Surface model of the collagen fibril as determined by two-stage plastic replica[1]

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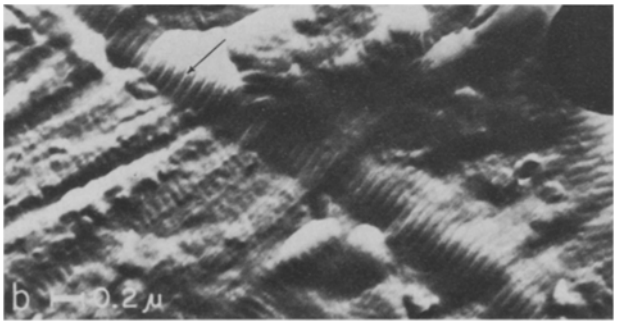
Summary. Two-stage plastic replicas of fresh and vacuum dried rat tail collagen were examined by electron microscopy. Microphotometer tracings made from the replica micrographs showed that the surface of the collagen has a periodic undulating topography which includes an approximately 20–40 Å deep depression located within the major band distance.

Previous workers[2-4] shadowed collagen fibrils in vacuum directly with gold, chromiun and platinum. They found a corrugated surface on the shadowed specimen with 2 elevations per major period D. The purpose of the present work was to determine whether the collagen banding phenomenon is reflected in the surface topography of the fibril as observed by surface replica techniques under ambiant conditions.

Materials and methods. Portions of fresh rat tail collagen, cleaned of extraneous tissue, were used immediately, vacuum dried at 10^{-5} torr for 24 h, or stained with NaPTA, sodium phosphotungstate, (pH 7.4). A small rectangular piece of acetylcellulose plastic was dipped into acetone and then placed on the collagen. After 30 min it was separated from the surface of the collagen and shadowed with 10 Å of germanium along the axial fibril direction at an angle of 30° to the surface. The specimen was then coated with a few hundred Å of silicon monoxide. The plastic was dissolved by floating it on acetone and the silicon replica was picked up on a 400 mesh grid. Latex spheres, placed on the replicas before shadowing, were used to calibrate the roughness of the collagen surface. Specimens were examined in the JEM-7A electron microscope at 100 kV accelerating voltage.

Results and discussion. Figure 1 shows micrographs of replicas of vacuum dried, fresh, and stained fresh fibrils, respectively. The sharp white line indicated by arrow in figure 1b was obtained by shadowing along the axis of the fibril from 1 direction, the standard technique. The microphotometer scan obtained from the negative of figure 1b was asymmetric and has been reported elsewhere[5]. (The direct micrograph of a collagen fibril also has an asymmetry or directionality to it.) A double shadowing technique was used to eliminate the possibility that the apparent asymmetry in surface topography was an artifact of the single shadowing. Replicas of vacuum-dried collagen fibrils were shadowed from the 2 opposite directions. The microphotometer scan of the negative of the double shadowed replica in figure 2a was also found to be asymmetric in nature, as shown in figure 2b.

Fig. 1. Electron micrograaphs of 2-stage replicas of a) vacuum-dried collagen, b) fresh unstained fibrils, and c) fresh stained fibrils.

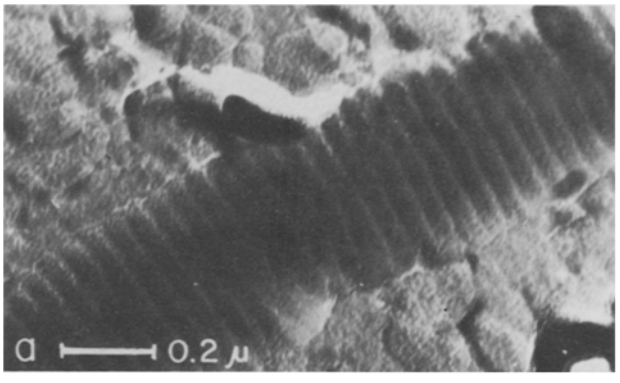


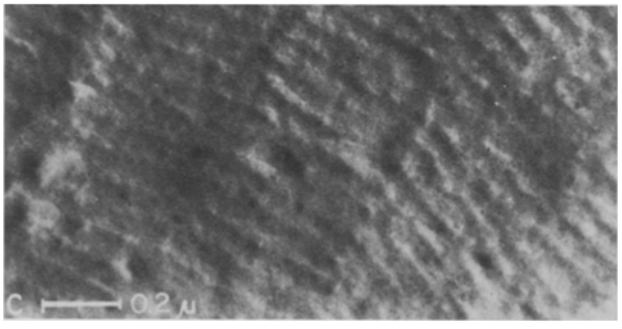
Figures 2a, b, c, and d have been aligned vertically so that the surface and bulk patterns can be correlated. Figure 2a shows a replica of the vacuum-dried collagen fibril shadowed from 2 opposite directions parallel to the fibril. The depth of the surface depression corresponding to the white line in figure 2a is equal to about 20–40 Å. Since double shadowing in our case approximately doubles the width of the shadow of a particular feature, that feature would have to be rather thin, when compared with the length of the shadows. It also would have to be an elevation on the replica. A thin depression on the replica would not give rise to this phenomenon, nor would a wide elevation or a wide depression.

Figure 2b is a microphotometer scan of an electron micrograph negative of the replica of the collagen fibril shown in figure 2a. It shows the percent transmission as a function of distance along the fibril axis. Thus the peaks corresponding to minimum transmission correspond to the whitest bands in figure 2a. The distance D between peaks of minimum transmission and the distance D_1 and D_2 between the peaks and troughs are indicated.

Figure 2d is a direct TEM micrograph of a collagen fibril stained with NaPTA. The transverse bands of fine structure show the axial asymmetry of the collagen fibril. The width D_1 of the dark band, the width D_2 of the light band, and the 'band repeat distance' D were measured on figure 2b and on the TEM micrograph in figure 2d. It was found that to within the experimental precision,

$$(D_1/D)_{\text{replica}} = (D_1/D)_{\text{TEM}} \text{ and } (D_2/D)_{\text{replica}} = (D_2/D)_{\text{TEM}}$$





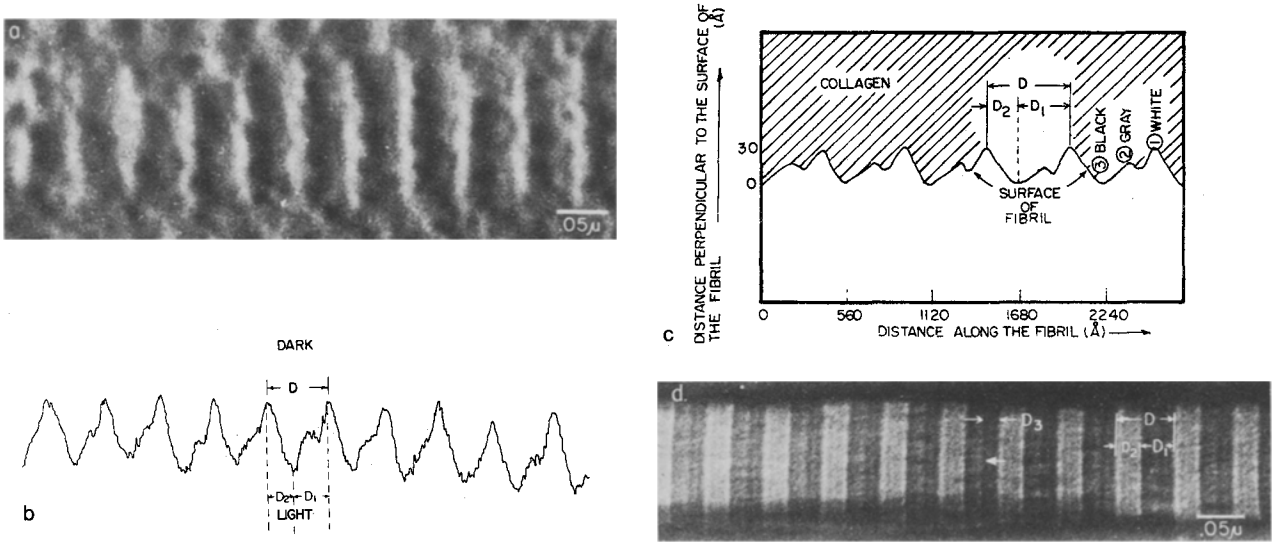


Fig. 2. *a* Replica of vacuum-dried collagen shadowed from 2 opposite directions parallel to the axis of the fibril. *b* Microphotometer scan of an electron micrograph negative of a replica of a collagen fibril as in *a*, linear in percent transmission through the film. The 'peaks' are due to the germanium 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