FIBRINOGEN UPTAKE BY THROMBI:
EFFECT OF THROMBUS AGE

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The uptake of radiolabeled fibrinogen in canine thrombi was determined at varying times after thrombus induction by electric current. The greatest thrombus/blood ratio was achieved when fibrinogen was administered 4 hr after thrombus induction but definite thrombus fibrinogen uptake was still observed when the tracer was administered up to 72 hr after thrombus induction. There was continued fibrinogen accumulation despite a decrease in weight of older thrombi suggesting that net thrombus propagation is not necessary for labeled fibrinogen uptake. Our results suggest that the fibrinogen uptake test may be useful for the diagnosis of deep vein thrombosis for several days after the onset of thrombosis.

The fibrinogen uptake test is an accurate means of detecting newly forming thrombi in the deep veins of the lower extremities (*1*) and has been used to define the incidence of venous thrombosis in various high-risk patient groups (*1–3*). The value of this test in instances of established thrombosis is somewhat more controversial and has been evaluated clinically with differing results (*1,4*). Further, based on the assumption that fibrinogen would not accumulate in preformed thrombi, the uptake of radiolabeled fibrinogen has been used to estimate the age of coronary artery thrombi in patients dying from acute myocardial infarction (*5*).

In the present study, we have employed an animal model of deep vein thrombosis to evaluate the relationship of thrombus age and labeled fibrinogen uptake.

METHODS

Twenty-four mongrel dogs weighing 15–25 kg were used in the studies. Deep vein thrombosis was induced by alteration of the intima with an electric current (*6–8*). This method is superior to those which require surgical manipulation of the vein since external radioactive monitoring over the site of thrombosis may be performed subsequently without interference from the deposition of fibrin in the healing surgical wound. With the dog under intravenous sodium pentobarbital anesthesia (30 mg/kg), an external jugular vein was exposed and incised. A vinyl catheter, containing a stainless steel wire that also served as an electrode, was introduced into the jugular vein and passed under fluoroscopic control into a femoral vein. Only the tip of the guide wire was exposed; the remainder was enclosed in the catheter. The wire was connected to the anode of a variable power supply and the cathode was connected through an ammeter to a skin clamp on the leg bearing the catheter. A direct current (5 mA, 2 volts) was applied for 1 hr. The guide wire and catheter were then withdrawn, the jugular vein was ligated, and the incision closed. Two dogs served as controls; the catheter was inserted and left in place for 1 hr but no current was applied.

Canine fibrinogen was labeled with either ^{125}I or ^{131}I by the iodine monochloride (*9,10*) or enzymatic method (*10,11*). The preparations contained an average of 0.5 atoms per molecule of fibrinogen and had an isotopic clottability, determined by the method of Regoeczi (*12*), ranging from 75 to 80%. Gel chromatography using a Sepharose-4B column has shown that fibrinogen iodinated by either method chromatographs as a single peak very similar to that of authentic unlabeled fibrinogen (*11,13*). A typical chromatogram is shown in Fig. 1. Labeled fibrinogen (100 μCi; 200–600 μg) was injected intravenously either during thrombus induction or 2, 4, 8, 24, 72, or 96 hr after anode withdrawal.

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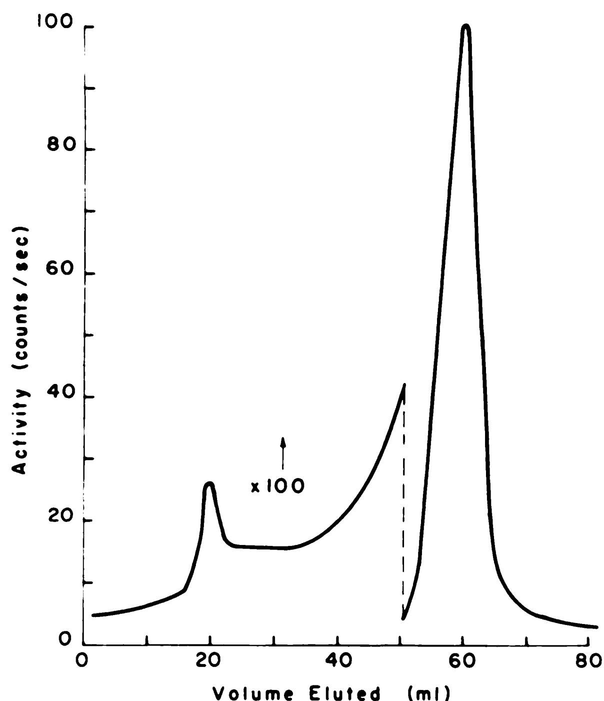

FIG. 1. Sepharose-4B gel chromatography of fibrinogen labeled by enzymatic method. Greater than 99.5% of activity is eluted with same retention time as authentic fibrinogen with small amount being eluted earlier and no evidence of free iodide.

External scintillation counting was performed on a limited number of animals using a 3-cm NaI(Tl) crystal detector with a 4.4-cm straight-bore lead collimator. Counts were obtained over both femoral regions and the precordium at 1, 2, 3, and 24 hr after fibrinogen injection. At least 2,000 total counts were accumulated at each site. The fibrinogen uptake in the experimental limb was calculated as follows:

$$\frac{\text{(Net cpm experimental limb)} - \text{(net cpm control limb)}}{\text{(Net cpm precordium)}} \times 100$$

The animal was reanesthetized 24 hr after labeled fibrinogen injection. A venogram was performed in the experimental limb with 10 ml of meglumine diatrizoate to demonstrate the size and location of the thrombus. The femoral vein was then exposed and the thrombosed vein identified, ligated, and resected. The venous segment was then opened and the thrombus was washed with 0.9% saline solution. A blood sample was obtained at the time of thrombus removal. The blood and thrombus were weighed and counted in a NaI(Tl) crystal well scintillation counter. The thrombus/blood ratio was calculated as cpm/gm thrombus ÷ cpm/gm blood.

RESULTS

Thrombi readily detectable by venography were induced in all dogs when the current was applied. Most of the thrombi (16/22) were partially occlusive; the remainder totally occluded the vein. The extent of thrombotic occlusion was not related to thrombus age. All the thrombi adhered to the vein wall. The relationship of thrombus weight to thrombus age is shown in Table 1. Thrombi removed 96–120 hr after induction were significantly smaller than those removed between 24 and 32 hr. In the two control dogs there were no detectable thrombi by venography or surgical exploration.

Fibrinogen uptake into the experimental thrombi was noted in all groups (Table 2). The greatest accumulation occurred when the labeled fibrinogen was injected 4 hr after thrombus induction. Thereafter, the thrombus/blood ratio declined but even at 96 hr, two of three thrombi had greater activity per unit weight than blood.

External monitoring was performed in four dogs. Fibrinogen was injected either during thrombus induction (two dogs), 4 hr later (one dog), or 8 hr later (one dog). The fibrinogen uptake percentage determined by external monitoring rose progressively with time after fibrinogen injection and at 24 hr ranged from 30 to 35%. The thrombus/blood ratios in all four of these dogs exceeded 5.3.

TABLE 1. THROMBUS WEIGHTS

Thrombus age (hr)	n	Weight (mg) Range	Mean	s.d.
24–32	13	16–678	219	172
48	3	34–236	149	104
96	3	86–114	100	14*
120	3	64–96	76	17*

* Significantly different compared with 24–32 hr group at $p < 0.05$.

TABLE 2. THROMBUS ACCUMULATION OF LABELED FIBRINOGEN

Time of fibrinogen injection after thrombus induction (hr)	Thrombus age (hr) at time of removal	Thrombus/blood ratio† n	Mean	Range
0*	24	3	7.1	5.3 – 8.0
2	26	3	9.4	8.4 –10.4
4	28	4	23.0	20.0– 26.0
8	32	3	7.2	5.7 – 8.2
24	48	3	4.6	4.3 – 5.0
72	96	3	4.0	2.1 – 6.2
96	120	3	2.3	0.62– 4.4

* Labeled fibrinogen injected during thrombus induction.
† Results expressed as cpm/gm thrombus ÷ cpm/gm blood.

DISCUSSION

Our results show that the greatest thrombus accumulation of radioiodinated fibrinogen occurs when fibrinogen is injected 4 hr after thrombus induction. Some accumulation of activity is seen through 96 hr but is more variable at later times. There have been few experimental investigations of the duration of fibrinogen uptake in evolving thrombi. By externally monitoring the uptake of ^{125}I-labeled human fibrinogen in induced canine venous thrombi, Martin, et al (*14*) demonstrated fibrinogen incorporation into thrombi up to 24 hr after their formation. Hackel, et al (*15*) found no thrombus accumulation of labeled fibrinogen injected 10–14 days after experimental induction.

The growth of experimental thrombi has been investigated by several workers. Zweifler (*16*) noted that thrombi removed immediately after induction by electric current were more fragile and smaller than those removed at 4 days. He did not study thrombus growth during the intervening time period. Bradham (*17*) noted that thrombi in dogs were slightly smaller at 6 hr than at 24 hr after induction. He found no further increase in thrombus size after 24 hr and at 10 days the thrombi had decreased in size. Our results are similar. Of thrombi removed 24–120 hr after thrombus induction, those removed at 32 hr were the largest and thereafter thrombus weight decreased.

We have previously observed thrombus/blood ratios of approximately 8 when fibrinogen labeled by the iodine monochloride method was injected just prior to thrombus induction (*10*). This result is similar to that obtained in the current study when fibrinogen was injected during thrombus induction. Since labeled fibrinogen is initially rapidly cleared from the circulation due to its equilibration in the extravascular space (*18*), the greatest thrombus uptake would be anticipated if the labeled fibrinogen were injected when the thrombus was most actively propagating. Under the conditions of these experiments, less thrombus fibrinogen accumulation would occur if thrombus propagation were less active during the 24-hr interval between tracer injection and thrombus removal. Our study suggests that fibrinogen uptake does continue for at least as long as 96 hr after thrombosis. This delayed fibrinogen uptake most likely reflects continued fibrin deposition in the thrombi. However, the observation that older thrombi are smaller suggests that active fibrinolysis also occurred during the period of observation.

External monitoring was performed in only four dogs but in each case the thrombus was easily detected. On the basis of these results and those from a previous study (*10*), we would expect that a thrombus/blood ratio of at least 3 is necessary for the thrombus to be detectable by external monitoring. Therefore, a positive fibrinogen uptake test could be expected if the fibrinogen were injected up to 72 hr after the onset of thrombosis. Some thrombi might also be detectable 96 hr after thrombosis. The detectability would also depend on the size of the thrombus and the radioactivity in the surrounding tissues.

Our results also suggest that radioiodinated fibrinogen should be used cautiously as a method of determining thrombus age. Erhardt, et al (*5*) found activity in the coronary artery thrombi of seven patients who died of myocardial infarction and had received labeled fibrinogen. They suggested that the thrombosis might be a secondary event in myocardial infarction since when the thrombi were serially sectioned, activity was distributed throughout the thrombus in each of six patients who had received fibrinogen within 15 hr of the onset of symptoms. In the remaining patient, fibrinogen was injected 47 hr after his symptoms began and only the ends of the thrombus showed fibrinogen accumulation. Our results, demonstrating that accumulation of labeled fibrinogen can continue for at least 96 hr after thrombosis, suggest that their conclusions may be invalid. The uptake they demonstrated may reflect continued deposition of labeled fibrinogen within a primary coronary arterial thrombus.

Whether our results are applicable to human subjects with deep vein thrombosis is uncertain. However, the experimental thrombi produced by electric current are histologically similar to human thrombi and may involve similar pathogenetic mechanisms (*8,19,20*). Furthermore, thrombi produced by electric current resemble human deep vein thrombi in their natural history (*21*). There is also clinical evidence to suggest that fibrinogen uptake will occur in established deep vein thrombi (*1*) and thus, extension of our findings to the clinical situation seems justified.

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REFERENCES

1. KAKKAR V: The diagnosis of deep vein thrombosis using the ^{125}I-fibrinogen test. *Arch Surg* 104: 152–159, 1972

2. WARLOW C, OGSTON D, DOUGLAS AS: Venous thrombosis following strokes. *Lancet* 1: 1305–1306, 1972

3. KAKKAR VV, HOWE CT, FLANC C, et al: Natural history of postoperative deep vein thrombosis. *Lancet* 2: 230–232, 1969

4. MAVOR GE, MAHAFFY RG, WALKER MG, et al: Pe-

ripheral venous scanning with ¹²⁵I-tagged fibrinogen. *Lancet* 1: 661–663, 1972

5. ERHARDT LR, LUNDMAN T, MELLSTADT H: Incorporation of ¹²⁵I-labeled fibrinogen into coronary arterial thrombi in acute myocardial infarction in man. *Lancet* 1: 387–390, 1973

6. LUTZ BR, FULTON GP, AKERS RP: White thromboembolism in man in the hamster cheek pouch after trauma, infection and neoplasia. *Circulation* 3: 339–343, 1951

7. SAWYER PN, PATE JW: Bio-electric phenomena as an etiologic factor in intravascular thrombosis. *Am J Physiol* 175: 103–107, 1953

8. DIDISHEIM P: Animal models useful in the study of thrombosis and antithrombotic agents. In *Progress in Hemostasis and Thrombosis*, Spaet TH, ed, New York, Grune & Stratton, 1972, pp 165–197

9. MCFARLANE AS: Labeling of plasma proteins with radioactive iodine. *Biochem J* 62: 135–143, 1956

10. COLEMAN RE, KROHN KA, METZGER JM, et al: An in vivo evaluation of *I-fibrinogen labeled by four different methods. *J Lab Clin Med* 83: 977–982, 1974

11. KROHN KA, WELCH MJ: Studies of radioiodinated fibrinogen. II. Lactoperoxidase iodination of fibrinogen and model compounds. *Int J Appl Radiat Isotop* 25: 315–323, 1974

12. REGOECZI E: Iodine-labeled fibrinogen: a review. *Br J Haematol* 20: 649–663, 1971

13. KROHN K, SHERMAN L, WELCH M: Studies of radioiodinated fibrinogen. I. Physicochemical properties of the ICl, chloramine-T, and electrolytic reaction products. *Biochim Biophys Acta* 285: 404–413, 1972

14. MARTIN DL, BUCKNER SA, SAMANI P: Localization of blood clot of varying ages by ¹²⁵I-labeled human fibrinogen (¹²⁵I-F). *Clin Res* 21: 832, 1973

15. HACKEL DB, ESTES EH, WALSTON A, et al: Some problems concerning coronary artery occlusion and acute myocardial infarction. *Circulation* Suppl IV 39, 40: 31–35, 1969

16. ZWEIFLER AJ: Standardized technique for the study of thrombus growth: Effect of heparin therapy. *J Lab Clin Med* 60: 254–260, 1962

17. BRADHAM RR: Propagation of induced venous thrombi. *Surg Gynecol Obstet* 133: 324–328, 1961

18. METZGER JM, SECKER-WALKER RH, KROHN KA, et al: Unsatisfactory biologic behavior of *I-fibrinogen labeled by the chloramine-T method. *J Lab Clin Med* 82: 267–275, 1973

19. SALAZAR AE: Experimental myocardial infarction: Induction of coronary thrombosis in the intact closed-chest dog. *Circ Res* 9: 1351–1356, 1961

20. STRACHAN CJL, GAFFNEY PJ, SCULLY MF, et al: An experimental model for the study of venous thrombosis in vivo. *Thromb Res* 5: 235–242, 1974

21. PITNEY WR: *Clinical Aspects of Thromboembolism.* Baltimore, Williams & Wilkins, 1972, p 7