Chemically determined mineral content of explanted porcine aortic valve bioprostheses: correlation with radiographic assessment of calcification and clinical data

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ABSTRACT Bioprosthetic valve calcification is usually assessed pathologically by gross inspection, radiographic studies, and histologic examination. Quantitation of mineral content by chemical assay has not been reported for failed clinical valves removed from adults. In this study, calcium determination by atomic absorption spectroscopy was done on 52 removed porcine valves after routine pathologic examination, including specimen radiography done by a standard technique. Specimens included 31 valves with calcific primary tissue failure, two calcified (but not overtly dysfunctional) valves removed simultaneously with failed valves, 14 nondeteriorated valves obtained at reoperation or autopsy after long-term implantation, and five valves removed within 1 month after insertion. Chemically determined mineral content varied widely among patients and duration of function. Valves with calcific failure had 113 ± 68 μg/mg calcium overall (mean ± SD) after 36 to 156 months (mean 87) of function. Almost all dysfunctional porcine valves with radiographically demonstrated calcific deposits had greater than 34 and 67 μg/mg calcium for mitral and aortic valves, respectively. Nondeteriorated valves (implanted 8 to 145 months, mean 57) had 5 ± 6 μg/mg calcium. Failed aortic valves had more calcium than failed mitral valves and valves with calcific stenosis more than valves with regurgitation caused by calcification with tearing. Correlation of semiquantitative radiographic grading with chemically determined valve mineral was good, indicating that radiographic assessment of calcification may be used reliably for clinical comparisons between valves. Circulation 76, No. 5, 1061–1066, 1987.

BIOPROSTHESES fabricated from glutaraldehyde-preserved porcine aortic valve or bovine pericardium are widely used to replace diseased human cardiac valves. Clinical regurgitation, stenosis, or both are frequently caused by primary tissue failure, necessitating reoperation with valve removal or causing death of approximately 25% of patients with porcine bioprostheses within 10 years after insertion.1–5 Calcification, with or without secondary cuspal tearing, is the most prominent pathologic factor.1, 2 Pericardial bioprostheses also fail by calcific degeneration.6–8

Assessment of calcification in explanted bioprosthetic valve specimens is usually done by gross inspection, histologic examination, and radiographic studies.9–11 Quantitative mineral determinations by chemical assay of accumulated calcium and phosphorus have been used widely in experimental studies12–15 and have been reported for a limited number of valves removed from children,2 but such data are not available for failed valves explanted from adults. The purposes of this study were to quantitatively determine mineral accumulation in removed dysfunctional and properly functioning porcine bioprostheses by chemical assay and to correlate these data with semiquantitative, non-destructive radiographic assessment of calcification and selected clinical variables.

Materials and methods
Fifty-two glutaraldehyde-preserved porcine aortic valve bioprostheses were studied. These valves were removed from adults over an interval of approximately 5 years, fixed, and stored in 10% neutral phosphate-buffered formalin. Fourteen nondeteriorated valves (six aortic and eight mitral, including 12 Hancock

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[Johnson and Johnson Cardiovascular, Anaheim, CA] and two Carpentier-Edwards [American Edwards Laboratories, Santa Ana, CA] porcine bioprostheses, implanted for 8 to 145 months (mean 57), were removed incidentally at autopsy (n = 2) or were obtained at surgery (seven with paravalvular leak, four at transplantation, one for other cause). In 31 valves (10 aortic, 21 mitral; 26 Hancock and five Carpentier-Edwards), primary tissue failure with calcification necessitated valve removal or caused death 36 to 156 months (mean 87) after insertion. Twenty-seven of these valves were regurgitant (eight aortic, 19 mitral) and four were stenotic (two aortic, two mitral). In two of the 31 patients with calcific valve failure, a functional aortic bioprosthesis was removed simultaneously with a dysfunctional mitral valve, 90 and 96 months after insertion. Thus 47 valves removed after 8 to 156 months function were examined. Five properly functioning valves removed at autopsy (four mitral, one tricuspid), implanted 0 to 24 days earlier, were used as controls; in no case was the cause of death valve-related. No valve studied had infective endocarditis or gross thrombus.

Prostheses were carefully examined grossly, photographed, and radiographed in axial projection at 35 kV for 1.0 min by a standard technique (Faxitron, Hewlett Packard, McMinnville, OR). Calcification of individual specimens was graded radiographically in three independent determinations: (1) by an observer during the routine surgical or autopsy pathologic evaluation of the individual specimens as they were accumulated, (2) by the same individual (observer 1) through sorting of radiographs into groups when all specimens had become available, and (3) sorting by a different individual (observer 2). In each case mineralization was graded as 0 = not present, 1+ = mild, 2+ = moderate, 3+ = more severe, and 4+ = most severe. Typical valve radiographs illustrating the major levels in the grading scheme are shown in figure 1.

Calcium content was measured chemically by atomic absorption spectroscopy of acid hydrolysates of finely minced cuspal tissue, oven dried to a constant weight.12,14,15 Concentrations are expressed as micrograms per milligram dry tissue weight. With this technique, the variation of multiple chemical determinations of the same specimen was small (less than 2%); thus standard deviations represent real patient valve heterogeneity. To assess the possible effects of formalin storage on measured valve calcium content, 10 separate specimens of freeze-dried, severely calcified glutaraldehyde-pretreated bovine pericardial tissue, previously implanted subcutaneously in 2 to 3-week-old rats and harvested 21 days after implantation, were analyzed for calcium content by atomic absorption spectroscopy, immediately after removal and after 7 and 30 days storage in 10% phosphate-buffered formalin (pH 6.9 to 7.1 at 25° C; Fisher Scientific, Springfield, NJ).

Results

Measured calcium contents of the four groups of valves are summarized in table 1 and plotted as a function of implant duration in figure 2. Valves removed after less than 3 years had insignificant mineral accumulation. One valve implanted for 36 months had 186 μg/mg calcium. At implant durations greater

FIGURE 1. Composite radiograph of calcified porcine aortic valve bioprostheses from this study demonstrating the various levels of mineralization, 1+ through 4+, and providing radiographic standards for examination. Top, Uncalculated (0), 1+, and 2+. Bottom, 3+, 3+, and 4+. All specimens illustrated are Hancock porcine valves except the bottom row, middle, which is a Carpentier-Edwards valve.
TABLE 1
Mineral content of bioprosthetic valvar tissue

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Duration (months)</th>
<th>Calcium (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short-term</td>
<td>5</td>
<td>&lt;1</td>
<td>0</td>
</tr>
<tr>
<td>Nondeteriorated</td>
<td>14</td>
<td>57 (8-145)</td>
<td>5 ± 6</td>
</tr>
<tr>
<td>Calcific</td>
<td>31</td>
<td>87 (36-156)</td>
<td>113 ± 68</td>
</tr>
<tr>
<td>Functional</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>accompanying</td>
<td>2</td>
<td>90, 96</td>
<td>30, 53</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

A Months, mean (range).

B μg/mg, mean ± SD.

C Removed simultaneously with failed valves.

than 3 years, calcium content varied widely among valves, some having no detectable mineral despite long-term function and others having substantial mineral accumulation. Calcium content varied from 1 to 7 μg/mg in 12 of the 14 functional valves implanted for 8 to 120 months to 239 μm/mg in a stenotic aortic prosthesis implanted for 99 months. Valves with calcific degeneration had a mean of 113 μg/mg calcium, with a mean duration of function of 87 months. Except for an aortic and a mitral valve with tears and radiographically observed minute calcific deposits (1+), but only 3 and 13 μg/mg calcium, respectively, all calcified and torn mitral valves had greater than 34 μg/mg calcium and all aortic valves had greater than 67 μg/mg. Two functional valves removed at cardiac transplantation had calcium content of 15 and 22 μg/mg after 59 and 145 months function, respectively. Two other valves removed incidentally (one at autopsy for death not related to the valve and one mitral prosthesis removed concurrently with aortic valve replacement) after 120 and 118 months, respectively, had 7 and 2 μg/mg calcium. Overall, functional valves had a calcium content of 5 μg/mg after a mean duration of 57 months. Each of the five valves implanted for 24 days or less had undetectable mineral content. Unimplanted porcine aortic valve tissue has previously been determined to have approximately 1 μg/mg or less calcium.\(^{12, 14}\)

Storage of calcified bovine pericardial tissue in neutral buffered formalin did not result in measurable loss in calcium. Calcium content was identical in aliquots of minced specimens derived from common source tissue, which were not stored in formalin (calcium content 156 ± 12 μg/mg), stored for 7 days (171 ± 12 μg/mg), or stored for 30 days (152 ± 8 μg/mg) (mean ± SEM).

The relationships between mineral content and both mode of failure and valve site are summarized in Table 2. Purely stenotic valves had more calcium than calcified valves with tears (mean 170 vs mean 105 μg/mg). Failed aortic valves (10 specimens) had more mineral than failed mitral valves (21 specimens) (145 vs 98 μg/mg calcium). Table 3 lists the mineral content of simultaneously removed aortic and mitral porcine valves. In five cases where both aortic and mitral valves were removed, there was no pattern indicating preferential mineral accumulation at either site. The func-

FIGURE 2. Calcium concentration of removed porcine valves, measured by atomic absorption spectroscopy, plotted against duration of function. Short-term, n = 5; nondeteriorated, n = 14; calcific degeneration, n = 31; accompanying failed valve, n = 2.

TABLE 2
Mineral content for different valve sites and modes of failure (31 valves with calcific degeneration)

<table>
<thead>
<tr>
<th>Valve site</th>
<th>n</th>
<th>Duration (months)</th>
<th>Calcium (μg/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitral</td>
<td>21</td>
<td>87 (36-156)</td>
<td>98 ± 60</td>
</tr>
<tr>
<td>Aortic</td>
<td>10</td>
<td>87 (62-124)</td>
<td>145 ± 77</td>
</tr>
<tr>
<td>Mode of failure</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stenosis</td>
<td>4</td>
<td>77 (68-89)</td>
<td>170 ± 56</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>27</td>
<td>89 (36-156)</td>
<td>105 ± 66</td>
</tr>
</tbody>
</table>

\(^{A}\) Months, mean (range).

\(^{B}\) μg/mg, mean ± SD.


TABLE 3
Mineral content of aortic and mitral valves implanted and removed simultaneously

<table>
<thead>
<tr>
<th>Duration (mo)</th>
<th>Calcium (μg/mg)</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>59</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>68</td>
<td>214</td>
<td>115</td>
</tr>
<tr>
<td>87</td>
<td>13</td>
<td>67</td>
</tr>
<tr>
<td>90</td>
<td>142</td>
<td>53</td>
</tr>
<tr>
<td>96</td>
<td>28</td>
<td>30</td>
</tr>
</tbody>
</table>

Figure 3 and table 4 summarize the correlation of chemically determined mineral content with radiographic calcification. Radiographic calcification varied from 0 (not detectable) to 4+ (most severe). Since the total number of valves with severe (3+ and 4+) calcification was small, they are combined for the purposes of this study. There was good correlation between radiographically measured calcification (among three determinations) and that measured by quantitative analysis. Variability in calcification within a specific radiographic classification reflects real variation in degree and distribution of calcific deposits among patients. All three determinations assigned the same radiographic grade in 28 valves (55%). In an additional 22 valves (42%), one determination differed from the other two; in almost all of these cases, the actual mineral content was intermediate between the means of the grades estimated.

Discussion

This study confirms by chemical assay that there is considerable heterogeneity among patients in the rate of mineral accumulation in implanted porcine bioprostheses, in agreement with the results of a previous study using radiographically assessed mineral. Indeed, this study demonstrates that mineral content varies considerably among valves at the time of removal necessitated by calcific failure. These results are consistent with actuarial data, which indicate that primary tissue failure caused by calcification is unusual less than 3 or 4 years after implantation but increases sharply in frequency

FIGURE 3. Correlation of measured calcium concentration with three individual determinations of radiographic grading of mineralization. A, Determination by an observer during the routine surgical or autopsy pathologic evaluation of the separate specimens. B, Determination by the same individual (observer 1) through sorting of radiographs into groups when all specimens had become available. C, Sorting as in B by a different individual (observer 2).
thereafter. Nevertheless, the results suggest that many patients without evidence of clinical prosthetic valvular dysfunction have minimal calcification of their bioprostheses, some despite long postoperative intervals.

Mean cuspal calcium concentration in valves removed because of calcific primary tissue failure was 113 μm/mg, after function for mean 87 months. In contrast, three functional valves implanted for 10 years or more had only 2 to 22 μg/mg calcium, emphasizing that not all porcine valves mineralize appreciably. Most valves removed for reasons other than calcific failure had less than 7 μg/mg calcium, only slightly more than unimplanted valves. The most calcified valve had 239 μg/mg calcium; removal of this valve was necessitated by stenosis. The calcium content of these clinical porcine valves compares closely with data of previous experimental studies using subcutaneous implants of porcine valve tissue and bovine pericardium in rats, in which approximately 115 μg/mg calcium is accumulated in 21 days and the saturation calcium content after long implantation times is 200 to 225 μg/mg calcium.

Experimental calcified pericardial specimens stored in neutral phosphate-buffered formalin had no significant loss of tissue calcium content after storage for up to 30 days. It is likely that no measurable loss would occur even after prolonged (years) storage, barring physical disintegration, since no significant solubilization of calcium phosphate mineral should be possible because of the insolubility of calcium phosphate crystalloids in the presence of an excess of phosphate.

Previous studies suggested that mitral porcine bioprostheses fail more frequently than aortic valves and that mitral valves accumulate more radiographically detectable mineral than aortic valves implanted simultaneously in the same patient. In combined aortic-mitral valves removed from five patients in this study, no pattern specific to valve site was discerned in the chemically determined mineral content. In contrast, failed mitral valves had less mineral than failed aortic valves. Perhaps mitral valves have less mineral at failure because they are subjected to higher mechanical stresses during closure than aortic bioprostheses, and thus tears that precipitate clinical deterioration of the patient occur with less mineral. Nevertheless, these data cannot be used to reveal relative trends in the natural course of mineral accumulation in valves implanted in the two sites, since these data reflect the mineral content only of failed valves. It is not surprising that stenotic porcine valves have more mineral than regurgitant valves, since valves should insidiously continue to accumulate calcific nodules either until accumulation is massive, causing stenosis, or until a tear intervenes, leading to regurgitation. All stenotic valves had at least 115 μg/mg calcium.

The marked variability among patients and valves in the propensity toward calcification may reflect an unpredictable interaction of various important host and implant factors that affect mineralization. Some of these determinants have been identified in previous studies. The rate of bioprosthetic tissue calcification is clearly dependent on host metabolic factors. For example, renal failure and young age potentiate mineralization. However, since all patients from which valves were studied are adults, this investigation does not permit consideration of the effect of age on calcification. An important determinant of the propensity of bioprosthetic tissue to calcify is the tissue preparation method. For example, pretreatment of tissue with an aldehyde cross-linking agent is a prerequisite for mineralization. Furthermore, clinical and experimental studies strongly suggest that dynamic mechanical stress and strain promote calcification.

The results of this study have implications for prevention of bioprosthetic valve calcification. Recent studies indicate that it may be possible to inhibit calcification of bioprosthetic valves by either therapeutic administration of pharmacologic agents, such as diphosphonates, or pretreatment of the cuspal tissue before implantation by these or other chemicals. In this respect, the lower limit of mineral content that provokes calcific failure may guide therapeutic strategies. In the present study, calcific dysfunction was generally associated with at least 34 and 67 μg/mg calcium for mitral and aortic valves, respectively. This suggests that calcific failure will be unlikely unless calcium accumulation exceeds these values. Thus preventive approaches restricting calcium uptake below these levels should be largely efficacious in preventing clinical dysfunction.

This study shows that determination of severity of mineralization by a standard radiographic technique predicts actual mineral concentration reasonably well. The less than perfect correlation is due to at least three factors. First, although assessment of radiographic mineral in the present study was done by an experienced cardiac pathologist, each valve was initially evaluated independently of the others in the course of routine surgical or autopsy examination of valves over a 5 year period. Second, since valves were sectioned for histologic analysis after radiography but before chemical mineral determination, and since chemical assay reflects a mean value of mineral in remaining tissue, it is possible that the tissue remaining was not
absolutely representative of all tissue originally present. Finally, radiographic assessment of mineral is a composite subjective determination taking into consideration both intensity and distribution of calcific deposits; thus discrepancies between chemical and radiographic assessments may reflect subtle differences in features of mineral morphology among valves. Nevertheless, these data confirm that radiographic assessment of calcification done by a reproducible technique provides semiquantitative data, which, although not precise, are sufficiently accurate to be reliable for comparisons between valves.

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References
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