Platelet Deposition on Vascular Grafts

The Accuracy of in Vivo *Quantitation and the Significance of* in Vivo *Platelet Reactivity*

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An *in vivo* platelet imaging system utilizing indium-111-labeled platelets and technetium-99m-labeled red cells was used to serially study and compare platelet deposition on autologous external jugular vein grfts, autologous arterial grafts, polytetrafluoroethylene (Gore-tex®) and two Dacron® (Meadox and USCI) small diameter (4 mm) vascular grafts implanted end-to-end in canine carotid and femoral arteries. This method of quantitating platelet deposition was validated by correlating deposition measured *in vivo* with deposition measured directly on explanted grafts ($r = 0.94$, $p < 0.01$). Platelet accumulation on all grafts was greatest immediately after implantation and declined over time. None of the artery or vein grafts thrombosed, and they had the lowest level of platelet deposition at all times. Platelet deposition on Gore-tex grafts was significantly less than on USCI Dacron grafts from 24 hours to 1 month after implantation. There was no statistical difference in 1-month patency among the synthetic graft groups. Synthetic grafts that thrombosed during the first month accumulated significantly more platelets immediately after operation than did those grafts that remained patent. Patent Dacron grafts with low levels of platelet deposition had less thrombotic debris at explantation on the luminal surface than did those grafts with high levels of platelet deposition. Differences in initial platelet deposition appeared to be more a function of platelet reactivity within each dog rather than the material used in graft construction.

PROLONGED RELIEF of ischemic symptoms following cardiac and peripheral vascular reconstruction is dependent on a patent vascular graft. Unfortunately, many of the available prostheses, both biologic and synthetic, lack the necessary characteristics to maintain long-term function. Many factors have been implicated in the genesis of graft failure, including errors in surgical technique, the native thrombogenicity of the graft material, the cleanliness and morphology of the surface, hemodynamic alterations in blood flow, and a mismatch of the mechanical properties of the graft and artery. Despite extensive attempts to manipulate each of these factors, only modest improvements in long-term graft patency have been achieved in animals and man.

Recent evidence suggests that the platelet may play an important role in graft failure and that platelet deposition, if excessive, may result in acute graft thrombosis or promote late graft failure through the stimulation of anastomotic neointimal fibrous hyperplasia.[1-3] To define further the role of platelets in graft thrombosis, we employed ^{111}In-labeled platelets and ^{99m}Tc-labeled red cells in a dual-isotope imaging system and now report our experience using this technique to image and quantitate platelet deposition on small diameter arterial grafts implanted into dogs. Specifically, our efforts were directed toward assessing the accuracy of this system in quantitating platelet deposition, how platelet deposition varies as a function of the type of prosthesis and duration of implantation, and the effect of platelet deposition on graft patency.

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Materials and Methods

Selection and Description of Graft Material

Autologous external jugular vein grafts (N = 10), autologous carotid and femoral arterial grafts (N = 8), expanded polytetrafluoroethylene (Gore-tex®) (N = 26), and two Dacron® velour grafts, USCI (N = 17) and Meadox (N = 14), were chosen for platelet deposition studies. All grafts were 5 cm long; synthetic grafts had an internal diameter of 4 mm. The internal diameter of vein grafts ranged from 5–7 mm. Autologous artery grafts were 2–4 mm in internal diameter. USCI grafts (C. R. Bard, Inc., Billerica, MA) were composed of weft knit noncrimped external velour Dacron (internal pile height 0.12 mm,

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external pile height 0.25 mm, wall thickness 0.5 mm, mean water porosity 1900 ml/min/cm^2). The Microvel® Meadox grafts (Meadox Medicals, Inc., Oakland, NJ) were made of noncrimped knitted Dacron velour (internal pile height 0.19 mm, external pile height 0.36 mm, wall thickness 0.83 mm, mean water porosity 1900 ml/min/cm^2). Gore-tex expanded polytetrafluoroethylene grafts (W. L. Gore and Associates, Inc., Flagstaff, AZ) had a wall thickness of 0.61 mm, internodal fiber length of 22 microns, and were nonporous to water at a pressure of 300 mmHg.

Surgical Technique

Nineteen mongrel dogs, weighing 20 to 30 kg (8 male, 11 female), were used. Anesthesia was obtained with intravenous (IV) administration of pentobarbital at an initial dose of 30 mg/kg. Smaller doses were given at intervals as needed. Each animal was intubated, placed on a Harvard respirator, and given IV lactated Ringer's solution containing 5% dextrose at 15 ml/kg/hr. After the neck and groin were aseptically cleaned, the carotid and femoral arteries were exposed bilaterally and mobilized over a 10 cm distance. External jugular veins were harvested unilaterally from 10 dogs and placed in cold saline solution prior to autologous grafting. Autologous carotid and femoral artery grafts from two dogs were similarly harvested and immediately implanted as described below. Both USCI and Meadox grafts were preclotted using the four-step method of Sauvage.[4] Gore-tex grafts were kept dry until implantation. After IV heparin administration (100 units/kg), the grafts were implanted into the carotid and femoral arteries end-to-end with 7-0 Prolene® (Ethicon, Inc., Somerville, NJ) by one surgeon using the triangulation technique of Carrel.[5] The order in which grafts were implanted was varied randomly. Thirty-eight grafts were implanted in the femoral position (12 Gore-tex, 9 Meadox, 10 USCI, 4 artery, and 3 vein grafts). The carotid position contained 37 prostheses (14 Gore-tex, 5 Meadox, 7 USCI, 4 artery, and 7 vein grafts). One dog received only three grafts. Blood flow was restored to all arteries simultaneously 15 minutes after injection of ^{111}In-labeled platelets. Labeled red cells were infused after wound closure (1 to 1.5 hours after administration of labeled platelets). Antibiotics (cefamandole 250 mg, Eli Lilly and Co., Indianapolis, IN) were administered before operation, at the end of the imaging procedure, and once daily for 2 days. Graft patency was assessed weekly by palpation, Doppler examination (Parks Model 1010, Parks Electronics Laboratory, Beaverton, OR), and, in some experiments, by digital intravenous angiography.

Scintigraphic Imaging and Analysis

Prior to induction of anesthesia, 25.9 ml of blood was collected from each dog in a syringe containing 4.1 ml of modified acid-citrate-dextrose (ACD) solution (E. R. Squibb and Sons, Inc., Princeton, NJ). Platelets were isolated and labeled with ^{111}In-8-hydroxyquinoline as described by Heaton and associates.[6] Autologous red cells were obtained from the same sample of blood used for platelet isolation and labeled with ^{99m}Tc by a modification of the method described by Schwartz and associates.[7]

After infusion of labeled platelets (300 to 600 μCi) and red cells (1–2 mCi), the animals were positioned under a large field-of-view scintillation camera (Searle Radiographics, Des Plains, IL), fitted with a medium-energy, parallel-hole collimator, and interfaced to a digital computer (model V-76, Varian Data Machines, Palo Alto, CA). Single channel analyzers on the camera were adjusted to the 140 KeV photopeak of ^{99m}Tc and 247 KeV photopeak of ^{111}In. Separate 100,000 count images of the carotid and femoral regions at each photopeak were collected, digitized, and stored in the computer for later processing. All animals were imaged 2 hours after graft placement and again at 24 hours, after injection of a second dose of labeled red cells. At 2 weeks and 1 month, dogs were similarly studied 24 hours after platelet infusion. Dogs surviving beyond 1 month were imaged at 2 months and then at varying intervals up to 9 months after implantation.

Quantitation of platelet deposition on each graft was accomplished with a dual-isotope subtraction method previously described by Powers and colleagues.[8] It was assumed that, in the region of the graft (ROG), the scintigraphically measured radioactivity from ^{111}In-labeled platelets (In_{ROG}) was equal to the activity associated with labeled platelets in the circulating blood pool (In_{BP}) plus the excess activity associated with any platelets adherent to the vascular graft or adjacent arterial wall (IE).

$$In_{ROG} = In_{BP} + IE.$$

Using a nonmanipulated artery in the same field of view and body plane (subclavian or distal femoral artery for carotid or femoral grafts respectively) as a reference region (REF), we determined the normal ratio in each region of ^{111}In activity to ^{99m}Tc activity (In_{REF}/Tc_{REF}). In_{BP} in the region of the graft was calculated by multiplying the measured ^{99m}Tc-red cell activity in the region of the graft (Tc_{ROG}) by the ratio of ^{111}In activity to ^{99m}Tc activity in the reference region.

$$In_{BP} = Tc_{ROG}\,(In_{REF}/Tc_{REF}).$$

IE was then derived as:

$$IE = In_{ROG} - In_{BP}.$$

IE was expressed as a percentage of In_{BP} such that

$$\%IE = (In_{ROG} - In_{BP})/In_{BP} \times 100.$$

TABLE 1. *Patency* Versus *Time Postimplantation*

Time of Graft Thrombosis	Artery		Vein		Gore-tex		Meadox		USCI	
	Carotid (N = 4)	Femoral (N = 4)	Carotid (N = 7)	Femoral (N = 3)	Carotid (N = 14)	Femoral (N = 12)	Carotid (N = 5)	Femoral (N = 9)	Carotid (N = 7)	Femoral (N = 10)
0–24 hours	0	0	0	0	1	1	0	0	0	3
24 hours–2 weeks	0	0	0	0	4	2	1	4	2	1
2 weeks–1 month	0	0	0	0	2	2	1	2	0	1
Patency (1 month)	100%		100%		54%		43%		59%	

One-month patency and the interval during which graft thrombosis occurred. There was no statistical difference in patency among the synthetic grafts.

Analysis of %IE data was performed after pooling results from the carotid and femoral regions for each type of graft.

Platelet Aggregation Studies

In vitro platelet aggregation studies were performed on 13 animals. Blood (9 ml) was collected prior to anesthesia in 1 ml of 3.8% sodium citrate (pH 7.35). The blood was centrifuged at 1100 × g for 2 minutes to produce platelet-rich plasma (PRP). An aliquot of PRP was further centrifuged at 15,600 × g for 1 minute to produce platelet-free plasma (PFP). Aggregation studies were performed with a dual channel aggregometer (Payton Associates, Buffalo, NY) immediately after centrifugation. Light transmission was set 100% for PFP and 0% for each sample of PRP. Aggregation studies were performed with arachidonic acid (250 nM) (NuChek Prep, Inc., Elysian, MN) as a stimulus. Aggregation was expressed as per cent change in light transmission at 3 minutes.

Graft Retrieval Studies

Four dogs, all with three or more thrombosed grafts, were sacrificed during the first 2 weeks. Five dogs were sacrificed at 1 month, five dogs at 2 months, and five dogs at 3 to 9 months after graft implantation. Prior to sacrifice, the dogs were anesthetized as described, given IV heparin (100 units/kg), and the grafts removed. Explanted grafts were opened longitudinally, gently rinsed with normal saline and the lumenal surface photographed. The degree of neointimalization of the flow surface in 18 patent synthetic grafts (7 Gore-tex and 11 Dacron) was estimated by the calculating per cent thrombus-free surface (%TFS) as described by Kenny et al.[9] To determine the ability of this dual-isotope platelet imaging technique to accurately quantitate platelet deposition, 34 patent synthetic or autologous vein grafts studied 2 hours to 9 months after implantation during this study and a previous investigation[10] were explanted immediately after *in vivo* imaging. Explanted grafts were processed as above and placed in a scintillation counter (Beckman Model 8000, Beckman Instruments, Inc., Palo Alto, CA) where actual indium-associated platelet activity (IE, *in vitro*) was directly measured. The amount of circulating indium activity (In_{BP}, *in vitro*) was similarly determined in a 5 ml sample of venous blood. %IE (*in vitro*) was then calculated by dividing IE (*in vitro*) by the value for the blood pool activity that would be expected in the volume of blood contained within the graft (0.63 ml). The correlation between %IE measured *in vitro* and *in vivo* was calculated by standard linear regression. Twelve patent synthetic grafts explanted 3 to 9 months after implantation were fixed in 2.5% gluturaldehyde in cacodylate buffer. These grafts were fixed in 1% osmium tetroxide for 1 hour. Each sample was then dehydrated in ascending grades of alcohol and critical-point dried. After the grafts were mounted and sputter coated with gold (125 A thick), a Philips 501 (Eindhoven, Netherlands) Scanning Electron Microscope was used to evaluate lumenal topography.

Statistical Analysis

The chi square test with Yates' correction[11] was used for comparison of graft patency rates and to determine the significance of differences between dogs in *in vitro* platelet aggregation studies. Statistical evaluation of %IE and %TFS data was performed using the Mann-Whitney rank test.[12]

Results

Patency

None of the autologous vein or artery grafts in either the carotid or femoral positions failed during the entire study period (Table 1). There was a high rate of graft thrombosis (40–60%) among all the synthetic grafts. There was no difference in synthetic graft patency with respect to position (carotid or femoral). The 30-day patency rates were not significantly different between the synthetic groups. Sixty-three per cent of the synthetic graft failures occurred during the initial 2 weeks and 27% of the thromboses occurred during the second 2 weeks after implantation. Ten dogs with 35 patent grafts were followed beyond 1 month (12 Gore-tex, 4 Meadox, 7 USCI, 8 artery, and 4 vein grafts). Three grafts, in two dogs, failed after

1 month; one Meadox graft at 3 months; and 2 Gore-tex grafts at 6 weeks and at 3 months.

Platelet Imaging

Platelet scintiphotos of all grafts revealed that platelet deposition was greatest immediately after operation and initially appeared uniform over the entire length of the prostheses. Serial images of platelet deposition on synthetic grafts indicated that platelet deposition gradually became localized to the midportion of the grafts with less deposition at the anastomoses (Fig. 1). The ^{99m}Tc-red cell images were helpful in evaluating patency and correlated well with the presence of antegrade pulsations or pulsatile Doppler signals in the grafts (Fig. 2).

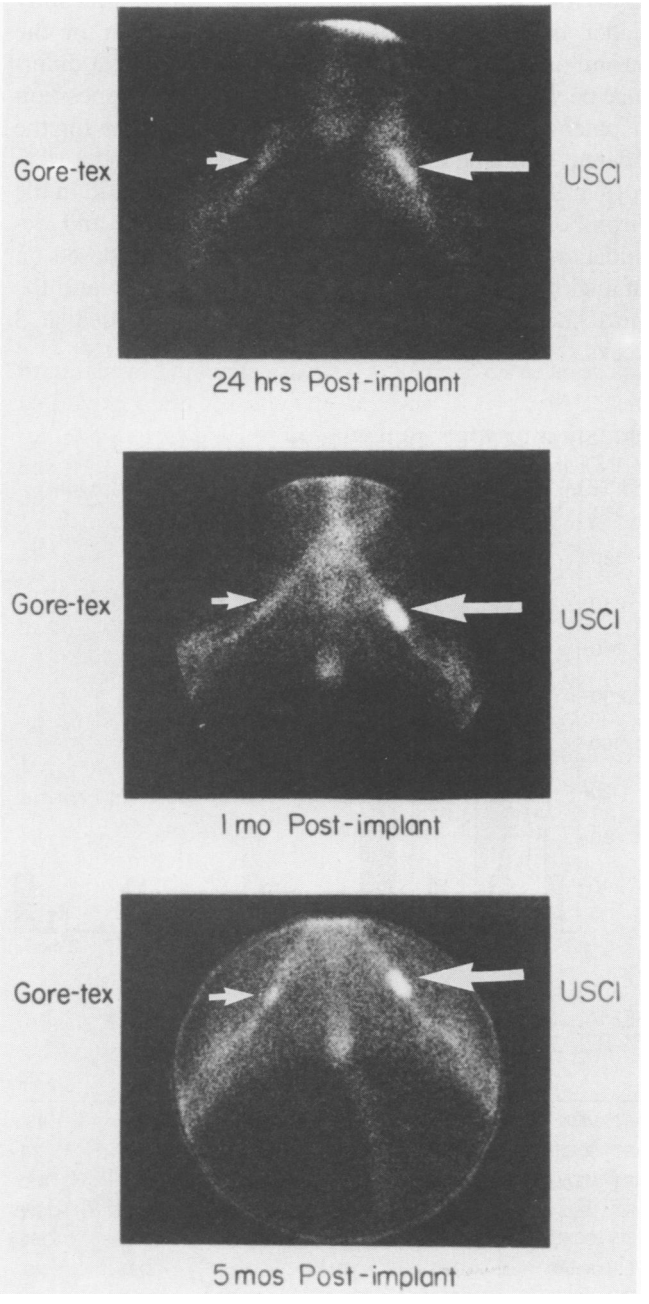


FIG. 1. Serial ^{111}In-labeled platelet images of the femoral region of a dog studied at 24 hours, 1 month, and 5 months postimplant. On the left is a Gore-tex graft (small arrows) and on the right a USCI graft (large arrows). Note that platelet deposition is greater on the USCI graft at all times. Deposition is initially uniform over the length of both prostheses but appears to localize to the midportion of the grafts on subsequent images.

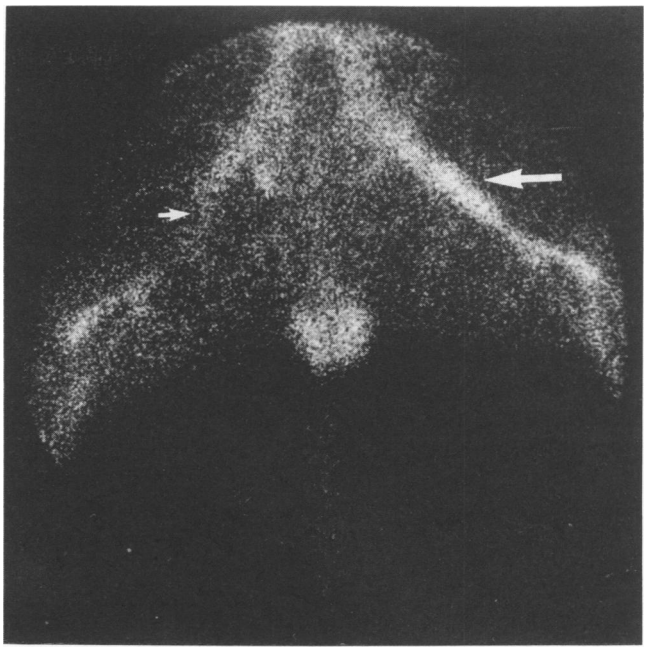


FIG. 2. Blood pool image with ^{99m}Tc-labeled red cells from the femoral region of a dog 2 weeks after graft placement. Blood pool activity is noted in the vein graft (large arrow) on the right but is not seen on the left (small arrow) indicating graft thrombosis.

Quantitation of Platelet Deposition

The correlation of %IE measured *in vitro* and *in vivo* is shown in Figure 3 ($r = 0.94$, $p < 0.01$). Platelet deposition, as measured by %IE, in carotid and femoral grafts is shown in Fig. 4. Deposition on all grafts was highest immediately after implantation and then decreased over time, with the greatest decline in platelet deposition occurring during the interval from 24 hours to 2 weeks. Vein and artery grafts accumulated fewer platelets than synthetic grafts at all time points. The deposition on Gore-tex grafts was consistently less than on either of the Dacron grafts up to 2 weeks following implantation and remained less than on USCI grafts even at 2 months. After 2 weeks %IE on Meadox grafts decreased to values near those for Gore-tex and became consistently less than those obtained from USCI grafts. There was no significant difference in platelet deposition among any of the grafts with respect to position (*i.e.*, femoral *vs.* carotid).

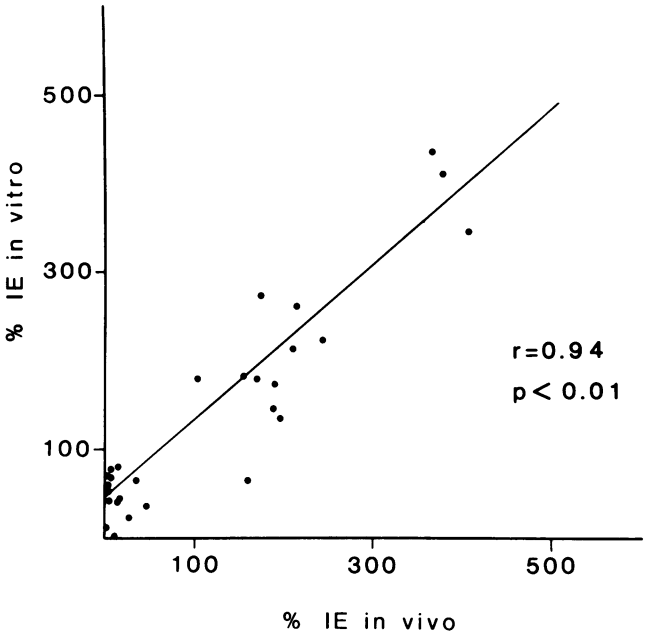
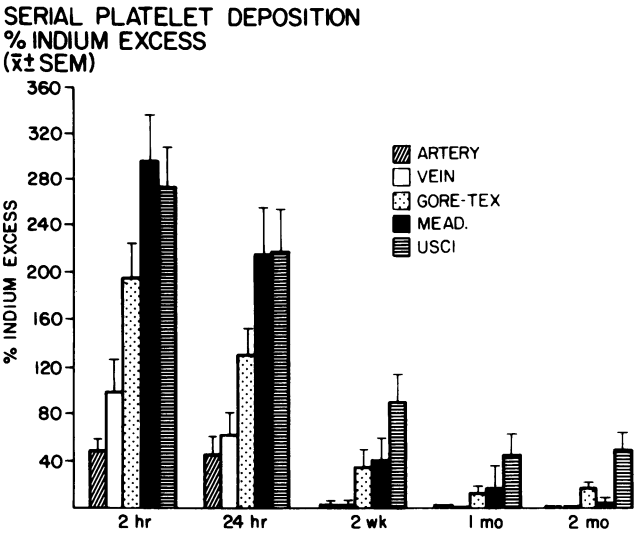


FIG. 3. Correlation of %IE measured *in vivo* and *in vitro* (N = 34). Both synthetic and autologous vein grafts were used in this analysis.

Statistical comparison of %IE on each type of graft at each time interval is shown in Figure 4. %IE at all imaging intervals was significantly less for artery and vein grafts when compared with that for the synthetic grafts except Meadox at 2 months. There was no difference in %IE between artery and vein grafts at any study point. Platelet deposition on Gore-tex grafts was statistically less than on USCI grafts during the interval from 24 hours to 1 month. Gore-tex grafts accumulated significantly fewer platelets than the Meadox grafts only at 24 hours. There was no difference in platelet deposition on Meadox and USCI grafts at any imaging interval.

To determine what effect early platelet deposition had on 1-month patency, grafts patent or thrombosed at 1 month were compared in terms of their 2-hour and 24 hours postimplant %IE (Table 2). Synthetic grafts that thrombosed during the 1-month study period accumulated two to three times more platelets on early platelet scintigrams than did those grafts that remained patent ($p < 0.025$ for all). Because of the high rate of early graft thrombosis, insufficient data were available to make a similar analysis at 2 weeks or 1 month.

Nine dogs had 28 of the 31 synthetic grafts (90.3%) that were patent at 1 month. Twenty-four of the 26 grafts (92.3%) that thrombosed during the first month were in the remaining eight animals. This observation led us to examine several variables in these two subsets of animals (which we have labeled thrombosis-resistant and thrombosis-prone, respectively). There were no differences between the groups with respect to dog breed, age, sex distribution, duration of operation, diet, or medications. Table 3 shows these two groups of dogs compared in terms of graft patency and platelet deposition on vein and synthetic grafts. %IE on the synthetic grafts at 2 hours and 24 hours after implantation in the group of thrombosis-prone dogs was 2–3 times greater than %IE on similar grafts in the thrombosis-resistant group. One-month patency for each type of synthetic graft was significantly higher in the thrombosis-resistant group than in the thrombosis-prone group. There was no significant difference between the two groups in either platelet deposition or patency (100%) for vein grafts. Patency rates for the different synthetic grafts within each of the subgroups were not significantly different. Three grafts thrombosed in the thrombosis-resistant group. One was infected and occluded at 2 weeks, one was noted to be thrombosed on an angiogram done immediately after operation and the third had initially elevated %IE and thrombosed at 3 weeks.



SERIAL COMPARISON OF PLATELET DEPOSITION (% IE) ON EACH GRAFT TYPE

	2 hr	24 hr	2 wk	1 mo	2 mo
ART : VEIN	NS	NS	NS	NS	NS
ART : GORE-TEX	<.025	<.05	<.0005	<.025	.025
ART : MEAD.	<.025	<.01	<.005	<.025	NS
ART : USCI	.025	<.01	<.0005	<.0005	<.025
VEIN : GORE-TEX	<.025	<.05	<.0005	<.025	.025
VEIN : MEAD.	<.025	<.01	<.005	<.025	NS
VEIN : USCI	.025	<.01	<.0005	<.0005	<.025
GORE-TEX : MEAD.	NS	<.05	NS	NS	NS
GORE-TEX : USCI	NS	<.025	<.01	<.005	NS
MEAD. : USCI	NS	NS	NS	NS	NS

FIG. 4. For each graft type, %IE (% Indium Excess ($\bar{X} \pm$ SEM)) was calculated after combining data from the carotid and femoral regions. Statistical comparisons of %IE data are shown in the table.

TABLE 2. *Early-Platelet Deposition* Versus *1-Month Patency*

Graft	Time postimplant	%IE ($\bar{X}$ ± SEM) Patent (1 month)	(N)	Thrombosed (1 month)	(N)	Significance (p)
Artery	2 hr	51 ± 8	(8)	None		—
	24 hr	48 ± 16		None		—
Vein	2 hr	98 ± 26	(8)	None		—
	24 hr	63 ± 19		None		—
Gore-tex	2 hr	118 ± 11	(14)	271 ± 48	(12)	<0.0005
	24 hr	89 ± 14		211 ± 32		=0.001
Meadox	2 hr	178 ± 54	(6)	382 ± 39	(8)	=0.025
	24 hr	101 ± 28		307 ± 53		=0.001
USCI	2 hr	184 ± 37	(10)	413 ± 33	(7)	=0.005
	24 hr	177 ± 38		335 ± 70		=0.025

Grafts patent or thrombosed at 1 month compared in terms of their 2-hour and 24 hours postimplant %IE.

Seven of the 13 dogs that received *in vitro* platelet aggregation studies were in the thrombosis-resistant group. Aggregation of platelets in response to arachidonic acid was not noted in any of these dogs. Platelets from four of the six dogs in the thrombosis-prone group aggregated on exposure to the stimulus. Arachidonic acid failed to stimulate platelet aggregation in the other two dogs from this group. This difference in response to arachidonic acid between the two groups was statistically significant ($p < 0.02$).

Evaluation of Explanted Grafts

Gross examination of grafts explanted during the first month postimplant indicated that the predominant cause of occlusion was an amorphous thrombus that was more adherent to the suture lines than to the body of the graft (Fig. 5). All of the synthetic grafts explanted after 1 month demonstrated varying degrees of a perianastomotic neointimal fibrous ridge. Two of the three synthetic graft failures occurring after 1 month were thought to be a result of this anastomotic hyperplastic reaction (Fig. 6). Two vein grafts had gross evidence of anastomotic neointimal fibrous hyperplasia (ANFH), one explanted at 1 month and another at 9 months after implantation. Interestingly, the vein graft explanted at 1 month had evidence of marked platelet deposition at the anastomosis on the 2-week platelet image (Fig. 7). None of the autologous artery grafts exhibited significant perianastomotic irregularities.

Formation of a neointimal pannus growing across each anatomosis was noted in patent synthetic grafts explanted after 2 weeks, and was more developed in grafts implanted for 2 months or longer (Fig. 8). The degree of neointimal coverage as estimated by %TFS varied among the grafts, but no prostheses demonstrated complete neointimalization (Table 4). Patent Gore-tex grafts generally had higher values for %TFS at explanation than did Dacron prostheses, but these differences were not statistically significant. The level of platelet deposition (%IE) between these two groups of grafts was significant ($p < 0.05$). The value for %IE in the Dacron grafts ranged from 5 to 226. The Gore-tex grafts in this group had a %IE that varied from 0 to 42%. To further evaluate the influence of neoin-

TABLE 3. *Platelet Deposition and Patency in Thrombosis-Prone* Versus *Thrombosis-Resistant Dogs*

Graft	Time Postimplant	%IE ($\bar{X}$ ± SEM) Thrombosis-Prone	Thrombosis-Resistant	Significance (p)
Vein	2 h	112 ± 40	79 ± 37	N.S.
	24 h	62 ± 28	63 ± 31	N.S.
	Patency (1 month)	100% (5/5)	100% (5/5)	N.S.
Gore-tex	2 h	296 ± 46	110 ± 11	<0.0005
	24 h	211 ± 32	81 ± 14	=0.001
	Patency (1 month)	8% (1/12)	93% (13/14)	<0.001
Meadox	2 h	382 ± 17	169 ± 46	=0.05
	24 h	317 ± 60	114 ± 26	=0.001
	Patency (1 month)	0% (0/7)	86% (6/7)	<0.001
USCI	2 h	413 ± 33	196 ± 40	<0.005
	24 h	335 ± 70	177 ± 38	<0.025
	Patency (1 month)	14% (1/7)	90% (9/10)	<0.001

Thrombosis-prone dogs were defined as having two or more occluded synthetic grafts at 1 month postimplant. The patency rates for the synthetic prostheses within each group were not significantly different. Only vein grafts had similar levels of platelet deposition and patency in both groups.

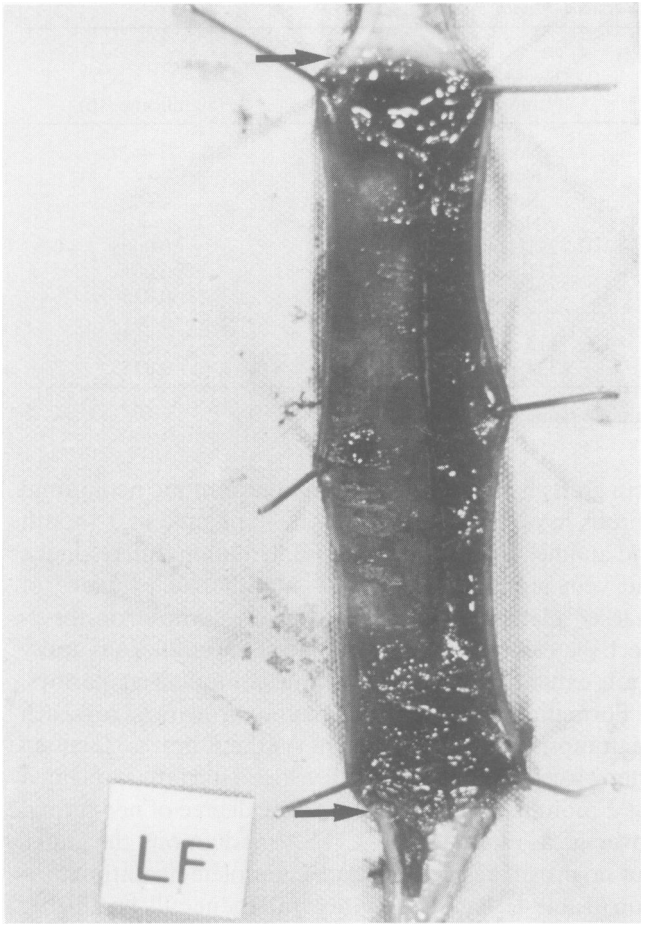


FIG. 5. A Gore-tex graft that thrombosed 1 week following implantation. Blood flow is toward LF. Arrows indicate the anastomotic regions where adherent, gelatinous thrombi are seen occluding the lumen.

timal coverage (%TFS) on %IE, the Dacron grafts were arranged according to a %IE greater than 50 or less than 50. These two groups of grafts were then compared to one another and to Gore-tex grafts in terms of platelet deposition (%IE) and neointimalization (%TFS) (Table 5). The Dacron prostheses with a %IE greater than 50 at explanation had significantly lower values for %TFS when compared to Gore-tex or to Dacron grafts with a %IE less than 50 ($p < 0.01$ and $p < 0.005$, respectively). There was no significant difference in %IE or %TFS between Gore-tex grafts and those Dacron grafts with a %IE less than 50. Scanning electron microscopic evaluation indicated the neointimal pannus areas to be composed of regularly arranged smooth sheets of endothelial cells while the mid-portion of the grafts were nonendothelialized and contained fibrin, red cells, and platelet aggregates (Fig. 9).

Discussion

Platelet tracing techniques have been instrumental in elucidating the platelet's role in arterial thrombosis. Early investigations most commonly used chromium-51 as a platelet label to study platelet survival and *ex vivo* platelet deposition on biologic and synthetic surfaces.[13] However, these methods required large volumes of blood for platelet labeling and were limited by the inability to image *in vivo* the low-abundance gamma photon of chromium-51. The use of indium-111 as a platelet label has greatly facilitated the *in vivo* study of the dynamic interaction between platelets and various types of vascular surfaces. Using platelet imaging techniques, several investigators recently have documented that deposition of ^{111}In-labeled platelets on arterial grafts is increased when compared with native vessels of the same caliber and that it can be reduced with platelet inhibitory drugs.[14,15]

To study objectively the effects of platelet inhibitors on platelet function *in vivo* and to compare platelet deposition on various types of vascular grafts, several methods of quantitating platelet deposition have been developed, but the accuracy of these techniques has yet to be proven. Platelet deposition measured by the dual-isotope platelet

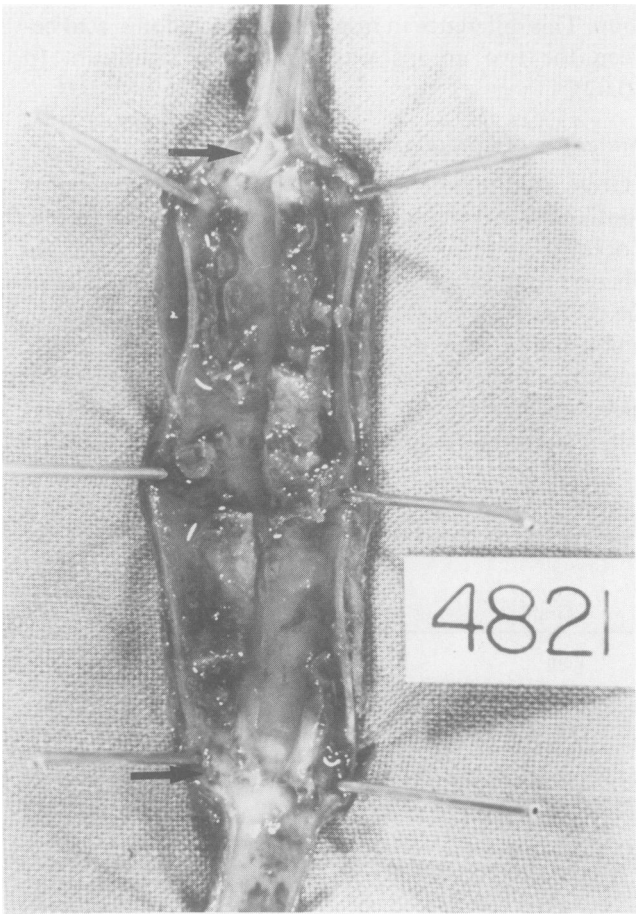


FIG. 6. A Gore-tex graft that thrombosed at 3 months. Bottom arrow indicates proximal anastomosis. An occlusive white fibrous ridge (top arrow) is present just beyond the distal anastomosis and appeared to cause graft failure.

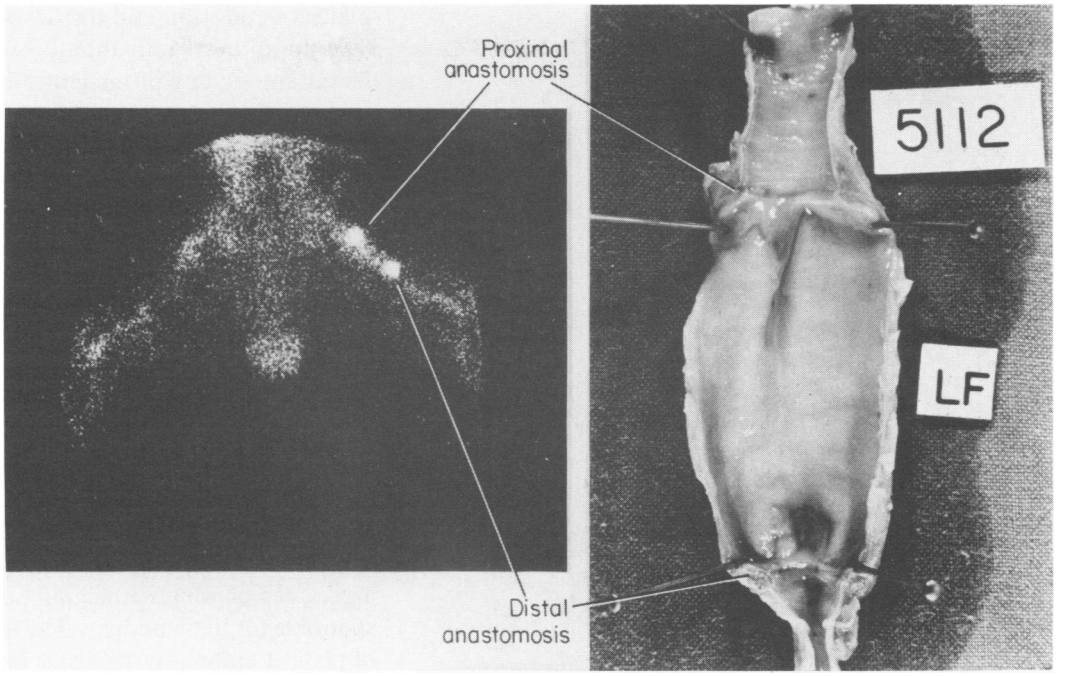


FIG. 7. Anastomotic ridges found in an autologous jugular vein graft explanted at 1 month correspond in location to areas of increased platelet deposition seen on the 2-week platelet image. Platelet scintigram at 1 month indicated no areas of excessive platelet deposition.

imaging method used in these studies correlated well with actual platelet deposition assessed by standard *in vitro* methods, thus allowing valid animal to animal comparisons with respect to graft type, location, and time after implantation. Additionally, blood pool images obtained with ^{99m}Tc-labeled red cells provided valuable information regarding patency in the carotid femoral regions. The lack of significant differences in platelet deposition between similar prostheses in the carotid or femoral artery position was not unexpected since both are similar in blood flow and size in the large dogs used in these studies.[16,17] External motion about the graft is a potential difference in these two locations. However, the grafts were relatively short in length (5 cm), and the femoral grafts were placed below the inguinal ligament where stretching or kinking of the graft should be minimized.

Restoration of arterial flow through the grafts after infusion of radiolabeled platelets resulted in extravasation of some radioactivity into the surgical wound and on the external surface of the grafts. The contribution of this extralumenal activity to the measured level of platelet deposition has not been well documented. The amount of platelet deposition found in the arterial autograft group reflects the background platelet activity produced by mobilizing, dividing, and anastomosing an artery. We found that the degree of excess platelet deposition produced in the arterial autografts was minimal when compared to the synthetic graft groups. This confirms that the elevated value for %IE found in these synthetic grafts primarily results from excess platelet deposition on the flow surface and not in the surgical wound.

Serial images of platelet deposition on synthetic grafts suggested that platelet deposition gradually became more prominent in the midportion of the grafts and was relatively less at the anastomoses. This localization of platelet deposition roughly corresponded with the time needed for anastomotic neointimal pannus ingrowth (1 to 2 months). Scanning electron microscopy indicated this pannus to be composed of endothelial cells while the midportion of the graft was lined with thrombotic debris. The reduction of platelet deposition on the pannus may be related to prostacyclin or thromboxane production by the surface endothelial cells as demonstrated in our laboratory and by the investigations of Clagget and colleagues.[18,19]

None of the synthetic grafts examined in the present study demonstrated complete neointimal coverage of the lumenal surface. The degree of neointimalization varied among the grafts but in all cases we found that a significant portion of the flow surface was composed of adherent microthrombi. We have noted in previous investigations that the percentage of thrombus-free surface (%TFS) is a significant determinant of platelet deposition.[20] The observations in this project confirm our previous findings indicating that those patent Dacron grafts with a %IE <50 have a %TFS that is significantly greater than those prostheses with a %IE >50. Although all of the patent Gore-tex grafts evaluated for %TFS had values of %IE less than 50, we did not find significant differences in %TFS when comparing Gore-tex grafts with the entire Dacron graft group. However, when the Gore-tex grafts were compared to those Dacron grafts with a %IE greater than

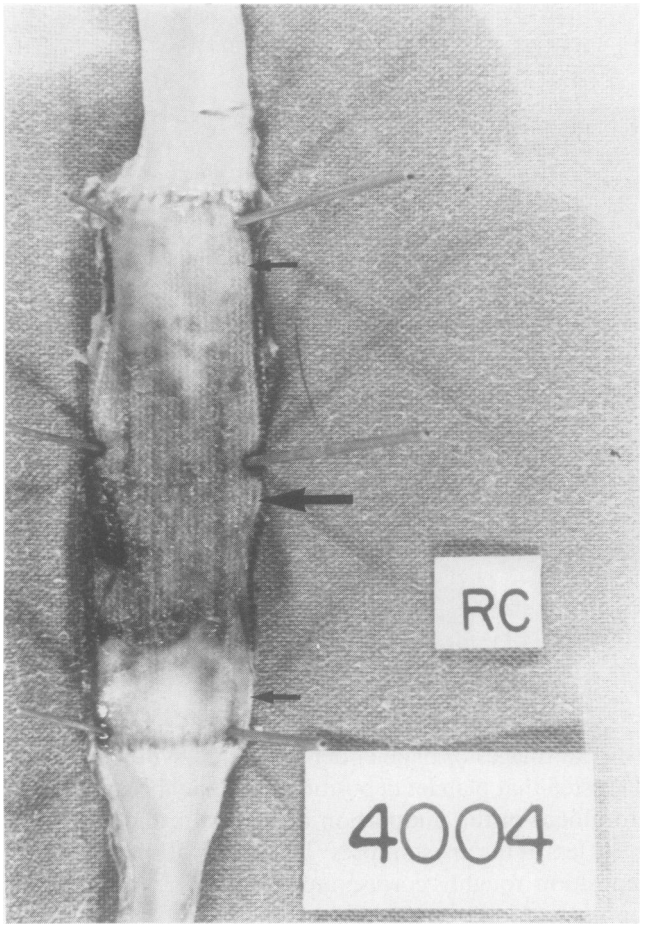


FIG. 8. A Meadox graft explanted at 3 months. The portions of the graft near each anastomosis have been covered by neointimal pannus ingrowth (small arrows). The midregion of the graft is lined by red microthrombi (large arrow). %TFS = 52.

50, we noted significant differences in both platelet deposition and thrombus-free surface, further supporting our impression that %TFS strongly influences platelet deposition. The fact that a subgroup of the Dacron grafts had values of platelet deposition and thrombus-free surface similar to those of Gore-tex grafts indicates that factors other than graft material or design are important in

TABLE 4. *Platelet Deposition* Versus *Thrombus-Free Surface in Patent Synthetic Grafts*

	GRAFTS		
	Dacron N = 11	Gore-tex N = 7	Significance
%TFS	51.6 ± 8.9	70.1 ± 9.4	N.S.
%IE	58.2 ± 19.5	13.7 ± 5.3	$p < 0.05$

$\bar{X}$ ± SEM.

Dacron and Gore-tex grafts compared in terms of thrombus-free surface (%TFS) at explanation and platelet deposition (%IE) immediately prior to explanation.

platelet deposition and the development of a neointima. The duration of implantation was not significantly different among any of the grafts evaluated for %TFS. The *in vitro* platelet reactivity did not seem to influence the %TFS; however, the numbers of grafts examined were too small to draw meaningful conclusions. The influence of persistently high levels of platelet deposition on long-term patency is unknown.

Christenson and workers reported that when platelets were infused prior to the re-establishment of blood flow, platelet deposition on PTFE grafts in canine carotid and femoral arteries peaked soon after cross clamp release and then declined.[21] Our data confirm this observation and indicate that platelet deposition on Dacron, autologous artery, and vein grafts follows the same pattern. The decline in ^{111}In activity on the grafts during the first 24 hours following implantation suggests that platelet adherence to the graft is not irreversible. Embolization of large platelet aggregates present at the graft surface may be partly responsible for these findings. However, we saw no evidence of platelet emboli in the distal vasculature on the platelet images.

A major goal of these investigations was to determine the significance of platelet deposition on graft function. Although platelet deposition was elevated on all grafts immediately after implantation, grafts that thrombosed during the first month initially accumulated two to three times more platelets than did those that remained patent. These findings emphasize the critical role of the platelet in the thrombosis of small diameter arterial prostheses and suggest the potential value of this dual-isotope platelet imaging technique in predicting which prostheses are at risk for failure. Only the vein or artery grafts had consistently low values for %IE and acceptable patency rates. Fonkalsrud and associates have shown that the endothelial cell damage associated with vein harvesting is repaired 4 to 10 days after implantation.[22] The low values for %IE on artery and vein grafts at 2 weeks corresponds well with these data, indicating that the endothelial surface has become functionally competent and can resist significant platelet deposition. The patency rates for Gore-tex and USCI grafts were similar in spite of values for %IE from 24 hour to 1 month that were significantly higher on USCI grafts. While there are other differences in these grafts that may influence patency, these data suggest that there may be a critical level of platelet deposition, unique to each type of prosthesis, above which thrombosis is likely. When evaluating the entire group of dogs, we were unable to demonstrate a significant difference in platelet deposition on Meadox grafts *versus* Gore-tex or USCI after 24 hours. We suspect that Meadox grafts, because of their similarity in construction, have a platelet affinity comparable to USCI. Our inability to demonstrate a difference in platelet deposition between Gore-tex and Meadox grafts

TABLE 5. *Platelet Deposition* Versus *Thrombus-Free Surface in Patent Synthetic Grafts*

	Grafts					
	Dacron					
	(A) %IE < 50 N = 6	(B) %IE > 50 N = 5	Gore-tex (C) N = 7	Significance A:B	A:C	B:C
%TFS	74.0 ± 5.7	24.8 ± 7.8	70.1 ± 9.4	$p < 0.005$	N.S.	$p < 0.01$
%IE	16.8 ± 4.1	108 ± 30.5	13.7 ± 5.3	$p = 0.0025$	N.S.	$p = 0.0025$

$\bar{X}$ ± SEM.

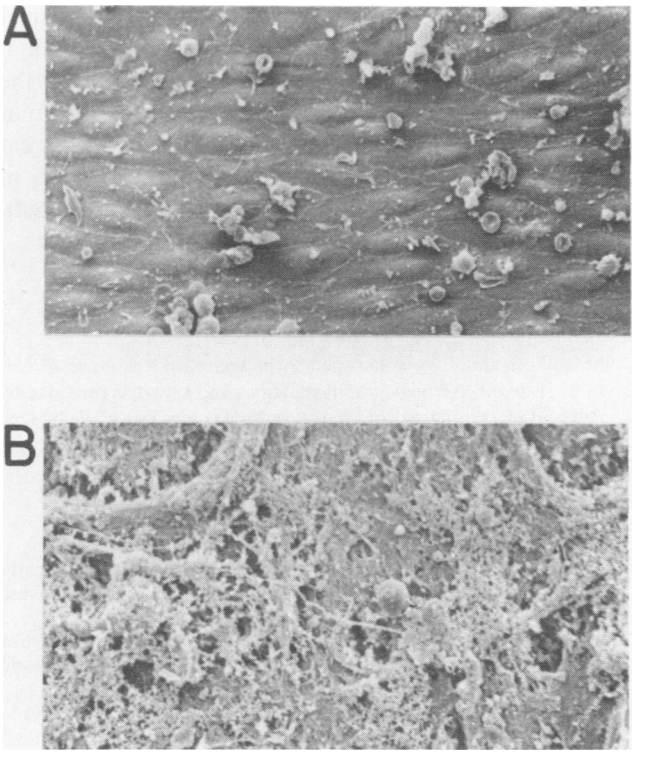
Because of the variability in platelet deposition on Dacron grafts, they were divided according to a %IE greater than 50 or less than 50 and compared in terms of %IE and %TFS to one another and to Gore-tex grafts (all Gore-tex grafts evaluated for %TFS had a %IE less than 50).

was probably due to the high rate of early graft failure that left only six Meadox grafts available for comparison at 1 month *versus* 14 for Gore-tex.

Blood flow, a factor known to be important in platelet deposition was not measured in this study. Others have shown that thrombosis of small diameter grafts in humans can result when blood flow falls below 70 ml/min.[23] Since blood flow in the carotid and femoral arteries of dogs of the size used in these studies is greater than 100 ml/min, we doubt that this was a major factor in graft occlusion. Additionally, Christenson and associates have reported that platelet deposition on PTFE grafts that eventually fail is elevated prior to a reduction in blood flow.[1]

The identification of thrombosis-prone and thrombosis-resistant dogs was not a planned objective of this project. However, we and others have noted in many studies using dogs with multiple synthetic grafts of varying types that, if one graft thrombosed, the other grafts in that animal would probably occlude as well.[24] Conversely, in other dogs, prostheses of any type rarely failed during the first month. Thus, it appeared that vascular graft occlusion was more related to the thrombogenic response of the dog rather than the material or manufacturing process used in graft production. In our investigations and those reported by others, only autologous tissue (arterial or venous) or synthetic grafts used in conjunction with platelet inhibitors or endothelial cell seeding techniques have enjoyed consistently high patency rates.[25-28] Data from this study support the impression that some dogs are thrombosis-prone while others are thrombosis-resistant and suggest that *in vivo* platelet deposition studies can distinguish these two groups. Significant differences between the groups were also noted in the *in vitro* platelet response to arachidonic acid. More extensive platelet function studies are needed to fully characterize the platelet reactivity of each dog, but our findings do indicate that such *in vitro* platelet studies may be important in identifying before operation those dogs that are thrombosis prone. Even though the thrombogenic response of the dog may have a greater influence on platelet deposition than does graft material or construction, the importance of graft design is apparent in that platelet deposition in the thrombosis-resistant dogs was greater 24 hours following implantation on Meadox and USCI grafts when compared to Gore-tex ($p < 0.05$ and $p < 0.01$, respectively). These differences in platelet deposition appear to have little effect on 1-month patency. Their significance with respect to long-term graft function is unknown.

Dogs in the thrombosis-prone group were responsible for 92.3% of the occlusions seen at 1 month in both groups. All of these failures were the result of thrombotic material



FIGS. 9A and B. Scanning electron micrographs demonstrating endothelial cells on the surface of the perianastomotic pannus (*A*), while the center of the graft is covered by fibrin, red cells, and platelet aggregates (*B*) (×320).

that had accumulated primarily at the anastomoses. In humans, this mechanism of thrombosis appears most important in acute graft failures while anastomotic neointimal fibrous hyperplasia (ANFH) is thought to be responsible for late occlusions. We noted gross evidence of ANFH only in grafts explanted after 1 month (primarily from the thrombosis-resistant group), and it was thought to cause only two graft failures—both of which were in a thrombosis-resistant dog and failed at 3 months after implantation. We speculate that ANFH occurs more commonly in the thrombosis-resistant dogs. The hemostatic systems of these dogs does not promote early graft failure, thus allowing time for ANFH to develop. In contrast, dogs with thrombosis-prone hemostatic systems quickly occlude vascular grafts with thrombotic material before significant ANFH forms. Preoperative identification of thrombosis-prone and thrombosis-resistant dogs may provide a better understanding of these two mechanisms of vascular graft thrombosis and what factors, such as platelet deposition, are important in their development.

In summary, we have documented the ability of dual-isotope platelet scintigraphy to accurately quantitate platelet deposition on vascular grafts. Platelet deposition in the surgical wound is minimal when compared to that found on synthetic prostheses and does not significantly influence the calculation of %IE. The degree of early platelet deposition critically influences the 1 to 2 month patency rate of any synthetic arterial prosthesis. Platelet deposition varies inversely with neointimalization. Initial platelet deposition appears to be more related to the thrombogenic response of the dog than to the type of material used in graft construction. These differences in canine platelet reactivity may account for the variability in both patency and the incidence of ANFH among published reports involving small diameter vascular grafts.

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