Tricatecholamide Analogs of Enterobactin as Gallium- and Indium-Binding Radiopharmaceuticals

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Isopropyl N-substituted tricatecholamide analogs of enterobactin have been found to form gallium and indium complexes with very high stability constants and to exhibit in vivo characteristics significantly different from gallium- or indium-transferrin and EDTA. The 3,4-DiP-LICAMS and TiP-MECAMS complexes were found to clear primarily through the kidneys, whereas the less polar 3,4-DiP-LICAM complex was eliminated through the liver. The rationale for developing new metal-binding analogs with larger organic groups attached to the amide nitrogens is discussed.

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Enteric bacteria such as *Escherichia coli* and *Salmonella typhimurium* are known to synthesize iron-chelating agents of low molecular weight, called siderophores, to satisfy their nutritional demands for iron (*1,2*). Enterobactin is a siderophore and is the most powerful ferric-ion-chelating agent known (*3,4*). Although its high log K of 52 is able to solubilize ferric iron and facilitate the transport of the metal into cells, enterobactin is a cyclic ester that hydrolyzes readily at physiological pH, and hence is not useful as a metal-complexing radiopharmaceutical. Development of 2,3-dihydroxybenzoylamide analogs of enterobactin has led to iron-binding ligands characterized by both high stability constants and resistance to hydrolysis in solution (*5,6*). These synthetic tricatecholate ligands are also kinetically and thermodynamically capable of removing iron from transferrin under physiological conditions (*7*), as well as from the iron-storage protein ferritin in the presence of ascorbic acid.

Nuclides of gallium and indium have found widespread use in nuclear medicine (*8*). Because these are Group III B elements with charges and complexing characteristics similar to those of ferric ion (*9,10*), we have investigated the applicability of synthetic tricatecholamide enterobactin analogs as gallium- and indium-binding radiopharmaceuticals. The three ligands studied are (a) 3,4-DiP-LICAMS [N,N″-bis(isopropyl)-N,N′,N″- tris(5-sulfo-2,3-dihydroxybenzoyl)-1,5,10-triazadecane]; (b) 3,4-DiP-LICAM [N,N″-bis(isopropyl)-N,N′,N″-tris (2,3-dihydroxybenzoyl)-1,5,10-triazadecane]; and (c) TiP-MECAMS [1,3,5-N,N′,N″-tris(isopropyl)-N,N′,N″-tris-(5-sulfo-2,3-dihydroxybenzoyl)triaminomethylbenzene] (Fig. 1). 3,4-DiP-LICAMS and TiP-MECAMS were studied to see whether the flexibility of the linkage between the three catecholamide groups of the former ligand would show biological behavior differing from that of TiP-MECAMS with its more constrained platform structure. The sulfonic acid groups attached to the aromatic rings were expected to alter the pharmacological characteristics of the complexes, since sulfonation increases the acidity of the phenolic oxygens, the stability to oxidation, and the water-solubility of catechols. The isopropyl substitution on the amide nitrogens was expected to decrease water-solubility and increase lipophilicity.

MATERIALS AND METHODS

Determination of stability constants. Stability constants for these ligands with gallium and indium were determined. Each ligand was synthesized, characterized,

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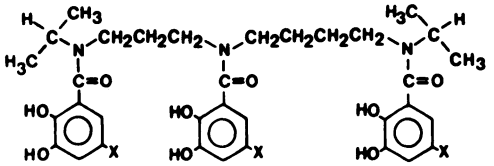
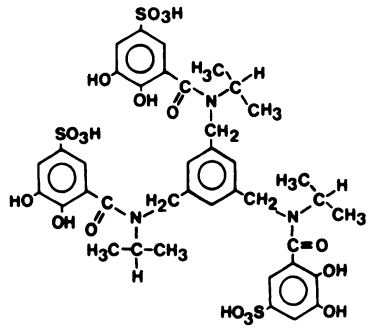


X=H: 3,4 DiP-LICAM

X=SO_3H: 3,4 DiP-LICAMS



TiP-MECAMS

FIG. 1. Chemical structures of tricatecholamide analogs of enterobactin.

and analyzed for purity according to reported techniques (*11*), and the stability constants of the gallium complexes were determined using Ga-67 in competitive exchange between the ligand and EDTA. Gallium-67 citrate was added to 0.1 *M* citrate buffer containing catecholate ligand and EDTA in varying ratios, and the pH adjusted to 7.0 using 1 *M* citrate. From sampling studies up to 10 days after mixing, it was found that exchange equilibrium was achieved within 48 hr. Therefore, after 48 hr of equilibration in sealed Pyrex test tubes at room temperature, the Ga-67 catecholamide and Ga-67 EDTA products that formed were separated by gel-permeation chromatography using a Sephadex G-10 column (1 × 145 cm) eluted with 0.075 *M* Tris-acetate buffer (pH 7.0). Fractions were collected (accounting for 95 ± 5% of the administered activity) and counted in a NaI(Tl) well counter, and the relative concentrations of Ga-67 EDTA and Ga-67 catecholamide were determined from the elution volumes for each complex. From the mid-exchange concentration ratio (the ligand concentration ratio that gave equal yields of Ga-67 EDTA and Ga-67 catecholamide), the stability constants were calculated.

In these competitive equilibrium experiments, citrate buffer was used because this organic acid forms soluble Ga^{3+} complexes up to pH 8 without hydrolysis to form a gallium hydroxide precipitate (*12,13*). Sodium citrate is necessary to maintain constant pH as well as ionic strength, so that activity coefficients can be ignored in the calculation of formation constants. In addition, the log stability constant for Ga citrate is equal to 10.02 (*14*), which is low enough so as not to interfere with the equilibria of the exchange processes occurring between Ga-EDTA (log K = 20.3) (*14*) and ligands having even greater gallium-binding affinity. Complete absence of Ga-67 citrate was seen in the gel filtration of all solutions studied, including those of Ga-67 catecholamide used separately to determine elution volumes.

The stability constants of the indium complexes were determined by Sephadex G-10 separation after competitive equilibration of In-111 in a solution of EDTA and ligand, as was done with the Ga-67 complexes. In addition, the decay characteristics of In-111 allow the use of perturbed angular correlation (PAC) experiments to study the complexing of this metal. This second method of analysis was used to study the agreement of the results from this technique with those obtained from gel-permeation chromatography, where exchange on the column may occur. The integral perturbation factor $\overline{\langle G_{22}(\infty)\rangle}$ is related to the rotational correlation times of the environment of the In-111 nucleus, such that a lower $\overline{\langle G_{22}(\infty)\rangle}$ indicates a slower rotational correlation time (*15,16*). The $\overline{\langle G_{22}(\infty)\rangle}$ was determined during complex formation using equipment described earlier (*17,18*). Indium-111-tagged 3,4-DiP-LICAMS, 3,4-DiP-LICAM, and TiP-MECAMS were studied by adding $^{111}InCl_3$ to 0.1 *M* citrate solutions (pH 7.0) containing various ratios of EDTA to catecholamide. The point of mid exchange was found from a plot of $\overline{\langle G_{22}(\infty)\rangle}$ against concentration ratio (Fig. 3) and the stability constants were calculated as in the gel-permeation separations.

Biodistribution data. Biological distributions for the three gallium complexes were determined using 150-g Sprague-Dawley rats with 10–20 μCi of the respective Ga-67 complex injected into the femoral vein. The distribution of In-111 3,4-DiP-LICAMS was studied and Ga-67 citrate was used for purposes of comparison. The animals were killed in groups of three or more at various times after injection (1,3,5,18, and 24 hr) and the major organs were removed, weighed, counted, and the percent injected dose/gram of tissue calculated.

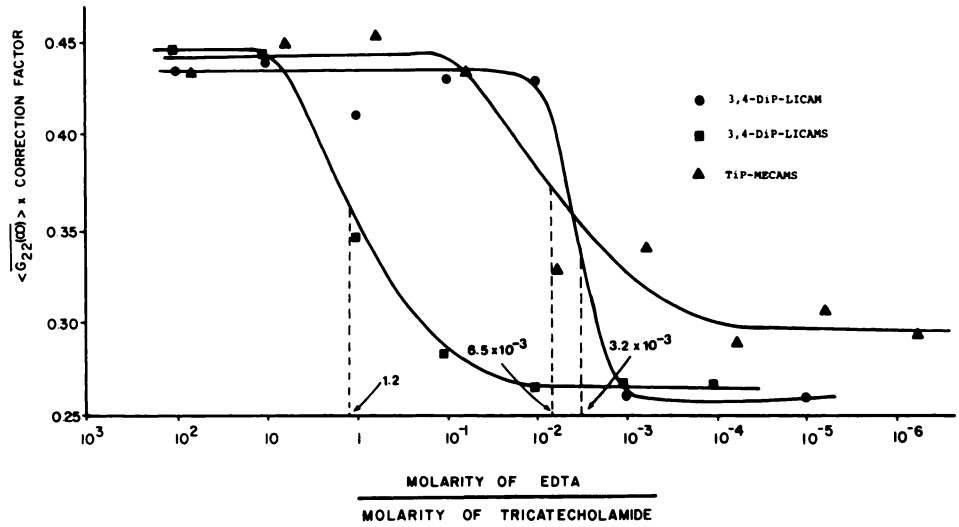


FIG. 2. Variation of integral perturbation factor of In-111 as a function of total ligand concentration ratio (in 0.1 *M* citrate, pH 7.0).

Biological clearance data. To investigate further the pharmacokinetics of these complexes in the rat, clearance data were collected based on the techniques of Konikowsky et al. (*19*). Male 150-g Sprague-Dawley rats underwent penile ligation, and 10–20 μCi of Ga-67-labeled EDTA, citrate, or tricatecholate were injected into the femoral vein. The animals were then killed, in groups of at least three, at 10, 20, 30, 60, and 120 min after injection. Blood concentration was determined for each time, and the cumulative urinary activity was found by quantitatively removing the intact bladder and contents. Similarly, the kidneys, liver, and intestines (plus contents) were removed and counted to give the cumulative activity per organ.

RESULTS

Determination of stability constants. The region of incomplete exchange between Ga-67 or In-111-labeled EDTA and catecholamide occurred sharply (within a range of log [EDTA]/[ligand] = ±1), so the concentration ratio at which mid exchange had been reached was easily found. This was true for the PAC exchange process, as seen by the relatively sharp change from $\overline{\langle G_{22}(\infty)\rangle} \cong 0.44$ for In-111 EDTA to $\overline{\langle G_{22}(\infty)\rangle} \cong 0.27$ for the In-111-labeled ligand (Fig. 2). At concentration ratios of EDTA-to-ligand greater or less than that of the exchange region, the $\overline{\langle G_{22}(\infty)\rangle}$ shows a flat response corresponding to either the In-111 EDTA or In-111 catecholate values. Similar exchange curves were found using the gel-permeation data, as seen in Fig. 3 for the In-111 case and Fig. 4 for the Ga-67 case.

Our value for $\overline{\langle G_{22}(\infty)\rangle}$ of In-111 EDTA (0.44 ± 0.02) differs from others reported in the literature for EDTA and its derivatives: $\overline{\langle G_{22}(\infty)\rangle}$ = 0.58 ± 0.02

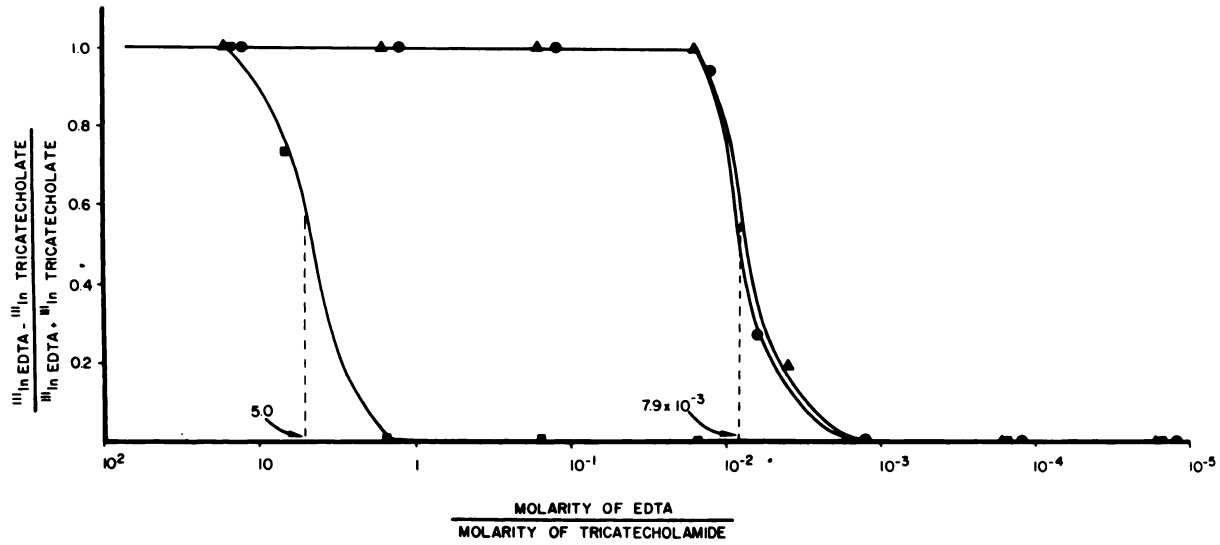


FIG. 3. Variation of In-111 complex concentration as a function of total ligand concentration ratio (in 0.1 *M* citrate, pH 7.0) as determined by Sephadex G-10 gel-permeation chromatography.

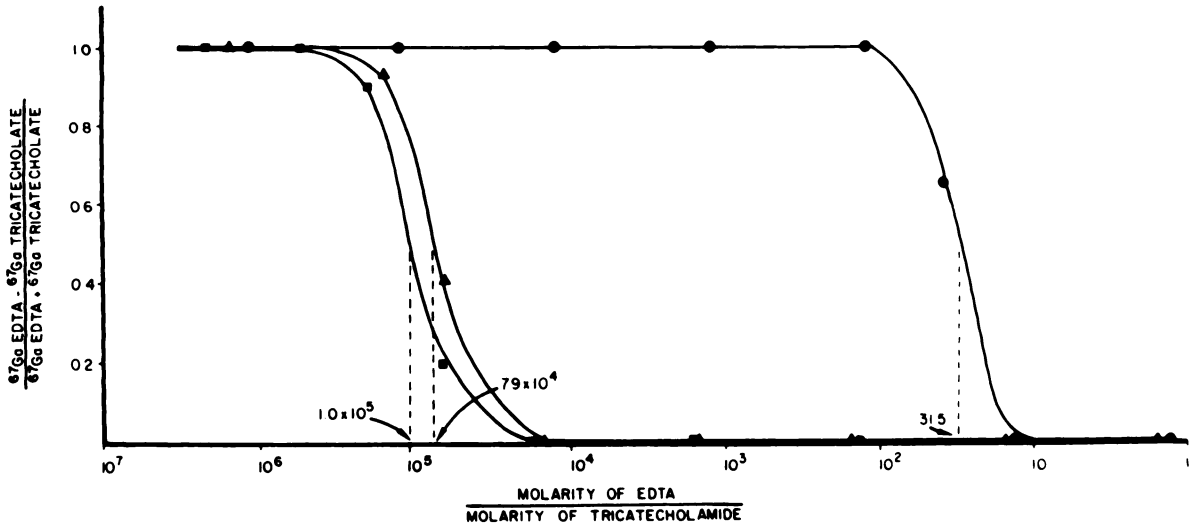


FIG. 4. Variation of Ga-67 complex concentration as a function of total ligand concentration ratio (in 0.1 *M* citrate, pH 7.0) as determined by Sephadex G-10 gel-permeation chromatographv.

(*20*,*21*) and 0.75 ± 0.02 (*22*). It is not certain whether these discrepancies are due to alterations in the pH of the In-111 EDTA solution or to differences in instrument calibration. In either case, what is important in this study is not the absolute value of $\overline{\langle G_{22}(\infty)\rangle}$, but rather the *change* from the $\overline{\langle G_{22}(\infty)\rangle}$ of In-111 EDTA to that of In-111 tricatecholate, for it is the ligand concentration ratio at this point that is used to calculate the indium-ligand formation constants. That our $\overline{\langle G_{22}(\infty)\rangle}$ for In-111 EDTA or In-111 tricatecholate is significantly different from those of In-111 citrate (0.41 ± 0.02) and $^{111}InCl_3$(0.10 ± 0.02) in this buffer solution allows us to conclude, with internal consistency, that we are actually measuring the exchange of In-111 from EDTA to the enterobactin analog.

The gel-permeation and PAC experiments were done in terms of the ratio R of *total* ligand concentrations. Since the tricatecholamide compounds are weak acids, a correction must be made for the fact that only a fraction of the ligands will be deprotonated and hence able to complex with gallium or indium cations. The exchange equilibrium involved at pH 7 is

$$ME^- + H_3L^{3-} \rightleftarrows ML^{3-} + E^{4-} + 3H^+,$$

where M is a gallium(III) or indium(III) ion, E^{4-} is hexadentate EDTA, and H_3L^{3-} is the triprotonated catecholamide species. E^{4-} at pH 7.0 can be calculated from the acidity constants of EDTA (pk_a = 0.0, 1.5, 2.0, 2.68, 6.11, 10.17) (*14*) to be equal to 3.18×10^{-4} $[E]_t$, the total concentration of EDTA species in solution. The distribution coefficient for the above exchange reaction is

$$K_x = \frac{[ML^{3-}][E^{4-}][H^+]^3}{[ME^-][H_3L^{3-}]} = \frac{K^*}{K_{ME^-}},$$

where K_{ME^-} is the formation constant for Ga-EDTA or In-EDTA, and K* is the proton-dependent stability constant of the catecholamide complex, given by

$$K^* = \frac{[ML^{3-}][H^+]^3}{[M^{3+}][H_3L^{3-}]}. \qquad (5)$$

Since K* has a third-order dependence on hydronium

TABLE 1. LOG STABILITY CONSTANTS FOR TRICATECHOLAMIDE ANALOGS OF ENTEROBACTIN*

Complex	Method	log K*	log K_f
In-111 3,4-DiP-LICAMS (n = 3)	PAC	5.1(4.0–6.2)	41.4(40.2–42.6)
In-111 3,4-DiP-LICAM (n = 3)		2.5(1.4–3.6)	38.8(37.6–40.0)
In-111 TiP-MECAMS (n = 3)		2.8(1.7–3.9)	39.1(37.9–40.3)
In-111 3,4-DiP-LICAMS (n = 2)	Gel permeation	5.7(4.6–6.8)	42.0(40.8–43.2)
In-111 3,4-DiP-LICAM (n = 2)		2.9(1.8–4.0)	39.2(38.0–40.4)
In-111 TiP-MECAMS (n = 2)		2.9(1.8–4.0)	39.2(38.0–40.4)
Ga-67 3,4-DiP-LICAMS (n = 2)	Gel permeation	5.8(4.4–7.2)	42.1(40.6–43.6)
Ga-67 3,4-DiP-LICAM (n = 2)		2.3(0.9–3.7)	38.6(37.1–40.1)
Ga-67 TiP-MECAMS (n = 2)		5.7(4.3–7.1)	42.0(40.5–43.5)

*In 0.1 *M* citrate pH 7.

TABLE 2. BIODISTRIBUTION OF Ga-67 3,4-DIP-LICAMS*

% I.D. per gram	Time after Injection (hr) 1	3	5	18	24
Blood	0.30(0.25–0.35)	0.27(0.20–0.34)	0.24(0.16–0.32)	0.02(0.01–0.03)	0.01(0.00–0.02)
Heart	0.17(0.15–0.19)	0.13(0.12–0.14)	0.13(0.11–0.15)	0.03(0.02–0.04)	0.04(0.03–0.05)
Lungs	0.27(0.26–0.28)	0.21(0.17–0.25)	0.18(0.13–0.23)	0.04(0.02–0.06)	0.06(0.05–0.07)
Liver	0.28(0.25–0.31)	0.42(0.34–0.50)	0.37(0.31–0.43)	0.18(0.12–0.24)	0.24(0.20–0.28)
Spleen	0.11(0.09–0.13)	0.16(0.11–0.21)	0.19(0.15–0.23)	0.05(0.04–0.06)	0.06(0.05–0.07)
Kidneys	17.56(14.68–20.44)	22.16(17.28–27.04)	13.91(12.95–14.87)	8.74(6.38–11.10)	11.14(6.47–15.81)
Muscle	0.08(0.04–0.12)	0.08(0.07–0.09)	0.07(0.06–0.08)	0.02(0.01–0.03)	0.03(0.02–0.04)
Bone	0.15(0.13–0.17)	0.22(0.15–0.29)	0.25(0.22–0.28)	0.01(0.00–0.02)	0.05(0.04–0.06)
Brain	0.01(0.00–0.02)	0.01(0.00–0.02)	0.01(0.00–0.02)	0.00	0.01(0.00–0.02)

* In 150-g Sprague–Dawley rats (n = 3 or more).

ion concentration, it is not generally useful for comparison with other complex stability constants. The acidity constants for these tricatecholamide ligands may be estimated from the bidentate analog N,N-dimethyl-2,3-dihydroxybenzamide (pk_a = 8.4, 12.1) (*5*).
From

$$K_{a1}\cdot K_{a2}\cdot K_{a3} = \frac{[H^+]^3[H_3L^{3-}]}{[H_6L]},$$

$$(10^{-8.4})^3 = (10^{-7})^3\frac{[H_3L^{3-}]}{[H_6L]},$$

$$[H_6L] = 10^{4.2}[H_3L^{3-}].$$

Since

$$K^* = \frac{[ML^{3-}][E^{4-}][H^+]^3}{[ME^-][H_3L^{3-}]}\cdot K_{ME^-}$$

and $[H^+] = 10^{-7}$, and at mid exchange $[ML^{3-}] = [ME^-]$, we substitute to obtain:

$$K^* = 10^{-21}\frac{[E^{4-}]}{[H_3L^{3-}]}\cdot K_{ME^-}$$

$$= 10^{-20.3}\frac{[E]_t}{[L]_t}\cdot K_{ME^-},$$

where $[E]_t$ and $[L]_t$ are the total concentrations of all EDTA and catecholamide species, respectively.

Similarly, from

$$K_4K_5K_6 = \frac{[H^+]^3[L^{6-}]}{[H_3L^{3-}]},$$

$$(10^{-12.1})^3 = (10^{-7})^3\frac{[L^{6-}]}{[H_3L^{3-}]},$$

$$[H_3L^{3-}] = 10^{15.3}[L^{6-}];$$

and the usual (proton-independent) formation constant

$$K_f = \frac{[ML^{3-}]}{[M^{3+}][L^{6-}]}$$

can be calculated from K* as

$$K^* = \frac{[ML^{3-}][H^+]^3}{[M^{3+}][H_3L^{3-}]},$$

TABLE 3. BIODISTRIBUTION OF Ga-67 CITRATE*

% I.D. per gram	Time after Injection (hr) 1	3	5	18	24
Blood	2.23(1.85–2.61)	1.42(1.27–1.57)	1.27(1.11–1.43)	0.30(0.28–0.32)	0.21(0.19–0.23)
Heart	0.80(0.75–0.85)	0.73(0.67–0.79)	0.57(0.49–0.65)	0.26(0.24–0.28)	0.26(0.21–0.31)
Lungs	0.98(0.83–1.13)	0.86(0.77–0.95)	0.76(0.45–1.07)	0.36(0.31–0.41)	0.30(0.27–0.33)
Liver	0.75(0.44–1.06)	1.08(0.96–1.20)	1.54(0.98–2.10)	1.32(1.12–1.52)	1.90(1.81–1.99)
Spleen	0.58(0.52–0.64)	0.98(0.90–1.06)	1.16(0.80–1.52)	1.75(1.17–2.33)	1.88(0.93–2.83)
Kidneys	0.83(0.74–0.92)	0.83(0.81–0.85)	0.87(0.64–1.10)	0.84(0.80–0.88)	0.82(0.79–0.85)
Muscle	0.23(0.13–0.33)	0.34(0.28–0.40)	0.23(0.21–0.25)	0.10(0.09–0.11)	0.08(0.05–0.11)
Bone	0.95(0.50–1.40)	1.35(1.23–1.47)	1.42(0.96–1.88)	2.44(2.18–2.70)	2.65(2.35–2.95)
Brain	0.10(0.07–0.13)	0.08(0.06–0.10)	0.06(0.03–0.09)	0.04(0.03–0.05)	0.04(0.03–0.05)

* In 150-g Sprague–Dawley rats (n = 3 or more).

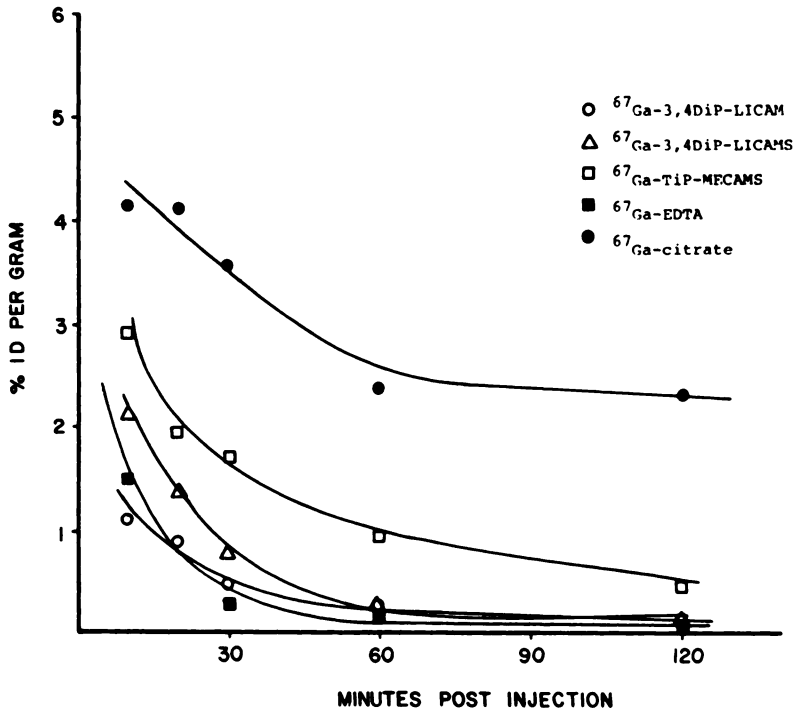


FIG. 5. Blood concentrations (in rat) of Ga-67-labeled complexes as a function of time.

$$= \frac{[ML^{3-}](10^{-7})^3}{[M^{3+}]10^{15.3}[L^{6-}]},$$

$$= \frac{[ML^{3-}]}{[M^{3+}][L^{6-}]} \cdot 10^{-36.3}$$

or $K_f = 10^{36.3}$ K* at pH 7.0. Table 1 lists the K* values—based on published values for the formation constants of Ga-EDTA (log K_1 = 21.1) and In-EDTA (log K_1 = 25.3) (*23*)—and the corresponding K_f values.

Biodistribution data. The tissue distributions in the rat for Ga-67 3,4-DiP-LICAMS are shown in Table 2. Results for In-111 3,4-DiP-LICAMS and Ga-67 TiP-MECAMS were the same within experimental error. The biodistribution for Ga-67 3,4-DiP-LICAM was also very similar, with the exception that the renal accumulation of activity never exceeded 6% of the injected dose per gram. Table 3 shows the results for Ga-67 citrate, studied as control. Each of the values represents the

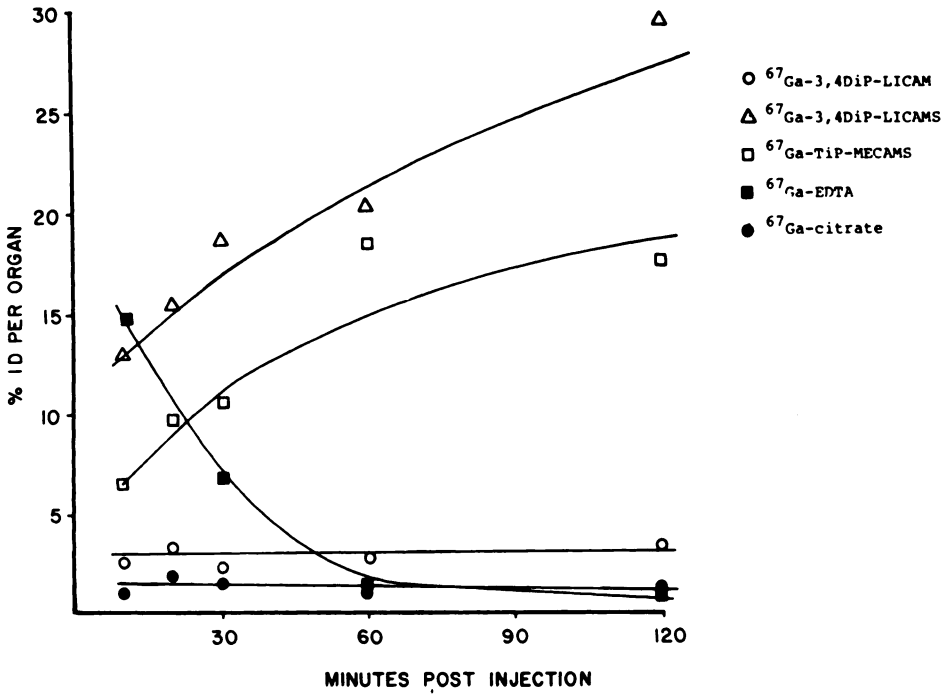


FIG. 6. Renal clearance curves of Ga-67-labeled complexes, in rat.

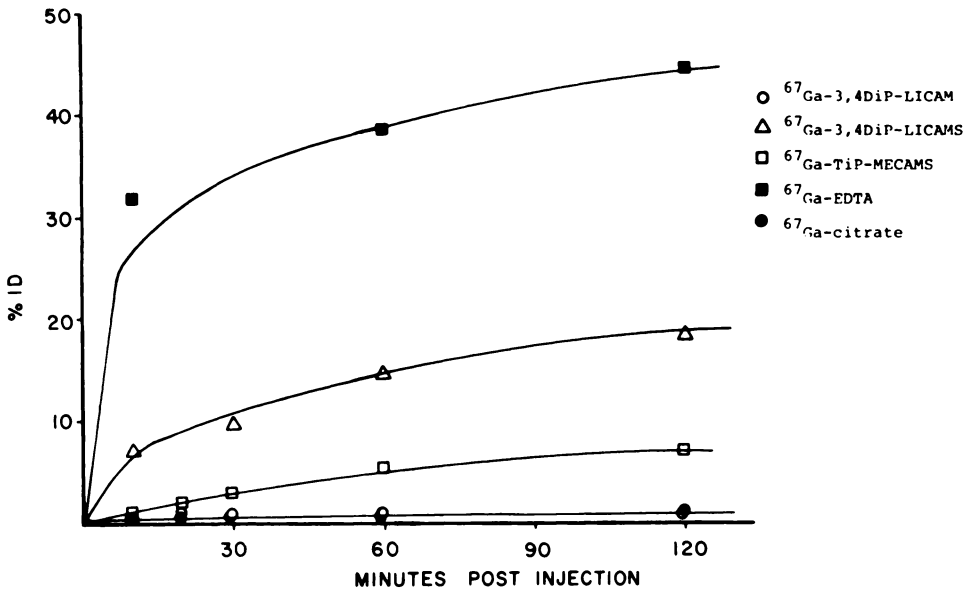


FIG. 7. Cumulative bladder activity curves for Ga-67-labeled complexes, in rat.

mean for at least three animals. As shown by these data, the in vivo distributions for all four tricatecholamide complexes differ markedly from the citrate complex. Whereas Ga-67 citrate (Table 3) shows high concentrations of activity in the blood, liver, and bone at 24 hr, the enterobactin analogs show significant decreases with time in these tissues. In vivo exchange of the Ga-67 complexes to form Ga-67 transferrin does not seem to be occurring, as is consistent with the high log K_f for the tricatecholate complexes.

Biological clearance data. Figure 5 shows the blood activity concentrations in rat as a function of time after injection. The points represent means of data obtained from three or more animals and indicate results for the three Ga-67 tricatecholate complexes as well as for Ga-67 citrate and Ga-67 EDTA. Blood activity is seen to decline faster for these synthetic complexes than for the citrate (thereby showing their in vivo integrity), but the clearance of the sulfonated complexes is slower than for Ga-67 EDTA.

Renal uptake and urinary excretion are shown in Figs. 6 and 7, respectively, while the liver and intestinal activity curves are shown in Figs. 8 and 9.

As seen in Fig. 8, the liver activity clears rapidly only for the unsulfonated Ga-67 3,4-DiP-LICAM complex. Ga-67 TiP-MECAMS shows a slight increase in liver activity with time, whereas the remaining complexes seem to clear from this organ as a function of the blood activity concentration. The elimination of Ga-67 3,4-DiP-LICAM from the liver into the intestines is seen by comparison of Fig. 9 with Fig. 8. The sharp rise in intestinal radioactivity corresponds closely with the rate of liver clearance. The relatively stable intestinal activity of Ga-67 TiP-MECAMS suggests that some of this complex also may be excreted through a hepatic route.

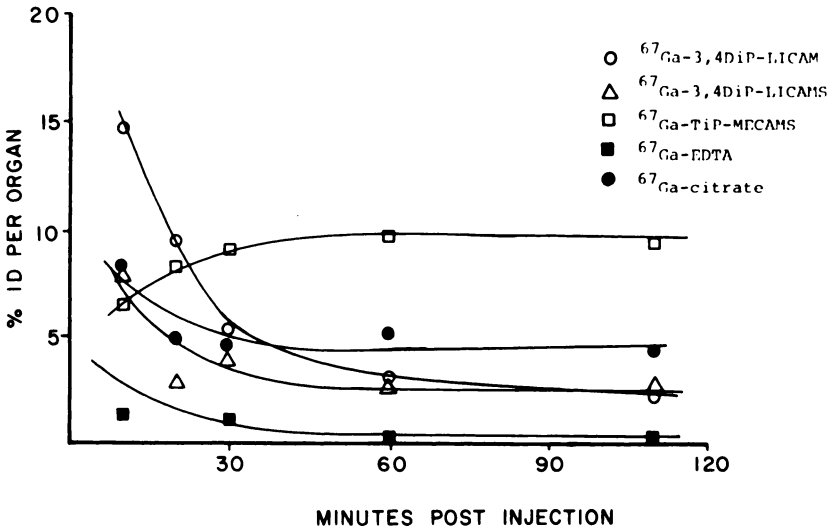


FIG. 8. Liver clearance curve for Ga-67-labeled complexes, in rat.

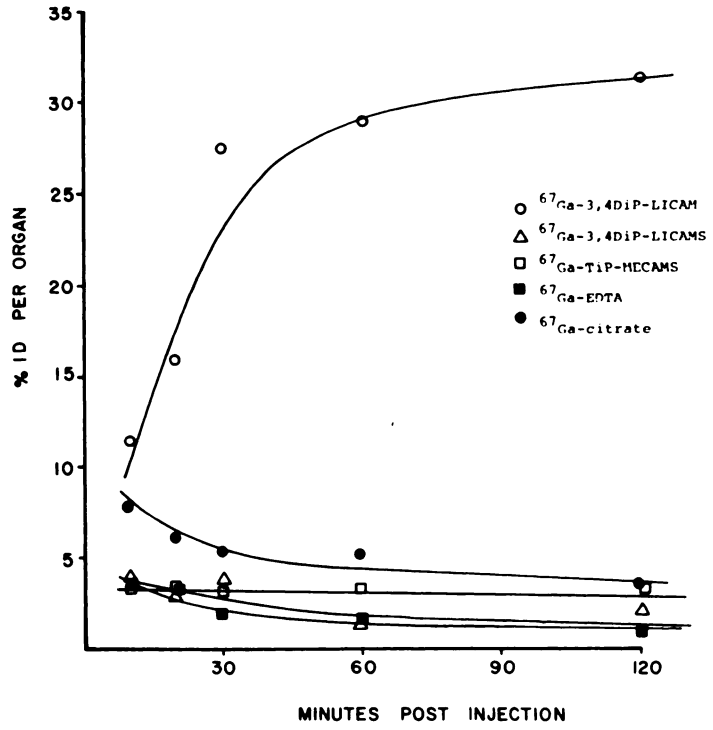


FIG. 9. Cumulative intestinal activity (in rat) for Ga-67-labeled complexes as a function of time.

DISCUSSION

As can be seen in Table 1, the log K_f values are very high, especially when compared with the formation constants of gallium- or indium-transferrin (log K_1 = 23.56 and 30.49, respectively) (*24*). Note that the sulfonated ligands complex gallium with greater affinity than the nonsulfonated LICAM derivative, whereas in the case of indium, only the 3,4-DiP-LICAMS derivative shows increased stability relative to 3,4-DiP-LICAM. This may be a result of steric hindrance caused by the additional isopropyl group of TiP-MECAMS, the effect of which is augmented in the case of the larger indium(III) cation. The sulfonation of 3,4-DiP-LICAMS increases K_f by two log units for both gallium and indium, showing how complex stability may be affected by the acidity of the aromatic hydroxyls. The very close similarity between the formation constants of gallium(III) and indium(III), which differ significantly in ionic radius (r_{Ga}^{3+} = 62 pm; r_{In}^{3+} = 81 pm), suggests that these ligands have a large degree of flexibility in coordinating cations with differing radii. The relaxation of steric constraints may also be because in "hard-hard" coordinative interactions (*25*), the stability of a complex results primarily from a favorable *entropy* change rather than *enthalpy* stabilization (*26*). There is good agreement between the PAC results and those of gel-permeation chromatography, implying that there are no gross artifacts being introduced by the latter analytical procedure.

In the biodistribution studies, the sulfonated complexes show high kidney localization and a steady drop in blood concentration with time, while Ga-67 3,4-DiP-LICAM shows low blood activity at 24 hr without concomitant renal accumulation. Thus, although sulfonation of the ligands does not dramatically alter the stability of the complex relative to competitive biological ligands, the radiopharmacologic properties are altered to a significant extent. A further comment directed to the structure-localization relationships of these complexes is that the Ga-67 3,4-DiP-LICAM complex shows no brain uptake, whereas cerebral localization would be expected in the case of a very lipophilic complex that is able to partition across the blood-brain barrier into the CNS. In view of previous work with ferric complexes (*5*), the gallium tricatecholates are probably of the form HML^{2-} at physiological pH and are therefore unable to diffuse into brain tissue because they are not neutral species (*27,28*). Apparently the isopropyl substitution on the amide nitrogen does not increase the net lipophilic character of the complex enough for entry into the central nervous system. N-substituted ligands having larger organic groups attached—such as 3,4-di(benzyl)-LICAM and 3,4-di(decyl)-LICAM—are currently being investigated with the goal of increasing the lipophilicity of these biologically stable gallium and indium complexes.

In the studies carried out to determine urinary excretion, Ga-67 EDTA shows the expected rapid drop in renal radioactivity as the complex is excreted into the bladder, which shows a cumulative activity of about 45% of the injected dose at 2 hr after injection. The two sulfonated complexes, Ga-67 3,4-DiP-LICAMS and Ga-67 TiP-MECAMS, show high kidney activity but relatively

slow urinary accumulation. This may suggest that tubular reabsorption of the water-soluble complexes is occurring, possibly a function of the lipophilic isopropyl N-substitution of the ligands. That sulfonation is requisite for renal sequestration is shown by the flat activity curve for Ga-67 3,4-DiP-LICAM (Fig. 6), which is slightly higher than that for Ga-67 citrate.

The utility of these complexes in the field of nuclear medicine is twofold in nature. Direct applications are (a) use of these complexes as Ga-68 radiopharmaceuticals, where the superior spatial resolution of positron-emission transaxial tomography can be used to assess renal blood flow and anatomical defects using Ga-68 TiP-MECAMS or Ga-68 3,4-DiP-LICAMS; or (b) to study hepatobiliary kinetics using Ga-68 3,4-DiP-LICAM. Indium-111 or In-113 m could also be used for the same purposes with single-photon instruments. In addition, current work on these ligands has shown that when they are administered after injection of Ga-67 citrate, they are able to sequester Ga-67 in vivo and follow the distribution kinetics of the catecholamide. This holds promise for the Ga-67 image-enhancement of tumors and abscesses, in which administration of catecholamide would decrease blood background as well as absorbed radiation dose to the patient. No acute toxicity has been found for these ligands, and work is currently under way to study analogs that show faster renal clearance, such as LICAMS or MECAMS in which the amido isopropyl group has been removed to increase water-solubility.

The long-range usefulness of these gallium- and indium-binding tracers is even more interesting because of the possibility of increasing the affinity of these complexes for nonexcretory organs. In this case, the tricatecholamide structure offers a thermodynamically stable metal-binding nucleus upon which structural alterations may be made. From this paper, one may see how relatively minor chemical changes alter the pharmacologic characteristics without attenuating the in vivo stability of metal binding. Merely sulfonating the catechol rings causes the renal path of excretion to be followed, whereas absence of this functional group allows for hepatic recognition and elimination into the bowel. Similar structure-activity relationships have also been reported for gallium complexes of dihydroxyanthraquinones (*29*). By making even more dramatic changes in tricatecholates, such as N-decyl or N-benzyl substitution, we expect that the affinity for nonexcretory organs will be enhanced. It should also be possible to attach LICAM(S) through the spermidine backbone to biomolecules and use substituted groups to alter the lipophilicity of the LICAM(S) derivative. In this way the lipophilicity of the bifunctional chelate could be matched to that of the biomolecule. Thus, by chemically modifying the polyamino portion of the tricatecholamide, molecules with considerably altered physico-chemical properties and strong metal-ion affinity may be developed, resulting in a variety of complexes having unique biological properties useful to nuclear medicine.

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