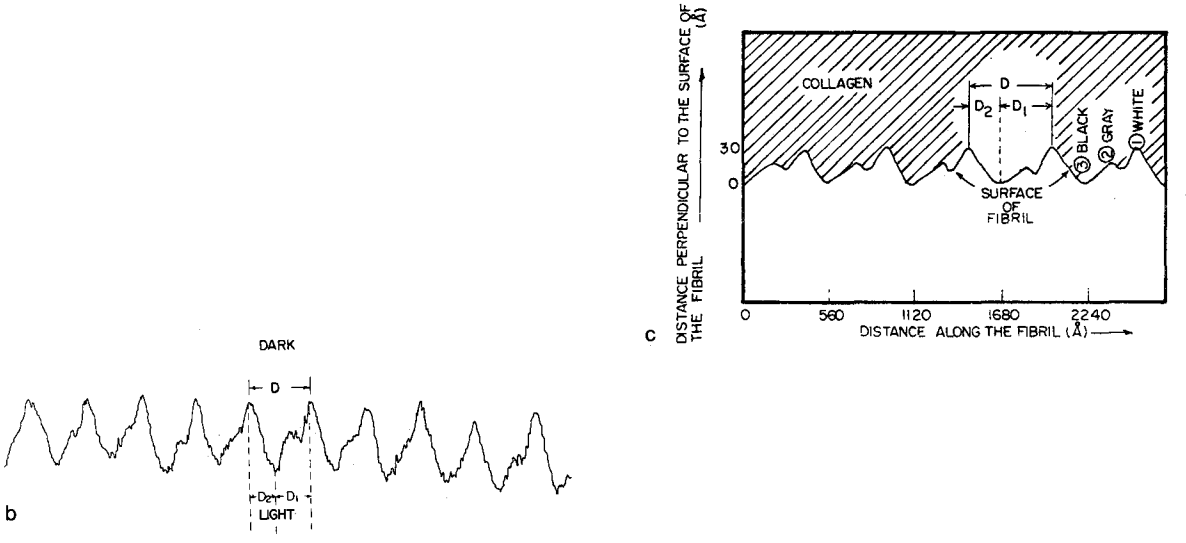


Fig. 2. *a* Replica of vacuum-dried collagen shadowed from 2 opposite directions parallel to the axis of the fibril. *b* Microphotometer scan of an electron micrograph negative of a replica of a collagen fibril as in *a*, linear in percent transmission through the film. The 'peaks' are due to the germanium shadows cast by the replica. *c* A preliminary contour model of the fibril surface based on the shadowed replicas in *a* and the microphotometer scan. Distance perpendicular to the surface has been greatly expanded relative to the distance parallel to the axis of the fibril. *d* Direct TEM micrograph of a collagen fibril stained with NaPTA.

Thus the gross features of the surface topography can be related to similar features in the TEM micrograph.

A model of the surface of a collagen fibril suggested by the replica density pattern is shown in figure 2c. The distances D, $D_1$, $D_2$ are indicated. The corresponding 'white', 'gray', and 'black' regions refer to the positive print of the replica in figure 2a. The shaded position of the figure represents the interior of the collagen fibril whereas the surface of the collagen is indicated by the arrow. With the linear correspondence assumed, the features in the direct TEM of the fibril (figure 2d) compare favorably with those of the proposed surface contour model, including the feature marked $D_3$.

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## Smooth muscle cells in the cusps of the aortic valve of pigs[1]

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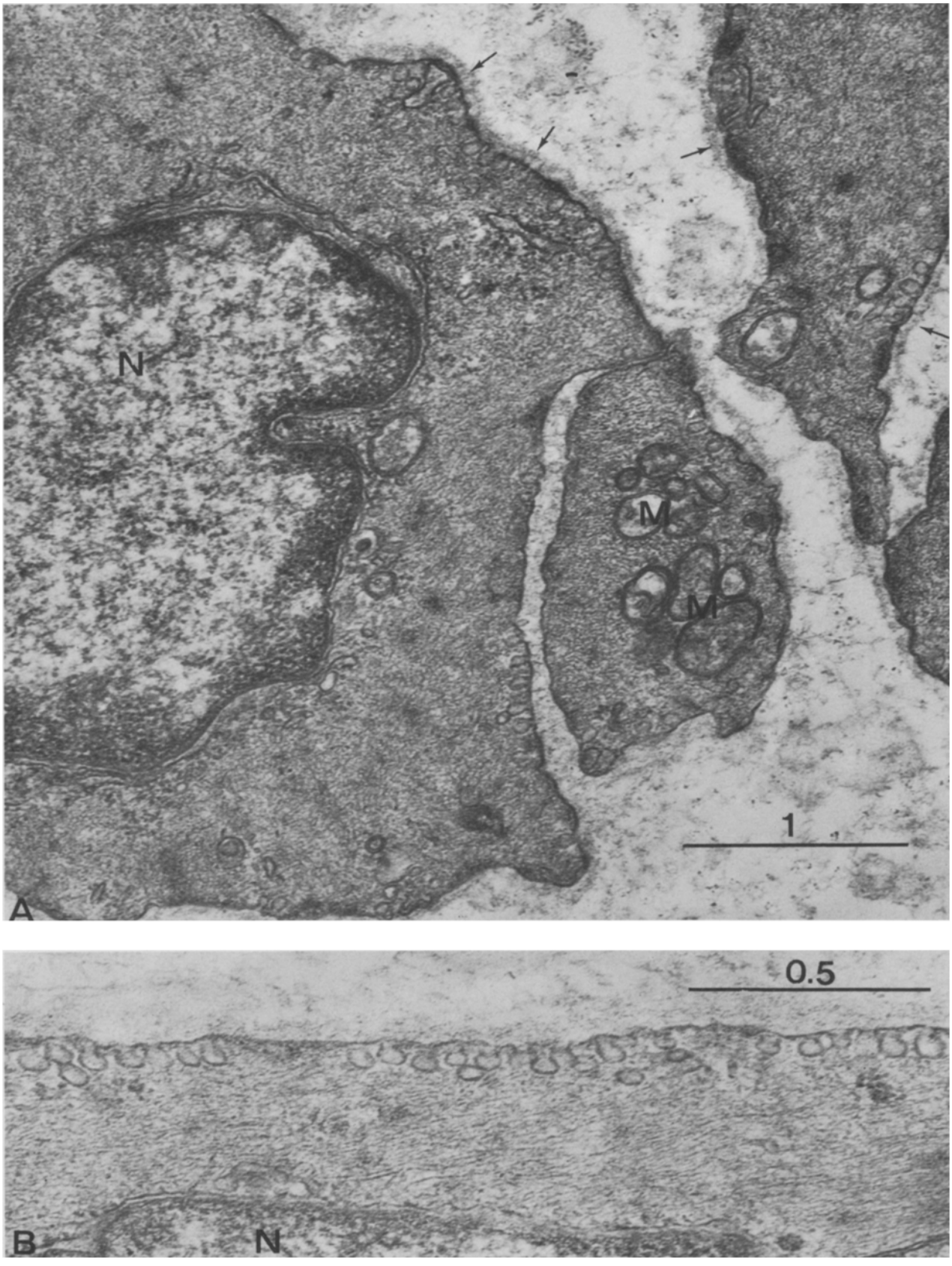
*Summary.* Evidence is presented that smooth muscle cells are consistently present in the aortic valvular cusps of pig.

The cusps of the aortic valve are considered to be outgrowths of the endothelium of the aorta. They originate in the embryonic life as swellings of the sub-endothelial connective tissue in the region of the bulbus cordis, where the main bulbar ridges will fuse into the aorto-pulmonary septum giving rise to aorta and pulmonary artery. In man it has been shown histologically[2] that each cusp consists of 5 layers of connective tissue, and is covered by an endothelium on either surface. The arrangement and orientation of collagen bundles within the connective tissue laminae has been investigated[3,4] in detail by light and electron microscopy, and has been related to the mechanical behavior of the valve during cardiac activity. Clark[3] has described characteristic arrays of collagen fibre bundles mainly circularly arranged, which account for the well-known appearance of grooves and ridges on the cusp's surface. To our knowledge, little attention has been paid to the cell types present in the connective tissue of the valvular cusps, but sparse fibroblasts and histiocytes have been recognized.

During an ultrastructural study of aortic valves conditioned for heterograft in man, we observed typical smooth muscle cells within the aortic cusps of pigs of either sex of about 1-year-old (b.wt 120 kg). The cytoplasm (figure) shows numerous thin filaments (about 5 nm in diameter) and a smaller number of thick filaments (about 15 nm in diameter). Inpocketings of the plasma membrane (caveolae) and a basal lamina are clearly visible. Patches of electron dense material (dense bands) are observed at the inner aspect of the plasma membrane and dense bodies are found in the sarcoplasm. These smooth muscle cells occur singly or in slender bundles. They lie parallel to the endothelial surface, but their orientation with respect to the free border of




Electron micrographs of smooth muscle cells of the valvular tissue. Fixation with 3% glutaraldehyde in collidine buffer, postfixation with 1% osmium tetroxide, Araldite embedding. *A* Cross section of 3 adjacent smooth muscle cells. The arrows point to the basal lamina. M, mitochondria. N, nucleus. The cytoplasm contains a large amount of filaments. *B* Portion of the cell surface with numerous typical caveolae.

the cusp is variable: they may run parallel or orthogonal to it. Their small size and limited number probably explain why they have not previously been reported. However, they are consistently present in all the specimens we have examined (5 cusps from 5 pigs), and they cannot be considered an occasional occurrence.

The presence of smooth muscle cells in the valvular cusps correlates with the embryonic origin of the cusps from the wall of the bulbus arteriosus, in the same way as the presence of striated muscle fibres[5] in the cusps of the atrioventricular valves correlates with the latters' origin and development. Further investigation is in progress to define the spatial arrangement of these contractile elements and their bearing on the mechanical properties of the valvular cusps.

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## The peritoneal leukocytes of the germ-free mouse[1]

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*Summary.* The cytology of the lavage-recoverable peritoneal cell population of germ-free mice is similar to that of conventional controls. The microbial status has no effect on the total counts, differential counts or $^3$H-thymidine labelling index of peritoneal leukocytes.

The germ-free mouse may be particularly useful for studying peritoneal inflammations in vivo[3,4], but only limited cytological information is available which characterizes the lavage-recoverable peritoneal cell population in germ-free animals[5,6]. The present study was therefore conducted as groundwork for an investigation of peritoneal macrophage stimulation in germ-free mice.

*Materials and methods.* Male and female NMRI strain mice were used at 8 weeks of age. Both the germ-free and the conventional animals were maintained in plastic isolators, and the germ-free mice were free from horizontally transmitted microbial associates including viruses[4].

Total leukocyte counts were obtained with an improved Neubauer chamber. Cells were recovered in Hanks' balanced salt solution and were immediately diluted in 2% (v/v) acetic acid containing a trace of toluidine blue and 15% (v/v) glycerol[7]. Differential counts were made from 0.5-µm sections of cell pellets which were prepared for transmission electron microscopy as described elsewhere[4]. Sections were stained in hot alkaline 1% (w/v) toluidine blue and 1000 randomly-chosen nucleated cell sections from each mouse were examined at a magnification of ×2500.

The $^3$H-thymidine labelling index was estimated in cells recovered by lavage 30 min following a single i.p. injection of 6-$^3$H-thymidine (sp.act. 20.7 mCi/mg; The Radiochemical Centre, Amersham, England). Each mouse received 20 µCi of $^3$H activity in 0.2 ml of Hanks' balanced salt solution. The cells were processed as for the differential counts, and 0.5-µm sections were hand-dipped into Ilford K-2 emulsion which was then exposed for 4 weeks at 4 °C. Developed autoradiographs were stained with alkaline 0.5% (w/v) toluidine blue and were scanned at ×2500 magnification. A nucleus was recorded as labelled if at least 5 developed silver grains were visible above it. 1000 nucleated cell profiles were examined from each mouse.

*Results and discussion.* Microbial status does not affect the total number, differential counts or $^3$H-thymidine labelling index of lavage-recoverable peritoneal leukocytes in healthy mice (table). Macrophages, lymphocytes and mast cells predominate in the peritoneal fluid whereas neutrophils comprised less than 0.1% of the recovered cell population in the present investigation and eosinophils were also rarely seen. Similarly, in earlier studies of germ-free mice, neither total counts[5] nor (probably) differential counts[6] were affected by microbial status. However, a smaller proportion of peritoneal leukocytes was reported as macrophages even during an experimental inflammation in the previous work[6] than is indicated in the table. Differential counts vary considerably among strains and age-groups of conventional mice and such variation evidently also exists in germ-free mice.

The small $^3$H-thymidine labelling indices shown in the table are consistent with earlier findings on conventional mice[8–10]. Radioactively-labelled nuclei were seen only within macrophages and lymphocytes but if a comparably small proportion of mast cells, neutrophils and eosinophils are in S phase, very few carrying $^3$H-thymidine would have been

Numbers and $^3$H-thymidine labelling index of leukocytes recoverable from the peritoneum of germ-free and conventional mice

| | Cell numbers (× $10^6$) | | Labelling index (%) | |
|---|---|---|---|---|
| | Germ-free mice | Conventional mice | Germ-free mice | Conventional mice |
| Macrophages | 3.5 ± 1.0 (10)* | 3.3 ± 1.6 (10) | 0.9 ± 0.3 (8) | 1.4 ± 0.4 (8) |
| Lymphocytes | 2.0 ± 0.4 (10) | 1.6 ± 0.3 (10) | 0.6 ± 0.3 (8) | 1.2 ± 0.4 (8) |
| Mast cells | 0.2 ± 0.07 (10) | 0.1 ± 0.03 (10) | - | - |
| Total recoverable leukocytes | 5.7 ± 1.5 (12) | 5.0 ± 1.0 (22) | 0.8 ± 0.3 (8) | 1.4 ± 0.4 (8) |

*Numbers in parentheses refer to the number of mice included in the determination. Means ± SD.