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Author
Yano, Y.

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Y. Yano, J. McRae, D.C. Van Dyke, and H.G. Anger
DONNER LABORATORY

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TECHNETIUM-99m-LABELED STANNOUS ETHANE-1-HYDROXY-1 1-DIPHOSPHONATE: A NEW BONE-SCANNING AGENT

Y. Yano, J. McRae, D.C. Van Dyke, and H.G. Anger

Donner Laboratory, Lawrence Berkeley Laboratory
University of California, Berkeley, California 94720

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The early detection of metastatic bone lesions will be greatly enhanced when the well-known advantages of ^{99m}Tc for scintigraphy (high yield of 140-keV photons, low radiation dose, and convenient availability from the 67-hour half-life parent, ^{99}Mo) are utilized in a bone-scanning agent.

Subramanian (1) has developed a ^{99m}Tc-polyphosphate complex for bone scanning. The studies of Francis and co-workers have shown that several diphosphonate compounds, by chemisorption on the surface of bone crystals, inhibit bone formation and resorption (2, 3). It became apparent to the authors of this paper that diphosphonate labeled with ^{99m}Tc would be a good bone-scanning agent, and the initial preparation and evaluation of a ^{99m}Tc-Sn-EHDP complex has been reported (4, 5). This study presents further evaluation of such a ^{99m}Tc-diphosphonate complex for bone scanning.

MATERIALS AND METHODS

Reagents. The following materials were used in the preparation: (a) sodium ethane-1-hydroxy-1,1-diphosphonate (EHDP)*, $CH_3COH(PO_3NaH)_2$; (b) reagent grade $SnCl_2 \cdot 2H_2O$ as a freshly prepared solution in either 0.01 N HCl or water, and (c) $^{99m}TcO_4^-$ in normal saline.† The reagent solutions were made up in sterile water, which was free of dissolved O_2 and saturated with N_2.

Sterile, evacuated serum vials of 6 ml capacity were used as the reaction vessels.

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* Supplied by M. D. Francis and A. J. Tofe of the Proctor and Gamble Co., Miami Valley Laboratories, Cincinnati, Ohio.

† Methyl-ethyl-ketone extracted; Mediphysics, Emeryville, Calif.

General method of preparation. In general the method of preparation was as follows: 10 mg of $SnCl_2 \cdot 2H_2O$ were dissolved in 100 ml of solvent. One ml of this solution was filtered through a 0.22μ Millipore filter into the reaction vessel, which contained the appropriate amount of EHDP dissolved in 1 ml of water. The desired activity of ^{99m}Tc, as $^{99m}TcO_4^-$ in 3 ml of normal saline, was then added to the reaction vessel. Adjustment to pH 6 was made with dilute NaOH when 0.01 N HCl was used as the solvent; no adjustment was required with water as the solvent. The final step in the preparation of ^{99m}Tc-Sn-EHDP was passage through a 0.22μ Millipore filter.

Variables to preparation method. The following parameters were investigated in the chemical preparation:

1. The molar ratios of EHDP to Sn, which ranged from 100:1 to 1:1.

2. The effect of using 0.01 N HCl or water as the solvent for $SnCl_2 \cdot 2H_2O$.

3. The effect of changing the concentrations of $SnCl_2 \cdot 2H_2O$ and EHDP.

4. The influence of the order of combining $SnCl_2 \cdot 2H_2O$, EHDP, and $^{99m}TcO_4^-$.

5. The determination of the site of Sn(II) uptake from ^{99m}Tc-Sn-EHDP by using ^{113}Sn as a tracer.

6. The stability in vitro in the presence of air compared to that in an "O_2-free" nitrogen atmosphere or in an evacuated vial.

Labeling Efficiency. The efficiency of the labeling reaction was evaluated by ascending paper chromatography using Whatman #1 paper strips in 85% methanol solvent. The activity remaining at the

origin was taken to be the ^{99m}Tc-Sn-EHDP complex. Although it is possible that other ^{99m}Tc-Sn complexes could also remain at the origin, the distribution in animals indicates the presence of a bone-seeking ^{99m}Tc-Sn-EHDP complex with only low liver and kidney uptake. High uptake would be indicative of the presence of additional ^{99m}Tc-Sn complexes.

Double isotope studies. Technetium-99m and ^{113}Sn were used to elucidate the role and site of Sn(II) uptake in rats for the ^{99m}Tc-Sn-EHDP preparation. Tin-113 as the chloride in 6 N HCl was reduced to Sn(II) in the presence of nickel under a CO_2 atmosphere. The preparation of the ^{99m}Tc-^{113}Sn-EHDP was carried out using the same amounts of Sn(II) tagged with ^{113}Sn and EHDP as for the standard preparation. Samples from the animal tissues and paper chromatogram were counted at both the ^{99m}Tc and ^{113}Sn gamma energy settings of the pulse height analyzer.

Procedures in rats.

1. The ^{99m}Tc-Sn-EHDP compound was evaluated in Sprague-Dawley rats that weighed from 190 to 290 grams. Each received the ^{99m}Tc-Sn-EHDP preparation in a volume of 0.25 ml injected by tail vein. Scintillation camera pictures using the multichannel or 1/8-inch pinhole collimator were obtained to visualize the distribution of ^{99m}Tc-Sn-EHDP at different times after i.v. administration. The rats were sacrificed from 5 min to 48 hr after injection, and the various tissues were counted in a well counter. The uptake of ^{99m}Tc-Sn-EHDP was calculated as a percent of injected dose either for the entire organ or per gram of tissue.

2. The uptake of ^{18}F and ^{99m}Tc-Sn-EHDP was compared in normal Sprague-Dawley rats. Each of 11 rats was injected by tail vein with 500 μCi of cyclotron-produced ^{18}F. They were sacrificed 3 hr later and the tissues handled in the same manner as with the ^{99m}Tc-Sn-EHDP preparation.

3. Preliminary toxicity studies of tin were done in rats by administering stannous and stannic tin as citrate and tartrate complexes at concentrations of 1.8, 4.7, 9, and 18 mg Sn/kg. These animals were followed for up to 60 days, then sacrificed, and a macroscopic examination was made of the various organs.

Procedures in dogs. The water and acid preparations of ^{99m}Tc-Sn-EHDP were evaluated separately using four male beagle dogs each weighing 8-12 kg. Acid and water preparations were compared in the same dog by separate injections a week apart. The anesthetized dogs received 1.0-2.0 ml of the preparation i.v. in the tongue. Two dogs were selected for data presentation on the basis of similar body weight. The scintillation camera or whole body scanner was used to visualize the in vivo uptake at 3 and 20 hr after administration. The rate of disappearance from the blood was followed by taking 1-ml blood samples periodically from 1 to 180 min after i.v. administration and counting them in a well counter.

The data were expressed as the fraction of the injected dose in the blood at times after administration, where:

$$\text{Fraction of injected dose} = \frac{\text{cpm/ml blood} \times 83.4 \text{ ml}^{*} \times \text{wt in kg}}{\text{total injected cpm}}$$

* 83.4 ml blood per kg body weight.
From: The Beagle as an Experimental Dog. A.C. Anderson, editor, Iowa State University Press, Ames, Iowa, 1970.

The urine was completely collected by catheterizing the bladder and flushing it with saline to determine the fraction of activity excreted for up to 245 min after administration.

RESULTS AND DISCUSSION

The percent uptake in rats of ^{99m}Tc from ^{99m}Tc-Sn-EHDP was not significantly affected by changes in the molar ratio of EHDP to Sn ranging from 50:1 to 5:1 or by the use of either acid or water for the preparation (Fig. 1). The variations shown at the 20:1 molar ratio were deemed not significant to the overall evaluation.

A comparison in rats of the uptakes at 3 hr of ^{18}F and ^{99m}Tc-Sn-EHDP indicates that the uptake of ^{18}F in the femur was about 65% greater than the uptake of ^{99m}Tc-Sn-EHDP, but the excretion of the diphosphonate in urine was about twice that of the ^{18}F excretion (Table 1). However, the fraction of the retained activity that was in the bone was comparable for both ^{18}F and ^{99m}Tc-Sn-EHDP.

Table 2 shows the percent uptake of ^{99m}Tc-Sn-EHDP in rats for various times after i.v. injection. The uptake in blood and soft tissues cleared rapidly, and within 1 hr the bone-to-blood ratio was 28: 1 while the bone-to-muscle ratio was 200:1. The bone uptake was maximum at 3 hr and remained with less than 10% loss of activity up to 48 hr later.

When the molar ratio of EHDP to Sn was maintained at 5:1 in a water preparation and their concentration in a standard volume was reduced first by a factor of 10 and then 100, there was a significant breakdown of the ^{99m}Tc-Sn-EHDP as well as poor labeling, as shown by the results in Table 3. When the concentration of EHDP and Sn was reduced to 1/10 of the standard preparation, there was a 10-fold

increase in kidney uptake and a 3-fold increase in gut uptake compared to the standard preparation. This indicates formation of ^{99m}Tc-Sn complexes. When the concentration was reduced to 1/100, there was 5 times the uptake in the kidneys, 23 times the uptake in the gut, with little bone uptake, which indicates a high free pertechnetate concentration from breakdown and/or poor binding to the diphosphonate.

Furthermore, when the standard preparation was diluted 100 times with physiological saline before injection, there was a decrease in bone uptake and an increase in soft tissue uptake. The breakdown of ^{99m}Tc-Sn-EHDP under these conditions appeared to be similar to that observed with a 1/100 concentration in a standard volume. The EHDP and Sn(II) concentration must be maintained above a minimum level (0.1 mg $SnCl_2 \cdot 2H_2O$ and 0.5 mg EHDP) in a standard 5 ml volume to promote good initial labeling and stability of ^{99m}Tc-Sn-EHDP.

Table 4 shows the distribution of ^{99m}Tc-Sn-EHDP in the whole organs of rats as affected by the order of combination of the chemical reagents: first, by adding in order EHDP, Sn(II), $^{99m}TcO_4^-$; and second, by adding in order Sn(II), $^{99m}TcO_4^-$, EHDP. By the first method the uptake in blood, liver, kidneys, and gut was relatively low at 3 hr, while the bone uptake was high. However, when the second method was used the bone uptake decreased while the blood, liver, and kidney uptake increased.

These data suggest that EHDP and Sn(II) might form a chelate that has a greater reducing potential than Sn(II) alone and that the Sn(II)-EHDP chelate will combine with the reduced ^{99m}Tc. On the other hand, when the Sn(II) and $^{99m}TcO_4^-$ were combined first, there was an

insoluble Sn-^{99m}Tc complex formed probably as the ^{99m}Tc coprecipitated with SnO (8) or as the SnOCl in the presence of the NaCl in the solution. The equilibrium condition may not allow the coprecipitate to be brought into solution and be chelated by the EHDP. This was reflected by the increased uptake in liver.

From the double isotope study with ^{99m}Tc-^{113}Sn-EHDP it can be seen that the uptakes of ^{99m}Tc and ^{113}Sn in the femur of rats were not exactly comparable (Table 5). It is probable that both the ^{99m}Tc in the reduced state and ^{113}Sn(II) are involved in a reaction which is similar to that reported with divalent tin and a transition metal (9,10). The compound ^{99m}Tc-^{113}Sn-EHDP appears to remain relatively intact in vivo, but the precise chemical structure remains to be investigated.

The stability of the ^{99m}Tc-Sn-EHDP in vitro was increased by evacuating the air from the reaction vessel, and replacing it with nitrogen. In such an atmosphere, the initial chemical binding efficiency ranged from 90-96% and the amount of ^{99m}Tc still bound to the Sn-EHDP after 6 hr was 80-90%. When the air was not removed, however, the preparation deteriorated within 2-4 hr with only about 65-75% of ^{99m}Tc still bound to Sn-EHDP.

The standard preparation of ^{99m}Tc-Sn-EHDP that is now being used has a 5:1 molar ratio of EHDP to Sn which contains 0.1 mg $SnCl_2 \cdot 2H_2O$, 0.5 mg EHDP, and 60 mCi of $^{99m}TcO_4^-$ in 5 ml of preparation. Water is the solvent for $SnCl_2 \cdot 2H_2O$ and the unadjusted pH is 4-5.

Figure 2, which is a composite of scintillation camera pictures of a rat taken with the 1/8-inch-diameter pinhole collimator, shows

the ^{99m}Tc-Sn-EHDP uptake to be primarily in the skeleton 3 hr after injection. There was no visible uptake in liver, spleen or stomach. Uptake in the kidneys was faintly seen and the bladder contained radioactive urine.

The blood clearance of the ^{99m}Tc-Sn-EHDP preparations from the beagle dogs indicated that the slowly disappearing component for the studies was very similar with a $T_{1/2}$ of 97 min (Fig. 2). There was about 5-6% of the dose remaining in the blood at 3 hr. At that time the fraction of the injected dose excreted in the urine ranged from 67 to 60% with a mean of 64%. These data indicate that there was no significant difference in the distribution in dogs of ^{99m}Tc-Sn-EHDP made by either the acid or water method.

Analysis of the blood clearance data by the computer method of Parker for a two-compartment model (7) gave an average bone clearance of 19.6% for ^{99m}Tc-Sn-EHDP when the ^{18}F clearance was taken as 100%. Although the amount of ^{99m}Tc-Sn-EHDP taken up in bone was significantly less than the uptake of ^{18}F in beagle dogs, the advantages of ^{99m}Tc for bone imaging are sufficient to overcome the lower bone uptake of the diphosphonate preparation.

The uptake of 5 mCi of ^{99m}Tc-Sn-EHDP in a dog 20 hr after i.v. injection as seen with the scintillation camera shows good bone uptake without visible uptake in soft tissue (Fig. 4).

The estimated radiation dose to a 70-kg patient from 10 mCi of ^{99m}Tc-Sn-EHDP is: $D_{\beta, \gamma}$ whole body, 0.148 rad; skeleton, 0.267 rad; kidneys, 3.68 rad; and bladder, 7.36 rad.

We assume $T_{1/2}$ (eff.) as 0.25 day in bone and 0.083 day in kidneys and bladder. The skeletal uptake is estimated to be 30% of the injected dose 3 hr after i.v. injection and the urinary excretion is about 70%.

Toxicity studies with Sn(II) and Sn(IV) in rats indicate an estimated safety factor of about 5×10^4 for 0.1 mg $SnCl_2 \cdot 2H_2O$ in a 70-kg patient. The therapeutic response level for EHDP in rats is about 1 mg/kg (9), which is about 700 times more than the 0.1 mg EHDP in 1 ml of ^{99m}Tc-Sn-EHDP (10 mCi) for a 70 kg patient. The proposed dose of Sn(II) is about 1/5 the amount being used in other radiopharmaceutical preparations (10).

SUMMARY

A method has been described for complexing ^{99m}Tc with Sn-EHDP to provide a bone-scanning agent that makes available the superior scanning characteristics of ^{99m}Tc. The concentrations of Sn(II) and EHDP as well as the order of addition of the reagents influence the in vivo distribution of the ^{99m}Tc-Sn-EHDP. In vitro stability was dependent upon having oxygen-free environment for the preparation. Animal studies and human studies now in progress indicate that ^{99m}Tc-Sn-EHDP will be extremely useful as a bone-scanning agent because of the high ratio of bone-to-soft-tissue uptake, high lesion-to-normal-bone uptake, rapid disappearance from blood and soft tissues, and low radiation dose to the patient.

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TABLE 1. PERCENT UPTAKE OF ^{18}F AND ^{99m}Tc-Sn-EHDP* IN WHOLE ORGANS 3 HR AFTER I.V. INJECTION IN RATS †

Organ	^{18}F ± S.D.	^{99m}Tc-Sn-EHDP ± S.D.
Blood/ml	0.004 ± 0.0007	0.034 ± 0.009
Lungs	0.020 ± 0.010	0.048 ± 0.021
Liver	0.030 ± 0.007	0.263 ± 0.090
Kidneys	0.021 ± 0.003	1.23 ± 0.37
Spleen	0.002 ± 0.0007	0.023 ± 0.022
Muscle of femur	0.009 ± 0.018	0.034 ± 0.003
Femur + marrow	3.53 ± 0.58	2.08 ± 0.47
Marrow (by diff.)	0.023 ± 0.006	0.036 ± 0.042
Gut‡	2.51 ± 1.10	2.92 ± 1.60
Carcass‡ (skeleton, muscle, skin)	75.2 ± 7.2	44.2 ± 5.4
Urine (by diff.)	19.0 ± 7.6	49.3 ± 5.5

* 5:1 molar ratio EHDP to Sn, water preparation.

† Each value is the mean of 11 rats.

‡ Counted with gamma camera, whole organ.

TABLE 2. PERCENT UPTAKE OF ^{99m}Tc-Sn-EHDP* IN RATS†

WITH TIME AFTER I.V. INJECTION

Organ	5 min	1 hr	3 hr	24 hr	48 hr
Blood/ml	1.08	0.054	0.012	0.003	---
Liver	2.15	0.37	0.13	0.10	0.084
Kidneys	8.49	0.81	0.56	0.49	0.46
Muscle/gm	0.14	0.008	0.003	0.001	0.004
Femur + marrow	0.68	1.52	1.72	1.67	1.62
Gut	3.84	0.854	1.27	0.153	0.167

* 5:1 molar ratio EHDP to Sn, water preparation.

† Each value is the mean of 3 rats.

TABLE 3. PERCENT UPTAKE OF ^{99m}Tc-Sn-EHDP PER GRAM OF RAT TISSUE FOR REDUCED CONCENTRATIONS OF Sn AND EHDP IN A 5:1 WATER PREPARATION 3 HR AFTER I.V. INJECTION*

	Concentration of Sn and EHDP		
Organ	Standard	1/10	1/100
Mean rat weight (g)	213	193	190
Blood/ml	.041	.607	.566
Heart	.018	.228	.158
Lung	.033	.365	.300
Liver	.044	.633	.741
Kidneys	.690	7.80	3.57
Spleen	.026	.329	.523
Muscle of femur	.024	.088	.062
Femur	2.63	2.38	.273
Gut†	1.80	5.50	41.2
Carcass†	41.2	32.6	28.8
Urine (by diff.)	53.3	37.9	14.8

* Each value is a mean of 3 rats.

† Percent of injected activity per total tissue.

TABLE 4. EFFECT OF ORDER OF COMBINATION OF EHDP, Sn(II), AND ^{99m}Tc UPON PERCENT UPTAKE IN WHOLE ORGANS OF RATS AFTER I.V. INJECTION

	Time after injection			
	3 hr		20 hr	
	Order of reagent addition			
Organ	EHDP-Sn(II)-^{99m}Tc†	Sn(II)-^{99m}Tc-EHDP†	EHDP-Sn(II)-^{99m}Tc†	Sn(II)-^{99m}Tc-EHDP‡
Blood/ml	0.047	0.430	0.008	0.087
Heart	0.015	0.125	0.004	0.030
Lungs	0.104	0.409	0.019	0.137
Liver	0.273	11.41	0.223	10.3
Kidneys	1.04	2.77	0.910	2.70
Spleen	0.020	0.280	0.015	0.304
Gut	0.698	1.72	0.672	1.41
Femur + marrow	2.11	1.22	1.62	1.13
Marrow (by diff.)	-	-	0.13	0.06
Femur	-	-	1.49	1.07
Carcass	33.5	33.3	30.4	26.8
Urine (by diff.)	62.1	53.3	64.5	56.7

* Acid preparation of 50:1, EHDP:Sn.

† Mean of 2 rats, 5.0 mg EHDP/kg rat.

‡ One rat, 5.0 mg EHDP/kg rat.

TABLE 5. PERCENT UPTAKE PER GRAM OF RAT TISSUE OF ^{99m}Tc AND ^{113}Sn FROM ^{99m}Tc-^{113}Sn-EHDP 3 HR POST-I.V. INJECTION*

Organ	^{99m}Tc	^{113}Sn
Blood	.046	.012
Heart	.027	.007
Thymus	.022	.005
Lungs	.059	.019
Liver	.113	.014
Kidneys	.368	.150
Spleen	.032	.013
Muscle of sternum	.022	.006
Sternum	.268	.357
Muscle of femur	.015	.006
Femur	2.33	2.43

* Average weight of rats = 204 g. Each value is the mean of 3 rats.

FIGURE CAPTIONS

FIG. 1. Percent uptake of ^{99m}Tc-Sn-EHDP in rat tissue 3 hr after i.v. injection of 5:1, 20:1 and 50:1 molar ratios of EHDP to Sn for acid and water methods of preparation.

FIG. 2. Scintillation camera picture of a rat 3 hr after intravenous administration of ^{99m}Tc-Sn-EHDP. The rat received 1 mg of EHDP. A 1/8-inch pinhole collimator was used.

FIG. 3. Disappearance of acid- and water-prepared ^{99m}Tc-Sn-EHDP from the blood of beagle dogs.

FIG. 4. Scintillation camera picture of a dog from posterior and lateral views 20 hr after i.v. administration of 5 mCi of ^{99m}Tc-Sn-EHDP. The dog received 1 mg of EHDP.

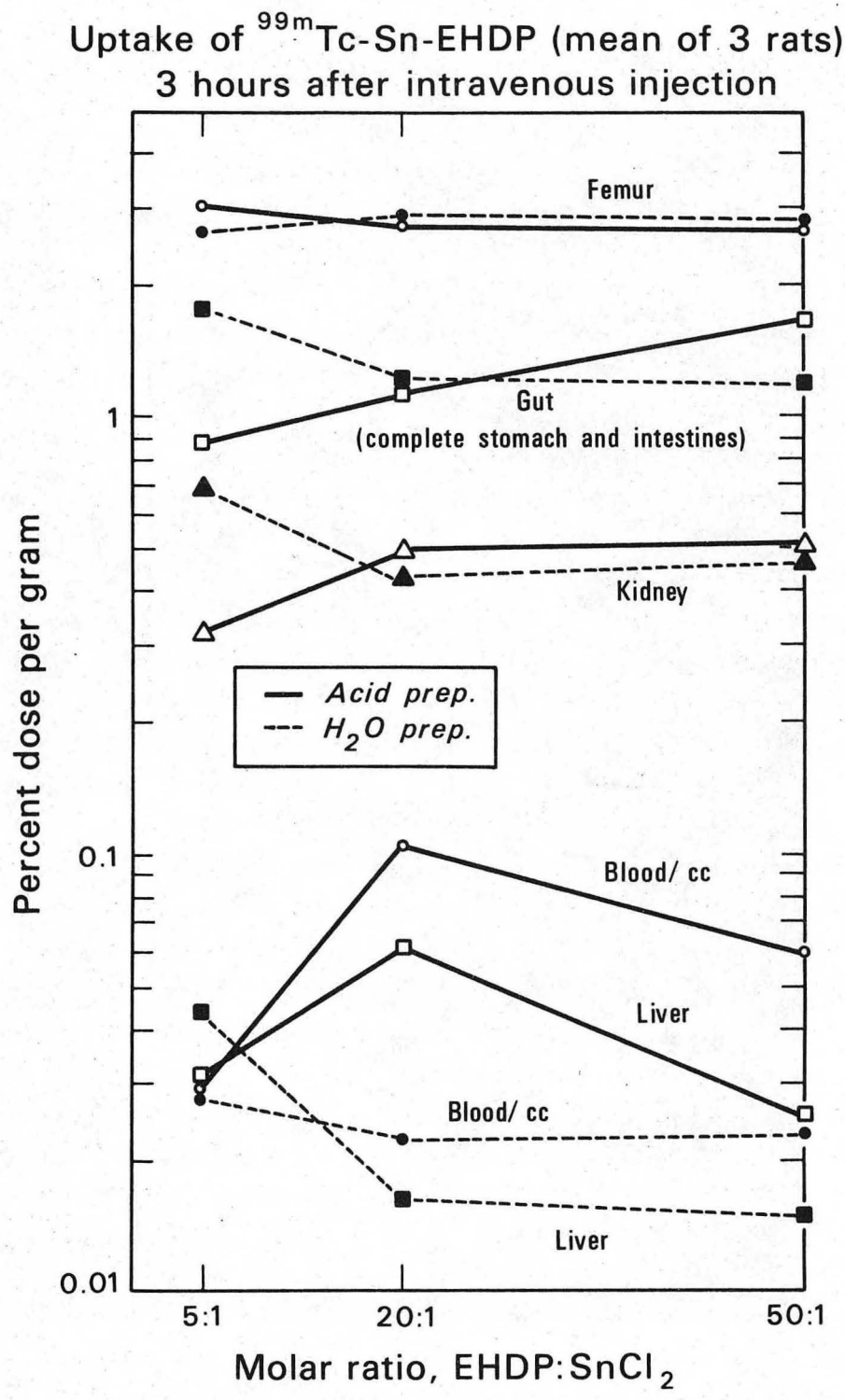

Fig. 1

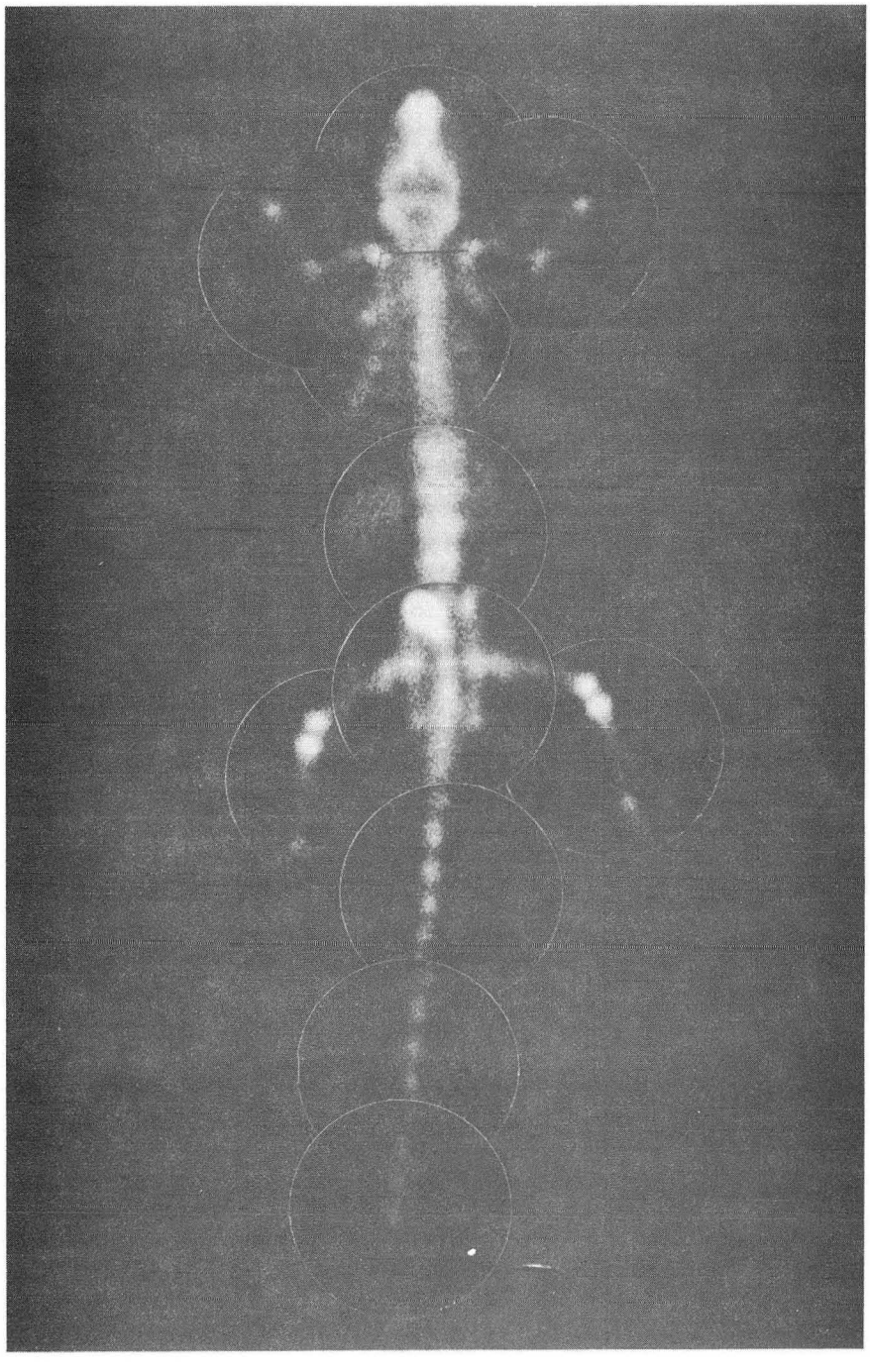

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Fig. 2

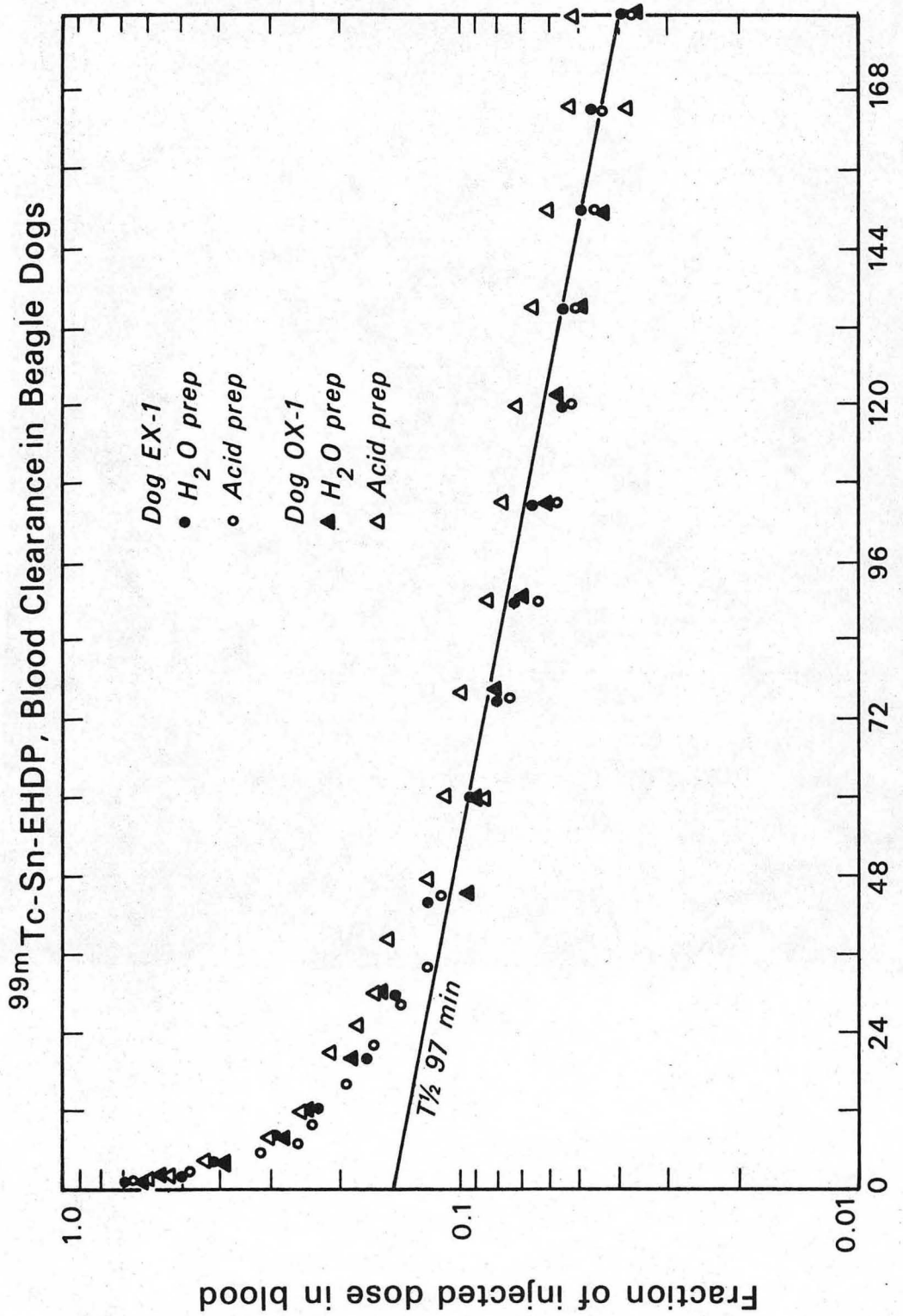

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Fig. 3

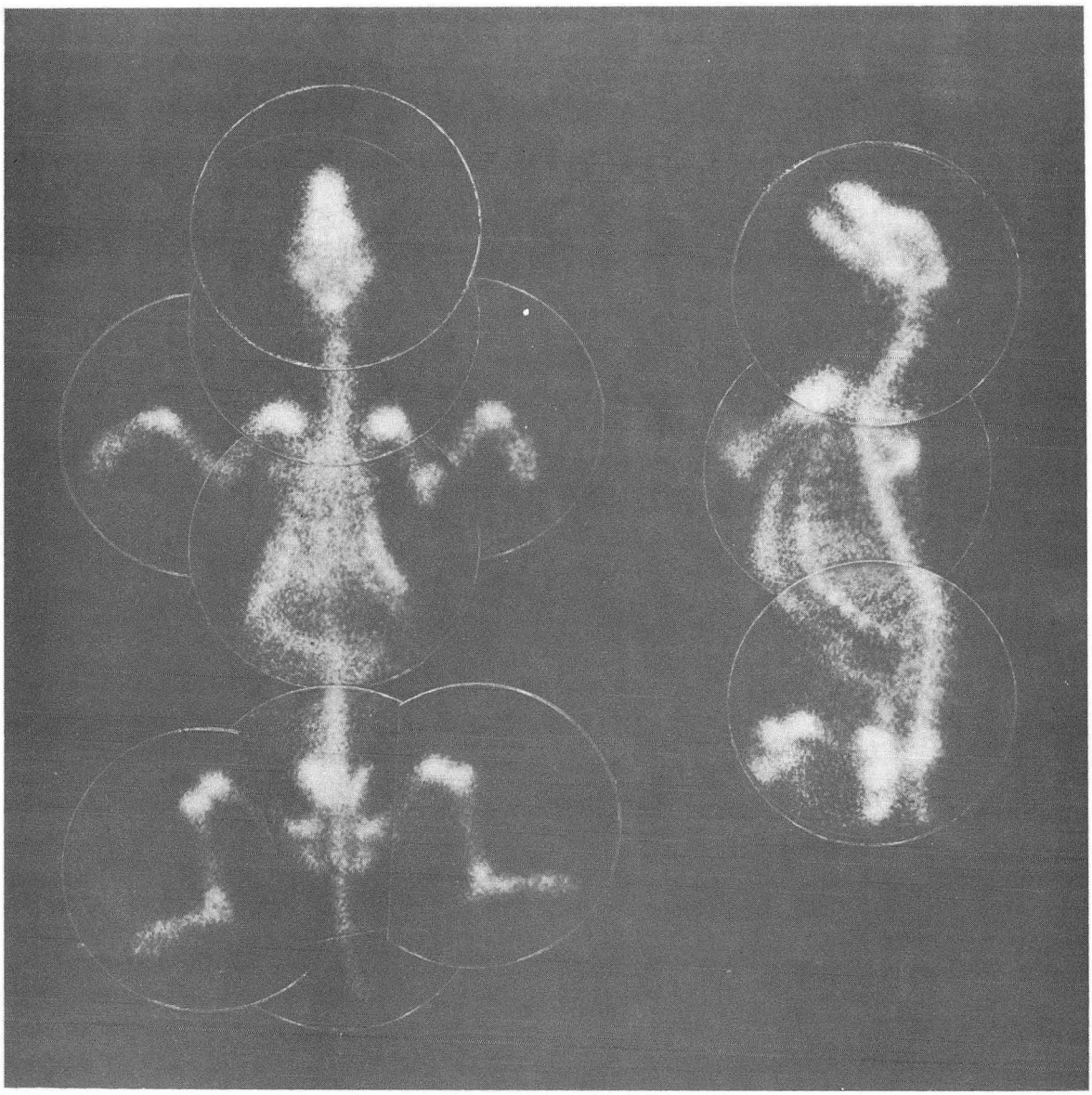

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Fig. 4

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