Radioiodinated Fibrinogen for Clot Detection in a Canine Model of Cervical Carotid Thrombosis

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Thrombus radiolabeling was studied in a canine model of cervical carotid thrombosis using I-131 fibrinogen prepared from autologous blood. The vector was iodinated by either the Hunter-Greenwood procedure using a chloramine-T/fibrinogen ratio of 12/1, or the iodine monochloride method. Both methods rapidly produced products that labeled the thrombi and permitted their detection by either probe counting or scanning. Post mortem specimens revealed a high ratio of uptake of radioactivity in the thrombi and arterial walls when compared with the blood or the opposite carotid. Of note was that the degree of uptake was independent of the amount of tracer injected or the method of radiolabeling. Also, the daily pattern of changes of counts and the proportion of uptake in the wall of the vessel, compared with the clot itself, were somewhat variable. This could have been due to technical factors, as well as to differences in the clotting and lytic systems of the different animals. An alternate Hunter-Greenwood procedure using a chloramine-T/fibrinogen ratio of 100/1 yielded a product that had twice the specific activity and similar clottability, and that maintained an adequate thrombus/blood ratio, although it was cleared much faster.

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A variety of radiolabeled compounds have been studied to develop noninvasive methods of diagnosing venous and arterial thrombi (*1–3*). Radioiodinated fibrinogen has been extensively evaluated in the detection of thrombophlebitis (*3,4*). It has also been possible to locate arterial lesions with radiolabeled fibrinogen both in vivo and in vitro (*5–8*). Ulcers at the carotid bifurcation in humans have been marked with In-113m fibrinogen (*9–11*). It has been shown that scanning can be used for detection when emitters of appropriate energy, such as I-131, are used (*1,12*).

The purpose of this project has been to evaluate the characteristics of autologous I-131 fibrinogen prepared by the chloramine-T (ChlT) procedure using chloramine-T/fibrinogen ratios (CT/F) of either 12/1 or 100/1, or by the iodine monochloride method (ICl). We then explored its ability to locate cervical carotid-artery thrombi by counting and scanning in a canine model developed for this purpose.

MATERIALS AND METHODS

Canine model of cervical carotid thrombosis. The intima was traumatized by a coaxial catheter technique (*13*). The outer catheter was made from 7.1 Fr radiopaque Teflon tubing and had a slight curve at its distal tip. The inner catheter was made from 4.1 Fr radiopaque polyethylene tubing. Within this was placed a 0.02-in. stainless steel wire stylet with a curved tip. The proximal portion of the Teflon catheter was fitted with a modified three-way stopcock adapter that allowed simultaneous irrigation and positioning of the polyethylene tubing and wire stylet.

With the dog anesthetized, the coaxial catheter was passed under fluoroscopic guidance from the femoral

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artery to a common carotid artery. The inner catheter was then passed 1 cm beyond the tip of the outer catheter and the wire stylet was advanced a few millimeters to press its stiff curved tip against the intima. The entire apparatus was then turned clockwise while it was slowly withdrawn several centimeters down the artery. The wire stylet was then pulled back into the polyethylene tubing, which was retracted into the Teflon catheter to prevent perforation of the vessel as the catheter was advanced to its original position. The vessel was traumatized this way ten times. The coaxial catheter was replaced with a 7 Fr polyethylene double-lumen balloon catheter whose tip was positioned in the artery just proximal to the traumatized segment. The balloon was inflated to occlude the vessel, and 2 ml thrombin solution (400 U) were injected distally. After 3–4 hr, the balloon was deflated and removed.

Preparation of radioiodinated fibrinogen. Blood was drawn from each experimental animal in EDTA vacutainers and fibrinogen was extracted and purified following the procedure of Frisbie et al. (*14*). The concentration of fibrinogen in the purified preparation was determined from its thrombin clotting time using a fibrometer. The purity of the preparation was assessed by electrophoresis on cellulose acetate at pH 8.6, using a barbital buffer of ionic strength 0.075 *M* (*15*). The purified product was radioiodinated as follows.

Chloramine-T procedure (16) with CT/F molar ratios of 12/1 and 100/1. The purified fibrinogen was iodinated according to the method of Frisbie et al. (*14*). The concentrations of chloramine-T used were 3 mg/ml and 25 mg/ml, respectively, such that 10 μl of the chloramine-T solutions used in conjunction with 1 mg of fibrinogen would give molar ratios of 12/1 and 100/1. After discarding the supernatant, the precipitate was dissolved in 0.8 ml of phosphate buffer.

Iodine monochloride (ICl) procedure. Fibrinogen was iodinated according to the method of McFarlane (*17*) with the following modifications. Five hundred μCi of carrier-free $Na^{131}I$ or $Na^{125}I$ in 10 μl of 0.1 *M* NaOH were mixed with 15 μl of a freshly prepared solution of iodine monochloride (iodine concentration = 400 μg/ml). Two hundred μl of an alkaline glycine buffer, and 1 mg of purified fibrinogen in 400 μl of 0.1 *M* phosphate buffer, pH 7.4, were added. Contents were gently mixed and allowed to react for 5 min. The iodinated fibrinogen was precipitated with 5 ml of a 30% (w/v) solution of ammonium sulfate. After centrifugation and removal of the supernatant, the precipitate was dissolved in 0.8 ml of phosphate buffer.

In vitro determinations. Isotopic clottability of the iodinated fibrinogen was measured according to a procedure modified from Regoeczi (*18*), and the specific activity was determined subsequently as μCi/mg clottable fibrinogen.

In vivo survival studies. The thyroid glands of the experimental animals were blocked with 500 mg sodium iodide given intravenously just as the lesion was made, and 200 mg potassium perchlorate administered by mouth for the next 2 days. Iodinated fibrinogen, 200–600 μCi, was injected into the anesthetized dogs 3–4 hr after vessel injury, at the time the balloon catheter was removed. Blood was drawn 15 min after the tracer infusion, and sampling was repeated at 1- to 10-hr intervals for up to 72 hr. Each time at least 1 ml of blood was collected in a 12- × 75-mm glass tube, and the radioactivity was determined by counting the blood sample in a gamma scintillation counter. The weights of these samples were subsequently obtained, and the counting ratio was adjusted to counts per minute per gram (cpm/g) of blood. The activity remaining in a blood sample at any given time was expressed as the percentage of radioactivity of the first blood sample drawn after tracer infusion. This was plotted as a function of time on a semilogarithmic graph.

Probe counting and scanning. Nine dogs were studied. Iodine-131 fibrinogen labeled by the ChlT method was used in five, and by ICl labeling in four. Counting and scanning studies began the day after the thrombosis was initiated and were continued daily, usually for two more days. All counts and scans were obtained with the animals anesthetized.

Counting was accomplished with a portable counting probe, using a 1-in. × 1-in. thallium-activated NaI crystal and a 1-in. lead collimator with a ¼-in. aperture. The window sill and top were at 339 and 389 keV. Two-minute counts were obtained bilaterally 1-in. below the thyroid cartilage, the level of the intimal injury. The raw counting data were normalized to the number of counts per μCi injected. The difference in normalized integrated counts for the thrombosed compared with the nonthrombosed side was analyzed for statistical significance.

A rectilinear scanner was employed for scanning. An easily discernible focal area of increased uptake was considered a positive scan.

Postmortem examination. The animals were usually killed 72 hr after injection of the radioiodinated fibrinogen. Both carotid arteries were removed after ligation at both ends to contain the enclosed blood or clot. The traumatized carotid was opened, and the clot and vessel wall were examined separately. The radioactivity of each specimen was measured in a gamma scintillation counter. The fibrinogen incorporation in the thrombus (cpm/g) was compared

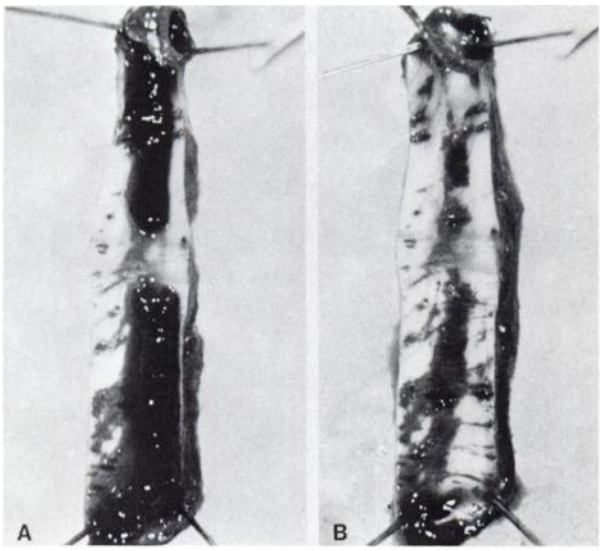

FIG. 1. (A) Carotid artery with thrombus in place. (B) Artery wall showing intimal trauma produced by catheter technique.

with the activity of the blood sample (cpm/g) collected from the animal just before it was killed. The thyroid glands were removed and counted to determine the amount of uptake of radioactive iodine.

RESULTS

Model. The dogs tolerated the in vivo procedure well, and no neurologic deficits were noted. The technique invariably produced a thrombus that persisted at least 3 days as verified by angiography. At autopsy the thrombi (Fig. 1A) and intimal injury (Fig. 1B) were obvious.

In vitro characteristics of labeled compounds. *Purity of fibrinogen preparation.* The protein electrophoretic pattern of the purified fibrinogen preparation, when compared with that of the original EDTA plasma, showed that fibrinogen was reasonably well separated from all other protein species except for a small residue of albumin. The purity of fibrinogen in 48 preparations ranged from 85 to 90%.

Clottability and specific activity. The clottable radioactivities of 48 iodinationed products produced by the three different procedures, and their respective specific activities, are summarized as follows.

Method of iodination	n	Clottability (%)	Specific activity (μCi/mg)
Chloramine-T (CT/F:12/1)	18	90 ± 5	221 ± 84
Chloramine-T (CT/F:100/1)	15	89 ± 6	544 ± 160
Iodine monochloride	15	91 ± 6	155 ± 62

In the two preparations using chloramine-T technique, an increase of chloramine-T concentration from 3 mg/ml (CT/F:12/1) to 25 mg/ml (CT/F: 100/1) appeared to have little effect on the isotopic clottability, which remained around 90%. On the other hand, an approximately twofold increase in specific activity was observed in the CT/F of the 100/1 preparation when compared with the CT/F of the 12/1 preparation. Iodination by the ICl technique yielded products with specific activity comparable with the chloramine-T method, where CT/F = 12.

In vivo survival studies. Figures 2 and 3 show the blood-clearance curves for fibrinogen iodinated by different techniques in normal dogs and in dogs with induced thrombi. These are biphasic curves, indicating a rapid initial redistribution and equilibration of fibrinogen followed by a slower decline associated with catabolic clearance. The half-life of each iodinated fibrinogen was determined by extrapolation of the second part of the curve to "zero" time and calculating the slope and intercept by regression analysis.

Method of iodination	Half-life (hr) Control dog Mean (range) (n)	Thrombosed dog Mean (range) (n)
Chloramine-T (CT/F:12/1)	48 (45.0–62.5) (4)	37 (30.3–52.0) (6)
Chloramine-T (CT/F:100/1)	27 (1)	25 (23.0–27.0) (3)
Iodine monochloride	47 (45.0–57.5) (6)	40 (34.0–48.5) (6)

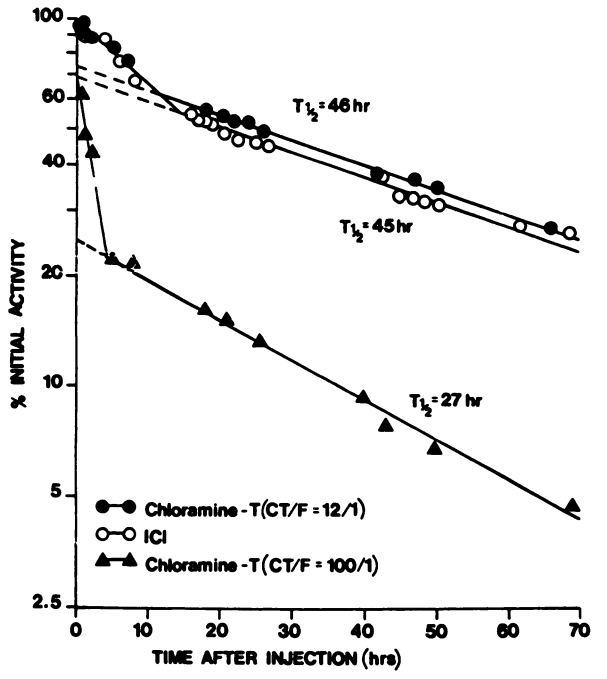

FIG. 2. Blood-clearance curves for iodinated fibrinogen in normal dogs. Iodinations were performed by chloramine-T procedure using CT/F ratio fo 12/1, ●—●; the same at 100/1, △—△; and ICl procedure, ○—○.

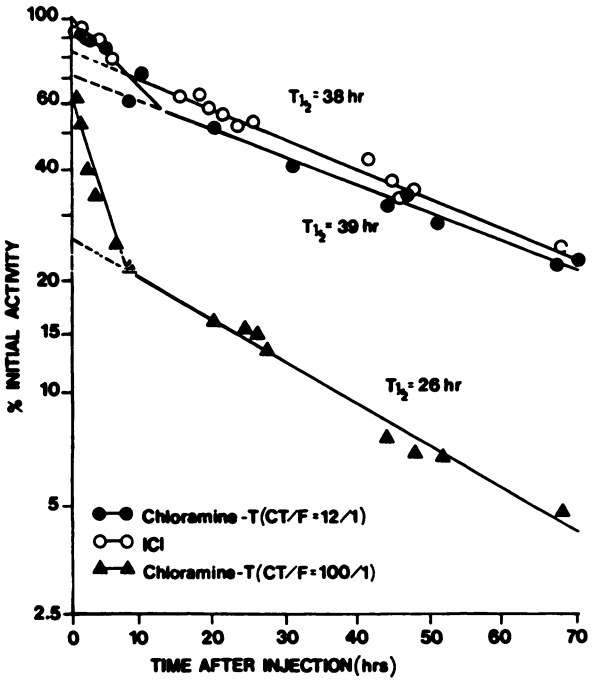

FIG. 3. Blood-clearance curves of iodinated fibrinogen in dogs with induced cervical carotid thrombi. Iodinations were performed by chloramine-T procedure using CT/F ratio of 12/1, ●—●; the same at 100/1, △—△; and ICI procedure, ○—○. Half-lives of the 12/1 and ICI preparations were significantly shorter than in normal dogs.

The half-lives of iodinated fibrinogen prepared by the chloramine-T procedure using a CT/F ratio of 12/1, and by the ICl method, are essentially identical when the two methods are compared in the normal control or in dogs with induced cervical carotid thrombi. With both preparations, however, the intravascular half-lives in normal dogs are significantly longer than in the dogs with thromboses. On the other hand, the preparation using chloramine-T CT/F ratio of 100/1 cleared from the circulation almost twice as quickly in control dogs as in dogs with thromboses.

Probe counting and scanning. The difference between the counts on the side with the lesion and the normal side was statistically significant at the 0.05 level in all nine dogs on all days studied, except for Dog 1 on Day 3 (Tables 1 and 2). Two interesting facts were observed. First, there was no consistent pattern of increase or decrease of counts on a day-to-day basis. Second, absolute counts did not correspond with the amount of tracer injected or the labeling method. In some cases, there was extravasation of blood into the carotid sheath at the time the intima was injured, but this did not seem to influence absolute counts or patterns of uptake.

Positive scans were observed in all nine dogs on all days studied, except perhaps for Dog 5 on Day 1 (Table 1). The extent of the clot was readily seen, usually most clearly on the second and third days (Fig 4). The thyroid glands were not seen.

Postmortem findings (Table 3). The thrombus/blood ratios, representing comparisons between the radioactivity (cpm/g) of the thrombi and the blood specimens drawn at 72 hr, were:

Method of iodination	n	Thrombus/blood ratio (range)
Chloramine-T (CT/F:12/1)	6	13.9 (9.5–20.0)
Chloramine-T (CT/F:100/1)	3	11.6 (6.5–17.4)
Iodine monochloride	6	11.2 (8.2–18.0)

The counts from the contralateral carotid with its contained blood were also measured. There was always much greater activity in the thrombosed specimens than in the nonthrombosed side or in the blood. The chloramine-T (100/1) method gave similar ratios. Of note, either the wall or the clot could show greater activity. As in probe counting, however, there was no consistent correlation between the labeling method, or the amount of tracer injected, and the absolute counts. The thyroid glands showed no appreciable accumulation of radioactivity.

DISCUSSION

Model. The model was designed for the purpose of studying the fibrinogen uptake test. Current models of stroke and cerebral ischemia (*19–21*) involve operative manipulation over the vessel and/or injection of foreign materials. These were not considered suitable for the purposes of this study because

TABLE 1. RESULTS OF SCANNING AND COUNTING

Dog No.	Method of labeling	Scanning day 1	Scanning day 2	Scanning day 3	Counting day 1	Counting day 2	Counting day 3
1	Chl T	+	+	+	+	+	−
2	Chl T	+	+	+	+	+	+
3	Chl T	0*	+	+	0	+	+
4	Chl T	+	+	+	+	+	+
9	Chl T	+	+	+	+	+	+
5	ICI	±	+	0†	+	+	0
6	ICI	+	+	0‡	+	+	0
7	ICI	+	+	+	+	+	+
8	ICI	+	+	+	+	+	+

+ = Positive.
± = Questionable.
0 = Not done.
* Flood.
† Problem with radio labeling, killed at 48 hr.
‡ Killed at 48 hr.

Results of scanning and counting radiolabeled thrombi using iodinated fibrinogen prepared by two different methods, both of which were shown to produce a satisfactory product.

TABLE 2. ANALYSIS OF COUNTING DATA

Dog No.	Side of lesion	Method of labeling	μCi	Day No.	Counts Right	Counts Left	Statistical significance
1	Right	Chl T	244	1	366	287	+
				2	2,822	2,446	+
				3	3,024	2,896	—
2	Right	Chl T	400	1	6,307	5,516	+
				2	7,126	4,914	+
				3	5,167	2,820	+
3	Left	Chl T	313	1	—	—	
				2	2,838	3,800	+
				3	2,032	2,647	+
4	Right	Chl T	540	1	11,189	5,326	+
				2	11,851	4,343	+
				3	8,829	3,819	+
9	Left	Chl T	500	1	3,671	5,483	+
				2	2,666	5,596	+
				3	2,323	7,293	+
5	Left	ICI	200	1	1,842	2,364	+
				2	1,164	1,416	+
				3	—	—	
6	Right	ICI	250	1	7,549	5,994	+
				2	9,894	4,374	+
				3	—	—	
7	Left	ICI	400	1	4,800	6,018	+
				2	4,333	4,540	+
				3	2,767	4,261	+
8	Left	ICI	430	1	5,588	6,401	+
				2	3,360	5,496	+
				3	3,092	7,242	+

Detailed analysis of counting data. Absolute counts did not correspond with method of labeling or amount of tracer injected. Also, day-to-day trends were variable with respect to highest counts.

the overlying traumatized tissues might take up radioiodinated fibrinogen (*22,23*) and because any foreign material might alter the nature of the clot. Our model was based on the so-called "Virchow's triad," which is that clotting within a vessel is promoted by injury to the vessel wall, stasis, and alteration of the nature of the blood (*19,24*). It meets the criteria of the characteristics of the ideal model of intravascular thrombosis, which is that it avoids extravascular trauma and is stable and reproducible (*25*).

Fibrinogen uptake test—Background. The use of I-125 fibrinogen to label intravascular thrombi for counting has been evaluated extensively in thrombophlebitis (*3,4*). By employing higher-energy emitters, images of such thrombi can also be produced (*1,12,26,27*). Using radiolabeled fibrinogen, thrombi have been detected in the hearts of humans (*8*), in coronary arteries in dogs (*7*), and on ulcers in atheromatous carotid plaques in humans (*9–11*). Important factors relating to detection include time after clotting begins, size and activity of the throm-

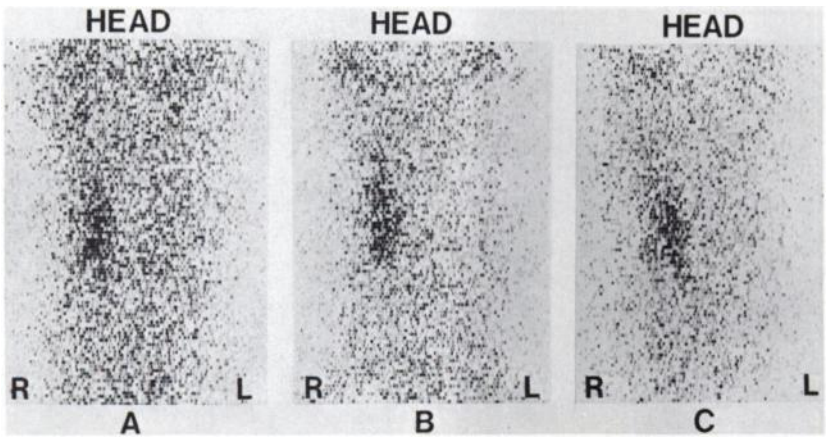

FIG. 4. Serial scans of a radiolabeled thrombus at 24 hr (A), 48 hr (B) and 72 hr (C). Thrombus becomes more easily detected on later scans as blood background fades.

TABLE 3. POSTMORTEM DATA

Dog No.	Method of labeling	μCi injected	CPM/g Clot	CPM/g Wall	Lesion side/ nonlesion side	Lesion side/ blood
1	Chl T	244	462,754	229,338	8.2	9.3
2	Chl T	400	1,221,374	582,072	9.7	11.0
3	Chl T	313	217,109	988,117	17.6	19.9
4	Chl T	340	1,544,999	349,114	7.0	20.1
9	Chl T	500	89,357	128,768	6.8	4.1
5*	ICl	200	151,476	153,243	16.5	9.6
6*	ICl	250	1,062,021	915,332	7.8	8.2
7	ICl	400	458,612	366,982	28.8	11.6
8	ICl	430	330,698	549,149	13.8	9.0

* Killed at 48 hr.

Data show that both methods of radiolabeling produced satisfactory products. Trends of uptake were variable, as were proportion of uptake in thrombi and in vessel walls. Thrombi were much "hotter" than blood.

bus, and the blood-to-thrombus ratio of the tracer (*2,28*). Uptake of label may occur throughout the thrombus (*6,23*) or especially at its distal end (*5*). A variety of explanations for patterns of uptake have been presented. Uptake will decrease as the thrombus disappears (*29–31*), unless significant inflammatory changes lead to persistent fibrin binding (*32*).

Fibrinogen uptake test—Current study. The simple extraction procedure of Frisbie et al. (*14*), combined with either the chloramine-T procedure using a CT/F ratio of 12/1 or the ICl method as outlined here, have made it possible to produce a fibrinogen preparation suitable for thrombus radiolabeling within 2 hr of blood sampling from the experimental animal.

Our data indicated that of the iodination techniques used in this study, the preparation using a chloramine-T to fibrinogen molar ratio of 100/1 appeared to yield the highest specific activity. Its in vivo half-life, however, was only about half those of the other two preparations, both in normal control dogs and in dogs with induced thrombi. The 100/1 preparation did exhibit an adequate thrombus/blood ratio with a background activity 1/10 that of the others'. Fibrinogen iodinated by the procedure may afford earlier and clearer clot detection if imaging is carried out with a scintillation camera. The almost identical clottabilities that we obtained for the preparations using CT/F ratios of 100/1 and 12/1 did not correlate with the differences noted in their half-lives, which emphasizes that in vitro clottability studies are not a sensitive index of the in vivo characteristics of radioiodinated fibrinogen (*33*).

Radioiodinated fibrinogen prepared by either a CT/F molar ratio of 12/1 or by the ICl method behaved similarly in vivo in control dogs and in dogs with thrombi. The shorter intravascular half-life exhibited by both preparations in the traumatized dogs may be attributed to a greater rate of fibrin formation at the injured sites than in normal states. In all animals infused with either the 12/1 or the ICl prepared fibrinogen, at least 60% of the initial activity remained in circulation when the fibrinogen catabolic clearance curves were extrapolated to zero time. This is at variance with the findings of Metzger et al. (*34*) who reported that less than 5% of the initial activity of the chloramine-T preparation, and greater than 50% of the initial activity of the ICl preparation, remained in the circulation to account for the catabolism of fibrinogen. A smaller chloramine-T/fibrinogen ratio used in our iodination may be one factor responsible for this difference.

One of the most interesting and unique aspects of the study has involved the findings from serial counting and postmortem examinations. Of note were the unpredictability of absolute tracer uptake, day-by-day trends in changes in count, and rates of uptake in the clot compared with those of the injured vessel wall. There may be a number of explanations for these findings. The probe counter was directed at the most active part of the clot as judged by a ratemeter and a howler. It may have been that the most active point was not always counted. Also, the speed of clotting and the speed of thrombolysis may have been variable from dog to dog, both because of local factors and because of individual differences in the dogs' clotting and lytic systems. These factors were not studied in this pilot project. The proportion of uptake between the wall and the clot was variable and may have been related to the degree of trauma, which could not be precisely controlled and was difficult to estimate. The high ratio of ipsilateral to contralateral vessel uptake was not reflected in probe counting, probably because of the vascularity of the tissue acting as the background and because of attentuation of counts by interposed tissues.

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