Platelet reactivity in vivo in dogs with arterial prostheses seeded with endothelial cells

G. Patrick Clagett, M.D., William E. Burkel, Ph.D., John B. Sharefkin, M.D., John W. Ford, B.A., Howard Hufnagel, B.S., David W. Vinter, M.S., Raymond H. Kahn, Ph.D., Linda M. Graham, M.D., James C. Stanley, M.D., and Peter W. Ramwell, Ph.D.

ABSTRACT This study was designed to assess platelet activity in vivo with vascular prostheses seeded with endothelial cells to determine the time course for development of thromboresistance and to test the ability of prostheses to produce prostacyclin. Sixteen dogs were randomly allocated to receive seeded (experimental group) or unseeded (control group) velour Dacron aortic prostheses. Serial measurements of platelet survival were performed to assess platelet interaction with prostheses in vivo, and platelet serotonin was monitored as an index of platelet release in vivo. After placement of prostheses, dogs in the experimental group had rapid normalization of platelet survival, with most having normal platelet survival at 4 to 8 weeks after surgery. In contrast, most control animals had reduced platelet survival throughout the 12 week period of study. Significant differences between groups in mean platelet survival were noted at 8 weeks after surgery (p < .005) and in mean platelet serotonin at 12 weeks after surgery (p < .05). Luminal surface production of 6-keto-PGF$_1$ from seeded prostheses was similar to aortic production and significantly greater (p < .05) than that of control prostheses. Gross thrombus was present on 6.0 ± 3.4% of the prosthetic surface in experimental animals in comparison to 26.6 ± 19.2% in controls (p < .005). The results of these studies document accelerated nonreactivity with platelets of seeded prostheses due to rapid coverage with endothelium possessing a normal ability to produce prostacyclin.


PLATELET INTERACTION with vascular prosthetic surfaces is an undesirable process that contributes to occlusive thrombosis and distal embolization.1-3 After placement of arterial prostheses in man and experimental animals, platelets rapidly and continuously accumulate on the surface.3-5 The process can be monitored quantitatively by serial measurement of platelet survival time or by imaging radiolabeled platelets on the surface.6 In dogs, measurement of platelet serotonin also reflects platelet interaction with prosthetic surfaces, and changes in platelet serotonin parallel changes in platelet survival.6 Studies in baboons and dogs have documented that platelet survival, which is reduced immediately after implantation, returns to normal within a variable period of months depending on the rate of growth of neointima.6-9 With almost all animal preparations, neointima extensively covers the surface,10 contains nearly continuous endothelium, and produces prostacyclin.8 In human beings, endothelialized neointima grows very poorly and is usually found only adjacent to anastomoses.11,12 Results of radioisotope platelet imaging in humans demonstrate that significant platelet accumulation on vascular prostheses continues for up to 10 years after placement.13,14

To improve thromboresistance of vascular prostheses, the technique of endothelial cell seeding has been developed.15 This process involves harvesting autogenous venous endothelial cells by mechanical or enzymatic means and adding them to blood used to precut prostheses.16,17 To date, all published work has been carried out in dogs. Most studies have focused on the morphologic appearance of endothelial seeded surfaces, and different groups of investigators have documented improved endothelial coverage of seeded prostheses in comparison to unseeded controls.16-19

From the Division of Surgery, Walter Reed Army Institute of Research, Washington, D.C.; Department of Surgery, Uniformed Services University of the Health Sciences, Bethesda, MD; Departments of Anatomy and Surgery, University of Michigan Medical School, Ann Arbor; and Department of Physiology and Biophysics, Georgetown University, Washington, D.C.

Address for correspondence: G. Patrick Clagett, M.D., Department of Surgery, University of Texas Health Science Center at Dallas, 5323 Harry Hines Blvd., Dallas, TX 75235.

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CIRCULATION
Morphologic studies, however, suffer from potential observer and sampling bias. Functional studies of prostheses seeded with endothelial cells are needed to assess this technique and, to date, only two have been reported. In one, enhanced patency of small-diameter Dacron arterial prostheses seeded with endothelial cells was documented.\textsuperscript{20} In the other study, our group demonstrated improved platelet survival in animals with large aortic Dacron prostheses seeded with endothelial cells.\textsuperscript{19} The present study extends these observations, documents rapid return to normal platelet survival in animals with aortic prostheses seeded with endothelial cells, and demonstrates that the neointimal surface of seeded prostheses produces prostacyclin in amounts equivalent to those produced by normal aorta under basal, unstimulated conditions.

**Methods**

**General design of study.** This project was shared between two institutions and was specifically designed to eliminate bias from all phases of the study. The study sought to compare prostheses seeded with endothelial cells (experimental group) with unseeded prostheses (control group) in dogs. Each institution was responsible for implantation of prostheses and the surgeon performing the operations was unaware of which prostheses were seeded with endothelial cells. The assignment of experimental and control prostheses was by random allocation. Sealed envelopes containing assignments were opened during implantation by a single member of the team who then added autogenous endothelial cells (which were harvested in all animals) to the blood used to precoat experimental prostheses. The assignment of experimental and control prostheses was not revealed until completion of the study and analysis of data.

The second institution was responsible for performing serial platelet function tests, harvesting prostheses, and assessing prostacyclin production by neointima. Members of this team were also unaware of which animals were experimental and which were control. Conversely, data generated by this team were not revealed to members of the first team during the course of the study.\textsuperscript{21} At completion of the study and after analysis of all data, the code was broken and the animals’ groups were revealed.

**Animal preparation and technique of endothelial seeding.** In conducting the research described in this article, we adhered to the Guide for Laboratory Animal Facilities and Care, as promulgated by the Committee on the Guide for Laboratory Animals Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences–National Research Council.

Sixteen adult dogs of either sex weighing 23 to 30 kg were randomly allocated into experimental and control groups. A single animal in the control group died shortly after operation, leaving seven in this group and eight in the experimental group. Preoperative values for hemoglobin, hematocrit, platelet count, and white cell count were normal in all animals. Platelet aggregometry was also performed preoperatively in all dogs, with adenosine diphosphate and collagen used for aggregating agents, and was found to be normal by criteria reported previously for dogs.\textsuperscript{5}

Autogenous endothelial cells were procured from 10 cm segments of external jugular veins. Immediately after excision, veins were secured and everted over a 5 mm diameter stainless steel rod and suspended in iced calcium, magnesium-free Hank’s\textsuperscript{3} balanced salt solution (BSS-CMF) (Hanks\textsuperscript{3} BSS-Eagle’s Medium, Grand Island Biological, Grand Island, NY). The veins were then washed of any adherent blood cells by spinning at 200 to 300 rpm in fresh BSS-CMF. After washing, the veins were incubated 10 min in 0.1% trypsin (Difco Laboratories, Inc., Detroit, MI) in BSS-CMF with 0.125% ethylene-diamine-tetraacetic acid (Fisher Scientific, Fair Lawn, NJ), pH 8.0, and then in 0.5% collagenase (Worthington, Inc., Freehold, NJ) in BSS-CMF for an additional 10 min at 37\textdegree C. A final wash with a jet of culture medium (Eagle’s Medium) resulted in the acquisition of an exfoliate containing sheets of five to 30 endothelial cells. The enzyme and wash solutions were centrifuged 2 min at 50 g to obtain an endothelial cell pellet. The sequential action of these enzymes provided a yield in the range of 0.5 to 1.5 \times 10^6 endothelial cells from each jugular vein. The derived cells were divided in thirds and suspended in individual 0.5 ml aliquots of culture medium.

Double velour Dacron prostheses (Meadox Medical Inc., Oakland, NJ), 25 to 30 cm long with a 10 mm inside diameter, were used for the thoracoabdominal aortic bypasses. The properties of this warp-knitted material include the following: inside pore, 0.15 mm; outside pore, 0.29 mm; wall porosity, 1300 ± 400 ml/min/cm\textsuperscript{2}; interstices greater than 30,000/2.5 mm\textsuperscript{2}; and pore size, 2 to 400 μm. The prostheses were precollotted in a standard manner. Three 10 ml aliquots of autologous blood were used for the first three steps. In experimental dogs, 0.5 ml of culture medium containing endothelial cells were added to each aliquot of blood. In control dogs, 0.5 ml of culture medium without cells were added to the blood aliquots. As a fourth step, clotted prostheses were flushed with 15 ml of autologous blood containing 5000 U of heparin sodium.

After precolloting, prostheses were immediately implanted. The details of the surgical procedure have been described previously.\textsuperscript{21}

**Platelet survival and platelet serotonin.** Platelet survival was measured with autologous platelets labeled with indium-111-oxine. The technique, based on that of Wilkinson et al.,\textsuperscript{22} has been specifically adapted to dogs and has been previously reported in detail.\textsuperscript{23} Briefly, 31.5 ml of blood were collected atraumatically and mixed with 3.5 ml of 3.8% trisodium citrate. After centrifugation at 200 g for 10 min at 32° C, 10 ml of supernatant platelet-rich plasma was removed and mixed with PGE\textsubscript{1}, at a final concentration of 200 ng/ml. Platelet-rich plasma was then centrifuged at 640 g for 10 min at 22° C and the platelet-poor plasma was removed. The platelet pellet was gently resuspended in 5 ml of Ringer’s citrate dextrose containing 200 ng/ml PGE\textsubscript{1}, 50 μCi of indium-111-oxine (Mediphysics Corp., Emeryville, CA) was added, and the platelets were incubated at 37° C for 7.5 min. Five milliliters of platelet-poor plasma was added to the platelet suspension, which was then centrifuged at 640 g for 10 min. The platelet pellet was resuspended in 5.3 ml of platelet-poor plasma and the suspension was injected into the animal. Blood samples of 10 ml were drawn at 2, 26, 50, 74, and 98 hr after injection and 4 ml aliquots were counted in a gamma counter on the fifth day after reimplantation of labeled platelets. In the remaining 6 ml of whole blood from the recovery samples, platelets were separated by differential centrifugation and washed with normal saline and 1% ammonium oxalate to lyse red cells; platelet-bound radioactivity was then counted. By means of this technique in dogs, mean platelet recovery of 46 ± 17% 2 hr after injection of labeled platelets was attained, and the average platelet-bound injected radiation was 93 ± 4%. With this method, platelet survival time in 38 healthy mongrel dogs was 5.06 ± 0.68 days (range 3.71 to 6.50).

Platelet survival was measured preoperatively and postopera-
tively at 2, 4, 8, and 12 weeks. Platelet survival curves were identical with either whole blood samples or samples from which red cells had been removed. Platelet survival times presented in this article were calculated with the latter. Platelet serotonin measurement in canine platelets has also been described and is based on the method of Crosti and Luchetti as modified by Holmsen and Weiss.

**Harvest of prostheses.** Four months after operation, animals were anesthetized and 200 U/kg heparin (Riker Laboratories, Inc., Northbridge, MA) was administered intravenously. Prostheses were surgically removed intact with 2 to 4 cm of adjacent aorta at proximal and distal anastomoses and thoroughly rinsed with lactated Ringer’s solution (Cutter Laboratories, Berkeley, CA). The animals were then killed with intravenous medication (T-61 Euthanasia Solution, American Hoechst Corp., Somerville, NJ). Prostheses were then sectioned transversely at the midpoint. The proximal half was kept in lactated Ringer’s solution for determination of prostacyclin production. The distal half was opened longitudinally, pinned to a paraffin block with the luminal side facing upward, and submerged in fixative, 2% glutaraldehyde, 4% paraformaldehyde in phosphate buffer (pH 7.45).

**Prostacyclin production.** To measure prostacyclin generation by prosthetic neointima and aorta, two techniques were used. In the first, 6-keto-PGF\(_{1\alpha}\) production from sliced rings of vascular tissue was measured. All periprosthesis tissue was removed, and a 4 cm long segment 1 cm distal to the proximal anastomosis and a 4 cm segment from the middle (near original point of transection) were removed, isolated, and cut transversely into small (1 to 2 mm) rings. A 3 cm length (measured in situ) of thoracic aorta from the same animal was also cut into rings. Careful measurements of lengths and diameters of all specimens were made so that 6-keto-PGF\(_{1\alpha}\) production could be related to surface area. Rings were incubated in 5 ml of Tris buffer (0.05M Tris-HCl, pH 7.50) for 15 min at 37\(^\circ\) C. The buffer was then removed and centrifuged at 12,000 g for 1 min, and aliquots were tested for prostacyclin aggregatory activity. Simultaneous aliquots were diluted 1:9 in Tris buffer and were quick-frozen at −60\(^\circ\) C for subsequent radioimmunoassay for 6-keto-PGF\(_{1\alpha}\).

The second method to assess prostacyclin production measured luminal surface generation of 6-keto-PGF\(_{1\alpha}\). A 4 to 5 cm portion of the prosthesis 6 to 7 cm distal to the proximal anastomosis was removed and gently rinsed with Tris buffer warmed to 37\(^\circ\) C. A 3 cm length of aorta was removed from the descending thoracic after ligation of the aortic wall of all intercostal branches. One end of the tubular sections of the prosthesis and the aorta were clamped with a straight bulldog vascular clamp, and both were placed in a beaker containing loosely packed saline-soaked sponges maintained at 37\(^\circ\) C. The vascular sections were propped upright in the manner of a cup and filled with Tris buffer warmed to 37\(^\circ\) C. Extreme care was taken to ensure that the segments were filled to the brim with no leakage and that accurate measurements were taken of the volume of buffer added to the segments. After incubation for 15 min at 37\(^\circ\) C, aliquots of buffer were removed, centrifuged at 12,000 g for 1 min, and quick-frozen at −60\(^\circ\) C for subsequent radioimmunoassay of 6-keto-PGF\(_{1\alpha}\). For each vascular segment, at least two incubations were measured.

For measurement of 6-keto-PGF\(_{1\alpha}\), 20 \(\mu l\) aliquots of the frozen supernatant were thawed and assayed by established radioimmunoassay techniques. The Upjohn Company supplied the 6-keto-PGF\(_{1\alpha}\) and antiserum against 6-keto-PGF\(_{1\alpha}\) was produced in rabbits in the laboratory of one of the authors. Cross-reactivity with other prostaglandins was less than 1%. Production of 6-keto-PGF\(_{1\alpha}\) was related to the surface and the results were expressed as nanograms of 6-keto-PGF\(_{1\alpha}\) produced per square centimeter of surface for 15 min at 37\(^\circ\) C.

**Morphologic studies.** For determination of luminal coverage with thrombus, color transparencies of the mounted, distal halves of prostheses were projected onto white paper. The luminal surface was traced and areas covered with gross thrombus were mapped. Planimetry of the tracing was then used to calculate percentage surface area covered by thrombus.

Paired samples of the luminal aspect of prostheses (from the midportion and near anastomoses) and attached aortas were studied by routine light microscopy and scanning electron microscopy.

**Statistical analysis.** All results are expressed as mean ± SD. Differences in mean values between experimental and control groups were assessed with Student’s paired t test. Additionally, Student’s paired t test was used in analyzing results of 6-keto-PGF\(_{1\alpha}\) measurements when comparisons were made between prosthetic neointima and native aorta. To calculate platelet survival time, computer-assisted least-squares techniques were employed as recommended by the Panel on Diagnostic Application of Radioisotopes in Hematology of the International Committee for Standardization in Hematology. The platelet radioactivities in the recovery samples were fitted to both linear and logarithmic decay functions, and the sums of the squares of the deviations of the data points from the fitted straight line and exponential curve were used to calculate a weighted estimate of the mean platelet survival time.

**Results**

**Platelet survival and platelet serotonin.** Serial platelet survival data are shown in table 1 and figures 1 and 2. Two weeks after placement of prostheses, four of the seven control animals and five of the eight experimental animals were available for measurement. Among controls, all had reduced values at this interval and

<p>| TABLE 1 |</p>
<table>
<thead>
<tr>
<th>Serial platelet survival in experimental and control animals</th>
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<tbody>
<tr>
<td>Before surgery</td>
</tr>
<tr>
<td><strong>Experimental</strong></td>
</tr>
<tr>
<td>Platelet survival (days)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Platelet survival (days)</td>
</tr>
</tbody>
</table>

*Data expressed as number of animals with normal platelet survival (defined as equal to or exceeding preoperative value)/number of animals studied.

*Significantly different from experimental value, p < .005.
mean platelet survival time was significantly different (p < .05) from preoperative values. In the experimental group, two of the animals had platelet survival that was equal to or exceeded preoperative values, and the mean difference was not significantly different.

At 4 weeks after surgery, five of the eight experimental animals had values equal to or exceeding preoperative platelet survival, and at 12 weeks platelet survival returned to preoperative values in all but one of these animals (table 1). In contrast, in only one of the seven control animals did platelet survival return to preoperative values throughout the entire postoperative period.

This analysis is potentially misleading, however, because the experimental group had shorter preoperative platelet survival times than did the control group (table 1, figures 1 and 2). Although the preoperative difference between the groups was not significant, bias favoring the experimental group may have been present because it would possibly be easier for animals with shorter baseline platelet survival to return to these levels than animals with longer preoperative values. To reduce bias, all platelet survival times were compared with values obtained for normal mongrel dogs in this laboratory (figure 2). With this comparison, all experimental by 8 weeks after surgery attained values

FIGURE 1. Serial mean ± SD platelet survival time in experimental animals with endothelial seeded prostheses (●) and in control animals (○).

FIGURE 2. Individual platelet survival determinations for experimental (●) and control animals (○). The shaded area represents mean ± 2 SD platelet survival time for 38 normal dogs.
that fell within the normal range in contrast to only three of the seven control animals (figure 2). At 12 weeks these relationships were maintained, although the data were more variable within groups.

In comparing mean values between the two groups, significant differences were seen only at 8 weeks after surgery when platelet survival in the experimental group was 4.92 ± 0.65 days vs 3.79 ± 0.61 for the control group (p < .005). The mean postoperative platelet survival, with values averaged across the entire postoperative period, was 4.83 ± 0.76 days for experimental animals and 3.83 ± 0.90 days for control animals (p < .05).

Platelet serotonin data are presented in table 2. The platelet serotonin level was modestly reduced in each group at 8 weeks after placement of prostheses. Measurements were not available at 2 and 4 weeks after surgery. At 12 weeks, the platelet serotonin level was normal in all experimental animals but remained reduced among control animals; differences between groups were significant (p < .05).

**Morphology.** At harvest of prostheses there were marked differences between groups in the amount of gross thrombus present on luminal surfaces. Experimental dogs had 6.0 ± 3.4% thrombus-covered surface compared with 26.6 ± 19.2% in control dogs (p < .005). On examination by scanning electron microscopy, thrombi were composed of abundant platelets, white cells, and fibrin. For all prostheses, surface covered with thrombus at time of harvest was inversely correlated with platelet survival measured before harvest (r = −.63, p < .02, n = 15).

Areas of the prostheses not covered with thrombus were composed of neointima, which was qualitatively similar in control and experimental animals. The cells lining the neointimal surface had the appearance of endothelium. They were flattened, polygonal, had distinct cell borders, and were continuous and identical to aortic endothelium. The subendothelium was composed of fibroblasts, intervening collagen, and scattered smooth muscle cells. There were no differences in the neointimal subendothelium of control and experimental dogs.

**Prostacyclin production.** Production of 6-keto-PGF1α by rings of vascular tissue and by the luminal surfaces of aortas and prostheses is shown in table 3. Rings of aortas from control and experimental animals produced two to three times the amount of 6-keto-PGF1α in comparison to rings from prostheses (p < .02). There were no significant differences between anastomotic and midprosthetic sites. Among experimental animals, these sites produced slightly more 6-keto-PGF1α than comparable sites in controls, but these differences were not significant.

**Aortic luminal surface production** of 6-keto-PGF1α under basal, unstimulated conditions was approximately 5% of that produced by aortic rings (table 3). In experimental dogs, luminal production by prostheses (all segments near middle of prostheses) was not significantly different from that of aortas. Among control animals, prosthetic luminal production was significantly lower than aortic production (p < .01) and was also significantly lower than that of comparable sites from experimental animals (p < .05).

**Discussion**

These studies show that animals with large aortic prostheses seeded with venous endothelial cells have longer postoperative platelet survival than unseeded controls. Most of the experimental animals had normal platelet survival 1 month after implantation. In contrast, most of the control animals had shortened platelet survival throughout the 12 week study period and showed little trend toward normalization of values.

Rapid normalization of platelet survival is distinctly unusual after placement of large arterial prostheses in dogs. Previous studies from our group have focused on evaluating prosthetic materials in the same animal preparation used in this study. After placement of wov-

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**TABLE 2**

Serial platelet serotonin values (μg/109 platelets) in experimental and control animals

<table>
<thead>
<tr>
<th></th>
<th>Before surgery</th>
<th>8 weeks</th>
<th>12 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental (n = 8)</td>
<td>1.78 ± 0.38</td>
<td>1.41 ± 0.22</td>
<td>1.83 ± 0.28</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>1.79 ± 0.47</td>
<td>1.28 ± 0.23</td>
<td>1.28 ± 0.43</td>
</tr>
</tbody>
</table>

*Significantly different from experimental values, p < .05.

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**TABLE 3**

Production of 6-keto-PGF1α (ng/cm²) by sliced rings and luminal surfaces of aorta and prostheses

<table>
<thead>
<tr>
<th></th>
<th>Aorta</th>
<th>Anastomotic sites</th>
<th>Midprosthetic sites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Production by rings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental (n = 8)</td>
<td>17.5 ± 8.0</td>
<td>7.4 ± 3.1*</td>
<td>8.9 ± 5.5*</td>
</tr>
<tr>
<td>Control (n = 7)</td>
<td>23.7 ± 8.1</td>
<td>6.4 ± 1.2*</td>
<td>5.7 ± 1.1*</td>
</tr>
<tr>
<td>Luminal production</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Experimental (n = 6)</td>
<td>0.99 ± 0.58</td>
<td>—</td>
<td>0.90 ± 0.72</td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>1.11 ± 0.60</td>
<td>—</td>
<td>0.15 ± 0.11b</td>
</tr>
</tbody>
</table>

*Significantly lower than aortic production, p < .02.

bSignificantly lower than aortic production, p < .01, and production by experimental prostheses, p < .05.
In Dacron prostheses, platelet survival remained depressed for 32 months, at which point prostheses were harvested and 60% of their surfaces were found to be covered with endothelialized neointima.\textsuperscript{8} In another study, velour knitted Dacron (similar but not identical to that used in the present study) and expanded polytetrafluoroethylene prostheses produced shortened platelet survival for 10.5 months and were found to be 57% and 34% surfaced, respectively, with neointima.\textsuperscript{9,30} With both of these prostheses, platelet survival remained markedly reduced at the time of harvest. In this same study, animals with standard weight knitted Dacron prostheses developed normal platelet survival 7.5 months after surgery and had endothelialized neointima covering 90% of the prosthetic surface. Until the recent study, standard weight knitted Dacron performed best in terms of rate of growth of neointima and normalization of platelet survival.

At the time of harvest, endothelialized neointima covered 94% of the surface of seeded prostheses in comparison to 73% of that of controls. These measurements were obtained 1 month after the final platelet survival determination and therefore cannot be directly related to normalization of platelet survival. Presumably, the extent of coverage was greater at harvest than when the final platelet survival was measured. Nevertheless, with these degrees of endothelialization one might expect to see progressive lengthening of platelet survival for both experimental and control animals instead of the plateaus reflected in the mean values in figure 1. Harker et al.\textsuperscript{7} demonstrated a close correlation between the degree of neointima development and prolongation of platelet survival in baboons with aortic prostheses. In all of our studies with dogs, we have not been able to obtain a similar degree of correlation.\textsuperscript{5,8,9,30} In dogs, platelet survival does not begin to appreciably lengthen until 60% to 70% of an aortic prosthesis is endothelialized,\textsuperscript{5,29} whereas, in baboons, Harker et al.\textsuperscript{7} found lengthening of platelet survival with only 30% coverage. The discrepancy may be due to species differences in platelets and mechanisms of shortened platelet survival. Canine platelets are more reactive with prosthetic surfaces than baboon platelets,\textsuperscript{30} and therefore shortened platelet survival might occur with comparatively lesser amounts of prosthesis exposed to blood. It would appear that in dogs, until 60% to 70% of a large aortic prosthesis is endothelialized, the thrombogenic stimulus remains sufficiently intense to override the discriminant ability of measurement of platelet survival to assess lesser degrees of endothelialization.

In light of these considerations, one can infer that the prostheses seeded with venous endothelium achieved rapid coverage (within 4 to 8 weeks) of at least 60% to 70% of their surfaces with endothelialized neointima. Some experimental dogs may have achieved maximal coverage within 2 weeks (figure 2). The time course for prolongation of platelet survival is in accordance with the results of serial morphologic studies demonstrating 70% to 80% coverage at 4 to 8 weeks in dogs with seeded prostheses.\textsuperscript{18,32}

As with platelet survival, reductions in platelet serotonin levels in dogs may reflect platelet interaction with prosthetic surfaces, and the two measurements complement each other.\textsuperscript{5} The mechanism underlying the fall in platelet serotonin level appears to be platelet serotonin release in vivo during reversible platelet-prosthetic interactions, with mild platelet damage limiting platelet reuptake of serotonin.\textsuperscript{5,31} Other mechanisms may also be involved, including selective consumption of dense, serotonin-rich platelets by the prosthetic surface or a shift in the age distribution of circulating platelets because of the stimulus for accelerated platelet turnover. In the present study, experimental animals had little reduction in platelet serotonin levels, and values were normal at 12 weeks after surgery and significantly higher than control values. This suggests less intense platelet interaction with prostheses after endothelial seeding.

The neointima from these prostheses was presumably lined by endothelial cells. The lining cells had light microscopic and scanning electron microscopic characteristics of endothelium. Previous, more detailed morphologic studies from our group using the same endothelial seeding technique as used in this canine preparation with identical prostheses have demonstrated that continuous, true endothelium is present.\textsuperscript{17,18,32} Techniques for morphologic evaluation in these studies included light, transmission, and scanning electron microscopy, complemented by immunofluorescent staining for factor VIII related antigen.

Prostacyclin production as assessed by 6-keto-PGF\textsubscript{1\alpha} generation by the luminal surface also suggests that true endothelium lined the experimental animals' prostheses. Pure surface production of 6-keto-PGF\textsubscript{1\alpha} by seeded prostheses under basal, unstimulated conditions was equivalent to that of the animals' native aortas and significantly higher than that of control prostheses. This suggests that the endothelium lining these prostheses can function normally and confer thromboresistance equivalent to normal aortic endothelium.

The values of 6-keto-PGF\textsubscript{1\alpha} generation by sliced rings of vascular tissue were 10- to 20-fold higher than
those obtained by measuring surface production, and there were no significant differences between experimental and control animals. This discrepancy stems from two sources. First, the ring technique measures subendothelial as well as endothelial contributions to prostacyclin generation. Subendothelial sources include capillary endothelium, smooth muscle cells, and possibly fibroblasts. Second, tissue trauma, which is inherent in this technique, has been shown to stimulate prostacyclin production in vascular tissue even when endothelium has been removed. "Since the subendothelial layers of neointima from experimental and control prostheses were morphologically identical and surface endothelial contribution to overall 6-keto-PGF₁α production was relatively small, it is not surprising that experimental and control prostheses could not be distinguished with this technique."

With the sliced ring technique, both experimental and control prostheses produced one-half to one-third the 6-keto-PGF₁α produced by aortic tissue. Further, anastomotic and midprosthetic sites were similar. These results are virtually identical to those reported previously by our group for neointima obtained from woven Dacron prostheses. The aortic wall is far richer in capillary endothelium, smooth muscle, and fibroblasts in comparison to neointima, and these results serve to emphasize the dominant contribution of the subendothelium when the sliced ring technique of measuring 6-keto-PGF₁α production is used.

Although successful in dogs, it is unknown whether endothelial seeding of vascular prostheses will work in human beings. Species differences in coagulation, thrombosis, healing, and endothelial growth preclude direct comparisons to human beings. If the technique can be adapted and shown to be successful in human beings, it may be possible to achieve thromboresistant prosthetic surfaces for a variety of devices and artificial organs. Human experimentation is both necessary and indicated.

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