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Amyloid deposits in aortic and mitral valves

A clinicopathological investigation of material from 100 consecutive heart valve operations

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Summary. The material from 100 consecutive aortic and mitral valve operations has been studied histologically with particular reference to the presence of amyloid deposits. Sixty seven per cent were positive (aortic 88%, mitral 45%).

The simultaneous occurrence of calcification of the valves and amyloid degeneration as well as of calcification and hyalinization was significant. Similarly there was significantly more amyloid in the older age groups, as well as a significant correlation between the degree of hyalinization of the valve and amyloid.

Thirty-two patients had previously suffered from rheumatic fever. The heart valves of these patients did not differ histologically from the others, whereas significantly more amyloid was observed in the stenotic mitral valves than in the mitral valves which were insufficient.

Key words: Amyloid deposits – Calcification – Hyalinization – Heart valves

Cardiac amyloidosis is a well-known condition which has been reported in incidences varying from a few per cent to more than 50% among the oldest age groups (Schwartz 1970; Westermark et al. 1977; Westermark et al. 1979). Virchow described the first case of cardiac amyloidosis in 1857. Lately Störkel et al. (1983) described 5 different groups of cardiac amyloid deposits.

The first report of amyloidosis of the heart valves was from Kann in 1922. A number of case reports of amyloid deposits in the heart valves have since appeared, but only few systematic studies have been published. Goffin (1980) found amyloid in 33 of 213 mitral and aortic valves removed due to "chronic changes" and Falk et al. (1981) described a close correlation between "calcific aortic stenosis" and amyloid aortic valve degeneration. Resently Iwata

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et al. (1982) and Cooper et al. (1983) have reported amyloid at a high incidence in surgically removed heart valves (44 and 53 per cent respectively).

The object of the present investigation has been to determine the frequency of amyloid deposits in aortic and mitral valves in consecutive surgical material, and to correlate amyloid degeneration with other histological changes in the valves in particular hyalinization and calcification and to clinical-physiological variables.

Material and methods

The material comprises tissue from 100 consecutive heart valve operations, carried out by the Department of Thoracic and Cardiovascular Surgery T, of the Odense University Hospital during the period January 1971 to August 1977.

In 43 men and 8 women the aortic valve was removed, while 14 men and 35 women were subjected to removal of the mitral valve. Four of the men had both the aortic and mitral valves removed. Thus the investigation comprises a total of 96 patients (43 women and 53 men). The average age was 51.6 years (range 6–71) without sex differences.

Thirty-two of the patients had previously suffered from rheumatic fever. Two had rheumatoid arthritis while five had earlier suffered from chronic inflammatory disease: pyelonephritis 2 – syphilis 2 – pulmonary TB one. None had clinical signs of primary or secondary amyloidosis, and none suffered from myelomatosis or other malignant diseases.

The valves removed were immediately fixed in 10% formalin for at least 24 h. Depending on the amount of material 2–6 sections were removed, which after the normal procedures and decalcification, if necessary, were embedded in paraffin. Six micron thick sections were stained with haematoxylin/eosin, elastin Van Gieson's connective tissue stain, PAS/alcian blue mucopolysaccharide stain at ph 2.7 and with alkaline Congo red, according to the method of Puchtler et al. (1962). Amyloid was considered present when the orange colour in Congo red showed green dichroism in polarized light. Hyalinasation was defined as areas in the connective tissue which were translucent and homogeneous and stained with eosin but not with the alkaline Congo red stain.

The occurrence of amyloid, hyalin and calcium phosphate was graded semiquantitatively. Slight (+): Area taken by amyloid roughly less than 10% of total area of the section. Moderate (++): Area of amyloid between 10 and 20% of total area. Severe (+++): Area taken by amyloid roughly more the 20% of total area of the section.

Small pieces of tissue from 5 heart valves were fixed for electron microscopy in 2,5% glutaraldehyde in cacodylate buffer, immediately after removal. They were rinsed in 10% sucrose in cacodylate buffer and post-fixed in 1% osmium tetroxide for two hours, after which they were dehytrated in alcohol and propylene oxide, embedded in Epon and cut on an ultramicrotome (Reichert). The ultrastructural studies were carried out on a Phillips EM 201 electronmicroscope.

Statistically the *chi-square-test* was employed on Tables 1, 2 and 3, while Fisher's exact test was used on Tables 4 and 5.

Results

Histological findings

Light microscopic examination revealed amyloid (congophilic areas, which in polarized light gave green dichroism), in 67% of the heart valves. In 12 only small focal deposits were observed (+), while moderate deposits were seen in 27 (++) and severe depostis in 28 (+++), (see Table 1).

Amyloid deposits were observed in 45 of the 51 aortic valves (88%) equally common in men and women. The amyloid could frequently be

Table 1. Simultaneous occurrence of amyloid and calcium phosphate deposits in mitral and aortic valves ($P < 0.001$)

		Calcium phosphate				
		0	+	++	+++	Total
A	0	22	8	2	1	33
M	+	0	5	4	3	12
Y	++	2	1	7	17	27
L	+++	0	2	4	22	28
O						
I	Total	24	16	17	43	100
D						

demonstrated admixed with or in a ring in close proximity to acellular granular calcium phosphate deposits (Figs. 1, 2). Calcium phosphate deposits were found in 46 of the 51 aortic valves. There was only one aortic valve with depositis of calcium phosphate without the simultaneous presence of amyloid degeneration.

Three of the patients subjected to removal of the aortic valve were below the age of 30 years. One of these had minimal deposits of amyloid and calcium phosphate. Among the 14 patients between the ages of 31 and 50 years amyloid was observed in 10 and calcium phosphate in 11, while all those above the age of 50 years had both amyloid and calcium phosphate in their aortic valves.

Amyloid was seen in 22 of the 49 mitral valves (45%), roughly equally in men (43%) and women (46%). Calcium phosphate deposits were rarer (61%) and of a lesser degree than in the aortic valves. The simultaneous occurrence of amyloid and calcium phosphate was not as constant. Thus two mitral valves with amyloid deposits were seen without simultaneous calcium phosphate deposits, and 11 valves with calcium phosphate were without amyloid. The topographic relation of amyloid and calcium phosphate deposits was as for the aortic valves (Fig. 3).

In 4 patients below the age of 30 years no deposits of calcium phosphate nor of amyloid were observed in the mitral valves.

The simultaneous occurrence of calcium phosphate and amyloid was, for the material as a whole, highly significant ($P < 0.001$), (Table 1).

Moderate or severe hyalinization of the connective tissue was found in 82 heart valves. Amyloid deposits (Fig. 4) as well as calcification was also seen in areas affected by hyalinization (Tables 2 and 3). For the material as a whole, there was a significant correlation between hyalinization of the connective tissue and amyloid degeneration ($P < 0.025$), (Table 3).

Mucoid degenerated areas had no topographic relation to the amyloid deposits in either the aortic or mitral valves. Inflammatory changes were rare, but cell rich areas could be seen occasionally containing both fibrocytes as well as lymphocytes and macrophages, frequently closely surrounded by foci

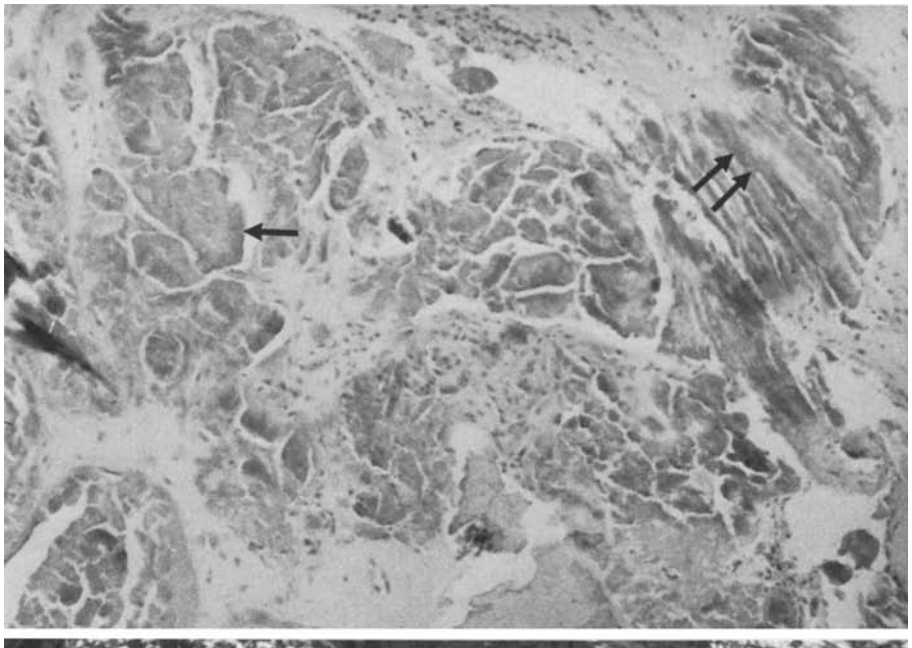

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Fig. 1. Amyloid deposits in calcific aortic valve. Admixed with calcium phosphate deposits (*one arrow*) and in the connective tissue around the calcium phosphate (*two arrows*). Alkaline Congo red × 95

Fig. 2. Same as Fig. 1 in polarized light × 95

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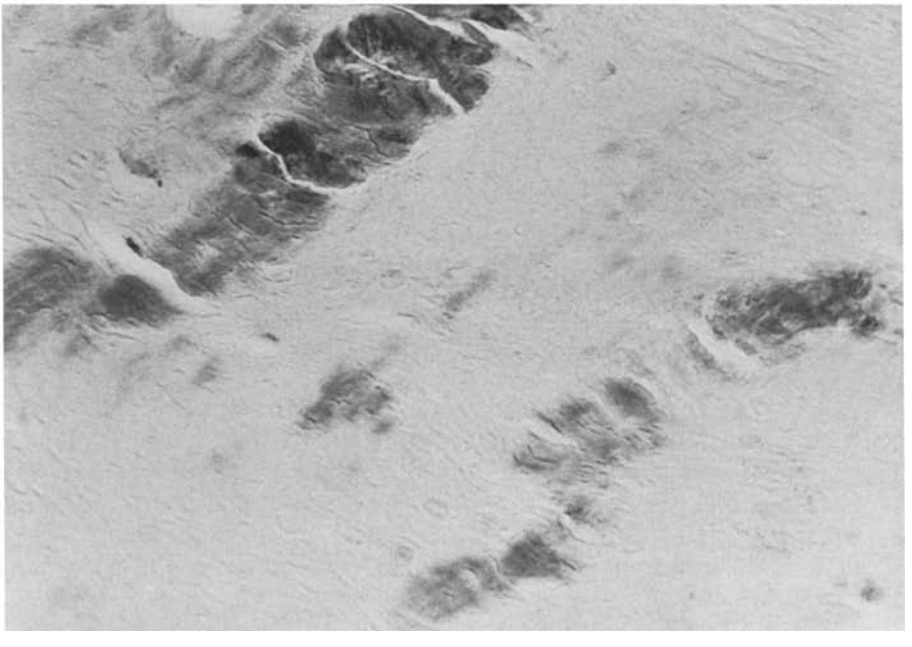

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Fig. 3. Amyloid deposits in calcific mitral valve. Alkaline Congo red × 240

Fig. 4. Amyloid deposits in haylinized heart valve. Alkaline Conge red × 240

Table 2. Simultaneous occurrence of hyalinization and calcium phosphate deposits in mitral and aortic valves ($P < 0.005$)

		Calcium phosphate				
		0	+	++	+++	Total
HYALIN	0	4	1	0	0	5
	+	7	1	1	4	13
	++	9	8	10	20	47
	+++	4	6	6	19	35
	Total	24	16	17	43	100

Table 3. Simultaneous occurrence of amyloid deposits and hyalinization in mitral and aortic valves ($P < 0.025$)

		Hyalin				
		0	+	++	+++	Total
AMYLOID	0	4	9	17	3	33
	+	1	0	8	3	12
	++	0	3	13	11	27
	+++	0	1	9	18	28
	Total	5	13	47	35	100

of amyloid. Congophilic material in the cytoplasm of fibrocytes and macrophages was seen in some valves.

Electron microscopy showed characteristic dense collagen connective tissue with innumerable, parallel and crossing bundles of collagen fibres.

Degenerative material, varying in amount from massive infiltration (Fig. 5) to small focal deposits could be observed; these deposits showed typical ultrastructurel features for amyloid and consisted of unbranched fibrils of a width from 8 to 12 nm, lying partly parellel to and partly crossing each other.

Further, fibrocytes containing similar fibrillar material in the cytoplasm could be observed.

Histological findings related to clinico-physiological variables

Thirty-two patients, corresponding to seven subjected to operation on the aortic valve (14%) and 25 on the mitral valve (51%), had earlier suffered from rheumatic fever. The valves from these patients did not differ histologi-

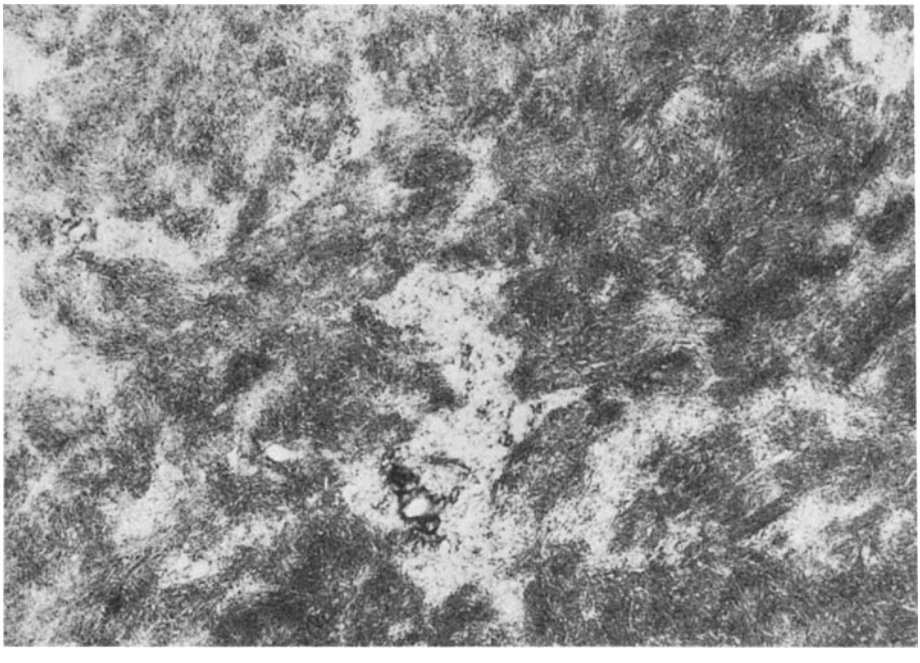

Fig. 5. Electron micrograph showing massive deposits of typical amyloid fibrils in heart valve (×18000)

cally from those of the other patients – in particular regarding the amount of amyloid and calcium phosphate.

It was possible, in 40 of the 51 aortic valves, to determine during operation whether they were bi- or tricuspid. Fourteen of these forty were found to be bicuspid (35%), of these 12 were from men and 2 from women. There was no difference in the average age with regard to bi- or tricuspid aortic valves, neither was there any significant difference as to the severety of the amyloid degeneration. All the bicuspid valves, with the exception of one, were the seat of calcium phosphate deposition. The systolic pressure gradient over the valve had been measured in 45 of the 51 aortic valves prior to operation. With Fisher's exact test there was a significant correlation between a systolic pressure gradient over 50 mm Hg and severer degrees of amyloid degeneration, hyalinization and deposits of calcium phosphate in the valve tissue (Table 4).

The mitral valves were evaluated peroperatively with regard to stenosis/insufficiency and for rupture of the cordae tendineae. Six of the valves were purely insufficient, 14 were purely stenotic while 29 were the site of both stenosis and insufficiency. In two cases there was also rupture of the cordae tendineae.

From Table 5 it can be seen that 12 of 14 mitral valves with stenosis only were the site of amyloid degeneration, while none of the valves with insufficiency only, contained amyloid. Only 10 of the 29 valves with a combination

Table 4. Degree of aortic stenosis in relation to amyloid deposits, hyalinization and calcium phosphate deposits

		Aortic stenosis (systolic pressure gradient in mm Hg)			
		<50	$\geqq 50$	total	Fisher's exact test
AMYLOID	None + slight degree	5	4	9	
	Mod + Severe degree	4	32	36	$0.02 > P > 0.01$
	Total	9	36	45	
HYALIN	None + Slight degree	5	4	9	
	Mod. + Severe degree	4	32	36	$0.02 > P > 0.01$
	Total	9	36	45	
CALCIUM	None + Slight degree	4	1	5	
	Mod. + Severe degree	5	35	40	$0.01 > P > 0.002$
	Total	9	36	45	

of insufficiency and stenosis had amyloid degeneration. Using Fisher's exact test one finds significantly more amyloid in the mitral valves with stenosis only ($P < 0.01$). No amyloid was found in the ruptured cordae tendineae, similarly no significant correlation was found between pulmonary capillary venous pressure and amyloid in the mitral valves (Table 5).

Discussion

Sixty-seven per cent of the heart valves in our material were the site of varying degrees of amyloid degeneration. The amyloid had characteristic light microscopic (Puchtler et al. 1962) and electron microscopic (Shirahama and Cohen 1967) properties.

In the aortic valves we found, using Fisher's exact test, a significant correlation between large amounts of amyloid, hyalin and calcium phosphate and a systolic pressure gradient of more than 50 mm Hg. Similarly most amyloid was found in the stenotic mitral valves. This has not been reported earlier, but Lachman and Roberts found in 1978 that the amount of calcific deposits in stenotic mitral valves correlated with the mean diastolic pressure gradient across the mitral valve ($P < 0.05$).

Table 5. Amyloid deposits in the mtiral valve in relation to stenosis, insufficiency, stenosis in combination with insufficiency, rupture of the cordae tendineae and pulmonary capillary venous pressure (PVC)

	Amyloid in mitral valve				
	0	+	++	+++	Total
Stenosis	2	2	8	2	14
Insufficiency	6	0	0	0	6
Stenosis + insufficience	19	5	2	3	29
Rupture of cordae tendineae	2	0	0	0	2
PCV (mm HG)					
Under 15	4	1	0	0	5
15–25	10	4	2	4	20
over 25	12	2	7	1	22

A survey of the literature shows that valvular amyloidosis until recently has been considered to be a relatively rare condition (Dahlin and Edwards 1949; Frederiksen et al. 1962; Lindsay 1946; Pomerance 1966; Symmers 1956). It was with the report of Goffin in 1980 that microscopically demonstrable amyloid in the heart valves was shown in a larger systematic investigation. Goffin found amyloid in 15.5% of his material, which consisted of mitral and aortic valves removed due to "chronic valvular disease". The amyloid was described as being localized to densely sclerotic and poorly vascularized scar tissue, frequently in close proximity to calcific deposits. In our investigation we have found significantly more amyloid in the severely hyalinized heart valves; similarly, there was a significant simultaneus occurrence of amyloid deposits and calcium phosphate deposits in the valves. There was also a correlation topographically between amyloid, hyalinization of the connective tissue and the presence of calcium phosphate. Falk et al. found in 1981 the constant presence of amyloid in calcific aortic valves.

It is well-known that calcification of both the aortic and mitral valves can lead to stenosis of the valves, and in some cases to insufficiency also (Bacon and Matthews 1959; Campbell 1968; Kitchin and Turner 1967; Lachman and Roberts 1978; Pomerance 1972; Roberts 1970). The pathogenesis of this calcification, particularly of the left side of the heart has on the other hand been the object of considerable discussion for many years.

In 1972 Pomerance divided aortic stenosis into three groups:
1) Calcification of congenitally malformed aortic valves,
2) Senile degenerative calcification and
3) Inflammatory stenosis.
ad 1: In our investigation, 35% of the aortic valves were bicuspid and all of these, with the exception of one, were the site of severe calcification.
ad 2: With regard to senile degenerative calcification, we found in our investigation that there was a significantly increased amount of calcium phosphate in the aortic valves from the older age groups.

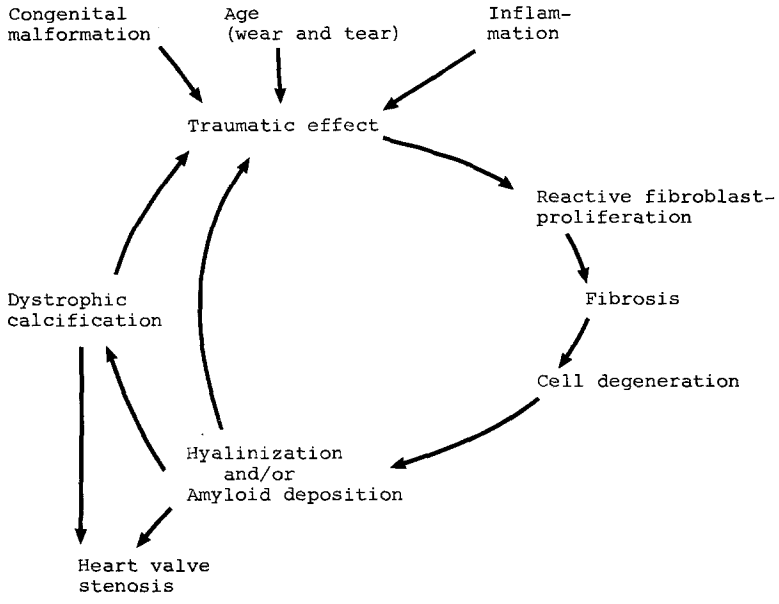

Fig. 6. The vicious circle of heart valve stenosis

ad 3: We did not find greater or smaller quantities of calcium phosphate in the heart valves from the 32 patients who had earlier suffered from rheumatic fever; similarly inflammatory changes in these heart valves were sparse. However, it should be pointed out that 51% of the patients subjected to operation for removal of the mitral valve had earlier suffered from rheumatic fever.

Sell and Scully (1965) have demonstrated that calcification of the mitral and aortic valves is preceeded by fibrosis and hyalinization of the connective tissue. We found significant simultaneus occurrence of heavy calcification and heavy hyalinization (Table 2). Kim et al. (1976) demonstrated, in agreement with this, that fibrocytes in the aortic valve degenerate, which leads to dystrophic calcification corresponding to cellular degradation products. These degradation products contain large quantities of cytofilaments. In 1979 Linder et al. showed that fibrocyte cytofilaments, both histochemically and ultrastructurally, have the same characteristics as amyloid. The deposition of these fibrocyte degradation products presumably results from an inhibition of the mechanisms responsible for the elimination of extracellular material (Cathart et al. 1970; Maury and Teppo 1982). The mechanism whereby cytoplasmatic filaments may become deposited extracellularly as amyloid has been discussed by Page et al. (1975) and by David and Buchner (1978).

We have demonstrated no causal relationship between amyloid, hyalin and calcification – only simultaneus occurrence (Tables 1, 2, 3). It is however well-known that in other localizations amyloid is found together with calcification (Ranløv and Pindborg 1966; Symmers 1956). We will now put forward the following hypothesis regarding the pathogenesis of stenosis of the mitral

and aortic valves: The process starts with the traumatization of the connective tissue of the valve, either as a result of congenital malformation (biscuspid aortic valve), wear and tear in connection with increasing age or due to rheumatic or non-specific inflammation. The trauma causes reactive fibroblast proliferation with the development of fibrosis, fibrocyte degeneration and hyalinization with accumulation of extracellular degradation materials, including cytofilaments, which are transformed to amyloid. The accumulation of these degradation compounds and dystrophic calcification of them leads to further traumatization of the valve (Pomerance 1977). The process is thus a vicious circle and will, within a number of years, end in symptom producing stenosis of the heart valves (Fig. 6).

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