THE ENDOTHELIAL LINING OF HOMOGRAFTS AND DACRON PROSTHESES IN THE CANINE AORTA

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The concept that an endothelial lining was essential to the maintenance of a patent aorta was shattered by the successful replacement utilization of homografts and prostheses for large segments of the human aorta. Obviously, immediately and for some time after insertion, there is no endothelial covering of either the homograft or prosthesis. The blood passing through the homograft is in contact with the substance of the graft; in the case of the prosthesis the blood is in contact with a coagulum that forms in the crevices of the fabric and on its inner surface. Conventional microscopic preparations in longitudinal and cross sections have not indicated with certainty whether or not an endothelial covering exists.[1] Furthermore, staining techniques used to demonstrate endothelium are difficult to apply to specimens preserved in formalin. The present study was undertaken to visualize, by special techniques, the lining of homografts and dacron prostheses in the canine aorta for an extended period.

MATERIAL AND METHODS

Three homografts and 6 dacron prostheses in canine abdominal aortas were available for study. These were 1 to 3.5 cm. long and had been in place for 3 years or longer. Immediately after sacrifice of the animals, the abdominal aorta, including the replacement segment, was removed. The aorta was opened longitudinally, and the inner surface was rinsed with tap water and then with physiologic saline or glucose in distilled water. The intimal surface was then covered with a silver nitrate solution, 0.25 per cent, rinsed with distilled water and exposed to either sunlight or sunlamp until the surface darkened. By this method the lining of the entire length of the homograft or prosthesis could be studied *en face* without the distortion of dissection, stretching or compression. This served to disclose the presence and shape of endothelial cells in continuity from the host aorta through the replacement. Thereafter the surface was covered with celloidin and the specimen placed in ethyl alcohol. Subsequently, pellicles were peeled from the silver-treated intima and were stained with hematoxylin and eosin. This revealed stained nuclei and additional cellular detail.

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Observations

In each of the 3 homografts, the inner surface of the graft was smooth, glistening, and similar to that lining the host aorta. At the proximal and distal anastomoses, the black silk sutures were clearly discernible beneath the transparent lining (Fig. 1). In *en face* silver preparations, delicate silver lines exhibited polygonal outlines with a mosaic pattern. Nuclei of endothelial cells were not evident (Fig. 2). The cell shapes varied somewhat in different regions. In the host portion and near the suture lines, the configuration was elongated and, in the main, paralleled the aortic axis. Towards the center of the homograft the spaces tended to become wider, with somewhat broader lines of silver deposit.

In the pellicle homograft preparations stained with hematoxylin and eosin, the borders of the endothelial cells were sharply outlined by the silver precipitate. The nuclei were clearly discernible, slightly oval, and were usually located in the center of the cell. Elongation of the nuclei paralleled that of the cells. Each endothelial cell was in contiguity with 4 to 8 others. Their configuration was polygonal, oval, or elongated, and with angular borders (Fig. 3). Occasional small polygonal spaces lacked nuclei. The silver-stained lines bordering the cells were delicate and finely granular, and exhibited short extensions into the cytoplasm. Delicate dispersions of silver granules formed a halo about the nuclei. Some cells had double nuclei and were of giant proportions.

In each of the 6 dacron prostheses, the inner surface, like that of the host aorta, was smooth and glistening. The fabric of the prosthesis and the proximal and distal black silk sutures were visible through a delicate lining (Fig. 4). In *en face* silver preparations, delicate lines formed a mosaic polygonal pattern with no nuclei visible. The outlined areas adjacent to the silk sutures were elongated and diamond shaped (Fig. 5). Those toward the center of the prosthesis were larger and polygonal, and the lines of silver deposit were somewhat broader.

In pellicle preparations stained also with hematoxylin and eosin, the cell borders were clearly outlined and the nuclei visible (Fig. 6). The cell shapes, nuclei, and the over-all pattern resembled those in the homografts, but the cells appeared somewhat larger.

Comment

The visualization of endothelial cells by silver staining of their borders and their *en face* examination, as described by O'Neill[2] and others,[3,4] have provided a means of studying the endothelial lining of blood vessels and the mesothelial covering of serous membranes. According to Poole, Saunders and Florey,[5] the staining of endothelial cell boundaries by

silver in rabbit aortas depends on the presence of chloride ions. Later, Florey, Poole and Meek [6] observed silver deposits on endothelial surfaces as well as at cell junctions. The endothelial lining of nylon grafts in the thoracic and abdominal aortas of dogs and goats for periods up to 594 days was investigated by Meijne.[7] He observed endothelium covering the suture lines and concluded that the endothelium over the nylon prosthesis originated from the adjacent aorta. The lining of crimped nylon (Edwards-Tapp) prostheses placed in the femoral artery of greyhounds was studied by Mackenzie and Loewenthal.[8] They calculated the average growth rate of endothelium to be 0.1 to 0.15 mm. per day. The most recent observations were made by Florey, Greer, Poole and Werthessen [9] on dacron prostheses in the abdominal aorta of baboons. The prosthesis was completely lined by endothelium at the end of 10 weeks.

In our studies of homografts and dacron prostheses, endothelial cells were visualized by an *en face* technique. The cells were believed to be undistorted, and to exhibit their natural shapes and relationships. The grafts were in place for a period of time exceeding that previously investigated by this method. The *en face* and pellicle preparations were essential for identifying the endothelial cells and their changes. These techniques combined with others may eventually reveal the nature of the intimate relationship between endothelium and the remainder of the vessel wall.

Summary

The endothelial lining of homografts and dacron prostheses in canine abdominal aortas was investigated. The replacements were 1 to 3.5 cm. long and had been in place for 3 years or longer. Silver-stained *en face* and pellicle preparations were used. The inner surface of both types of replacement were entirely covered by endothelial cells clearly identified by their silver stained borders. Cells were polygonal, varied in size and shape, and formed a mosaic pattern. In some areas in the replacements and at the suture lines there were varieties in size, shape, and nuclear configuration.

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LEGENDS FOR FIGURES

FIG. 1. A distal suture line in an aortic homograft, dog H3. The silk sutures are visible beneath the transparent surface. × 6.

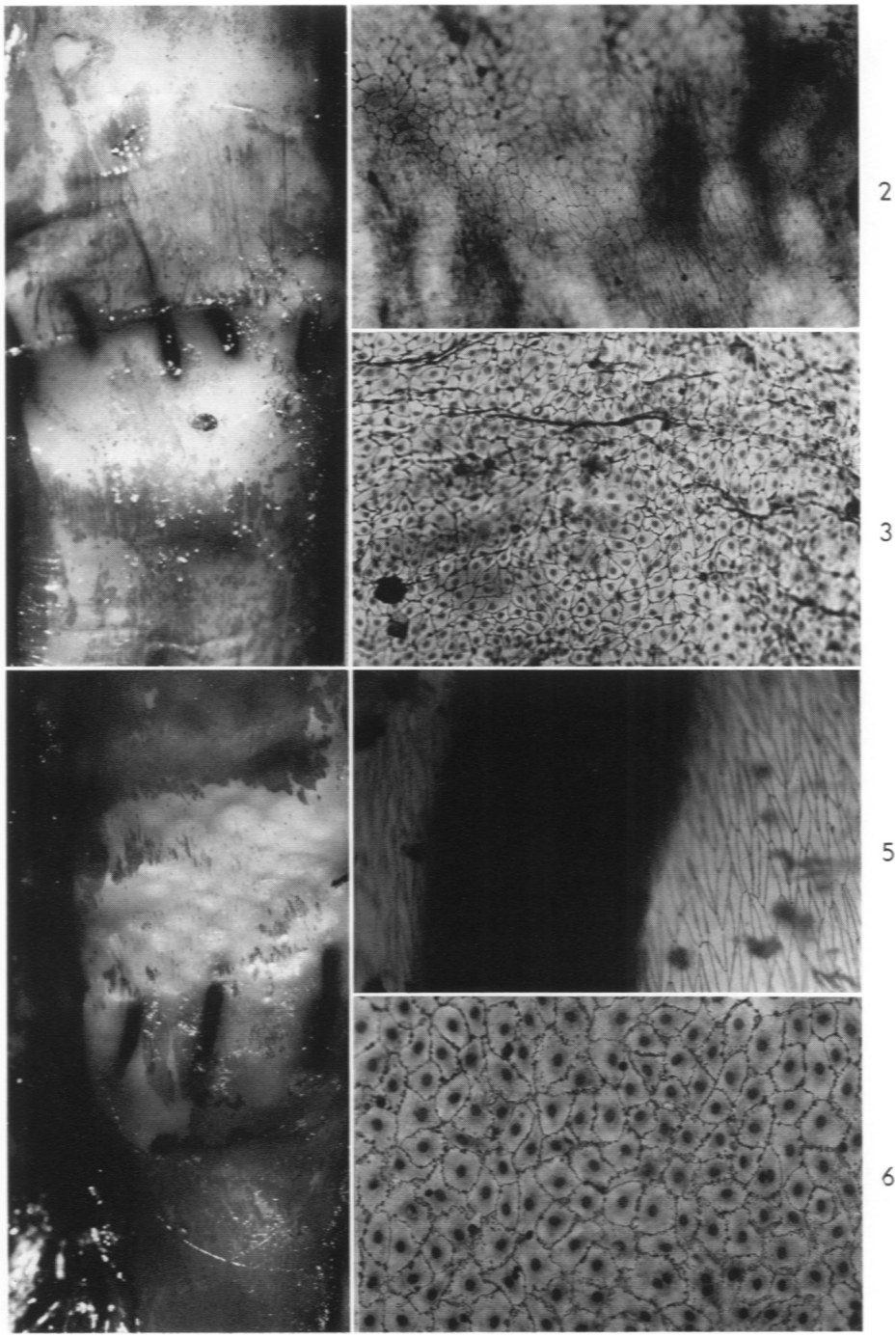
FIG. 2. An *en face* silver preparation of the endothelium lining the homograft, dog H3. Polygonal spaces are bordered by delicate silver lines and form a mosaic pattern. The shapes vary in different areas. × 100.

FIG. 3. The endothelium covering on the inner surface of a homograft, dog H3. This is a pellicle silver preparation from the center of the graft, stained with hematoxylin and eosin. The borders of endothelial cells are outlined by a silver precipitate, and nuclei are now clearly discernible. The endothelial cells are of about equal size; their shapes are polygonal, oval, or elongated, and borders are angular. Each cell is in contact with 4 to 8 adjacent cells. × 100.

FIG. 4. The distal suture line of a dacron aortic prosthesis, dog D5. The inner surface is smooth and glistening; the silk sutures and fabric of the prosthesis are visible through a delicate lining. × 6.

FIG. 5. An *en face* silver preparation, dog D5. Delicate lines of silver deposit form elongated diamond-shaped spaces adjacent to a silk suture. × 100.

FIG. 6. Endothelium covering the intimal surface of a dacron prosthesis, dog D6. The borders of endothelial cells are clearly outlined, and their nuclei are visible. Occasional cells have double nuclei. This is a pellicle, silver-treated preparation stained also with hematoxylin and eosin. × 150.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