PHARMACOKINETICS OF RADIOIODINATED STREPTOKINASE

Geoffrey Coates, Sally J. DeNardo, Gerald L. DeNardo, and Frederic A. Troy

University of California School of Medicine, Davis, California

The pharmacokinetics of radioiodinated streptokinase have been investigated in mice and dogs in order to explore further its potential usefulness as a radiopharmaceutical to detect thrombi and neoplasms. The purified streptokinase used in these studies showed no alteration in its physical or enzymatic properties following radioiodination.

In the mouse, radioiodinated streptokinase accumulated rapidly in the liver and at 4 hr, large amounts of free iodine were detected in the plasma. The plasma clearance curve in dogs was biexponential showing that 70% of the protein-bound radioactivity was cleared with a half-life of 15–25 min while the remaining 30% was cleared with a half-life of 60–80 min. Seventy percent of the plasma radioactivity appeared as free iodine after 4 hr. No change in clearance was obtained by preloading animals with unlabeled streptokinase.

Based primarily on the rapid plasma clearance, deiodination, and possible limitations of effectiveness of preloading with unlabeled streptokinase, the results of these studies are in accord with the conclusion that radioiodinated streptokinase may have restricted usefulness as a radiopharmaceutical for detecting thrombi and neoplasms. The full extent of its potential usefulness, however, awaits the provision of further in vivo and in vitro studies directed at testing a covalently modified enzyme that retains streptokinase activity but whose immunologic properties have been altered so that the enzyme is not as rapidly cleared from the plasma.

The use of radioiodinated fibrinogen in the detection of thrombotic disease is well established (*1–3*). The intravenous injection of fibrinogen, however, involves the risk of inducing viral hepatitis in the patient. In order to avoid this risk, radioisotopically labeled streptokinase has been suggested as a possible clot-detecting agent (*4,5*). Streptokinase, an extracellular proteolytic enzyme produced by hemolytic streptococci, activates the fibrinolytic process and is rapidly cleared from the plasma. The risk of viral hepatitis is not associated with this preparation. Preliminary studies by Siegel, et al (*4*) using ^{131}I-streptokinase and Dugan, et al (*5*) using ^{99m}Tc-streptokinase showed that this enzyme could be used in animals to localize preformed thrombi. Fibrinogen has also been found to localize in neoplasms (*6,7*) so that it is possible that labeled streptokinase might be useful for localizing neoplasms. In view of the potential of this enzyme, studies were initiated to explore its pharmacokinetics.

MATERIALS AND METHODS

Preparation of radioiodinated streptokinase. Purified streptokinase (Hoechst Pharmaceutical Co., Lot No. RM 564-3, "Streptase") was labeled with ^{125}I, ^{131}I, or ^{123}I by McFarlane's iodine monochloride procedure (*8*) as follows: to 1.66 mg of streptokinase [100,000 Christensen units (*9*)], dissolved in 0.5 ml of citrate buffer (0.02 *M* citrate, 0.15 *M* saline, pH 6.0), was added 0.5 ml of 0.8 *M* glycine buffer, pH 9.5. This was followed by the rapid addition with stirring of iodination-grade radioiodine and 50 μl (25 μg) of iodine monochloride dissolved in normal hydrochloric acid, pH 1.5. The reaction was terminated after 5 min by the addition of 0.1 ml of 0.1 *N* sodium iodide. All steps were carried out at 25°C. The labeled protein was separated from the free iodine by molecular sieve chromatography on a

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For reprints contact: Gerald L. DeNardo, Div. of Nuclear Medicine, University of California School of Medicine, Davis, Calif. 95616.

2- × 10-cm column of Sephadex G-25 gel eluted with citrate buffer. One-milliliter fractions were collected and radioactivity was determined by scintillation counting.

Determination of the optimum molar ratio of iodine monochloride to streptokinase. The effect on labeling efficiency of different molar ratios of iodine monochloride to streptokinase was determined by varying the amount of iodine monochloride from 2.5 μg to 30 μg while maintaining the amount of streptokinase constant at 1.66 mg (100,000 units).

Purity and molecular weight of unlabeled streptokinase. *Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis.* The purity and SDS-polyacrylamide molecular weight value for unlabeled and ^{131}I-labeled streptokinase were determined using essentially the procedure described by Weber and Osborn (*10*). Approximately 1.5 mg of protein was dissolved in 0.5 ml of 10 m*M* sodium phosphate buffer, pH 7.2, containing 1% SDS and 1% β-mercaptoethanol. The samples were then heated at 70°C for 10 min. Following denaturation in SDS, 25 and 50 μl aliquots of the protein solution were added to 10 μl of tracking dye (0.05% bromphenol blue) containing three crystals of sucrose. After mixing, the entire sample was layered over 7.5% polyacrylamide gels prepared as described by Weber and Osborn (*10*). Electrophoresis was carried out in sodium phosphate buffer, pH 7.2 containing 1% SDS and 1% β-mercaptoethanol in a two-compartment electrophoresis chamber at a constant current of 6 mA/gel. The tracking dye migrated through approximately 80% of the gel in 4.5–5 hr. Following electrophoresis, the gels were removed from the glass gel tubes and stained for 12 hr with 0.25% Coomassie blue dissolved in 50% methanol containing 7.5% acetic acid. The gels were destained in 5% methanol containing 7.5% acetic acid. The protein profile within the polyacrylamide gels was recorded by scanning the gels at 600 nm in a Model 2400 Gilford spectrophotometer. The radioactive profile of the ^{131}I-labeled streptokinase was determined by scintillation counting of unstained 1-mm gel slices previously fixed in 5% methanol and 7.5% acetic acid. The molecular weight of streptokinase was determined from a standard curve that was constructed by plotting the electrophoretic mobilities of a number of proteins against their known molecular weight, expressed on a semilogarithmic scale. The SDS-polyacrylamide gel mobility was calculated by dividing the distance of dye migration into the distance of protein migration.

Cellulose acetate electrophoresis. Electrophoresis of unlabeled and ^{131}I-labeled streptokinase dissolved in citrate buffer was performed on cellulose acetate strips for 45 min at 6 mA in 0.05 *M* sodium barbiturate buffer, pH 8.6. A control of human serum was run at the same time. The cellulose acetate strips were fixed in 20% sulfa-salacylic acid and stained for 10 min with Coomassie blue. The strips were destained in water for 2–3 hr. The distribution of radioactivity on unstained strips was determined by using a radiochromatographic strip scanner.

Analytical column chromatography. Approximately 0.5 mg of the ^{131}I-streptokinase in 1 ml of citrate buffer, obtained from the protein peak of the Sephadex G-25 column, was layered on a 1- × 30-cm column of Sephadex G-200 gel equilibrated with citrate buffer. Two milliliter fractions from the column were collected and radioactivity was determined by scintillation counting. A single peak, with a small shoulder accounting for less than 5% of the total radioactivity, was observed. Since a single protein band was observed on both cellulose acetate and polyacrylamide gel electrophoresis (see below), the shoulder probably represents aggregation.

Stability of labeled streptokinase. The stability of labeled streptokinase was determined using cellulose acetate electrophoresis in 0.05 *M* sodium barbiturate buffer, pH 8.6, for 10 min at 6 mA. Iodine-125-streptokinase was stored at 4°C for 5 days and at 35°C for 18 hr. Aliquots were removed daily and assayed electrophoretically. Under these conditions, no free iodine or other degradation products were observed.

Biologic characterization. *Clot lysis assay of labeled streptokinase.* The biologic activity of labeled streptokinase was determined by a modification of the clot lysis assay procedure as described by Christensen (*9*). One milligram of human fibrinogen (Cutter Laboratories) dissolved in 0.1 ml 0.05 *M* sodium barbiturate buffer, pH 8.6, was added to each of 14 test tubes. Five units of ^{131}I-labeled streptokinase in citrate buffer were added to Tube 1 and 5 units of unlabeled streptokinase dissolved in the same buffer were added to Tube 7. To each subsequent tube in the series, the amount of streptokinase added was increased by 5 units so that 30 units of labeled streptokinase were added to Tube 6 and 30 units of unlabeled streptokinase were added to Tube 12. Tubes 13 and 14 served as control tubes and received no streptokinase. The tubes were incubated for 5 min at 35°C and then 0.01 ml of 0.02 *M* calcium chloride and 10 units of bovine thrombin (Parke-Davis) were added to all 14 tubes. The resulting clots were observed and the time for subsequent lysis recorded.

In vitro clot incorporation of labeled streptokinase. The incorporation of streptokinase into clots prepared in vitro was determined by a modification of

the conventional clottability method (*11*). Fifty units of ^{131}I-streptokinase were added to 10 mg of fibrinogen dissolved in 1 ml of 0.05 *M* phosphate buffer, pH 6.2, and incubated for 5 min at 35°C. One hundred units of bovine thrombin and 0.1 of 0.02 *M* calcium chloride solution were added to form a clot as described above. Following incubation for 5 min at 35°C, the supernatant fluid was removed and the clot washed twice with phosphate buffer. The radioactivity in the clot and the supernatant fluid was then determined by scintillation counting. Radioactivity in the clot was expressed as a percentage of the total radioactivity originally added to the tube.

Mouse organ distribution. Approximately 0.1 mg of streptokinase labeled with approximately 5 μCi of ^{131}I was injected into the dorsal tail vein of 20–25 gm Balb C mice. Four mice were sacrificed by blunt transection of the cervical cord at 1, 2 and 4 hr after the injection of the radioactivity. The amount of radioactivity injected into each mouse was determined by counting the mouse immediately after injection with two opposed 2-in. sodium iodide detectors arranged so that the total counts varied less than 5% regardless of the location of the radioactivity within the field of view of the collimators. The mice were dissected and 10 μl of blood were obtained from the still-beating heart. The blood, lung, heart, liver, spleen, stomach, kidney, and bowel were weighed and the amount of radioactivity in each was determined by scintillation counting. The contents of the bowel were removed, and the bowel was weighed and recounted. The amount of radioactivity in each organ was expressed as a percent of the administered dose per gram of organ and percent of the administered dose in the entire organ.

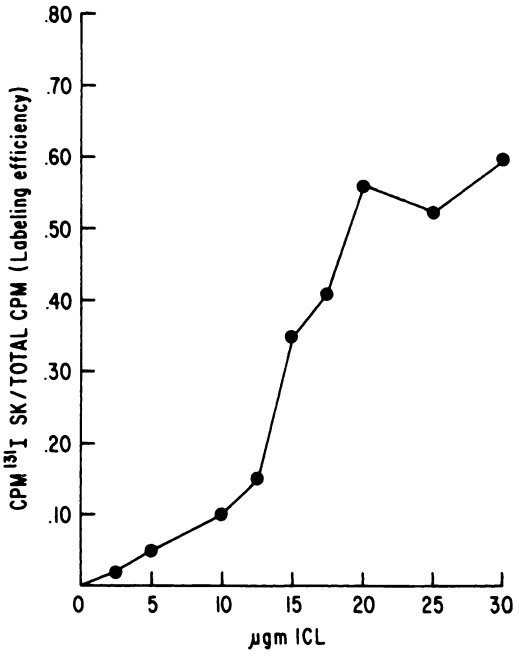

FIG. 1. Labeling efficiency of streptokinase. Abbreviations used: ICI, iodine monochloride; SK, streptokinase.

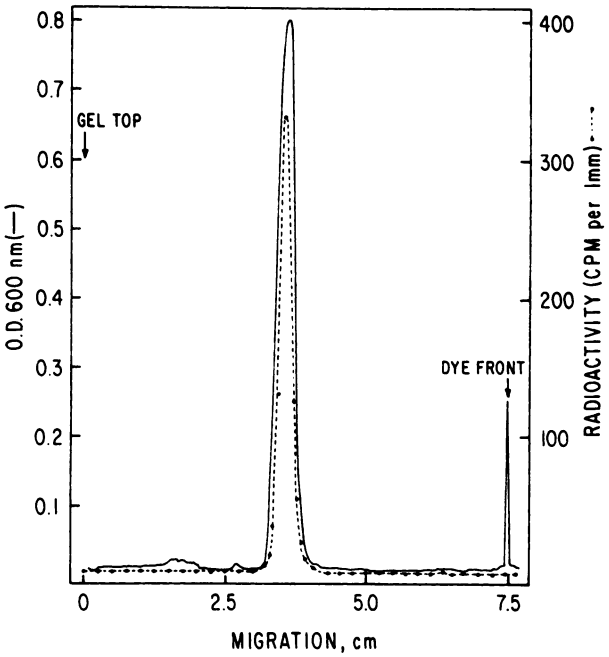

FIG. 2. ^{131}I-labeled and unlabeled streptokinase were electrophoresed in 7.5% polyacrylamide gels containing 1% SDS and 1% β-mercaptoethanol as described under Experimental Procedure. Solid line represents optical density profile of stained, unlabeled enzyme and dashed line represents radioactive profile of labeled enzyme.

Plasma clearance of labeled streptokinase. The plasma clearance of ^{131}I-streptokinase labeled with 3.4 atoms of iodine per molecule of streptokinase was obtained in five dogs ranging in weight from 20 to 30 lb. One dog was studied without preloading with unlabeled streptokinase, two dogs were studied after preloading with unlabeled streptokinase, and two dogs were studied both before and after preloading with unlabeled streptokinase. A sixth dog was studied using ^{131}I streptokinase labeled with 0.3 atoms of iodine per molecule of streptokinase.

Plasma clearance without preloading. Approximately 0.25 mg (15,000 units) of streptokinase labeled with 30–40 μCi of ^{131}I was injected into each dog through an intravenous catheter. Sixteen aliquots of blood were obtained during the 4 hr following the intravenous injection and 1-ml samples of plasma were counted in a well scintillation detector. One milliliter of 15% trichloracetic acid was then added to the plasma; the resulting precipitate was washed once and counted. The radioactivity in the liver of the dogs was monitored continuously after injection using a 2-in. diam sodium iodide scintillation detec-

tor attached to an analog ratemeter and strip chart recorder.

Plasma clearance after preloading. Each dog was injected with 0.16 mg (10,000 units) of unlabeled streptokinase per pound of body weight 20 min before the intravenous injection of approximately 0.25 mg (15,000 units) of ^{131}I-streptokinase. The procedure described before for determining the plasma clearance of streptokinase was then followed. Two dogs were studied both before and after loading with unlabeled streptokinase. In these dogs, the loading dose of streptokinase was given intravenously immediately following the 4-hr blood sample. The clearance rate prior to preloading was obtained using ^{125}I-streptokinase; ^{131}I-streptokinase was injected after the loading dose.

RESULTS

Determination of the optimum molar ratio of iodine monochloride to streptokinase. Increasing the amount of iodine monochloride added to the reaction mixtures from 2.5 to 30 μg resulted in a rapid increase in labeling efficiency to 58% with 25 μg of iodine monochloride and 1.66 mg of streptokinase; larger amounts of iodine monochloride increased the labeling efficiency only slightly (Fig. 1). Using a different batch of streptokinase (Lot No. RM 564-1 "Streptase") we were able to obtain a maximum labeling efficiency of up to 80% using 25 μg of iodine monochloride. The reason for this difference in labeling efficiency is not known. Routinely, 25 μg of iodine monochloride, which resulted in 3.2–3.4 atoms of iodine per molecule of streptokinase, depending on the labeling efficiency, were used for all labeling procedures.

Purity and molecular weight of streptokinase. As shown in Fig. 2, both the ^{131}I-labeled and unlabeled streptokinase used in these studies were homogenous as determined by SDS-polyacrylamide gel electrophoresis. Furthermore, since the radioactive profile of the labeled enzyme was superimposable on the protein profile of the unlabeled enzyme, these results clearly establish that no structural alterations were produced in the polypeptide chain during the labeling procedure. From the electrophoretic mobility observed in Fig. 2 (0.48), the molecular weight for streptokinase was determined to be 62,000. Cellulose acetate electrophoresis of labeled and unlabeled streptokinase also showed that the protein migrated as a single band. The streptokinase migrated immediately behind the albumin band of the control serum.

Biologic characterization. *Clot lysis assay of labeled streptokinase.* There was no appreciable difference in the ability of the labeled streptokinase to lyse clots when compared with the unlabeled streptokinase. This provides additional confirmation that the labeling procedure did not structually alter the enzyme.

In vitro clot incorporation of labeled streptokinase. In two separate experiments to determine the affinity of labeled streptokinase for clots prepared in vitro, 60% of the added radioactivity was found in the clot.

Mouse organ distribution study. The organ distribution studies revealed an early accumulation of radioactivity in the liver, 27% per gram and 24% per organ at 15 min with a subsequent decrease of radioactivity to 1.4% per gram and 2.4% per organ at 2 hr. As liver and blood radioactivity decreased, stomach radioactivity increased from 5% per gram and 1.3% per organ at 15 min to 19% per gram and 3.7% per organ at 4 hr. There was also an increase in bowel activity relative to blood at 2 hr (Table 1).

Plasma clearance of labeled streptokinase without preloading. The plasma clearance of labeled streptokinase in six dogs was described by a biexponential curve with a fast phase half-life of 15–25 min and slow phase half-life of 160–260 min (Table 2). The clearance of the protein-bound radioactivity in the precipitated fraction of the plasma was also biexponential with a fast phase half-life of 20–25 min representing 70% of the total radioactivity (Fig. 3)

TABLE 1. ^{131}I STREPTOKINASE DISTRIBUTION IN 25-GM BALB/C MICE

Organ	15 min		2 hr		4 hr	
	%/gm	%/organ	%/gm	%/organ	%/gm	%/organ
Stomach	5.0 (2–6.8)*	1.3 (0.8–2.3)	16.0 (13.5–19)	6.0 (2.8–10)	19.0 (10–22)	3.7 (2–5)
Liver	27.0 (16–42)	34.0 (22–49)	1.4 (1–1.7)	2.4 (1.7–3)	1.7 (1.4–2)	2.3 (2–2.4)
Kidney	38.0 (20–60)	12.0 (8.4–16)	4.0 (2.7–5.5)	1.4 (1–1.7)	4.8 (3.9–5.6)	1.5 (1.3–1.7)
Bowel full	2.0 (1.5–3)	6.0 (5–6.8)	1.3 (1–1.5)	3.9 (3–5)	1.4 (1.2–1.7)	2.8 (2.3–4)
Bowel empty	1.4 (0.9–1.6)	2.5 (2.4–2.6)	0.3 (0.1–0.5)	0.5 (0.2–0.9)	0.1 (0.1–0.1)	0.2 (0.1–0.2)
Blood	19.0 (12–25)		3.0 (2.8–3.1)		2.9 (1.8–4.2)	

* Mean and range of four mice.

TABLE 2. PLASMA CLEARANCE OF ^{131}I-STREPTOKINASE IN HEALTHY DOGS

Animal (No.)	Preloading with unlabeled SK	Total plasma ^{131}I		Protein-bound ^{131}I		Slow phase as % of total ^{131}I (%)
		$T_{1/2}$ fast phase (min)	$T_{1/2}$ slow phase (min)	$T_{1/2}$ fast phase (min)	$T_{1/2}$ slow phase (min)	
1	Before	17	250	—	—	36
2	After	24	160	—	—	50
3	Before	15	190	—	—	35
	After	15	600	—	—	35
4	Before	20	260	20	75	25
	After	22	220	24	75	28
5	After	25	160	25	60	32
6	Before*	24	200	25	80	30

* Labeled with 0.2 atoms iodine.

and a slow phase half-life of the protein-bound radioactivity of 60–80 min. Protein-bound radioactivity only accounted for 30% of the total radioactivity in the plasma by 4 hr after injection (Fig. 3). The nonprotein-bound iodine migrated like free iodine on cellulose acetate electrophoresis. After the intravenous injection of labeled streptokinase, radioactivity in the liver reached a maximum at 1 hr (Fig. 4). The clearance of radioactivity from the liver was very slow compared with that of the plasma.

Plasma clearance of labeled streptokinase after preloading. No change in the clearance was observed in any of the animals receiving a loading dose of unlabeled streptokinase prior to the labeled streptokinase (Fig. 5). The fraction of the total amount of radioactivity cleared with a fast half-life remained at 70% (Table 2).

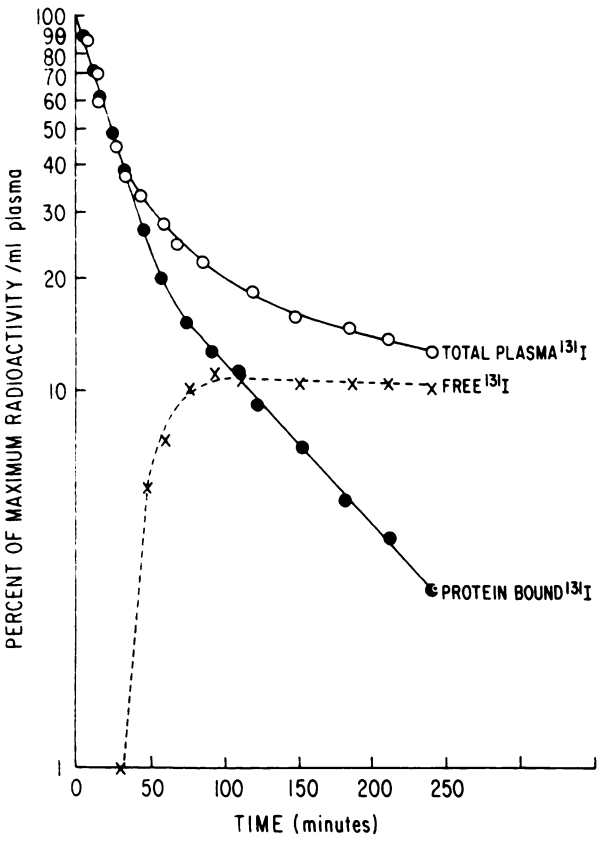

FIG. 3. Plasma clearance of total ^{131}I (○—○), protein-bound ^{131}I (●—●), and nonprotein-bound ^{131}I (X--X) after injection of ^{131}I-streptokinase into healthy dog.

DISCUSSION

Streptokinase is a product of hemolytic streptococcal metabolism and is therapeutically useful as a fibrinolytic agent (*12–16*). The findings by Gross (*17*) that ^{131}I-streptokinase localized in in vitro clots stimulated investigations into the use of streptokinase as an agent to detect thrombi. The details of the mechanism by which streptokinase localizes in clots is still unknown (*18*).

Before investigating the biologic behavior of streptokinase, it was essential to establish the purity of the enzyme and the absence of alterations potentially induced by the labeling process. With cellulose acetate electrophoresis, Sephadex gel column chromatography, and SDS polyacrylamide gel electrophoresis, unlabeled and radioiodinated streptokinase behaved like a single protein. The SDS molecular weight was 62,000. More importantly, the clot lysis assay and the clot affinity determination showed that the labeling procedure caused no loss in biologic activity. Twenty-five micrograms of iodine monochloride labeled the streptokinase with 3.2–3.4 atoms of iodine. This level of labeling did not affect the biologic activity of other biologically active proteins (*19*).

The organ distribution studies in mice revealed an early accumulation of radioactivity in the liver followed by loss of radioactivity from the liver and

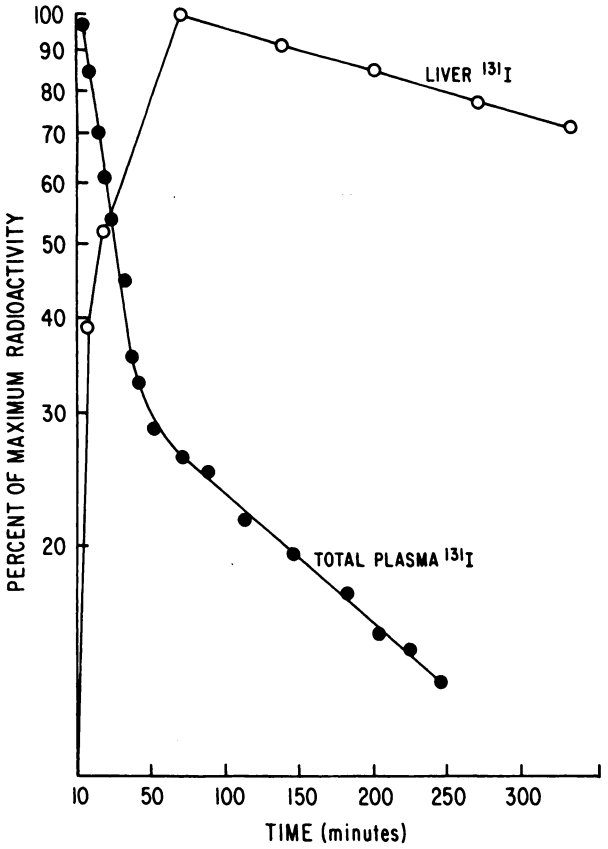

FIG. 4. Radioactivity in liver (○—○) and plasma (●—●) of normal dog after intravenous injection of ^{131}I-streptokinase. There is slow clearance of radioactivity from liver as compared with plasma.

blood and an increase in radioactivity in the stomach (Table 1). This increase in stomach radioactivity represented free iodine since, at 4 hr, most of the plasma radioactivity moved as free iodine on cellulose acetate electrophoresis. Plasma clearances of streptokinase labeled with 0.3 or 3.4 atoms of iodine per molecule were comparable and similar to results obtained by other workers (*20–23*); this suggests that the rapid in vivo deiodination of the streptokinase was not caused by damage to the protein by the labeling process. The rapid plasma clearance of streptokinase may be the result of the formation of an antigen–antibody complex since most humans and dogs have developed streptococcal antibodies that cross-react strongly with streptokinase (*9*). As the protein-bound radioactivity in the plasma decreased, an increase in nonprotein-bound radioactivity in the plasma and the liver was observed. This suggests that the antigen–antibody complex is cleared and deiodinated by the liver. Francis (*24*) has described similar hepatic clearance of other antigen–antibody complexes. This phenomenon may offer a reasonable explanation for the inconsistent localization of labeled streptokinase in thrombi reported by Siegel, et al (*4*).

Fletcher (*20*) found that the infusion of unlabeled streptokinase prior to the administration of labeled streptokinase dramatically altered the clearance of the labeled streptokinase from the plasma of humans and animals. This preloading technique is widely used in patients on streptokinase therapy for venous thrombosis (*25*) and could represent a method for preventing the undesired binding of labeled streptokinase by antibodies. In our experiments, loading with streptokinase did not produce any change in the plasma clearance rate of labeled streptokinase (Fig. 5). Although we did not measure the plasma streptokinase levels directly, these preloading experiments were carried out with a loading dose of 450 units of streptokinase per milliliter of plasma. This presumably represents a considerable excess of streptokinase relative to the antistreptokinase titers present in dogs since Christensen (*9*) reported a maximum antistreptokinase activity in dogs of 156 units/ml of plasma. The precise reason for the differences between our results and those of others is not known.

Rhodes, et al (*26*) have recently reported variable

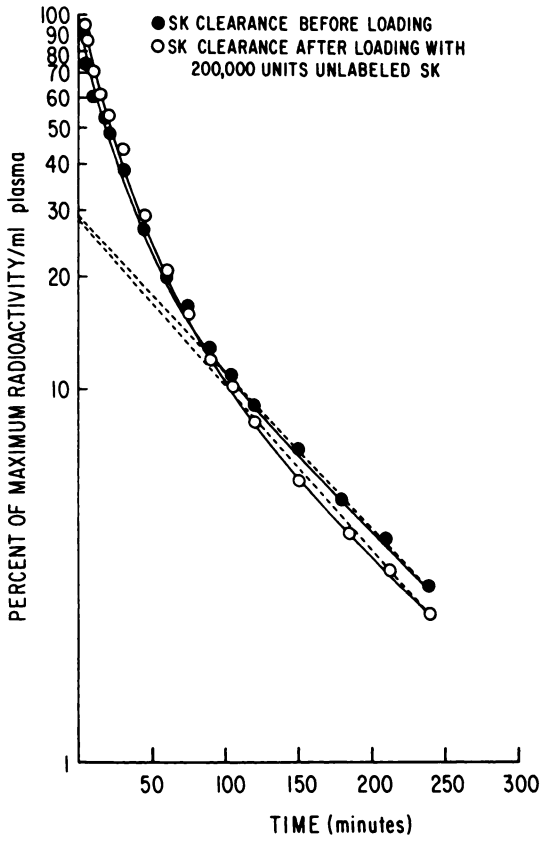

FIG. 5. Plasma clearances of ^{131}I-streptokinase before (●—●) and after (○—○) loading with 200,000 units of unlabeled streptokinase in 20-lb dog.

results in their studies on the localization of thromboemboli in dogs with labeled streptokinase. These workers have also pointed out the multiple variables involved in studies of this type and have emphasized the possible limitations of the dog as a model system. Although the extent of the usefulness of radioiodinated streptokinase as a radiopharmaceutical agent for detecting thrombi and neoplasms awaits the provision of further in vivo and in vitro experimentation, the results of our studies are in accord with the conclusion that it may have limited applicability. This conclusion is based primarily on the rapid plasma clearance and deiodination results as well as on the possible dangers and ineffectiveness of preloading with unlabeled streptokinase. These results do not exclude the possibility, however, that sufficient amounts of the labeled enzyme may be taken up by thrombi in vivo in spite of the rapid clearance, thereby permitting detection by scintigraphic techniques. Although it may be fruitful to pursue this possibility further, it is important to recognize that it has not been possible to establish it experimentally with any degree of reproducibility (*26*). If the rapid removal of labeled streptokinase from plasma is a result of immunologic clearance due to endogenous streptococcal antibodies, then a more promising approach may be to circumvent this limitation by the use of a streptokinase-like enzyme derived from a bacterial strain other than streptococcus. Presumably; the antibody titer of such an enzyme in humans would be markedly reduced if not absent. Alternatively, studies are currently in progress to modify covalently the streptokinase in an attempt to produce a protein that has altered immunologic properties but still retains streptokinase activity.

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