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The glutaraldehyde-processed porcine aortic heart valve is similar in its microscopic organization to the human aortic valve (1). It is important to understand the detailed histology of this bioprosthetic valve because distinct components of its leaflets are altered selectively when the valve is initially collected from the pig and stabilized with the commercial glutaraldehyde fixation process. Further structural modifications also occur after the commercially processed valve has been inserted into a patient where it undergoes progressive degeneration.

The Porcine Valve before Implantation

Structural components of the unprocessed valve

When a porcine valve is removed from the animal and immediately fixed by conventional methods, its leaflets reveal a series of distinct layers when they are examined by both light microscopy (Fig. 1) and transmission electron microscopy (Fig. 2). A contiguous monolayer of endothelial cells covers both surfaces of the leaflet, which would contact the circulating blood when the valve was in its normal anatomic position. The endothelial cells are joined together by intercellular junctions and supported by a basement membrane of varying complexity. On the inflow surface (the surface that faces the ventricular cavity when the valve is in its normal position), the basement membrane is thin and poorly defined. In contrast, the basement membrane on the outflow surface is thicker, reduplicated, and multilayered.

Beneath the basement membrane on the outflow surface of the leaflet lies a fibrosal layer composed of dense fibrous connective tissue. This layer contains typical fibroblasts, abundant collagen fibrils densely packed and sometimes arranged in parallel bundles, and an extracellular matrix which contains glycosaminoglycan molecules and proteoglycan complexes. The middle layer of the leaflet, called the spongiosa, is composed of a more loosely organized type of fibrous-elastic connective tissue. Fibroblasts, randomly oriented collagen fibrils, elastic fibers, proteoglycan molecules, and filaments with a small diameter (50A across) are found in this layer.

The layer subjacent to the basement membrane on the inflow surface, the ventricularis, is identified by the presence of numerous elastic fibers (2).

The principal structural proteins of the porcine valve are collagen and elastin. Collagen is dominant and comprises about 55% of the valvular tissue by dry weight, while elastin accounts for only 12% (3,4). In the porcine aortic valve, type I collagen, which is also found in bone, tendon, and skin (5), is present in the greatest amount (6,7). Type III collagen, which is common to blood vessels (5), is found to a lesser extent.

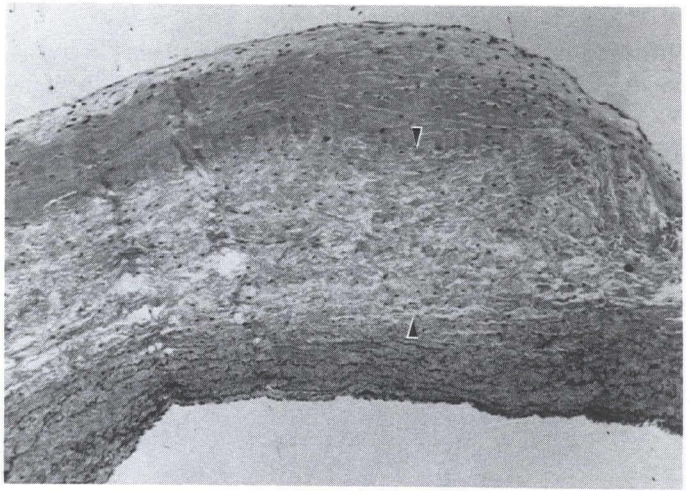

Fig. 1

Light microscopic appearance of a normal porcine aortic valve showing histologically distinct layers. Note the central spongiosa layer (arrow) X 100.

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The glutaraldehyde fixation process

Porcine aortic valves used to replace diseased human heart valves are processed by a commercial procedure that stabilizes them with glutaraldehyde. Glutaraldehyde is the preferred fixative because of its ability to form intrafibrillar collagen crosslinks interconnecting lysyl and hydroxylysl side chains (8). Initial structural preservation of the valve is achieved by placing it in a low concentration of glutaraldehyde in buffer at a physiologic pH. After partial fixation, the valve is stored in buffered glutaraldehyde.

The glutaraldehyde-processed valve has several characteristic structural alterations. One consistent finding (Fig. 2) is that endothelial cells are partially or completely lost from the outermost surface of the valve (9,10). This is particularly important since an intact endothelial cell lining represents the most effective thrombosis-resistant surface now known. Endothelial cell loss, however, may not be entirely related to exposure of the valve to the glutaraldehyde fixation. It may also be a direct response to autolytic changes that occur during the time between the removal of the valve from the pig and the fixation process.

After commercial fixation, the collagen and elastin fibrils of the leaflets retain their normal ultrastructural appearance, including the typical periodicity which identifies collagen fibrils. However, the spatial orientation of the fibrous framework is altered so that fibers are more widely separated, and consequently, more loosely arranged (9). In addition, the overall flexibility of the processed leaflets is somewhat reduced.

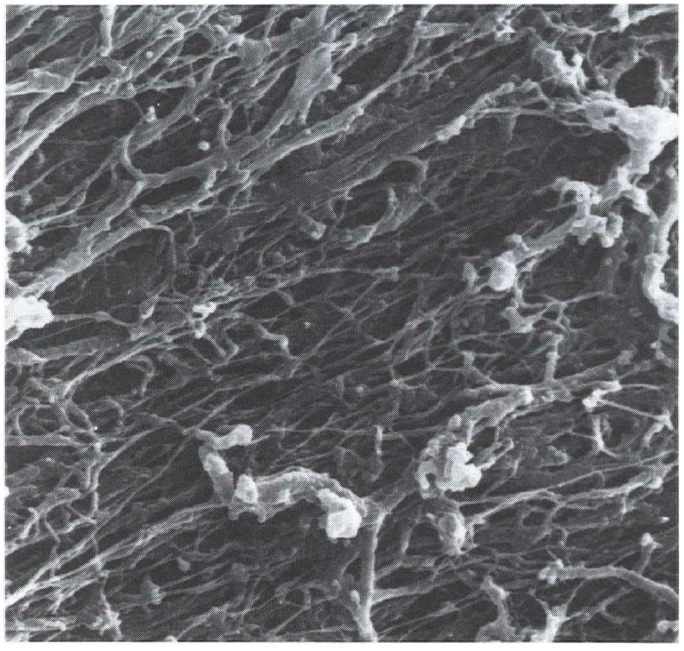

Fig. 2

Scanning electron micrograph of a glutaraldehyde-processed, unimplanted, porcine bioprosthetic valve showing the subendothelial fibrous network exposed after the loss of the endothelium (X 7,000).

In processed valves, the glycosaminoglycan (mucopolysaccharide) and/or the proteoglycan (mucopolysaccharide-protein complexes) content, particularly of the spongiosa layer, are decreased (9). This may be a significant modification as some of these molecular complexes have been found to inhibit calcification (11,12), which represents one of the major degenerative processes in this type of valvular bioprosthesis.

The integrity of the fibroblasts in the connective tissue of the spongiosa and fibrosal layers is also compromised after exposure to the glutaraldehyde stabilization process. Cellular alterations include 1) disruption of the plasma membrane; 2) interruptions of the internal organellar cytoplasmic membranes (2); and 3) mitochondrial vacuolation (Fig. 3). These ultrastructural findings signal cell death and suggest that these cells are no longer capable of performing their normal synthetic functions.

The Porcine Valve after Implantation

After the commercially processed porcine valve is inserted into a patient, many different events occur as this bioprosthesis interacts with the circulating blood and is subjected to the hemodynamic stresses associated with normal valve function.

Plasma proteins

Since most of the endothelium is lost from the surface of the leaflets during commercial processing, the circulating blood contacts subendothelial structures (basement membrane, collagen fibers, and elastin fibers) rather than a layer of endothelial cells. Plasma proteins were found to penetrate this relatively open, mesh-like surface and were deposited as fine, fibrillar material that surrounded the collagen fibers, particularly in the spongiosa layer (2).

Peripheral blood elements

Formed elements of the peripheral blood also interact with and are deposited onto the surface of the valve as it degenerates in vivo. Erythrocytes (10), leukocytes (2,9,10, 13-16), and platelets (2,9,10,13-21) were found on the exterior of degenerated porcine bioprosthetic valves after they had been inserted into patients for various lengths of time (Figs. 4,5).

Leukocytes

By means of stained sections and light microscopy or ultrathin sections and transmission electron microscopy, mainly adherent lymphocytes, plasma cells, macrophages, and giant cells were observed in contact with the exposed basement membrane or fibers (2,9,13,15). We suspected that these observations might not reflect the entire hematogenous cytopopulation of the degenerated bioprosthetic valve because only a limited area can be surveyed in a light microscopic or ultrathin section.

In order to more fully examine the types of cells present, we initiated a study which employs enzymatic digestion with collagenase to remove adherent cells from the surface of the leaflets of degenerated porcine valves that have been surgically removed. To date, we have studied two degenerated valves that had been inserted for seven and ten years each. The number of cells obtained from all three leaflets of each valve was 4×10^6 cells/ml and 5.1 $\times 10^6$ cells/ml, respectively. The isolated cytopopulations were centrifuged onto glass microscope slides. One of these preparations was stained with Leishman's stain, and a differential count of 500 cells was performed (Table I).

Although these data confirm the observations made by microscopic examination of sectioned material, namely, that lymphocytes, macrophages, and plasma cells were present in the highest percentages, they also demonstrated that granulocytes (neutrophils, eosinophils, and basophils) interacted with and adhered to the surface of the degenerating valve (14.6% and 22.4% of the "released cells"). Polymorphonuclear neutrophils were the most numerous type of granulocytes seen. Careful examination of individual, stained neutrophils revealed that: 1) large, darkly stained cytoplasmic granules occurred among the smaller sized, specific neutrophil granules; and 2) vacuoles were scattered throughout the cytoplasm. Both of these morphologic modifications are

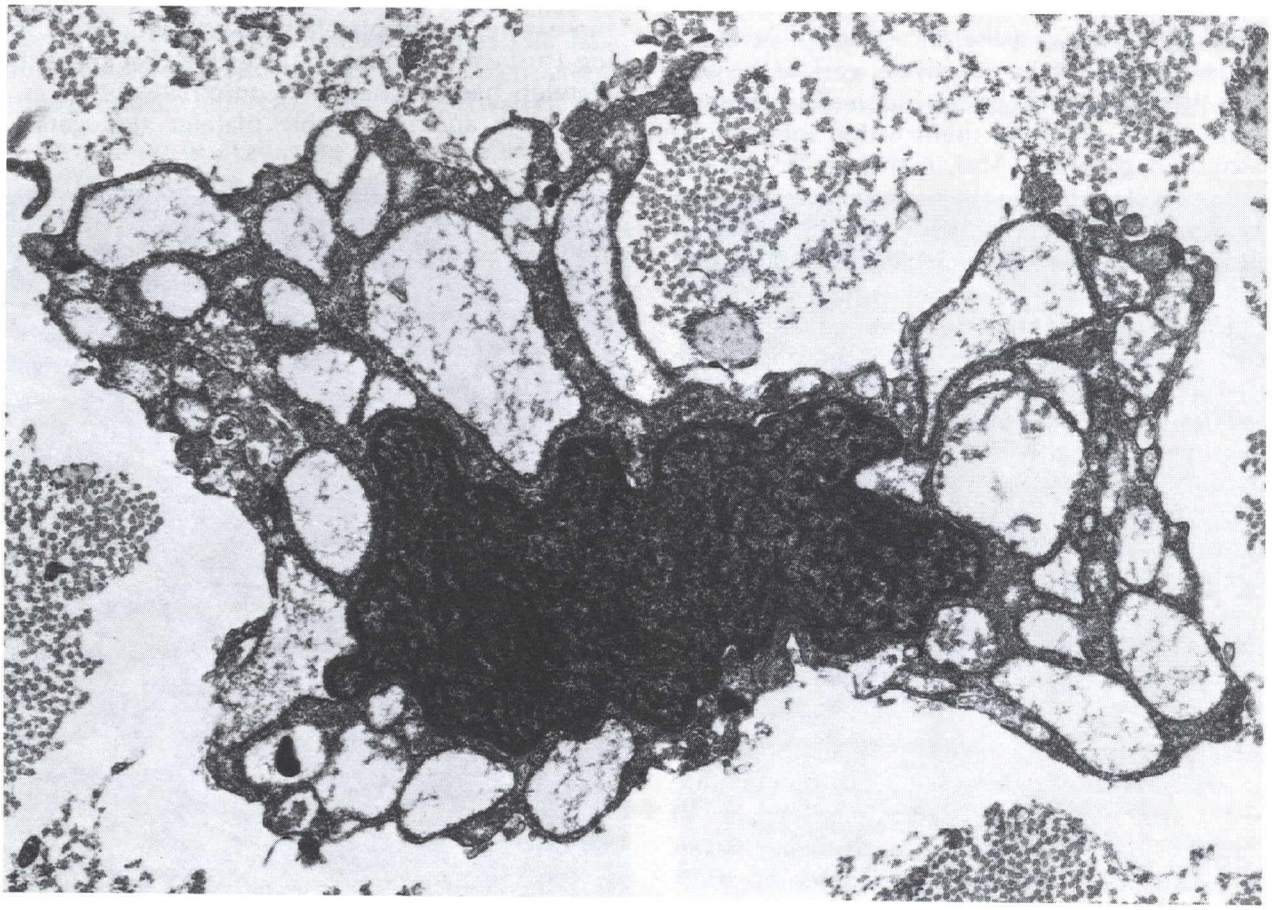

Fig. 3

Transmission electron micrograph showing evidence of cellular injury (cytoplasmic vacuolation and mitochondrial swelling) of the fibroblasts in the spongiosa layer after glutaraldehyde treatment (X 22,300).

TABLE I

Cytopopulation of the Surface of Degenerated Porcine Bioprosthetic Valves

Cell Type	Patient 1	Patient 2
Neutrophils	12.8	14.4
Eosinophils	1.4	6.8
Basophils	0.4	1.2
Lymphocytes	11.8	46.8
Plasma Cells	32.2	15.0
Macrophages	38.2	6.6
Giant Cells	1.2	0.8
Endothelial Cells	2.0	8.4

known to occur as a neutrophil participates in the process of phagocytosis, which is its major functional role (22).

Neutrophils may play a role in the process of progressive degeneration of implanted porcine bioprosthetic valves in the following ways. Adherent neutrophils might reduce the thickness of the fibrous network of the porcine valve by phagocytizing available small collagen or elastin fibers and digesting them within intracellular cytoplasmic phagosomes. Also, if the neutrophil encounters a particle too large for it to engulf, such as the network of exposed fibers on the surface of the leaflet, it can release its lysosomal enzymes extracellularly and deposit them onto the surface contacted, where enzymatic digestion can proceed (23). Although neutrophils do not constitute the major portion of the cells which adhere to the surface of the degenerated porcine valve, they may nonetheless operate as a major destructive force for the exposed connective tissue framework. Neutrophils contain the following releasable lysosomal enzymes: 1) a chymotrypsin-like enzyme which functions at neutrality and is capable of degrading protein-containing macromolecules such as the protein associated with the proteoglycan of the connective tissue matrix (24); 2) an elastase which specifically attacks elastic fibers (25,26); and 3) a collagenase which could selectively attack the collagen fibers (27).

Macrophages on the surface of degenerated porcine bioprosthetic valves may also damage the fibrous network. Because these mononuclear phagocytes are capable of ingesting particulate materials such as exposed collagen or elastin fibers, they too could engulf fibers and effect intracellular digestion via lysosomal enzymes (28). Collagenase and an elastase-like enzyme are also present in lysosomes of macrophages, and these too can either be released into phagosomes or secreted extracellularly (29). As is true of the neutrophil, the enzymatic potential of the lysosomes of the adherent macrophages could function both intracellularly and extracellularly to digest collagen and elastic fibers which constitute the framework of the porcine bioprosthetic valve. The enzymatic potentials of neutrophils and macrophages may therefore play a role in reducing the quantity and compromising the integrity of the fibrous component of the valve leaflet so that it ultimately succumbs to continuous hemodynamic demands and develops a tear (Fig. 7).

Platelets

Microthrombus formation and platelet deposits (2, 9,10,13-21) have been found on the surface of degenerated porcine bioprosthetic valves. In a previous study (10), we used scanning electron microscopy to examine the surfaces of degenerated valves that had been inserted in six patients from four to seven years. We demonstrated that individual platelets as well as platelet aggregates were present on the surface of five of six (83%) of these valves. Single platelets showed cytoplasmic spreading (surface activation), suggesting that the adherent platelets had released some internal substances. Both reversible and irreversible platelet aggregates were seen.

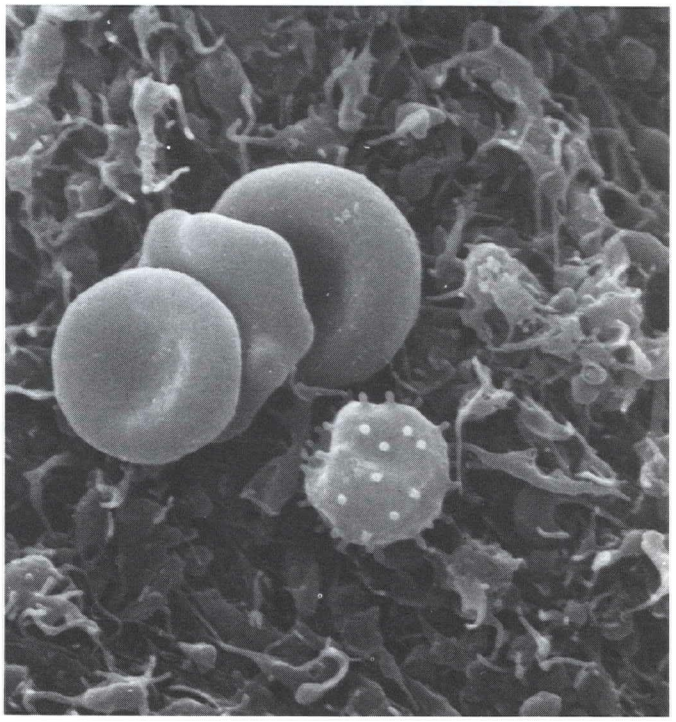

Fig. 4

Adherent erythrocytes on the surface of a degenerated porcine bioprosthetic valve (X 7,000).

It therefore became important to determine whether the reactivity of the circulating platelets was normal or abnormal in these patients. We measured the response of circulating platelets from six patients with degenerated porcine valves just before their valves were removed (29). We then evaluated the degree of cytoplasmic spreading exhibited by single, adherent platelets and the amount of aggregation shown by the platelet populations in vitro. We used a test system in which platelets were exposed to an activating surface (Formvar film) under standardized conditions. Circulating platelets from five of the six patients were hyperactive (Fig. 8). Each patient who had an abnormal in vitro platelet response also had platelets deposited onto the surface of the excised valve. In one patient, the platelets reacted normally in vitro, and no platelet deposits were seen on the surface of his degenerated valve. These observations demonstrated that platelets from patients with degenerated porcine bioprosthetic valves show increased reactivity. The deposition of platelets onto the surface of the degenerated valves correlated with a hyperactive response of the circulating platelets. Others (30) have found that platelet survival was decreased in ten patients after they received a porcine bioprosthetic valve.

Another method of evaluating the degree of platelet activation is to assay the plasma concentration of certain products released only by platelets, namely, platelet factor 4 and β-thromboglobulin (31). When we measured the plasma levels of these substances in two patients with degenerated porcine bioprosthetic valves, we found that the concentrations of both substances were abnormally elevated. The levels of platelet factor 4 were 12.91 ng/ml and 14.67 ng/ml with corresponding levels of 117.96 ng/ml and 83.09 ng/ml for β-thromboglobulin, respectively. The average normal value for platelet factor 4 is 2.98 ± 1.04 ng/ml, and the average normal value for β-thromboglobulin is 25.8 ± 11.7 ng/ml.

The accumulated information to date seems to suggest that platelets are deposited on the exposed subendothelium of degenerated porcine bioprosthetic valves, and this is accompanied by an increased level of reactivity of circulating platelets. Therefore, the porcine bioprosthetic valve may be more thrombogenic than is clinically recognized (17,32-35).

Platelets may also participate in the overall degeneration process in the following ways: 1) platelets deposited onto the valve may add thickness and therefore reduce the pliability of the leaflet; 2) microthrombi may break off and remove incorporated fibers, thereby reducing

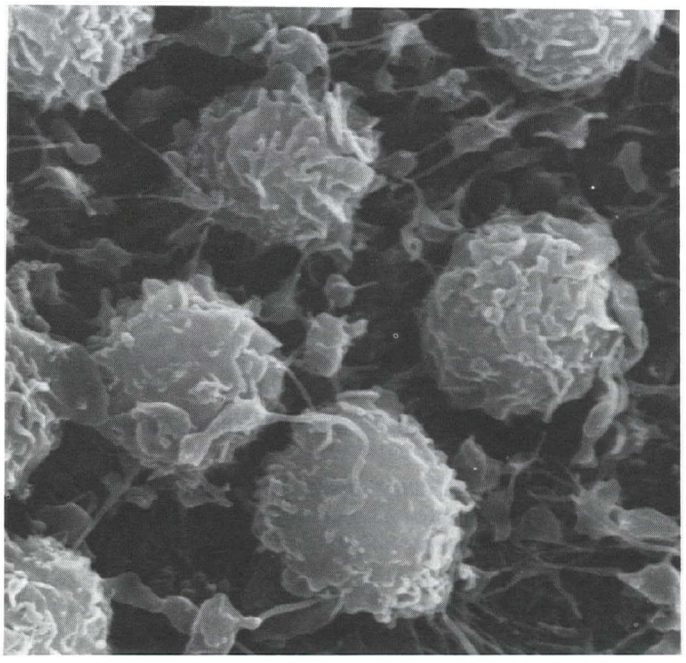

Fig. 5

Activated leukocytes showing numerous folds and ridges on the exterior as they interact with the surface of a degenerated valve (X 7,000).

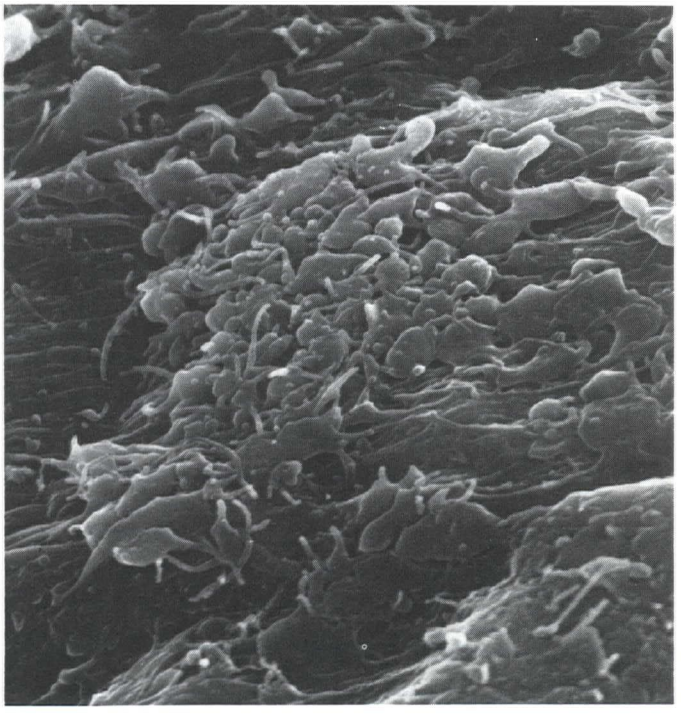

Fig. 6

Single platelets and platelet aggregates deposited onto the surface of a degenerated porcine bioprosthetic valve (X 7,000).

the mass of the fibrous framework; and finally, 3) microthrombi may serve as a nidus for calcification.

Collagen

Morphologic studies have clearly shown that individual collagen fibers on the framework of the porcine valve leaflet are changed as the degeneration process develops. They demonstrate atypical staining, fraying, and tufting of their ends. Some fibers are destroyed so completely that only short, thread-like fibrils intermixed with granular material remain (2). At present, the exact mechanism(s) responsible for depleting the collagen fibers are unknown.

Calcification

Calcification of the leaflets of degenerated valves is frequently found both grossly and microscopically (21,36-38). This process of calcium deposition occurs more rapidly in children. Although the mechanisms responsible for such calcification are currently unknown, some factors which could contribute to the calcification process have been suggested: 1) altered collagen fibers, cell debris, bacterial remnants, and surface-attached thrombi serve as sites for the deposition of calcium salts; 2) the repeated flexing motion of the valve cusps damages the integrity of the already altered fibrous framework of the leaflet, thereby encouraging the deposition of calcium; 3) removal of calcification inhibitors, such as proteoglycan, in the extracellular matrix of the connective tissue promotes calcification; or 4) the selective absorption of calcium-binding proteins into the interior of the valve leaflet allows calcium to accumulate.

In conclusion, although the porcine bioprosthetic valve is widely used to replace damaged natural valves in humans, many basic problems relating to the mechanisms by which these valves undergo degeneration in vivo remain to be defined. Information about the morphologic and biochemical characteristics of this valve is accumulating, but additional work is necessary to determine how the various factors (cellular, enzymatic, calcium deposition) interrelate and how the degeneration of this type of bioprosthetic valve is initiated.

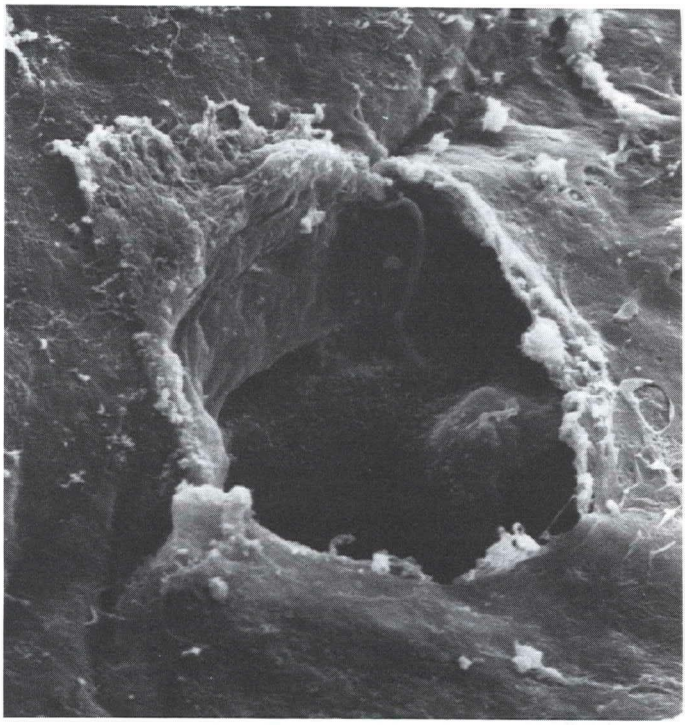

Fig. 7

Small perforation through the leaflet of a degenerated porcine bioprosthetic valve (X 420).

References

1. Gross L, Kugel MA. Topographic anatomy and histology of the valves in the human heart. Am J Pathol 1931;7:445-73.
2. Ferrans VJ, Spray TL, Billingham ME, Roberts WC. Structural changes in glutaraldehyde-treated porcine heterografts used as substitute cardiac valves. Am J Cardiol 1978;41:1159-84.
3. Bashey RI, Torii S, Angrist A. Age-related collagen and elastin content of human heart valves. J Gerontol 1967;22:203-8.
4. Jimenez SA, Bashey RI. Solubilization of bovine heart-valve collagen. Biochem J 1978;173:337-40.
5. Eyre DR. Collagen: Molecular diversity in the body's protein scaffold. Science 1980;207:1315-22.
6. Morris SC, McClain PE. Heterogeneity in the cyanogen bromide peptides from striated muscle and heart valve collagen. Biochem Biophys Res Comm 1972;47:27-34.
7. Mannschott P, Herbage D, Weiss M, Buffevant C. Collagen heterogeneity in pig heart valves. Biochem Biophys Acta 1976;434:117-83.
8. Korn AH, Feairheller SH, Filachione EM. Glutaraldehyde: Nature of the reagent. J Mol Biol 1972;65:525-29.
9. Ferrans VJ, Spray TL, Billingham ME, Roberts WC. Ultrastructure of Hancock porcine valve heterografts: Pre- and post-implantation changes. Circulation (Suppl I) 1978;58:10-18.
10. Riddle JM, Magilligan DJ Jr, Stein PD. Surface morphology of degenerated porcine bioprosthetic valves four to seven years following implantation. J Thorac Cardiovasc Surg 1981;81:279-87.
11. Howell DS, Pita JC. Calcification of growth plate cartilage with special reference on studies of micropuncture fluids. Clin Orthoped Related Res 1976;118:208-22.

12. Howell DS, Pita JC, Marquez JF, Gatter RA. Demonstration of macromolecular inhibitor of calcification and nucleational factor in fluid from calcifying sites in cartilage. J Clin Invest 1969;48:630-41.

13. Yarbrough JM, Roberts WC, Reis RL. Structural alterations in tissue cardiac valves implanted in patients and in calves. J Thorac Cardiovasc Surg 1973;65:364-75.

14. Spray TL, Roberts WC. Structural changes in porcine xenografts used as substitute cardiac valves. Am J Cardiol 1977;40:319-30.

15. Ashraf M, Bloor CM. Structural changes in Hancock porcine heterograft after various durations of implantation. Am J Cardiol 1978;41:1185-90.

16. Spray TL, Roberts WC. Structural changes in Hancock porcine xenograft cardiac valve bioprostheses. Adv Cardiol 1978;22:241-51.

17. Horowitz MS, Goodman DJ, Fogarty TJ, Harrison DC. Mitral valve replacement with the glutaraldehyde-processed porcine heterograft. J Thorac Cardiovasc Surg 1974;67:885-95.

18. Johnson AD, Daily PO, Peterson KL, LeWinter M, DiDonna GJ, Blair G, Niwayama G. Functional evaluation of the porcine heterograft in the mitral position. Circulation 1975;50-51 (Suppl I):40-48.

19. Hetzer R, Hill DJ, Kerth WJ, Wilson AJ, Adappa MG, Gerbode F. Thrombosis and degeneration of Hancock valves: Clinical and pathological findings. Ann Thorac Surg 1978;26:317-22.

20. Theine G, Bortolotti V, Panizzon G, Milano A, Gallucci V. Pathological substrates of thrombus formation after heart valve replacement with the Hancock bioprosthesis. J Thorac Cardiovasc Surg 1980;80:414-23.

21. Fishbein MC, Gissen SA, Gollins JJ Jr, Barsamian EM, Cohn LH. Pathologic findings after cardiac valve replacement with glutaraldehyde-fixed porcine valves. Am J Cardiol 1977;40:331-7.

22. Hollander JL, McCarty DJ Jr, Astorga G, Castro-Murillo E. Studies on the pathogenesis of rheumatoid joint inflammation. I. The "R.A. cell" and a working hypothesis. Ann Intern Med 1965;62:271-80.

23. Weissman G. Leukocytes as secretory organs of inflammation. Hosp Proc 1978;13:53-62.

24. Krane SM, Dayer J, Goldring SR. Consideration of possible cellular events in the destructive synovial lesion of rheumatoid arthritis. In: Ziff M, Velo GP, Gorini S, eds. Advances in inflammation research. New York: Raven Press, 1978:1-16.

25. Plow EF. Leukocyte elastase released during blood coagulation. J Clin Invest 1982;69:564-72.

26. Yasutake A, Powers JC. Reactivity in human leukocyte elastase and porcine pancreatic elastase toward peptide 4-nitroanilides containing model desmosine residues. Evidence that human leukocyte elastase is selective for cross-linked regions of elastin. Biochemistry 1981;20:3675-9.

27. Weissman G, Smolen JE, Korchak HM. Release of inflammatory mediators from stimulated neutrophils. New Eng J Med 1980;303:27-34.

28. Davies P, Allison AC. Secretion of macrophage enzymes in relation to the pathogenesis of chronic inflammation. In: Nelson DS, ed. Immunobiology of the macrophage. Academic Press, 1976:427-62.

29. Riddle JM, Magilligan DJ Jr, Stein PD. Platelet reactivity in patients with degenerated porcine bioprosthetic valves. Thromb Res 1981;22:185-94.

30. Joge JA, Lee G, Rose A, DeNardo S, Gorang A, Weiner C, DeMaria AN, Mason DT. Platelet kinetics following valve replacement: Comparison between porcine and mechanical valves. Circulation 1979;59-60 (Suppl II):222.

31. Brown TR, Ho TTS, Walz DA. Improved radioimmunoassay of platelet factor 4 and β-thromboglobulin in plasma. Clin Chem Acta 1980;101:225-33.

32. Johnson AD, Daily PO, Peterson KL, LeWinter M, DiDonna GJ, Blair G, Nigayama G. Functional evaluation of the porcine heterograft in the mitral position. Circulation 1975;51-52 (Suppl I):40-48.

33. Zuhdi N. The porcine aortic valve bioprosthesis: A significant alternative. Ann Thorac Surg 1976;21:573-5.

34. Stinson EB, Griepp RB, Oyer PE, Shumway NE. Long-term experience with porcine aortic valve xenografts. J Thorac Cardiovasc Surg 1977;73:54-63.

35. Cevese PG, Gallucci V, Morea M, Volta SD, Fasoli G, Cessarotto D. Heart valve replacement with the Hancock prosthesis: Analysis of long-term results. Circulation 1977;56 (Suppl II):111-116.

36. Piatt MR, Mills LJ, Estera AS, Hillis LD, Boja LM, Willerson JT. Marked thrombosis and calcification of porcine heterograft valves. Circulation 1980;62:862-9.

37. Ferrans VJ, Boyce SW, Billingham ME, Jones M, Ishihara T, Roberts WC. Calcific deposits in porcine bioprostheses: Structure and pathogenesis. Am J Cardiol 1980;46:721-34.

38. Rose AG, Forman R, Bowen RM. Calcification of glutaraldehyde-fixed porcine xenograft. Thorax 1978;33:111-4.