Endothelial Lining of Dacron Prostheses of Porcine Thoracic Aortas.* (27510)

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Aortic homografts and prostheses have been investigated by conventional gross and microscopic methods. These studies have failed to reveal an endothelial lining of the inner surface in contact with the blood stream (1). However, the absence of any endothelial lining has not been established. Recently, special methods of visualizing the endothelium of blood vessels have been applied to the study of the lining of vascular prostheses. It has been demonstrated in goats(2), dogs (3) and baboons(4) that aortic prostheses in place for an extended period become completely lined with endothelium. It was observed by Wesolowski(5) that structural alterations in vascular prostheses occur more rapidly in young pigs than in other animals used for similar experiments. The inner surfaces of knitted crimped dacron prostheses of porcine thoracic aortas, some with and some without impregnation with an absorbable modified thiolated gelatin compound,† were studied to determine the length of time needed for coverage by endothelium.

Material and methods. Knitted, crimped dacron prostheses, 4 to 6 cm long, were used to replace like segments of thoracic aortas in 17 young growing pigs weighing 7 to 16 kg. Of the prostheses, 10 were impregnated with gelatin and were left in place for 7, 7, 14, 15, 30, 42, 49, 78, 78 and 139 days. The remaining 7 were not impregnated and were left in place for 7, 7, 14, 14, 23, 30 and 43 days. At the end of the experimental period each prosthesis with its adjacent host aorta was removed in continuity and opened longitudinally. The intimal surface was rinsed, treated with 0.4% silver nitrate solution, submerged in formalin 10% and then exposed to sunlamp until the surface darkened. The inner surface was then studied "en face" to ascertain the presence or absence of endothelial cells over the replacement. The specimen was next dehydrated in graded ethyl alcohol solutions, the surface covered with celloidin (8% in ether and ethyl alcohol), and the specimen was placed in ethyl alcohol 30%. Subsequently, pellicles were peeled from the silver treated intima and were stained with hematoxylin and eosin to reveal the nuclei and additional detail of the endothelial cells.

Observations. Of the 9 prostheses that were left in place for 7 to 23 days, 4 were impregnated with gelatin (7,7,14 and 15 days)

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FIG. 1. Gross appearance of the inner surface of a knitted crimped dacron prosthesis replacing a segment of the porcine thoracic aorta in place for 7 days (P-46). The thin transparent covering is uniform throughout the length of the prosthesis.

FIG. 2. Inner and cut surfaces of dacron prosthesis of porcine thoracic aorta in place for 42 days (P-35). The prosthesis appears as a wavy line of even width. The luminal tisssue is composed of an inner compact zone and a loose zone adjacent to the fabric.

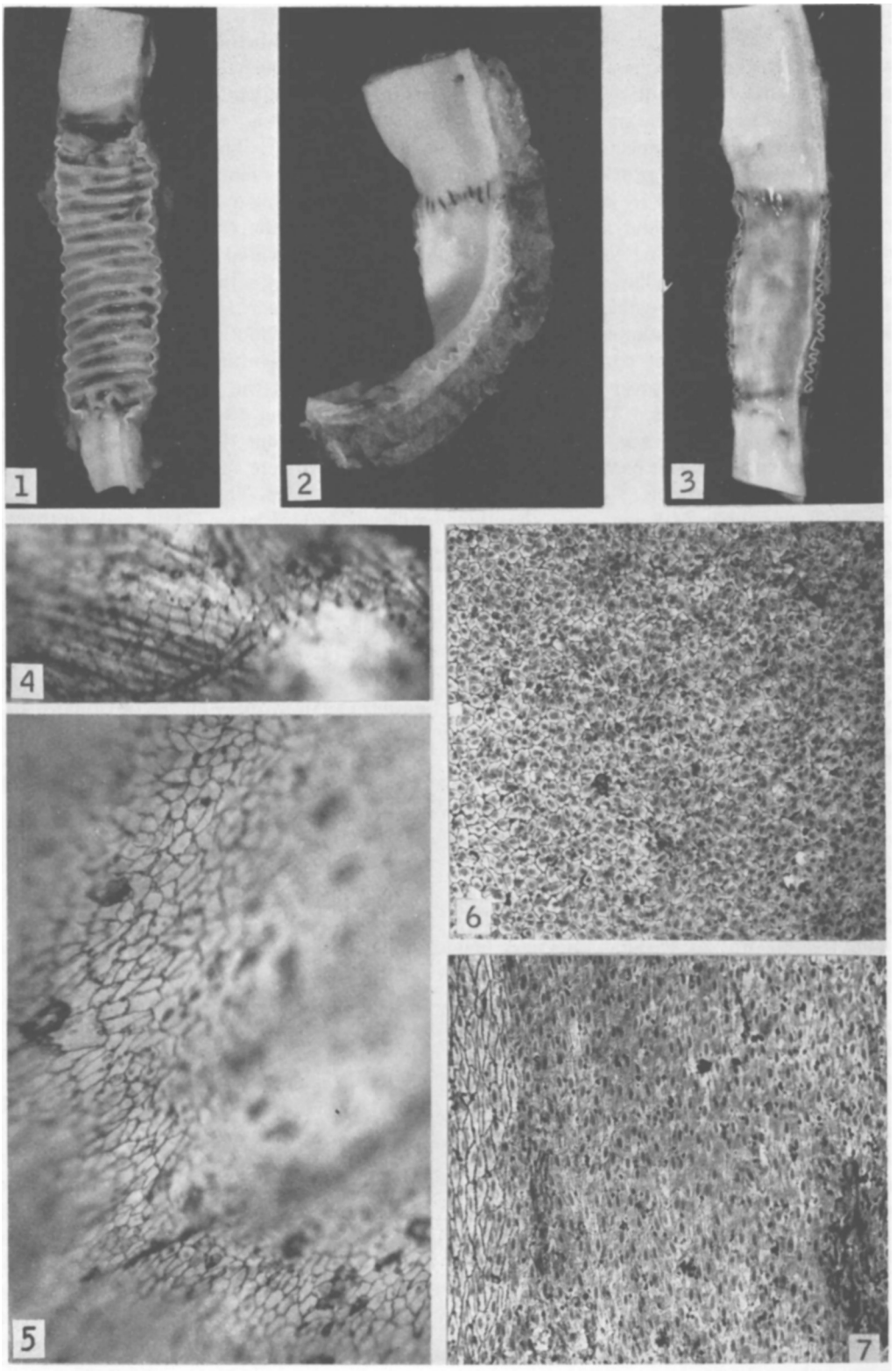
FIG. 3. Inner and cut surfaces of dacron prosthesis of porcine thoracic aorta in place for 78 days (P-28).

FIG. 4. "En face" silver preparation from P-46 shown in Fig. 1. The delicate silver lines border polygonal spaces in a mosaic pattern. Knitted fibers of the dacron are seen underneath X 100.

FIG. 5. "En face" silver preparation of prosthesis in place for 15 days (P-41). The delicate silver lines forming polygonal spaces slope from the crest of a "hill" into a "valley" X 100.

FIG. 6. Pellicle silver preparation stained with hematoxylin and eosin from P-35 shown in Fig. 2. The endothelial cells are clearly discernible with nuclei within each cell X 100.

FIG. 7. Pellicle silver preparation stained with hematoxylin and eosin from P-28 shown in Fig. 3. The orientation of endothelial cells parallels the long axis of the prosthesis X 100.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and 5 (7, 7, 14, 14 and 23 days) were not. Grossly there was no noticeable difference between the prostheses that were impregnated and those that were not. The prostheses were firmly adherent to the surrounding structures and encased in connective tissue. On the luminal surface the proximal and distal suture lines were covered by a thin transparent substance overlying and adherent to the contours of the underlying structures. The circular or semicircular ridges and the knitted individual fibers of the prostheses were clearly visible. In 3 of the prostheses in place for 7 days the thin transparent covering was continuous from the aorta over the prostheses just past the suture lines. The central portion of these prostheses was covered by an apparent coagulum. The pattern of the fabric was clearly discernible. In one non-impregnated prosthesis, 4.5 cm long, the thin transparent covering was uniform throughout the entire length (Fig. 1). In 3 of the prostheses in place for 14 or 15 days the transparent covering of the surface was more or less uniform, while in one it was interrupted by areas of apparent granulation tissue. The inner surface of the 23-day-old prosthesis was smooth and glistening. The pattern of the prosthesis was still discernible through a thin covering.

All of the 8 prostheses that were in place from 30 to 139 days were enclosed in a sheath of more or less dense connective tissue (Fig. 2, 3). On the cut surfaces the prosthesis appeared as a wavy line of even width. The luminal tissue was of varying thickness and was composed of an inner compact zone and a loose zone adjacent to the fabric. The intimal surface was glistening and similar to that of the host aorta, though less smooth. The prosthesis appeared gray-white, while the host aorta had an ivory hue. At the proximal and distal anastomoses black silk sutures were visible through a thin lining. Elsewhere the layer of newly formed tissue covered the pattern of the prosthesis so that the crimped ridges of the fabric were not evident.

In "en face" silver preparations of areas covered by endothelium, delicate silver lines bordered polygonal spaces of varying sizes and shapes in the familiar mosaic pattern. In 3 of the 4 prostheses that were in place for 7 days, the endothelium was contiguous from the host aorta over the prostheses for a length of 0.1 to 0.2 cm, while in one the entire length of the prosthesis was covered with endothelium (Fig. 4). The orientation of the polygonal spaces was mostly, but not entirely, parallel with the long axis of the prosthesis. Corresponding to the crimps of the dacron, the surface was elevated or depressed, resembling hills and valleys. In one of the 4 prostheses that were in place 14 or 15 days, the mosaic pattern of the endothelium was interrupted in areas though continuity could be demonstrated through the length of the prosthesis. In the remaining 3, the pattern was uninterrupted throughout the prosthesis. The polygonal spaces were more uniform than in the 7-day prostheses. The slopes of the "hills and valleys" were of lesser height and depth (Fig. 5).

In all the 30- to 139-day prostheses the mosaic pattern was alike and the elongation of the cell outlines paralleled the long axis. The network of silver lines was less frequently interrupted, was more delicate, and was more uniform than in the younger prostheses. The elevations and slopes were absent though occasional slight suggestion of them remained.

Pellicle preparations from areas that disclosed endothelium "en face" revealed in all prostheses delicate wavy dark lines bordering the cells. In the center of each was a round or oval nucleus. The endothelial cells of the host intima and those of the prostheses were similar. Each cell was in contact with 4 to 8 other cells (Fig. 6). Over occasional areas of the prostheses some endothelial cells were 2 or more times the size of others and were not elongated. The larger cells had larger nuclei. In the older prostheses the elongation of the cells parallel to the axis was clearly discernible (Fig. 7).

Comment. The growing pig has proved to be a suitable subject for observations on aortic prostheses, particularly on the endothelium. Under our experimental conditions, the prostheses became encased in connective tissue. The lining of the prostheses with endothelium proceeded rapidly and apparently at a rate faster than that observed in adult

animals of other species that have been used for this purpose.

In the porcine thoracic aorta, endothelium commenced to cover the crimped dacron prosthesis soon after its implantation. The entire length of a dacron prosthesis, 4.5 cm long, was completely covered with endothelium in 7 days.

Summary. Prostheses of knitted, crimped dacron, some with and some without gelatin impregnation, were placed in the thoracic aortas of 17 young pigs. The prostheses replaced excised segments of aortas, 4 to 6 cm long, and were left in place for 7 to 139 days. "En face" and pellicle silver preparations revealed complete endothelial covering of the surface of a prosthesis after 7 days.

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Ovulating Hormone Release in Gonadotrophin Treated Immature Rats.* (27511)

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It has been reported that the gonadotrophin of pregnant mare serum (PMS) is capable of producing ovulation in the immature rat (1-3). Evidence exists that the ovulation brought about by PMS is due to a certain inherent luteinizing property of the PMS molecule, and to the ability of PMS to cause release of endogenous gonadotrophin(3). Recently Strauss and Meyer, working in this laboratory, obtained data which supports the concept that ovulation caused by a single injection of PMS in 30-day-old immature rats is due to release of ovulating hormone from the animal's own pituitary. Their data, obtained from barbiturate injection and hypophysectomy, strongly indicates that ovulating hormone release occurs between 2:00 and 4:00 p.m. on the afternoon of the second day after PMS injection.

In attempting to use the 21-day-old female rat to assay luteinizing hormone by the superovulation method of Zarrow(4), considerable difficulty was encountered because the priming dose of PMS often caused ovulation. It became of interest to determine whether the ovulation was due to release of pituitary gonadotrophin during a precise period such as Strauss and Meyer had found in the 32-day-old rat.

Materials and methods. Female rats 21 days of age and weighing 45-50 g were obtained from Holtzman Co. and Sprague-Dawley, Inc., Madison, Wis. On the following day between 7:30 and 8:00 a.m. a single dose of 1.375 Cartland-Nelson Units of PMS (Upjohn Gonadogen) was injected subcutaneously. The animals were killed 3 days later between 8:00 a.m. and 1:00 p.m. The oviducts were removed and the ova counted by the method of Rowlands(5), except that the ova in the egg mass were separated mechanically.

In the experiments involving barbiturate blockage of ovulation, 167-250 mg/kg of an aqueous solution of sodium barbital was injected intraperitoneally at 1:30 or 5:30 p.m. on the day before autopsy. In one experiment, human chorionic gonadotrophin

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