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EVALUATION OF RADIOLABELED RUTHENIUM COMPOUNDS AS TUMOR-LOCALIZING AGENTS*

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ABSTRACT

This work introduces a new class of radiopharmaceuticals based on ruthenium-97. The excellent physical properties of Ru-97, the high chemical reactivity of Ru, the potential antitumor activity of several Ru coordination compounds, and BLIP production of Ru-97, provide a unique combination for the application of this isotope in nuclear oncology. A systematic study was undertaken on the synthesis, characterization, and evaluation of a number of ruthenium-labeled compounds. In a variety of animal tumor models, several compounds show considerable promise as tumor-localizing agents when compared to gallium-67 citrate. The compounds studied (with Ru in different oxidation states) include ionic Ru, a number of hydrophilic and lipophilic chelates, and various ammine derivatives.

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INTRODUCTION

Diagnosis of cancer at early stages using noninvasive nuclear medicine procedures is highly desirable but so far has shown limited potential because of the lack of suitable radiopharmaceuticals based on radionuclides with optimal physical and chemical properties. Even though gallium-67 citrate is presently the agent of choice, it does not concentrate selectively in tumors, the background levels remain high, and its imaging characteristics are far from ideal. As clinical trials have progressed, it has become evident that gallium-67 citrate concentrates in a variety of normal structures and in several diverse benign and malignant pathologic lesions. This lack of specificity has made the interpretation of gallium-67 scans difficult.

In general, slow kinetics of uptake of the radiopharmaceuticals by cancerous tissue necessitates the use of intermediate half-life nuclides, and for good quality scans, a radiolabel with optimum physical and biological properties is required. Ruthenium-97 is a potential radionuclide for applications in nuclear oncology because of its excellent physical properties -- an essentially monoenergetic gamma emission of 216 keV, and a half-life of 2.9 days -- and the high chemical reactivity of the element ruthenium (1). Because of the relatively high fission yield of ruthenium nuclides, various studies of biological interest have been reported in the literature. Usefulness of various ruthenium nuclides in nuclear medicine has been pointed out by some authors (2); possible application of ruthenium-97 was first suggested by Subramanian et al in 1970 (3). Therapeutic potential of ruthenium compounds has also been described (4,5).

A method was recently developed for an efficient production of clinically useful quantities of ruthenium-97 using the Brookhaven Linac Isotope Producer (BLIP) (6). In an ongoing program at Brookhaven, systematic studies were undertaken to exploit the chemistry of ruthenium for the design of new radiopharmaceuticals for a number of applications in nuclear medicine. A variety of representative agents (some developed and others under active development) is described in Table 1.

A major thrust has recently been on the development of tumor-localizing agents. Several classes of compounds (with ruthenium in various oxidation states) including ionic ruthenium, hydrophilic and lipophilic chelates and a variety of ruthenium-ammine derivatives were prepared using ruthenium tracer, and evaluated in normal and tumor-bearing animals. Several of these compounds have shown antitumor activity in recent NCI animals screens (5). Results with the more promising ruthenium-labeled tumor-localizing agents are reported.

MATERIALS AND METHODS

Most initial investigations were done using ruthenium-103 due to its convenient 39.6 day half-life. Ruthenium-103 chloride was obtained as a carrier-free solution in 3.5N hydrochloric acid from the Oak Ridge National Laboratory. Ruthenium-97 was prepared as needed at the BLIP from proton spallation of high purity (> 99.9%) rhodium foils (6). The target was bombarded with 200-MeV protons from the Linac. After the bombardment, the target was transferred

to a processing hot cell and dissolved by a.c. electrolysis in 6N hydrochloric acid (0.3A/cm^2, ~15 hr). After evaporating the solution to near dryness, radiochemical separation of ruthenium-97 was achieved by distillation of RuO_4 from a sulfuric acid medium in the presence of an oxidizing agent (permanganate). The distillate was collected in ice-cold ethanol/hydrochloric acid (1:1). Recovery of ruthenium from the target was almost quantitative.

The complex-forming reactions were optimized with regard to different variables — total ligand, time of reaction, temperature, pH, etc. In general, due to slow initial kinetics, a heating period of 10 to 30 min at 90° was included in most preparations as a last step. In-vitro analysis of all agents was performed using paper and thin-layer chromatography, gel filtration, cellulose acetate electrophoresis, and spectrophotometry and/or elemental analysis, when appropriate. A typical method of preparation was as follows. To a suitable quantity of the tracer (^{103}Ru or ^{97}Ru, both available in 3.5N HCl) neutralized to pH 2, an appropriate amount of the ligand was added, and following an incubation period of 5-20 min, the pH was raised to between 5 and 7.5. The resulting solution was heated on a boiling water bath for 10-30 min. In some preparations, 0.05-50 mg ruthenium was added to study the carrier effect on biological distribution. The complexes with 1,10-phenanthroline derivatives were prepared by reducing the ruthenium solution with hydroxylammonium chloride in the presence of the ligand, and subsequent heating for 30 min after pH adjustment. Oxine (8-hydroxyquinoline) 7-carboxylic acid acetate was crystallized from a solution of oxine 7-carboxylic acid in acetic acid. Radiosynthesis of potassium pentachloroaquoruthenate(III), potassium tris-oxalatoruthenium(III), chloropentaammineruthenium(III) chloride, cis-dichlorotetraammineruthenium(III)chloride, cis-dichloro bis(ethylenediamine)-ruthenium(III) chloride, and several other ammine complexes was carried out using suitably modified literature procedures (7).

Organ distribution data on the various compounds were obtained in normal mice, rats and hamsters. Biodistribution studies in tumor-bearing animals were carried out in the following tumor models: (i) EMT-6 sarcoma in Balb/c mice (8), (ii) squamous-cell carcinoma in Fisher CDF/female rats (9), and (iii) Greene melanoma in Golden Syrian hamsters (10).

RESULTS

For most ruthenium complexes prepared without added carrier, cellulose acetate electrophoresis in a borate/NaOH/KCl buffer of pH 8 was found to be the analytical method of choice. Paper and thin-layer chromatography also provided good resolution. Gel filtration generally did not give satisfactory separations. The ammine compounds were characterized spectrophotometrically and by elemental analysis.

Blood clearance data on various ruthenium compounds were obtained in normal dogs. Ruthenium chloride (pH 2, a mixture of 3 and 4 oxidation states) clears (Figure 1) from the blood slowly and at 30 minutes after i.v. injection, ca. 40% of the activity remains in circulation. At later time periods, most of the activity remains diffused over various parts of the body and the muscle uptake is quite high (~20% in mice). Upon injection of hydrophilic chelates

such as ruthenium citrate, ruthenium tartrate or ruthenium-DTPA, kidneys are visualized immediately. Blood clearance is very rapid, and within 5-10 minutes, most of the compound leaves the blood via the kidneys and the bladder. Ruthenium phosphate complexes clear rapidly from the blood and localize in bone. Complexes with oxine, phenanthroline, and iminodiacetic acid derivatives such as HIDA and PIPIDA, are extracted by the hepatobiliary system with very little excretion in the urine.

Table 2 describes representative data on the tissue uptake of several ruthenium compounds in EMT-6 sarcoma-bearing mice. The results are expressed as per cent injected dose uptake per g by the tissue; gallium-67 citrate is included for comparison. Data on ruthenium complexes with citrate, tartrate, diethylenetriaminepentaacetic acid (DTPA), ethylenediaminetetramethylene phosphonate (EDTMP), and various phenanthroline derivatives were also obtained but are not described in this table since tumor uptake was insignificant with these compounds. Evaluation of the promising compounds in the other two animal tumor models is presently being carried out. Compounds 2-6 in this table have shown chemotherapeutic potential in recent NCI animal screens (5).

Tumor-to-tissue ratios at various time intervals after injection are shown in Table 3. Values for gallium citrate are also included for comparison. The whole body retention is shown in the last column for all the compounds. Several compounds, as noted, were synthesized using stable ruthenium carrier and the ruthenium dose corresponded to between 2 and 6 mg/Kg body weight. Table 4 describes the tumor concentration index (TCI) of various compounds. This index which provides a useful correlation between the tumor uptake and the mean body concentration is defined as the ratio of per cent injected dose per g of the tumor to per cent injected dose per g remaining in the whole body at any given time period.

DISCUSSION

Ruthenium (Z=44) is a member of the second triad of the group VIII transition metals. It is a highly reactive element and its chemistry is quite complicated. It displays as many as ten oxidation states in its compounds, the common ones being Ru(II), Ru(III), Ru(IV) and Ru(VIII). The high reactiveness of ruthenium and the desirable physical characteristics of ruthenium-97, a pure gamma emitter, provide a valuable combination for application to nuclear medicine. The chemistry of ruthenium can be manipulated much as desired to obtain useful compounds for specific applications. The 2.9 day half-life of ruthenium-97 makes many studies and procedures possible, not otherwise practical with technetium-99m. Efficient and economical production (>100 mCi per day) of ruthenium-97 in the BLIP facility at Brookhaven has been found to be feasible using the rhodium-103(p,2p5n)ruthenium-97 reaction, and this should make widespread use of this nuclide possible subsequent to the development of suitable radiopharmaceuticals.

In the area of tumor-localizing agents, ruthenium-97 appears to show substantial promise. A number of ruthenium coordination compounds have shown antitumor activity in recent animal screens; several of these appear useful as tumor localizing agents when labeled with ruthenium-97. It is likely that dif-

ferent ruthenium agents follow different localization mechanisms and in order to develop successful tumor avid compounds, a whole series of ligands would have to be studied. Mechanistic studies on model compounds have recently been initiated which would undoubtedly aid in the design of more specific agents.

The preliminary results in the EMT-6 sarcoma in mice demonstrate the potential of a number of ruthenium compounds as tumor-scanning agents. The tumor uptake of ruthenium compounds described in Table 2, expressed as per cent dose per gram, ranges between 1.5 and 6.8, and compares favorably with the gallium uptake (3.5-7.1). The general body background of cis-dichlorotetraammine-ruthenium(III) chloride and Ru-oxine 7-carboxylic acid acetate is much lower than that of gallium citrate; the other compounds are comparable to gallium in this respect. The tumor-to-blood (T/B) and tumor-to-muscle (T/M) ratios with several compounds are high enough for delineating tumors by imaging. The uptake of gallium citrate by the tumor reaches a plateau at about 24 hr; thereafter, significant loss of the activity is seen without comparable excretion from the body. This results in a decrease in the value of the tumor concentration index (Table 4). This index reaches a maximum value at different time periods with different compounds. The TCI of seven most active compounds at 24-96 hr ranges between 1.33 and 2.68 and is comparable to or better than that of gallium citrate 1.45-1.81). Using various important criteria, ruthenium-oxine 7-carboxylic acid acetate (TCI max 72 hr 2.68; T/B 2.4; T/M 7.2) and cis-dichlorotetraammineruthenium(III) chloride (TCI max 96 hr 2.0; T/B 4.4; T/M 4.2) look particularly promising when compared to gallium citrate (TCI max 24 hr 1.81; T/B 4.2; T/M 13.4). The other compounds have favorable tumor uptake; however, the blood and body background remains quite high. By exploiting the chemistry of ruthenium, it may be possible to reduce nontarget uptake of these and other compounds and thus improve the tumor-to-background ratios. Chemical modification of the compounds is also expected to increase their tumor specificity thus making them as good or better than gallium citrate. Another approach that presently is being evaluated is the use of mixed-ligand chelating agents (11) or other competitive preparations (12) to selectively reduce the blood and normal tissue background without affecting the tumor uptake. A variety of other ruthenium compounds, in addition to those described here, are presently under investigation.

In summary, results so far on the tumor uptake of various ruthenium compounds have been very encouraging and the potential for developing highly specific tumor-localizing agents based on ruthenium-97 appears very great. Use of this nuclide could represent a distinct advantage over most presently used radioscintigraphic agents for tumor localization.

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TABLE 1

RADIOLABELED RUTHENIUM COMPOUNDS AND THEIR POTENTIAL APPLICATIONS IN NUCLEAR MEDICINE

Compounds	Application
Simple salts (ionic)	
Ruthenium chloride	
Potassium tris-oxalatoruthenium(III)	Tumor localization;
Potassium pentachloroaquoruthenate(III)	myocardial agents
Hydrophilic chelates	
Tartrate	Kidney agents;
Citrate	tumor localization;
DTPA	cisternography
Glucoheptonate	
Lipophilic chelates	
8-hydroxyquinoline(oxine)	Hepatobiliary
Oxine 7-carboxylic acid	agents; labeling cells
Oxine 7-carboxylic acid acetate	and platelets, etc.; study
1,10-phenanthroline derivatives	of cellular transport
β-diketones	mechanisms; brain perfusion;
Lidocaine iminodiacetic acid(HIDA) and analogs	tumor localization
Dimethylsulfoxide	
Phosphate compounds	
Pyrophosphate	Bone agents (for normal
EHDP	uptake and for physiological
EDTMP	studies); agents for
MDP	myocardial-infarct localization
Ammine compounds	
Chloropentaammineruthenium(III)chloride	Tumor localization
cis-Dichlorotetraammineruthenium(III) chloride	
cis-Dichlorobis(ethylenediamine)-ruthenium(III) chloride	
Trichlorotriammineruthenium(III)	
Ammineruthenium(III) complexes	
Purines, pyrimidines, nucleosides, nucleotides	Tumor localization
Colloidal Preparations	
Ruthenium-sulfur colloid	Lymphoscintigraphy

TABLE 2. TISSUE UPTAKE OF VARIOUS PROMISING RUTHENIUM COMPOUNDS AND OF GALLIUM-67 CITRATE IN EMT-6 SARCOMA-BEARING MICE (% DOSE PER G)[a] (n=6)

Compound	Time (hr)	Tumor	Blood	Muscle	Liver	Kidney	Whole Body Retention (% Dose)
1. Ruthenium chloride	24	5.42	4.27	1.57	6.11	12.24	55.25
	48	6.10	3.20	1.15	9.17	10.48	51.67
2. $K_2[RuCl_5(H_2O]$[b]	24	6.76	5.46	2.06	10.89	11.76	61.86
3. $K_3[Ru(Ox)_3]$ X H_2O[c]	24	5.97	3.81	2.03	5.22	6.32	57.77
4. $[Ru(NH_3)_5Cl]Cl_2$[d]	24	3.29	1.97	0.79	2.37	5.89	25.70
	96	2.30	0.67	0.80	1.63	3.81	17.40
5. cis-$[Ru(NH_3)_4Cl_2]Cl$[e]	24	5.38	5.18	1.54	5.12	9.23	49.61
	72	5.93	2.10	1.32	4.14	7.21	43.80
	96	4.92	1.15	1.20	3.23	5.35	37.06
6. cis-$[Ru(en)_2Cl_2]Cl$[f]	24	5.97	5.62	1.69	5.76	11.15	52.80
7. Ru-Oxine 7-CA acetate[g]	24	2.38	2.34	0.39	3.03	2.88	19.01
	72	1.68	0.72	0.24	1.49	1.46	9.61
	96	1.48	0.47	0.21	1.54	1.38	8.42
8. Gallium-67 citrate	24	7.09	1.81	0.53	9.30	9.21	59.58
	48	5.05	0.51	0.47	9.49	8.03	50.68
	96	3.54	0.36	0.37	8.19	7.14	38.29

[a] Compounds 2-6 were synthesized using stable ruthenium carrier; ruthenium dose 2-6 mg/Kg body weight

[b] Potassium pentachloroaquoruthenate(III)

[c] Potassium tris-(oxalato)ruthenium(III)

[d] Chloropentaammineruthenium(III) chloride

[e] cis-Dichlorotetraammineruthenium(III) chloride

[f] cis-Dichloro bis(ethylenediammine)ruthenium(III) chloride

[g] 8-Hydroxyquinoline 7-carboxylic acid acetate

TABLE 3. TUMOR-TO-BLOOD AND TUMOR-TO-TISSUE RATIOS OF VARIOUS RUTHENIUM-103-LABELED COMPOUNDS IN EMT-6 SARCOMA BEARING MICE (n=6)

Compound[a]	Time Post Injection, Hr	Ratio, Tumor to Blood	Muscle	Liver	Kidney
Gallium-67 citrate	24	4.2	13.4	0.8	0.8
	48	10.7	11.0	0.6	0.6
	96	10.3	10.6	0.4	0.5
Ruthenium chloride	24	1.0	2.9	0.6	0.4
	48	1.9	5.3	0.7	0.6
$[Ru(NH_3)_5Cl]Cl_2$	24	1.7	4.2	1.4	0.6
	48	1.9	4.5	1.2	0.5
	96	3.5	4.1	1.4	0.6
cis-$[Ru(NH_3)_4Cl_2]Cl$	24	1.1	3.5	1.1	0.6
	48	1.7	4.0	1.3	0.7
	96	4.4	4.2	1.5	0.9
$K_2[RuCl_5(OH_2)]$	24	1.3	3.3	0.6	0.6
$K_3[Ru(Ox)_3]$	24	1.6	3.0	1.1	0.9
cis$[Ru(en)_2Cl_2]Cl$	24	1.1	3.6	1.1	0.5
Ru-oxine 7-CA Acetate	24	1.0	6.3	0.8	0.8
	48	1.7	7.2	1.0	1.0
	96	3.2	7.7	1.0	1.2

[a]For abbreviation and other explanations, see Table 2

TABLE 4. TUMOR CONCENTRATION INDEX[a] OF VARIOUS RUTHENIUM-103-LABELED COMPOUNDS IN EMT-6 SARCOMA (Balb/c mice)

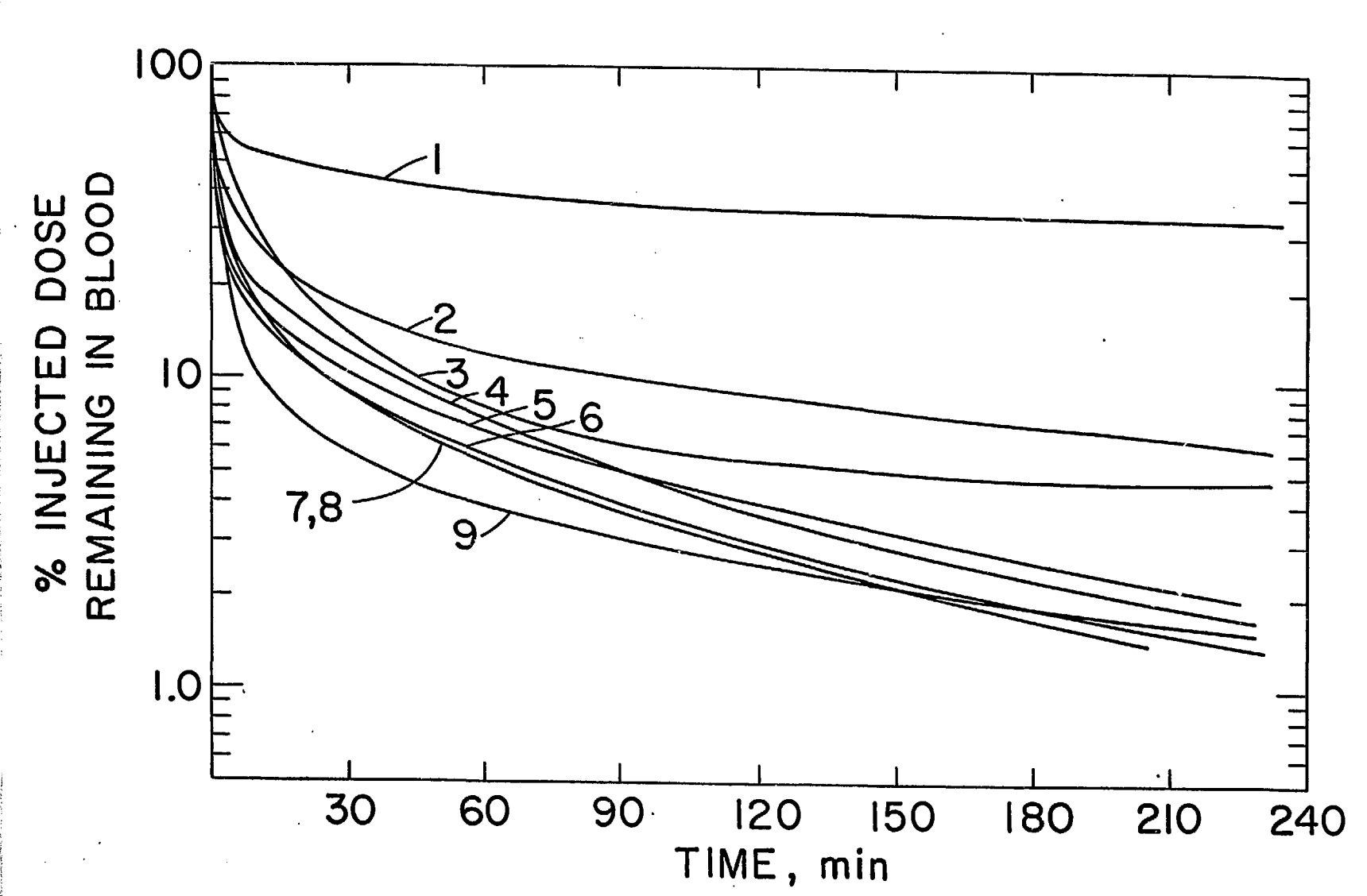
Compound[b]	TCI 24 hr	48 hr	72 hr	96 hr
Ru-Oxine 7-CA acetate	1.90	2.39	2.68	2.50
Gallium-67 citrate	1.81	1.48	1.57	1.45
cis-$[Ru(en)_2Cl_2]Cl$	1.70	-	-	-
$[Ru(NH_3)_5Cl]Cl_2$	1.70	1.46	1.37	1.49
$K_2[RuCl_5(OH_2)]$	1.65	-	-	-
$K_3[Ru(Ox)_3]$	1.64	-	-	-
cis-$[Ru(NH_3)_4Cl_2]Cl$	1.56	1.74	2.03	2.00
Ruthenium chloride	1.33	1.86	-	-

[a]Tumor concentration index (TCI) is defined as the ratio of per cent injected dose per g of the tumor to per cent injected dose per g remaining in the whole body at any given time period. For inter-species comparisons, and for normalizing for different animal body weights, TCI could be expressed as per Kg body wt.

[b]For abbreviations and other explanations, see Table 2

FIGURE LEGEND

FIG.1. Blood clearance of several ruthenium complexes in dogs. Technetium-99m-DTPA and technetium-99m-MDP are included for comparison. (1) Ruthenium chloride, pH 2; (2) Ru-EHDP; (3) Ru(II)tris-(3,4,7,8-tetramethyl 1,10-phenanthroline)dichloride; (4) Technetium-99m-DTPA; (5) Ru-citrate; (6) Ru-EDTMP; (7) Ru-MDP; (8) Technetium-99m-MDP; (9) Ru-DTPA.


100
10
1.0
% INJECTED DOSE
REMAINING IN BLOOD
1
2
3
4
5
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9
30
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180
210
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TIME, min