Surface Topography of Stenotic Aortic Valves by Scanning Electron Microscopy

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SUMMARY Surface features of 19 stenotic aortic valves from patients undergoing valve replacement were investigated by scanning electron microscopy. Villi, prominent on five valves, were distributed either singularly or in clusters and differed in shape. Endothelial cells had microvilli and bulbous surface projections. Endothelial disruption with a focal loss of endothelial cells was uniformly observed. Erythrocytes were found scattered over the exposed subendothelial surface or enmeshed within fibrin networks on 11 of the valves. Activated leukocytes were seen on four valves and showed veil-like projections as well as microvilli. Platelets, observed on three valves, displayed pseudopodial formation and hyalomeric spreading, signifying an increased degree of membrane response. Most platelet aggregates were composed entirely of dendritic forms (reversible aggregates), but a few also contained spread forms (irreversible aggregates). Focal deposits of crystalline material, presumably containing calcium, were observed in areas of endocardial disruption.

THE MICROARCHITECTURAL DETAILS of aortic valve leaflets removed from both normal human subjects and dogs have been displayed with the scanning electron microscope. The exterior of canine aortic valve leaflets was found to be covered by a smooth layer of endothelium that had tight, intercellular junctions and intercellular pores. Likewise, the cusps of normal human aortic valves obtained either at autopsy or after surgery showed smooth surface undulations and numerous evenly spaced hillocks corresponding to endothelial cell nuclei. This layer of single endothelial cells was continuous and cell junctions were identified as fine lines running in valleys between nuclei. Gross and Kugel confirmed the multilaminated organization of the human aortic valve cusp as originally delineated by light microscopy and found sublayers within the major divisions. Missirlis and Armeniades found marked differences in the surface topography of the aortic valve when leaflets were fixed in a stressed state as opposed to the relaxed state. In addition, these authors noted that the aortic side of the normal leaflet when stressed had a rough surface, with striations arranged circumferentially, while the ventricular surface was smoother, with very fine striations that extended radially.

Although several topographic studies of the normal aortic valve are available, the literature to date contains only a single report that depicts pathologic changes of the human aortic valve at the level of the scanning electron microscope. Only one surgically resected aortic valve was surveyed in this study.

In this study, we describe in detail the altered endothelium, exposed subendothelium and deposited peripheral blood elements associated with stenotic aortic valves.

Materials and Methods

Valves from 19 patients who underwent valve replacement because of aortic stenosis were included in our study. Deposits of calcium in the region of the aortic valve were observed in 68% (13 of 19) during image-intensification fluoroscopy. The age of this patient group was 55 ± 4 years (mean ± SEM) (range 19-70 years); only five patients were younger than 40 years old.

During the surgical procedures, the valves were handled as gently as possible, but the surgical procedure and method of handling the tissue were not altered from usual surgical practice. Immediately upon removal, the valve was rinsed with glutaraldehyde, and thereafter the specimen was handled little and only with forceps on the edges. Sections for scanning electron microscopy were not made near the margins of the incisions where tissue distortion was likely to have occurred. Frequently, portions of the valves were removed in fragments, and we could not distinguish with certainty between the aortic and ventricular surfaces.

Sample Preparation

The entire surgical sample was placed immediately in 0.2 M cacodylate-buffered 3% glutaraldehyde fixative. Each piece of tissue was examined using a Zeiss OPMI 1 operation microscope; two pieces of the excised leaflets were usually selected for study. The selected portions were fixed for an additional 24 hours, washed overnight in 0.2 M cacodylate-buffered sucrose, dehydrated in progressing concentrations of ethanol and dried using the freon method of critical point drying. Finally, each sample was glued onto an aluminum stub and its surface was coated with a thin film of gold-palladium using the sputtering technique. The topographic features of the samples were surveyed using an ETEC (ETEC Corp., Hayward, California) scanning electron microscope operated at 20 kV. Samples were examined in both flat and tilted positions.

Results

Various surface features were observed. Villus-like projections were a prominent microarchitectural detail on five of the valves (26%). They were distributed either singularly or were found clustered...
together in a small group within a focalized area. Individual projections had different shapes: A few were short and had a relatively uniform width and a blunt, rounded end, but most were longer and tapered to a pointed end (fig. 1).

Individual endothelial cells, present in areas where the endothelial lining was either discontinuous or continuous, displayed different external characteristics. These included normal microvilli (fig. 2) and occasionally an abnormally altered surface characterized by the extrusion of rounded masses (bulbous cytoplasmic extensions) that varied in size (fig. 3). In addition, the shape of adherent endothelial cells varied from the typical spindle form to cells whose cytoplasmic margins were spread so that the nucleus was evident as a central bulge and the overall outline became essentially circular (fig. 2).

Disruption of the endothelial lining was a uniform finding (100%). Its integrity was interrupted by the loss of scattered, single endothelial cells from an otherwise continuous lining (fig. 4) or by denuding by patchy sloughing of groups of endothelial cells (fig. 5). We were able to delineate various stages in the process of detachment. What appeared to be the earliest phase
Denudation of patches of endothelial cells (magnification × 370).

Denudation consisted of separation of an endothelial cell from its adjacent neighbors at its lateral margins and loosening of the polar intercellular junctions. As the process continued, the underside of the cell detached from the subendothelium and the unattached portion often appeared to fold over the fixed part of the cell (fig. 6).

In areas where the endothelium was missing, the fibrillar texture of the subendothelium was evident (fig. 7). With the resolving power of the scanning electron microscope we could not determine if the basement membrane was present or absent.

Erythrocytes were seen on the surface of 11 of these diseased valves (58%). In some instances, they were lying free and contacted the exposed subendothelium (fig. 8); in others, many erythrocytes were enmeshed within a fibrin network that covered a section of denuded subendothelium (fig. 9). Erythrocytes generally retained their typical biconcave shape (fig. 9), but were occasionally distorted by fibrin strands (fig. 10) or the projection of numerous processes (poikilocytosis).

Leukocytes were a predominant surface feature of four of these stenotic aortic valves (21%) and were also
distributed either singularly or in small groups (fig. 11). The exterior of the leukocytes either displayed numerous, short, blunt microvilli (fig. 12) or veil-like extensions (fig. 13). The leukocytes whose surfaces were covered by microvilli were probably polymorphonuclear neutrophils, because this appearance is frequently associated with this type of white blood cell. The veil-like projections are known to be less specific as this form of membrane activity is found in conjunction with both activated neutrophils and phagocytic monocytes.

Blood platelets were observed on the surface of three of the valves examined (16%). These peripheral blood elements were always found in contact with the exposed subendothelium (fig. 14). Most single platelets showed the extension of several pseudopodia of varying lengths (fig. 15). In a few instances, cytoplasmic spreading had occurred between the bases of adjacent pseudopodia (fig. 16). These morphologic modifications — pseudopodial formation and spreading — signal an increased state of membrane response. In addition to scattered single platelets, a few platelet aggregates were also present. These were generally small, loosely associated and composed of...
dendritic-type platelets (fig. 17). Aggregates containing spread-type platelets, implying irreversibility, were rarely seen.

Occasionally, a focalized accumulation of crystalline material, presumably calcium-containing deposits, was visualized with the scanning electron microscope as an identifiable surface feature (fig. 18). The morphology of these deposits differed. One type of deposit had various shapes and consisted of a tightly packed mass containing both granular material and apparent crystalline arrangements with angular edges (fig. 19). In contrast, another type of deposit was composed of loosely packed spherules, some smooth-surfaced and others rough-surfaced (fig. 20).

**Discussion**

Prominent villi, not obvious on gross examination, were visualized with the scanning electron microscope, and apparently correspond to Lambl's excrescences. They have been described previously on both aortic and mitral valves by light microscopy and scanning electron microscopy. The origin of these processes is controversial. Opinion is divided as to whether they
arise in association with thrombus formation or are an expression of wear and tear during the normal aging process. Our findings in this study do not provide us with sufficient information to choose between these two possibilities.

One possible explanation for the various degrees of endothelial injury may be changes in the flow dynamics as a consequence of the diseased valve. Fry has shown in an experimental model that the histology of endothelial cells changes in response to increased blood velocity gradients. Disruption of the integrity of the normally continuous endothelium would probably have marked pathologic implications, because this anatomic arrangement has at least two important functions: 1) to provide a barrier that selectively regulates the transfer of substances with different molecular sizes between the circulating blood and the subendothelium, and 2) to separate the circulating peripheral blood elements from subendothelial components.

Interaction between the peripheral blood elements, the altered endothelium and the exposed subendothelium was variable. The entrapment of erythrocytes within fibrin nets may not be a com-
pletely passive relationship because in microangiopathic hemolytic anemia, schistocyte formation reportedly occurs because fibrin strands "cut" erythrocytes into small fragments.\textsuperscript{18}

Leukocytes were occasionally seen on the valve surfaces. Although the interaction between the leukocytes and endothelium is not fully understood, potential attractants in the system under investigation might include substances emanating from damaged or stimulated endothelial cells, products of local ongoing proteolysis and material released from other peripheral blood elements.\textsuperscript{16} The specific type of leukocyte involved cannot be determined by exclusively viewing the external topography. When activated, exudative neutrophils display a range of membrane activities, including surfaces covered by dominately microvilli or numerous folds. The latter may mimic the plasma membrane configuration of phagocytic mononuclear leukocytes.\textsuperscript{17}

With endothelial denudation and exposure of subendothelial components, platelet deposition would be expected to occur. The available subendothelium, however, may not be as attractive as one might predict, because the uncovered fibers of collagen or elastin may remain masked by a continuous sheet of basement membrane. The dimensions of basement membrane are too small to be displayed by scanning electron microscopy.\textsuperscript{18} If the basement membrane remained intact, platelets would probably not adhere, because the valvular basement membrane, at least in the rabbit, has a low level of platelet attraction.\textsuperscript{19} The affinity of platelet adherence for vascular components, arranged from the highest to the lowest degree, is as follows: collagen fibers, basement membrane, microfibrils and elastin.\textsuperscript{20} If elastin fibers were exposed instead of collagen, platelets would be less reactive. We could not distinguish the two types of fibers in our study.

In a previous light microscopy study, microthrombi with evidence of organization were found on 10 of 19 calcified and stenotic aortic valves (53\%).\textsuperscript{21} In this scanning electron microscopy study, if platelet deposition, fibrin formation and villi are accepted as stages of thrombosis, 14 of 19 of these stenotic aortic valves (74\%) would have been involved in thrombotic episodes.

Little information about the cellular mechanism of calcification in the human aortic valve is available at the ultrastructural level. Calcified spheres and needle-shaped crystals were noted as participants in this process at the level of the transmission electron microscope.\textsuperscript{22} These entities may correspond to the spheres and crystalline arrangements we viewed by scanning electron microscopy.

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Circulation. 1980;61:496-502
doi: 10.1161/01.CIR.61.3.496

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1980 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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