-preserved collagenous fibrils and remnants of original cellular elements. In one area, in the vicinity of the valvular surface, which was rough and not covered by endothelial cells, masses of straight nonbranching amyloid fibrils were found (Figures 7 and 8). Calculated from the microscopic enlargement as well as from the periodicity of collagenous fibers (64 nm), the amyloid fibrils had a thickness of 8 nm. On cross-section (Figure 7) the fibrils consisted of electron-dense rings with a lucent core, and they appeared to be surrounded by an electron-lucent mantle of varying thickness. In between the amyloid fibrils membrane-bound vacuoles of varying electron density were found (Figure 7). Moreover, some viable-looking cells were seen in this area that had the features of fibroblasts and macrophages. Some of these macrophages showed an evident topographical relationship to the amyloid mass (Figure 8).

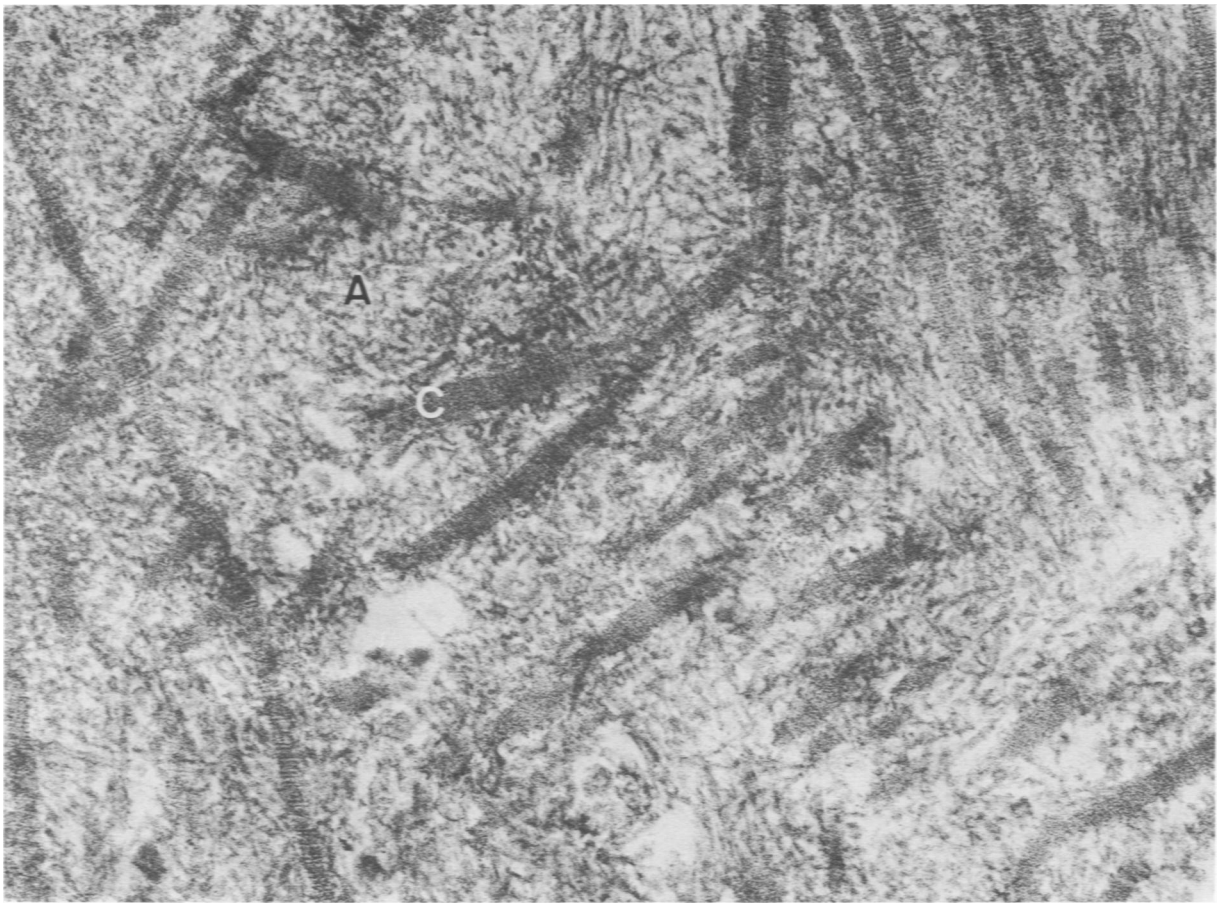

Figure 5—Electron micrograph of a Carpentier-Edwards porcine bioprosthesis implanted for 50 months in the mitral position in a 13-year-old girl (Case 4). Nonbranching fibrils with a mean diameter of 10 nm are disposed at random in a matrix of porcine collagenous fibers. (×75,000)

Frequency and Correlation Studies of Amyloid in Porcine Bioprostheses

Amyloid deposits were detected in 10 of 30 implanted porcine bioprostheses (33.3%). As shown on Table 2, the distribution of the positive cases indicates three trends: 1) Eight of 22 cases with prosthetic valve dysfunction were amyloid-positive, whereas only 2 of the 8 normally functioning bioprostheses contained amyloid. 2) No amyloid was observed in the group of 5 valves with less than 33 months of implantation. After that period, the proportion of positive valves was 10 of 25 (40%) with a peak frequency between 33 and 42 months. 3) The appearance of amyloid in bioprostheses was unrelated to aging, although the median age of the patients in the group with amyloid-laden bioprosthesis was somewhat lower on average than in the amyloid-free group.

Larger groups of patients will be required to establish the significance of these findings. Sex distribution, site of implantation, and type of porcine bioprosthetic valve (Hancock versus Carpentier-Edwards) were not statistically different in the two groups.

Presence of Amyloid in Bioprosthetic Valves and Patient Morbidity Factors

In none of the 31 patients was systemic or cardiac amyloidosis, chronic inflammatory conditions, cancer, or immunocytic dyscrasia suspected. Three of the 10 patients with an amyloid-laden bioprosthesis died in our hospital, but postmortem examination failed to disclose any other amyloid deposition. Retrospective analysis showed that the predisposition for amyloid deposition in the prosthetic valve was not influenced by the underlying cause of the native valvular disease.

Discussion

Our investigations have brought to light the hitherto unsuspected evidence of amyloid deposits in bioprosthetic valves implanted in patients. Amyloid was

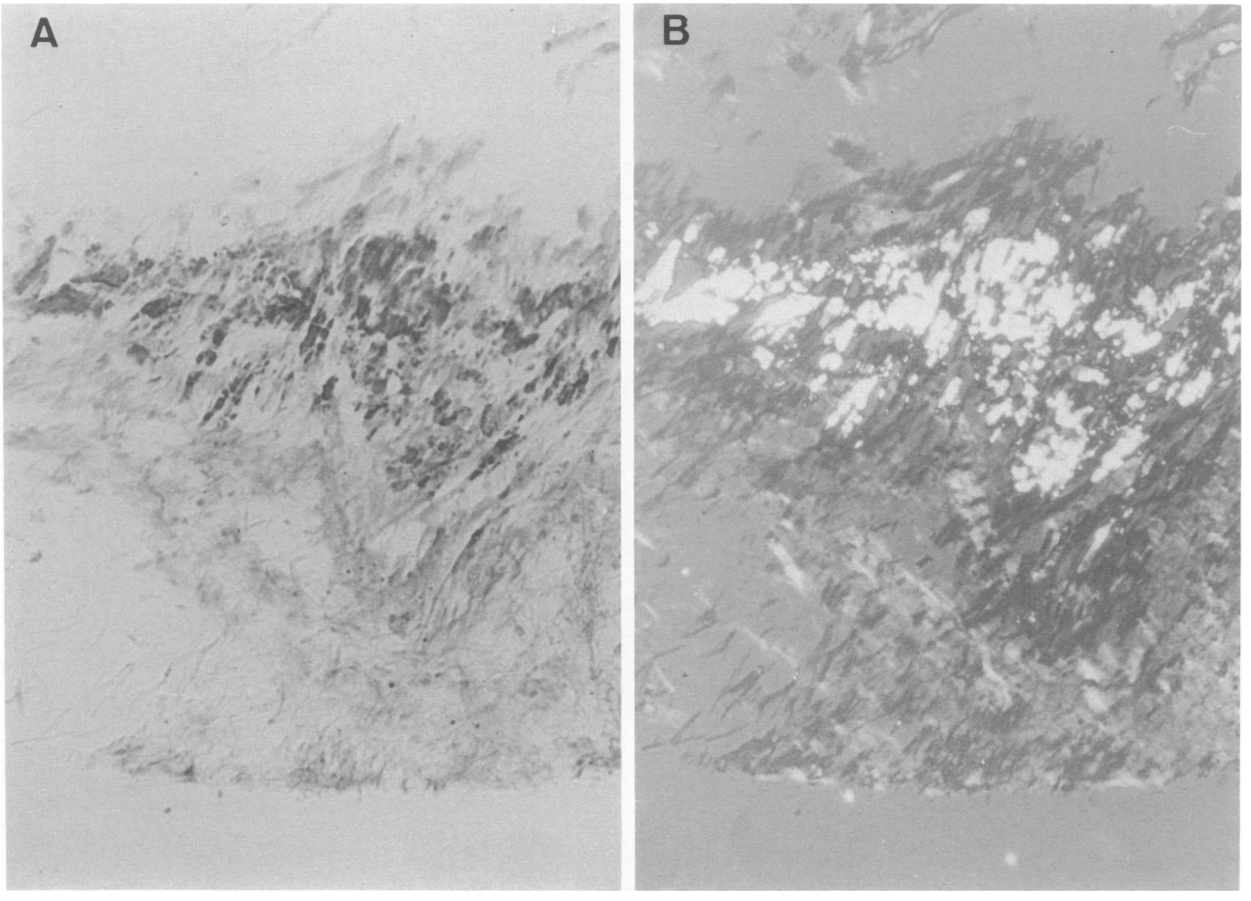

Figure 6—Photomicrograph of a Carpentier-Edwards porcine bioprosthesis implanted for 82 months in the mitral position in a 63-year-old woman (Case 10). Alkaline Congo red staining of a fresh-frozen section showing massive amyloid deposition in the fibrosa. **A**—Under ordinary light. **B**—Under polarized light. (×63)

not detected prior to 33 months of implantation and was most often present in bioprostheses with important morphologic changes and functional abnormalities.

In the 30 porcine bioprostheses, amyloid was disclosed with a frequency of 33.3%. This new location of amyloidosis was observed in devitalized and deteriorated heterologous bioprostheses, independent of the patient's condition or age. Histologically, the amyloid deposits occurred frequently in the annular and basal parts of the cusps and in some cases in the vicinity of infiltrated human macrophages. It may be of interest to note that the paraannular regions of the cusp are reportedly subjected to the strongest mechanical stress during valve functioning *in vivo*.[10] The ultrastructural study of 3 porcine bioprosthetic cusps leaves no doubt that the collagen bundles are disorganized by polymeric amyloid fibrils: the collagen fibers are obviously separated by a microfibrillar unstriated and well-stained material. In Case 10, their cross-section shows a characteristic electron-dense ring with a lucent core, as has been described in human[11] and animal amyloid.[12] The fibrils are most often not in parallel or in continuity with the collagen fibers. They cannot be a degradation product of the fibers. Neither do they resemble collagen Type III microfibrils, which should occur in small bundles. Furthermore, most collagen fibers seem well-preserved and of adequate caliber.

Although amyloid should impair the elasticity of the cuspal tissues, considering the hardness of its β-fibrillar ultrastructure, its localized character and microscopic size do not seem to add significantly to the stiffness and fragility of partly calcified bioprostheses.

In the series of amyloid-laden porcine bioprostheses, the dissimilar results of the permanganate and DMAB methods suggest that bioprosthetic amyloidosis is protean in nature. Consequently, 8 cases have been classified into three categories of amyloid:

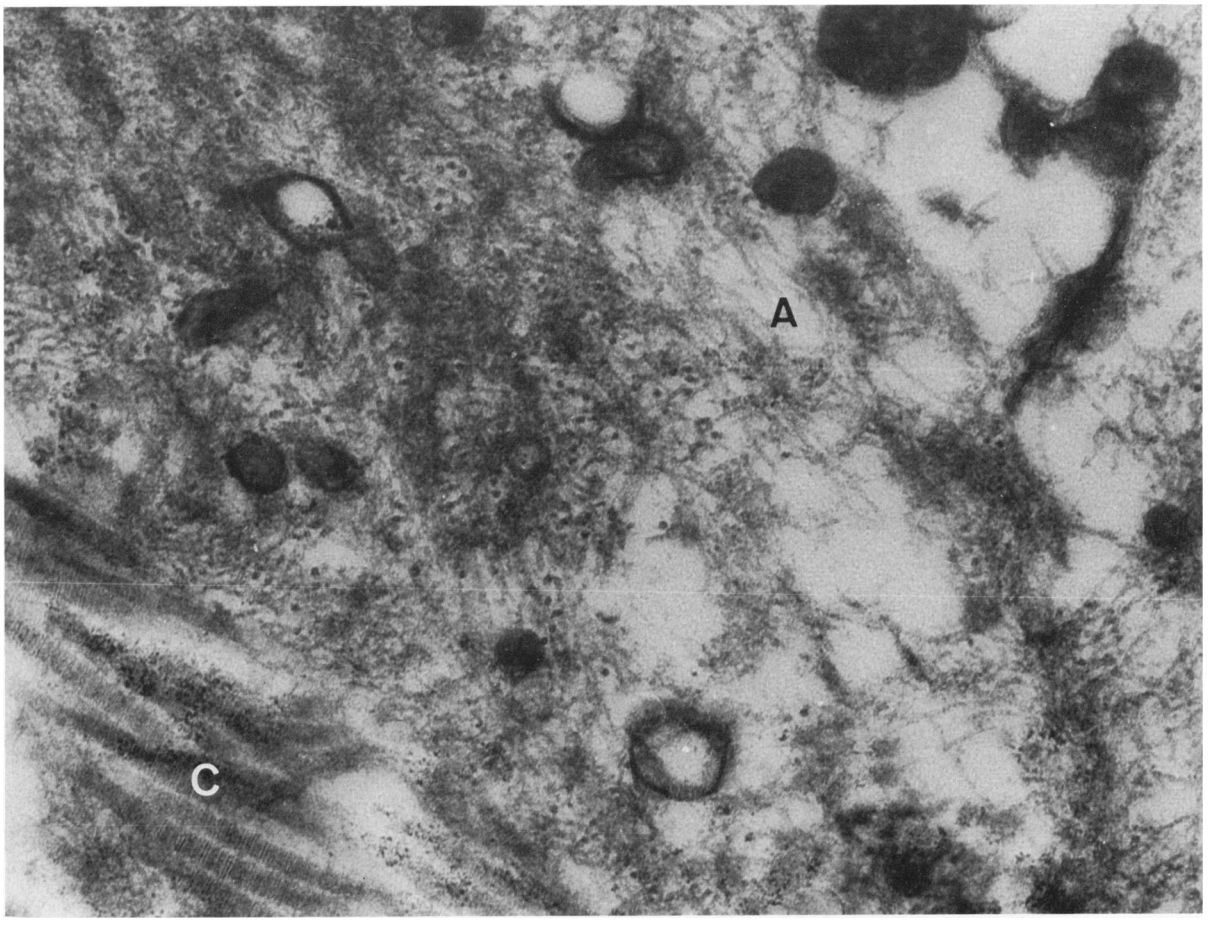

Figure 7—Electron micrograph of the superficial area of same bioprosthetic cusp as shown in Figure 6 (Case 10). Masses of crisscrossed nonbranching amyloid fibrils are deposited between collagen fibers. *A*, amyloid fibrils; *C*, collagen. (Lead citrate, ×60,000)

1) Permanganate-Sensitive and Tryptophan-Positive Amyloid

According to Wright et al,[8] permanganate sensitivity indicates secondary (reactive) amyloid fibril protein A (AA) with SAA as its blood-borne precursor protein.[13,14] Cases 1, 3, 5, 8, and 9 form this category.

2) Permanganate-Resistant and Tryptophan-Positive Amyloid

This protein or proteins share the histochemical features of other types of amyloid such as primary or myeloma-associated immunoglobulin light chain amyloid fibril proteins (AL) and senile cardiac amyloid. They also might be related to blood-borne precursors, such as light chains of immunoglobulins[15] and human prealbumin,[16] respectively. The second category includes Cases 2, 4, and 10.

3) Permanganate-Resistant and Tryptophan-Free Amyloid

As far as the peculiar Congophilic deposits of Case 7 constitute real amyloid fibrils—and this needs confirmation—they share the histochemical features of the endocrine or APUD types of endocrine tissue amyloid fibrilproteins,[17] isolated atrial type of senile cardiac amyloid (IAA),[18] and certain forms of cerebral amyloid.[19] Of interest is that these types are thought to be related to local precursors.[20-22] In this respect, amyloid deposition within devitalized myocardial cells of the porcine muscular annulus resembles IAA deposits, which are reportedly found in atrial muscle cells.[23]

Case 6 is difficult to classify, considering the ambiguous result of the permanganate method. The approximate 50% sensitivity of the amyloid deposits might indicate a mixed protein composition with AA and another type of amyloid.

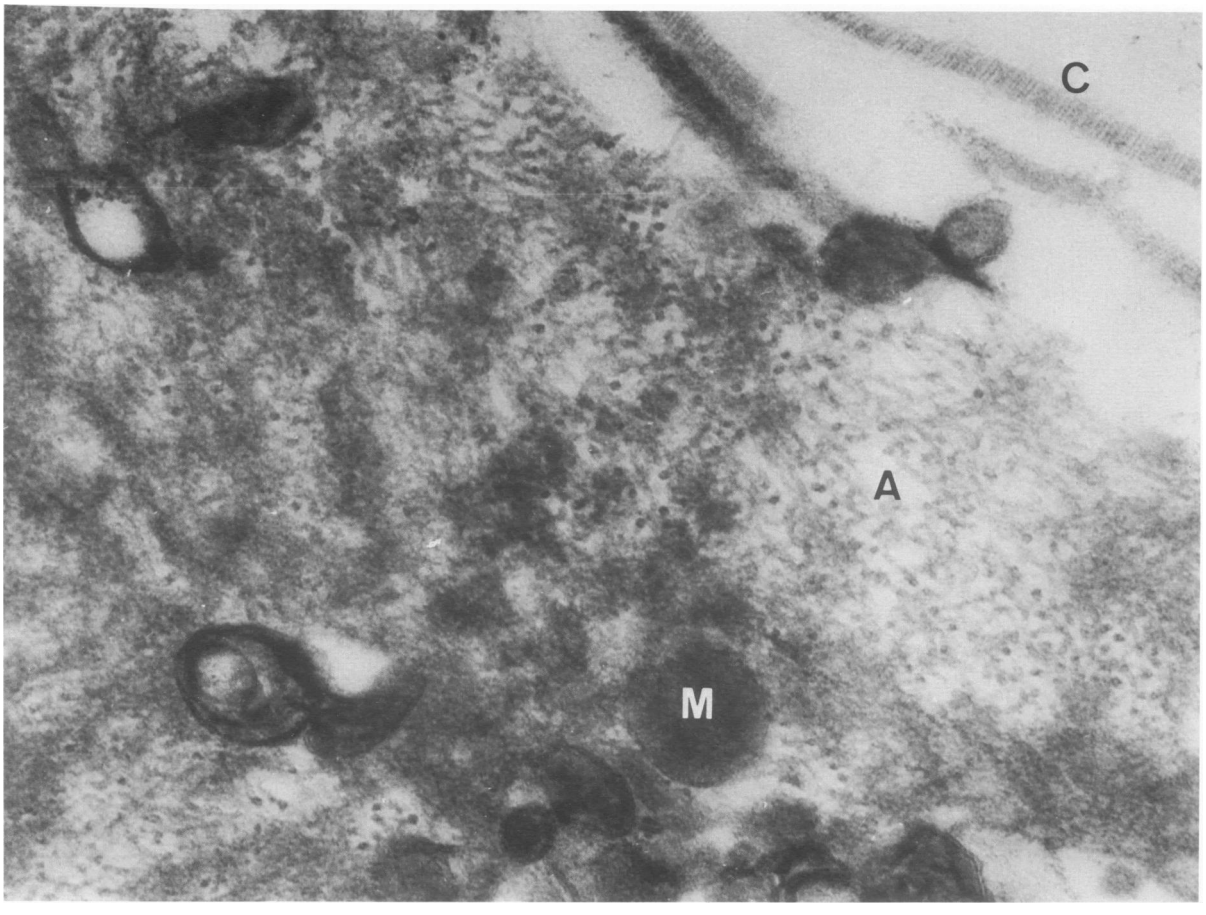

Figure 8 – Electron micrograph of the same porcine bioprosthesis under a stronger magnification. On cross-section the amyloid fibrils show electron-dense ringlike structures with an electron-lucent core, and they appear to be surrounded by an electron-lucent mantle. Between the amyloid fibrils remnants of electron-dense membrane-bound vesicles are prominent (*M*). *A*, amyloid fibrils; *C*, collagen. (Lead citrate, × 102,000)

According to recent studies dealing with porcine bioprosthetic valves, the substantial decrease in the content of proteoglycans of the spongiosa of fresh porcine aortic valves during glutaraldehyde fixation creates a void in the tissue.[2] This void is likely to facilitate the accumulation in the depth of the cusp and its annulus of the plasma proteins and other components of human blood, which have insudated through the numerous microscopic slits and erosions of the surface during long-term implantation.

It is possible that specific serum precursors of amyloid fibril proteins, circulating in the patient's blood flow, penetrate the damaged cuspal tissues, together with other plasma proteins, calcium and fibrin, and that these proteins get trapped in the loosened collagenous matrix of the cusp. One can further speculate that the insudated proteins are submitted to a denaturation process, resulting eventually in the formation of amyloid fibrils. This transformation could be mediated by lysosomal enzymes produced by the macrophages penetrating the damaged surface of the bioprosthesis. The role of macrophages in the formation of amyloid fibrils AA and AL has indeed been suggested by Glenner and co-workers in the majority of cases of the acquired systemic amyloidoses.[14] Levels of SAA are likely to be increased before and at the time of surgery and in young patients with recurrent rheumatic fever. Unfortunately, our retrospective clinical study did not provide any information regarding specific serum precursors.

In any case, we feel that amyloid deposition is the result, rather than the cause of the structural and/or functional abnormality of the bioprosthesis.

An alternate explanation for the presence of amyloid deposits in porcine bioprosthetic valves, ie, that the deposits are of porcine origin and were already present in the valve at the time of manufacturing, seems very unlikely for two reasons: 1) according to the veterinary literature, amyloidosis is a rare finding in swine, with most cases occurring as a result of na-

Table 2—Synopsis of 30 Porcine Bioprosthetic Cardiac Valves

	Age (yr)	Sex		Implantation site		Duration of implantation (mo)	Valve function		Type of prosthesis	
		Male	Female	Mitral	Aortic		N	AN	C E	Hancock
Amyloid-positive valves (10 cases)	9–63 mean = 35½	5	5	7	3	33–82 mean = 49½	2	8	6	4
Amyloid-negative valves (20 cases)	11–69 mean = 40	9	11	16	4	1⅓–79 mean = 47½	6	14	9	11
Total = 30 cases	9–69 mean = 39	14	16	23	7	1⅓–82 mean = 48	8	22	15	15

N, normal; AN, abnormal, CE, Carpentier-Edwards.

tive and experimental infections[23]; 2) Congo red staining of the three unimplanted glutaraldehyde fixed porcine bioprostheses and further hematoxylin and eosin staining of 7 bioprostheses implanted for 24 hours to 35 days did not disclose proteinaceous material showing any resemblance to amyloid (Y. Goffin, unpublished observation).

If it is confirmed that these amyloid fibrils are of human origin, this will be the first reported study on the presence of human amyloid in heterologous bioprostheses (no citation found in *Index Medicus* in the period 1967 to 1981).

Conclusions regarding the pathogenesis of amyloidosis in porcine bioprostheses and classification into three categories of amyloid proteins remain speculative even if the latter categorization is based on permanganate and tryptophan studies. Histochemical studies with antibodies to specific amyloid fibril proteins should be undertaken in an attempt to classify more precisely the amyloid in our tissues. As a further step, enzyme histochemistry might be used for the study, of the possible role of the lysosomal enzymes of macrophages in the deposition of amyloid fibrils.

The presence of permanganate-resistant tryptophan-positive amyloid deposits in the annulus of a sclerotic autologous fascia lata valve seems to represent a somewhat different form of localized amyloidosis, even though its definite chemical type and precise mechanism of deposition are unknown. Its histologic and histochemical features, its local association with sclerosis, and the absence of amyloid deposits elsewhere at postmortem examination suggest further that localized amyloidosis of sclerotic fascia lata valves represents a particular location of so-called dystrophic amyloidosis of human heart valves, recently described in surgical specimens as a common complication of chronic sclerocalcific valvulopathies.[6,24–26]

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