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^{125}I Versus ^{75}Se Labeling of Fibrinogen for Measuring Turnover of Canine Fibrinogen[1] (38101)

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The turnover of fibrinogen in normal persons is usually said to be about 2 mg/dl plasma per hour based on disappearance rates of radioiodine-labeled fibrinogen with half-times of 4–5 days (1). Brodsky *et al.* (2) reported comparable disappearance rates when subjects labeled their own fibrinogen endogenously after receiving [^{75}Se]methionine. However, inspection of their data (their Fig. 3) shows that in seven normal subjects the half-time of ^{75}Se-labeled fibrinogen averaged 6.5 days. From this, one can calculate a mean turnover of about 1.1 mg of fibrinogen/dl plasma per hour, or about half that observed when ^{125}I- or ^{131}I-labeled fibrinogen is used.

We have found a similar discrepancy in dogs when ^{125}I-labeled canine fibrinogen was compared with cohort labeling with [^{75}Se]-methionine. This is a report of the discrepancy with a suggested explanation.

Materials and Methods. Labeled tracers. ^{125}I-Labeled fibrinogen was kindly furnished by Dr. J. F. Jeffries (Abbott Laboratories, North Chicago, IL).[2] Its use has been described previously (3). Essentially, a solution of the labeled protein is received frozen in Dry Ice; it is thawed and separated into 0.5-ml samples that are promptly refrozen. Single tubes are thawed for each experiment, and the preparation is centrifuged to free it of small amounts of insoluble material. The doses given to dogs approximated 50 μCi and 3 mg of clottable fibrinogen. Samples of blood were collected twice daily from each dog. Fibrin clots were obtained by addition of 100 U of thrombin to platelet-poor citrated plasma diluted 10-fold with saline. The clots were thoroughly rinsed with saline and blotted before radioisotope assay. The observed radioactivity was plotted semilogarithmically against time.

[^{75}Se]Methionine was purchased from Squibb (Sethotope). Specific activities exceeded 100 mCi/mg. It was diluted with sterile saline to about 100 μCi (1 μg) per dose for standard tests. After intravenous administration, peak labeling of plasmatic fibrinogen occurred well within the first 24 hr, so the semilogarithmic plot was begun 24 hr after injection. Thrombin-induced clots from platelet-poor plasma were assayed for ^{75}Se. Since less than 3% (at 24 hr: mean, 2.79; SD, 0.69) of the plasmatic radioactivity was in the fibrinogen, it was important that the clot be virtually free of radioactive contaminants. We found that, after washing and blotting, the clot from undiluted plasma to which ^{131}I-labeled albumin had been added always contained less than 0.1% of the total plasmatic ^{131}I. After a preliminary 10-fold dilution of plasma, before addition of thrombin, there was even less ^{131}I contamination.

With both the ^{125}I-labeled fibrinogen and ^{75}Se-labeled fibrinogen, half-times and disappearance rates were calculated from lines visually fitted to the data. Multiplying the fractional rate, $0.693/t_{\frac{1}{2}}$, by the concentration of fibrinogen in plasma gives the turnover rate if the intravascular and extravascular pools of

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[2] Fibrinogen was precipitated from dog plasma with 8% ethanol and washed twice with an ethanol–glycine citrate buffer (Blombäck, B., and Blombäck, M., *Arkiv. Kemi* 10, 415 [1956]) Radioiodination was done by stepwise oxidation, with NaOCl of iodide to iodine while the fibrinogen was mixing with the iodide at neutral pH. Unreacted iodine was removed by ion exchange. Labeling was less than 1 atom I per molecule fibrinogen. The labeled fibrinogen was more than 90% clottable.

fibrinogen remain unchanged and if the rates of synthesis and of destruction stay constant during the study (4). Because these studies were on normal dogs, the assumptions seem reasonable. Turnover rates are expressed as mg fibrinogen/dl plasma per hour.

Dogs. Mongrels weighing about 20 kg were used. They had passed a 24-day quarantine period. Plasma fibrinogen was determined daily during each study, and all values in each dog were averaged. ^{125}I-Labeled fibrinogen or [^{75}Se]methionine was injected intravenously early in the morning to permit adequate blood sampling. Results in nine dogs receiving the labeled fibrinogen were compared with those in seven other dogs receiving the labeled methionine. Two dogs were given both preparations concurrently and fibrin clots were assayed for ^{125}I and ^{75}Se by differential counting; one of these dogs was normal and the other was receiving a continuous infusion of a very weak preparation of canine thromboplastic emulsion.

Exchange transfusions. Three pairs of dogs were studied. One of each pair was given a large dose of [^{75}Se]methionine intravenously (360–450 μCi; 2.4–4.2 μg). From 1 to 5 days later, 450 ml of its blood was removed and transfused into an untreated dog in exchange for 450 ml of blood from the untreated dog. Each dog received 2000 U of heparin intravenously just before donating its 450 ml of blood into a Fenwall blood donor bag containing 75 ml of USP formula A ACD solution (0.8% citric acid, 2.2% sodium citrate, and 2.45% dextrose) as anticoagulant. Each dog immediately received the blood from the other dog. No ill effects were observed in any of the three experiments. Disappearance rates of ^{75}Se in plasmatic fibrin were determined in all six dogs.

Results. As previously reported (3), the mean (±SD) half-time of disappearance of ^{125}I-labeled canine fibrinogen in nine normal dogs was 38.5 ± 5.8 hr. Calculated turnover rates averaged 4.79 ± 0.72 mg/dl plasma per hour.

When [^{75}Se]methionine was injected into seven normal dogs, half-time of disappearance averaged 119.4 ± 30.7 hr, yielding a turnover rate of 1.96 ± 0.63 mg/dl plasma per hour, or slightly less than half as much as with the ^{125}I-labeled fibrinogen.

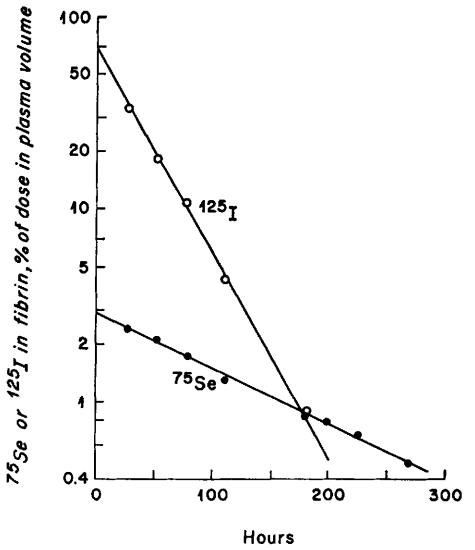

FIG. 1. Disappearance of ^{125}I and ^{75}Se from plasma (measured in fibrin clots) after concurrent intravenous infusion of ^{125}I-labeled fibrinogen and [^{75}Se]-methionine. The turnovers of fibrinogen are 6.34 mg/dl plasma per hour with ^{125}I ($t_{\frac{1}{2}}$ = 28 hr) and 1.72 mg/dl per hour with ^{75}Se ($t_{\frac{1}{2}}$ = 103 hr).

In the two dogs—one normal and one receiving an infusion of weak thromboplastin—given ^{125}I-labeled fibrinogen and [^{75}Se]methionine concurrently, turnover rates were as follows: normal dog (Fig. 1), 6.34 mg/dl per hour with ^{125}I and 1.72 mg/dl per hour with ^{75}Se; infused dog, 6.16 mg/dl per hour with ^{125}I and 4.13 mg/dl per hour with ^{75}Se.

Results in the three pairs of dogs undergoing exchange transfusions are summarized in Table I. Disappearance rates in the dogs receiving the labeled methionine were calculated from samples taken beginning 24 hr after injection of label. In the dogs receiving the radioactive blood transfusions, the ^{75}Se disappeared exponentially from the time of the transfusion. Only slight differences were observed between the paired dogs.

Discussion. When disappearance rates of plasmatic fibrinogen were measured in normal dogs, the rates were regularly faster when ^{125}I-labeled canine fibrinogen was used than when [^{75}Se]methionine was injected and the dogs labeled their own fibrinogen. Apparent fibrinogen turnover rates were slightly more than twice as great with use of the ^{125}I preparation. The same order of difference was observed in

TABLE I. Disappearance Rates of ^{75}Se in Fibrin Clots in Three Pairs of Dogs.[a]

	Pair		
	1	2	3
Dose of [^{75}Se]methionine:			
μCi	360	450	450
μg	4.2	3.3	2.4
Interval between injection of dose and blood donation (hr)	29	75	125
Donor dog			
Plasma fibrinogen (mg/dl)	489	378	357
Disappearance $t_{\frac{1}{2}}$ (hr)	124	113	147
Turnover rate (mg/dl hr)[b]	2.73	2.32	1.68
Recipient dog			
Plasma fibrinogen (mg/dl)	359	235	321
Disappearance $t_{\frac{1}{2}}$ (hr)	85	61	102
Turnover rate (mg/dl/hr)[c]	2.94	2.67	2.12

[a] [^{75}Se]Methionine given to "donor" dog; then exchange transfusion of 450 ml of blood was performed between donor and recipient.

[b] Mean, 2.24 mg/dl/hr.

[c] Mean, 2.58 mg/dl/hr.

two dogs receiving the two labels simultaneously.

The difference could be explained by unphysiologically rapid degradation of the iodinated fibrinogen or by significant reutilization of ^{75}Se continuously entering the fibrinogen pool. Because infusion of blood from dogs that had received the [^{75}Se]methionine into other dogs led to fibrinogen turnover rates differing only slightly between the two groups, reutilization of the labeled methionine is a minor problem in fibrinogen turnover studies. Iodinated fibrinogen preparations may not behave normally *in vivo* (5), even though their clottability may still be normal (6). Therefore, it seems likely that in these studies the ^{75}Se data are the more valid.

Summary. Apparent turnover of fibrinogen in normal mongrel dogs was observed to be consistently greater when ^{125}I-labeled fibrinogen (canine) was used than with the Brodsky technique ([^{75}Se]methionine): 4.79 vs 1.96 mg/dl plasma per hour, respectively. When the labeled amino acid was given intravenously to three dogs and their blood was transfused into three other dogs 1–5 days later, only slight differences in fibrinogen turnover were observed between blood donors and recipients, suggesting negligible reutilization of [^{75}Se]methionine. It would seem that the ^{125}I-labeled fibrinogen was degraded rapidly *in vivo* because of some intrinsic alteration in the labeled material.

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