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Thrombocytopenia and Altered Platelet Kinetics Associated with Prolonged Pulmonary-artery Catheterization in the Dog

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The authors studied platelet kinetics in healthy dogs subjected to pulmonary-artery (Swan-Ganz) catheterization. Ten dogs with a mean weight of 11.0 ± 1.12 kg (SD) were equally divided into experimental and control groups. A fraction of platelets from each dog was harvested, labelled with ^{51}Cr, and reinfused. Daily blood samples for determinations of radioactivity and platelet count were obtained. From the third to fifth day after platelet labelling, experimental dogs underwent catheterization with Swan-Ganz catheters.

During 48 hours following catheterization, platelet counts decreased 64.2 ± 15.2 per cent from baseline. There was no corresponding change in the control dogs. Calculated platelet survival times (based on decay in platelet radioactivity over time) were 102 ± 6.5 hours for the experimental group and 139.8 ± 16.3 hours for controls ($P < 0.01$). Postmortem examination of one sample dog demonstrated thrombus formation along the length of the catheter and in the pulmonary artery.

Significant thrombocytopenia with decreased platelet survival is associated with the use of Swan-Ganz catheters in dogs. The mechanism has not been defined. This observation adds an important consideration to the interpretation of platelet kinetics observed in many pathologic states in animal models. (Key words: Blood: hemostasis; platelets. Complications: thrombocytopenia. Equipment: catheters, Swan-Ganz. Monitoring: pressure, pulmonary arterial.)

THROMBOCYTOPENIA develops frequently in critically ill patients, and often contributes to morbidity and mortality.[1] Flow-directed balloon-tipped (Swan-Ganz) catheters, commonly used in critically ill patients, are known to be thrombogenic in man.[2,3] To evaluate the kinetics of the platelet consumption associated with these catheters and the extent to which this contributes to thrombocytopenia, we studied healthy dogs subjected to 48 hours of pulmonary-artery catheterization.

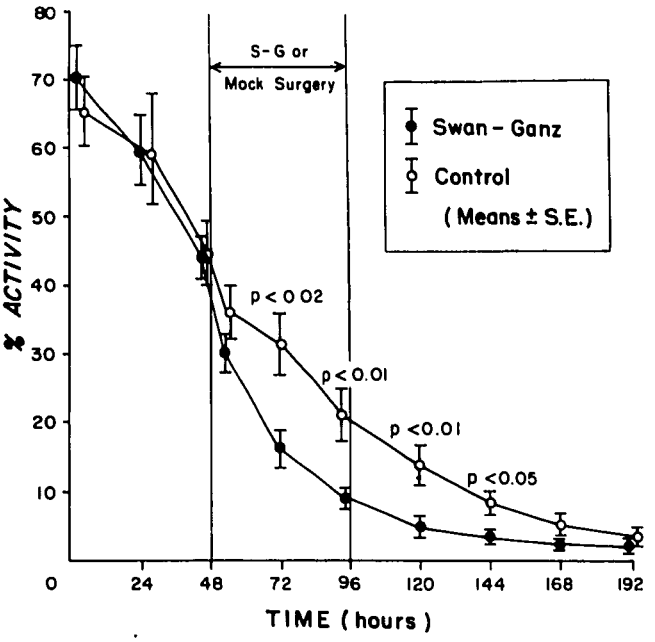

FIG. 1. Composite platelet survival curve based on data from all dogs in the experimental (*solid circles*) and control (*open circles*) groups. The steeper slope of the line for the experimental group beginning at the time of catheterization indicates more rapid platelet consumption.

Materials and Methods

Ten healthy male mongrel dogs weighing 9.3–14.1 kg (mean ± SD:11.0 ± 1.12) were divided into experimental and control groups; each dog was studied over a period of nine days. On day 1, each dog was anesthetized with thiopental (20 mg/kg), the trachea was intubated, and anesthesia was maintained with halothane (1 per cent) in oxygen. The animals breathed spontaneously at an end-tidal CO_2 of 5 per cent. A total of 90 ml of whole blood was drawn from an external jugular vein into three plastic syringes each containing 5 ml of anticoagulant citrate dextrose solution, USP, formula A.§ Platelets were labelled with radioactive chromium in the following standard fashion.[4] The platelet-rich fraction was obtained by differential centrifugation and incubated for one hour with 1.0 mCi ^{51}Cr.¶ The platelets were washed once with platelet-poor plasma, resuspended, and injected. A sample of the injectate was saved for radioactivity

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§ Fenwal Laboratories, Deerfield, Illinois.

¶ New England Nuclear, Boston, Massachusetts: 1 mCi/ml.

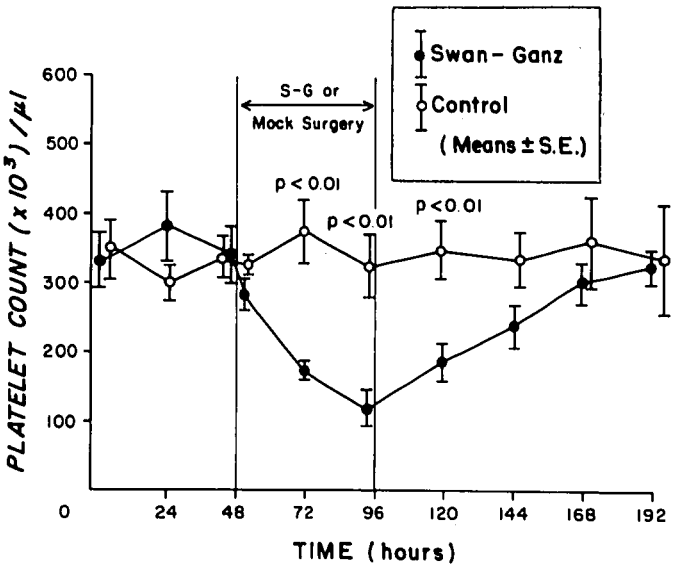

FIG. 2. Mean peripheral blood platelet counts (±SE) for experimental (*solid circles*) and control (*open circles*) animals. The progressive decline occurring in experimental animals represents a 64.2 ± 15.2 per cent (SD) reduction from baseline, with return toward baseline resulting from removal of the catheter.

counting, and an accurate weight and specific gravity of the injectate were obtained prior to injection. Half an hour after injection, a 3-ml sample of heparinized whole blood was obtained via a peripheral vein for radioactivity counting. Anesthesia was then discontinued, approximately four hours after induction. Every 24 hours thereafter, 3-ml blood samples were taken from a peripheral vein of the awake animal for radioactivity counting. All radioactivity counting was done in triplicate in a gamma well counter.** In addition, we performed daily platelet counts in triplicate by phase-contrast microscopy (method of Brecher *et al.*[5]). On day 3 (48 hours after platelet labelling), the dogs were again anesthetized with thiopental and halothane; dogs in the experimental group underwent cutdown of the right groin and insertion of a 7-Fr, double-lumen Swan-Ganz catheter†† via the femoral vein, with ligation of the distal femoral vein. The catheter was advanced into the pulmonary artery, with confirmation of the final position by recording the pressure tracing on a Grass polygraph [average depth of insertion: 48.8 ± 2.7 cm (SD)]. The catheter was flushed with 5 ml of saline solution, 0.9 per cent. The end of the catheter at the groin was occluded with a heavy silk ligature tied tightly at the point of entry of the catheter into the vein, with the remaining catheter external to the vein cut off. The wound was closed and anesthesia discontinued. Dogs in the control group underwent the same anesthetic and surgical procedures for the same length of time (approximately an hour), with ligation of the femoral vein, but without insertion of the catheter. Blood samples for platelet count and radioactivity determinations were obtained six hours after the surgical procedure in addition to the other daily samples. On day 5 (96 hours after platelet labelling), the catheters were removed from the experimental animals, using thiopental–halothane anesthesia; control animals underwent anesthesia with opening and closure of the groin wound, the procedure requiring 45 min for animals in either group. On day 9 (192 hours after platelet labelling), blood volumes of both control and experimental animals were determined by injecting radioactive iodinated (^{133}I) serum albumin, 0.1 mCi.‡‡ The RISA was diluted, weighed, and counted in the gamma well counter prior to injection; three 1-ml samples of whole blood were obtained for counting half an hour after injection.

** Intertechnique Model 4000.

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TABLE 1. Calculation of Platelet Consumption and

	Blood Volume (ml)	Pre-S-G Platelet Count (/µl × 10⁵)	Pre-S-G Total Platelets (×10¹¹)	Post-S-G Platelet Count (/µl × 10⁵)	Post-S-G Total Platelets (×10¹¹)
Experimental					
Mean	970	3.41	3.35	1.20	1.28
± SD	248.2	.760	1.230	.581	1.029
Control					
Mean	981	3.35	3.30	3.25	3.20
± SD	155.8	.604	.872	1.006	1.230
Significance of difference (experimental *vs.* control) by Student *t* test	*N.S.*	*N.S.*	*N.S.*	$P < 0.01$	$P < 0.05$

Rectal temperature was recorded daily. Hematocrit, body weight, and leukocyte count were obtained just prior to and 48 hours after catheterization. Blood cultures were obtained after 48 hours of catheterization.

One additional animal was sacrificed 48 hours after catheterization for observation of clot formation around the catheter and pulmonary artery. This dog received 3000 U heparin, iv, 15 min prior to sacrifice by exsanguination.

The catheter tip from this dog was fixed in 1 per cent glutaraldehyde, dehydrated in graded alcohols and amyl acetate, and finally subjected to critical-point drying. A molecular layer of gold was deposited on the surface and the specimen was examined in a JEOL JSM50-A scanning electron microscope. Photomicrographs at 1000× magnification were obtained.

A segment of left main pulmonary artery from the same dog was fixed in Bouin's solution, sectioned, stained with hematoxylin and eosin, and examined under light microscopy for evidence of thrombus formation.

Decay of platelet radioactivity is expressed by the following equation: Percentage activity at time

$$t = \frac{\text{counts } ^{51}\text{Cr/ml blood at time t} \times \text{blood volume}}{\text{total counts injected initially}} \times 100$$

Plotting percentage activity as a function of time (t) gives a platelet survival (decay) curve; typically, activity immediately after injection of the labelled platelets is about 60 per cent due to initial splenic uptake of injected platelets ("per cent recovery").[6] Calculation of platelet survival time was done in a standard fashion[4] using the four points on the platelet survival curve (per cent activity *vs.* time) obtained just prior to Swan-Ganz catheter insertion and during the two days when the catheter was in place (per cent activity 48, 54, 72, and 96 hours after labelling) in the experimental group, and at the same time intervals in the control group.

Changes in total numbers of platelets for all dogs during the interval of catheterization (experimental) or sham operation (control) were calculated. Total circulating platelets immediately prior to insertion and just before removal of the catheters (or sham operation) was derived by multiplying blood volume times peripheral blood platelet count. The net change in platelets is the difference in total platelets during the interval of catheterization (or between the two sham surgical procedures). The percentage change in radioactive labelled platelet activity during the time of catheterization (or sham operation):

$$\left(\frac{\text{Activity pre-catheterization} - \text{activity post-catheterization}}{\text{Activity pre-catheterization}} \times 100\right)$$

multiplied by the number of total platelets before catheterization estimates the number of total platelets consumed during that time interval. Effective production is the sum of the total platelets consumed plus the overall net change in total platelets.

Unpaired Student t tests were used to compare means of the two populations, with $P < .05$ considered statistically significant.

Results

The platelet survival curves for the control and experimental animals, with each curve derived from the average percentage radioactivity (±SE) for the five dogs in the group, are almost identical in appearance until 48 hours, the time of catheterization in the experimental group (Fig. 1). Thereafter, the survival curve for the experimental group shows a more rapid decay (steeper slope). Mean platelet survival times

Production (S-G = Swan-Ganz Catheterization)

Net Total Platelets (Post-S-G − Pre-S-G) (×10¹¹)	Per Cent Activity Pre-S-G	Per Cent Activity Post-S-G	(Per Cent Pre-S-G − Per Cent Post-S-G) / Per Cent Pre-S-G × 100 = Per Cent Δ	Consumption = Per Cent Δ × Total Pre-S-G (×10¹¹)	Effective Production = Net + Consumption (×10¹¹)
−2.07	43.2	8.5	80.3	2.66	0.59
.678	5.81	3.80	8.54	.910	.671
−.094	44.6	21.8	51.7	1.70	1.61
.443	10.96	7.60	8.206	.456	1.641
$P < 0.0005$	*N.S.*	$P < 0.01$	$P < 0.0005$	$P < 0.05$	$P < 0.05$

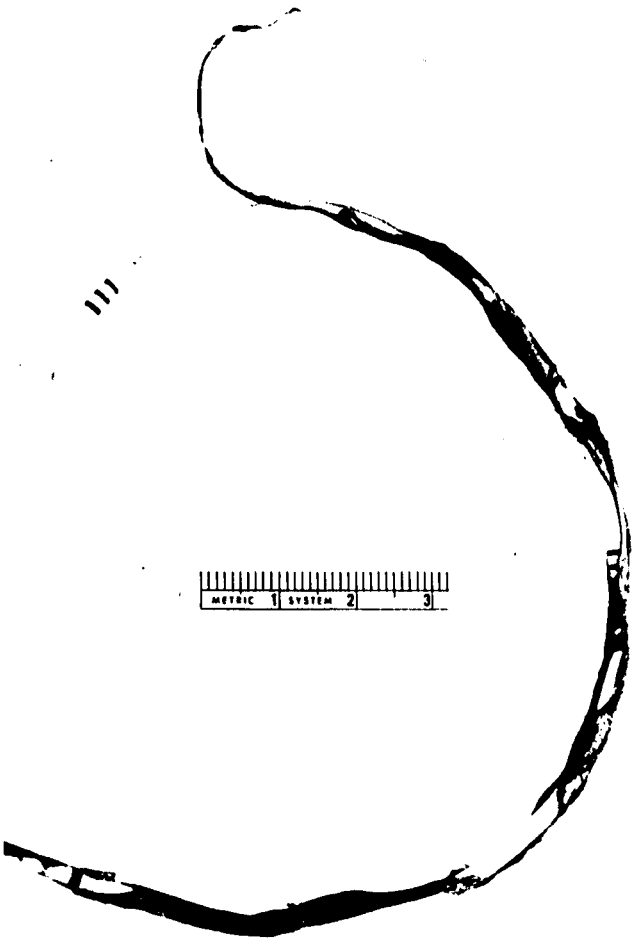

FIG. 3. Organized thrombus surrounding the portion of the catheter that lies in the inferior vena cava, taken after 48 hours of catheterization from a dog systemically heparinized just prior to sacrifice.

calculated from the four points on the curve obtained just prior to and during catheterization were 102 ± 6.5 hours (SD) for the experimental group and 139.8 ± 16.3 hours for the control group, significantly different at $P < 0.01$.

Average peripheral blood platelet counts in the two groups were the same at the beginning, but a decrease in the experimental group began to become apparent as early as six hours after catheterization, was significant after 24 hours, and continued until removal of the catheter (fig. 2). The nadir of the curve after 48 hours of catheterization (96 hours after platelet labelling) represents a 64.2 ± 15.2 per cent (mean ± SD) decline from the 48-hour baseline value. Within the next four days, platelet counts returned to baseline.

The decrease of total platelets (table 1, column 6) in the experimental group ($2.07 \pm .678 \times 10^{11}$) was significantly larger than that in the control group ($.094 \pm .443 \times 10^{11}$) ($P < 0.0005$). The total number of platelets destroyed (column 10) in the experimental group ($2.66 \pm .910 \times 10^{11}$) was significantly greater than that in the control group ($1.70 \pm .456 \times 10^{11}$). Effective production (column 11) in the experimental group ($.59 \pm .67 \times 10^{11}$) was significantly less than that in the control group ($1.61 \pm 1.641 \times 10^{11}$).

Table 2 shows average body weights, hematocrits, and leukocyte counts for the dogs in both groups just prior to and after 48 hours of catheterization or sham operation. There was no significant difference between the groups. Cultures of blood from all dogs were sterile.

Figure 3 shows organized thrombus surrounding the portion of the catheter that lay in the inferior vena cava of the dog that was systemically heparinized just before sacrifice after 48 hours of catheterization. Thrombus could be seen surrounding the entire length of the catheter, extending into the pulmonary artery, where it was firmly fixed to the arterial wall. The presence of polymorphonuclear leukocytes within the thrombus indicated its age to be at least 12–24 hours. A scanning electron photomicrograph of this catheter surfaced showed deposition of fibrin, erythrocytes, and platelets.

TABLE 2. Changes in Body Weight, Hematocrit, and Leukocyte Count after Catheterization or Sham Operation

	Body Weight (kg)		Hematocrit (Per Cent)		Leukocyte Count ($\times 10^3$/mm^3)	
	Pre-catheterization	Post-catheterization	Pre-catheterization	Post-catheterization	Pre-catheterization	Post-catheterization
Experimental						
Mean	11.0	11.0	41.2	38.8	9.8	14.5
± SD	1.9	1.9	2.4	3.6	3.9	3.4
Control						
Mean	10.6	10.7	39.4	37.8	9.9	14.2
± SD	1.0	1.1	1.8	3.1	3.9	3.4
Significance of difference (experimental *vs.* control) by Student *t* test	*N.S.*	*N.S.*	*N.S.*	*N.S.*	*N.S.*	*N.S.*

Discussion

The peripheral blood platelet count represents the balance between platelet production in the marrow and consumption and dilution in the peripheral blood, with production, consumption, and blood volume being constant at steady state.[6] Thrombocytopenia results from increased platelet consumption (or sequestration), reduced production, or hemodilution. Hemodilution does not explain the large decrease (60 per cent) of platelet counts in our experimental dogs, since other indices of increased plasma or total water volume (*i.e.*, hematocrit and body weight) did not change during catheterization.

Increased platelet consumption is evident from the steeper slope of the ^{51}Cr platelet survival curve of the catheterized dogs. The absolute magnitude of total platelets consumed is dependent upon the size of the existing pool of circulating platelets, as well as the number of newly formed platelets (ongoing marrow production during the time of catheterization) that are involved in the consumption process. Our calculation of total consumption only approximates the extent of this process. This calculation may be an underestimate, since it does not include the consumption of newly formed unlabelled platelets, a number that we did not directly measure.

The calculated "effective production" reflects the marrow's ability to restore the peripheral platelet count to its normal level. It is unlikely that the presence of a catheter in the circulation could alter the activity of the marrow [which is chiefly under hormonal (thrombopoietin) control], and actual platelet production at least as likely in the experimental group as in the control group.[7] Calculated "effective production" (net platelets plus consumed platelets) is underestimated to the same extent as is total platelet consumption (see above). That the "effective production" in the experimental group was less than that in the control group restates the phenomenon that consumption is of a greater magnitude than production. Eventually, a new steady state would arise whereby production and consumption would equalize at a lower peripheral platelet count. In all likelihood, the marrow would actually increase platelet production to compensate for the increased losses of platelets.

Many platelets taken out of the circulation appear at clot on the catheter. Swan-Ganz catheters are fabricated from polyvinylchloride, a known thrombogenic material.[2,8,9] The clot formation seen along the catheter and pulmonary artery from one representative dog is similar to that found in man.[2] However, catheter-induced thrombocytopenia has not been described previously. Foreign surfaces have been shown to increase platelet adhesiveness, resulting in clot formation and predisposition to subsequent adhesion, causing continued deposition of platelets with time.[9,10] Foreign surface-induced platelet activation increases platelet phagocytosis by the reticuloendothelial system.[6] This could account for the continuous decline in platelet count during catheterization. Such a mechanism remains unproved.

Despite species differences, human and dog platelets adhere similarly to polyvinylchloride.[8,11–15] That catheter-induced thrombocytopenia occurs in man remains conjectural.

This study also emphasizes that interpretation of platelet kinetics in dogs subjected to pathologic states (*e.g.*, shock) must take into account the abnormalities induced by catheterization.

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References

1. Ellison N: Diagnosis and management of bleeding disorders. ANESTHESIOLOGY 47:171–180, 1977
2. Hoar PF, Stone JG, Wicks AE, et al: Thrombogenesis associated with Swan-Ganz catheters. ANESTHESIOLOGY 48: 445–447, 1978
3. Foote GA, Schabel SI, Hodges M: Pulmonary complications of the flow-directed balloon tipped catheter. N Engl J Med 290:927–931, 1974
4. Belcher EH, Berlin NI, Eernisse JG, et al: Recommended methods for radioisotope platelet survival studies. Blood 50: 1137–1144, 1977
5. Brecher G, Schneiderman M, Cronkite EP: The reproducibility and constancy of the platelet count. Am J Clin Pathol 23:15–26, 1953
6. Harker LA, Finch CA: Thrombokinetics in man. J Clin Invest 48:963–974, 1969
7. McDonald TP, Clift R, Lange RD, et al: Thrombopoietin production by human embryonic kidney culture. J Lab Clin Med 85:59–66, 1975
8. Sawyer PN, Stanczewski B, Garcia L, et al: Experimental and clinical evaluation of a new catheter material. Trans Am Soc Artif Intern Organs 22:527–537, 1976
9. Wilner GD, Casarella WJ, Baier R, et al: Thrombogenicity of angiographic catheters. Circ Res 43:424–428, 1978
10. Buruille YA, Leonard EF, Litwak RS: Platelet–platelet interactions and non-adhesive encounters on biomaterials. Trans Am Soc Artif Intern Organs 21:609–616, 1975
11. Addonizio VP, Edmunds LH, Colman RW: The function of monkey (*M. mulatta*) platelets compared to platelets of pig, sheep and man. J Lab Clin Med 91:989–997, 1978
12. Grabowski EF, Didisheim P, Lewis JC, et al: Platelet adhesion to foreign surfaces under controlled conditions of whole blood flow: Human *vs.* rabbit, dog, calf, sheep, pig, macaque, and baboon. Trans Am Soc Artif Intern Organs 23:241, 1977
13. Dodds WJ: Platelet function in animals: Species specificities. Edited by G deGaetano, S Garattini. New York, Raven Press, 1978, pp 45–59
14. Calkins J, Lane KP, LoSasso B, et al: Comparative study of platelet aggregation in various species. J Med 5:292–296, 1974
15. Sinakos Z, Caen JP: Platelet aggregation in mammalians (human, rat, rabbit, guinea-pig, horse, dog). A comparative study. Thromb Diath Haemorrh 17:99–111, 1967