Gallium-68 1,1,1-Tris (5-Methoxysalicylaldiminomethyl) Ethane: A Potential Tracer for Evaluation of Regional Myocardial Blood Flow

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Reaction of tris(acetylacetonato)gallium(III) with 1,1,1-tris(5-methoxysalicylaldiminomethyl)ethane, H_3[(5-MeOsal)$_3$TAME], affords a neutral six-coordinate complex, Ga[(5-MeOsal)$_3$TAME], for which the x-ray crystal structure is reported. The ^{67}Ga and ^{68}Ga complexes of H_3[(5-MeOsal)$_3$TAME] and the bis(salicylaldimine) of triethylenetetramine were prepared and characterized by paper chromatography and electrophoresis. The biodistribution of lipophilic ^{68}Ga[(5-MeOsal)$_3$TAME] was determined following intravenous injection in rats. Myocardial images obtained by positron emission tomography from three dogs injected with ^{68}Ga[(5-MeOsal)$_3$TAME] show a correlation between ^{68}Ga uptake and regional myocardial blood flow. Single-pass coronary extraction studies in open-chest dogs indicate that ^{68}Ga[(5-MeOsal)$_3$TAME] behaves neither as a freely diffusible tracer nor as a microsphere analog.

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A major obstacle to widespread use of positron emission tomography (PET) for diagnostic imaging is that most positron emitters must be cyclotron-produced at the site where they are to be used. Gallium-68(^{68}Ga) is one of the few positron emitters available from a parent-daughter (^{68}Ge → ^{68}Ga) generator system (*1,2*), and possesses a half-life sufficiently long (68 min) (*3*) that the chemical synthesis of a diverse array of radiopharmaceuticals should be possible. Thus the preparation of suitable ^{68}Ga radiopharmaceuticals could facilitate the use of PET even in locations lacking an in-house cyclotron.

The use of PET for evaluation of regional cerebral and myocardial blood flow is well documented (*4–11*), and in the current work we are developing ^{68}Ga radiopharmaceuticals as alternatives to cyclotron-produced agents for use in these measurements. The most obvious requirement a gallium complex must meet if it is to be a useful radiopharmaceutical is the ability to resist hydrolysis of the Ga(III) salt in aqueous solution at physiological pH. Other properties that a gallium complex should possess, if it is to be a useful blood-flow tracer capable of crossing the blood-brain barrier, include (*11–13*): lipophilicity, electrical neutrality, and relatively low molecular weight. The final requirement that must be met by a gallium radiopharmaceutical to be used in vivo is kinetic inertness towards exchange with the plasma protein transferrin, which binds the Ga(III) ion with very high affinity (log K_1 = 20.3) (*14*). In practice, this last requirement necessitates the coordination of the Ga(III) ion by a polydentate chelating ligand.

Metal complexes with Schiff's-base ligands have been described extensively in the chemical literature (*15*). The

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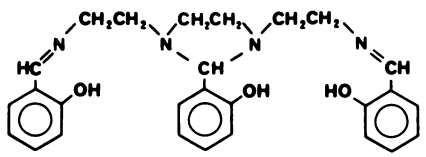
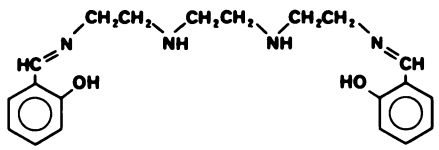
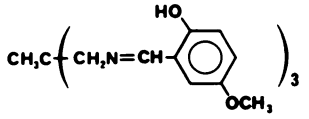


$H_3[(sal)_3TETA]$



$H_2[(sal)_2TETA]$



$H_3[(5\text{-}MeOsal)_3TAME]$

FIGURE 1
Selected salicylaldimine ligands

salicylaldimines have received the greatest attention due, in part, to their high stability. We have begun an investigation of some polydentate salicylaldimine ligands (Fig. 1) to determine their suitability for use as chelating agents for the preparation of gallium and indium radiopharmaceuticals. We report here our synthesis and characterization of the gallium complex of 1,1,1-tris(5-methoxysalicylaldiminomethyl)ethane, Ga[(5-MeOsal)$_3$TAME],[11] and our evaluation of the suitability of the ^{68}Ga complex of this ligand for use in myocardial imaging by positron emission tomography.

MATERIALS AND METHODS

General

Literature methods were used for the synthesis of Ga(acac)$_3$ (*16,17*) (acacH ≡ acetylacetone); 1,1,1-tris-(aminomethyl)ethane (*18*) (TAME); and $H_3[(sal)_3$-TETA] (TETA-triethylenetetramine) (*19*). Iodine-125 (^{125}I)iodoantipyrene, $^{67}GaCl_3$, and the $^{68}Ge \rightarrow {}^{68}Ga$ generator were obtained commercially.* Partition coefficients were measured by vortex mixing of 1 ml octanol and 1 ml Tyrode's buffer, pH 7.35 (*20*), containing a 1-μl sample of the radiolabeled compound in aqueous solution. Following centrifugation at 1800 *g* for 5 min, the octanol and aqueous phases were sampled and counted in a well counter. Partition coefficients, P, are reported as counts per gram of octanol divided by counts per gram of water. In all cases, back extraction of the octanol phase with Tyrodes buffer gave a P value within 10% of the originally measured P, indicating the absence of ionic impurities. The distribution of radioactivity on paper strips 2.5 cm wide following chromatography or electrophoresis was determined using a radiochromatogram scanner interfaced to a strip-chart recorder.

The commercial [I-125]iodoantipyrene was purified by extraction immediately before use. Thus, [^{125}I]iodoantipyrene was extracted into 2 ml chloroform from 2 ml water, and the chloroform layer separated and evaporated to dryness under a stream of nitrogen. The iodoantipyrene was redissolved in 0.2 ml ethanol for use as described below.

Synthesis of H_3[(5-MeOsal)$_3$TAME]

To 1 g $CH_3C(CH_2NH_2)_3$ in hot ethanol was added 0.43 g 2-hydroxy-5-methoxybenzaldehyde$^+$ dissolved in hot 95% ethanol. The mixture was heated in a boiling-water bath for 10 min then allowed to cool slowly. The yellow product deposited on cooling was recrystallized from hot ethanol. Melting point 123–124°C. ^{1}H NMR at 60 MHz in ^{2}H-chloroform: δ(ppm) 1.08 (s 3H) C-CH_3; 3.53 (s 6H) N-CH_2; 3.70 (s 9H) OCH_3; 6.70 (multiplet 3H), 6.88 (multiplet 6H) $-C_6H_3$; 8.17 (s 3H) N=CH; 12.80 (s 3H) O-H $\cdots$ N. IR (KBr disk) ν(C=N) 1633 cm^{-1}. The electron-impact mass spectrum shows the parent ion ($C_{29}H_{33}N_3O_6$) at m/e = 519.

Synthesis of Ga[(5-MeOsal)$_3$TAME]

To 0.150 g H_3[(5MeOsal)$_3$TAME] in 75 ml hot ethanol was added 0.105 g Ga(acac)$_3$ in 10 ml ethanol. The mixture was heated to 65° C for 1 hr and then allowed to cool slowly, depositing the yellow crystalline product. The crystalline solid could not be redissolved in any of the common solvents: ethanol, acetone, dimethylsulfoxide, dimethylformamide, benzene, hexane, tetrahydrofuran, pyridine, acetonitrile, chlorocarbons. Yield: 0.163 g, 97%. Melting point >320°C. Analysis (Galbraith Laboratories): found: C, 59.43; H, 5.33; N, 7.03; Ga, 11.80%; calculated for $C_{29}H_{30}N_3O_6Ga$: C, 59.41; H, 5.16; N, 7.17; Ga, 11.89%. Infrared spectrum (KBr pellet): ν(C=N) 1632 (s); 1650 (sh); 1610 (m) cm^{-1}. The parent ion peak, $C_{29}H_{30}N_3O_6{}^{69}Ga$, is observed at m/e = 585 in the electron-impact mass spectrum, along with M + 1, M + 2, and M + 3 peaks of expected relative intensities.

For the preparation of ^{68}Ga[(5-MeOsal)$_3$TAME], 30 mCi ^{68}Ga in 2.5 ml of 1*N* HCl was evaporated to dryness by heating in a test tube under a flow of nitrogen. To the remaining ^{68}Ga was added 0.4 mg H_3[(5-MeOsal)$_3$-TAME] in 0.5 ml ethanol containing 0.002 wt% acetylacetone. After thoroughly rinsing to remove as much

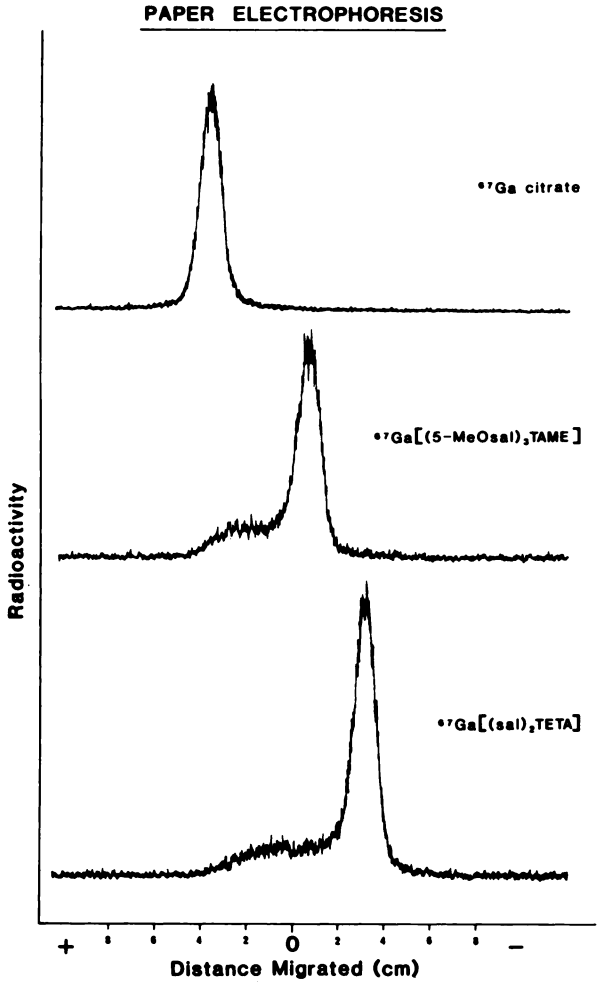


FIGURE 2
Migration of radioactivity on paper strips following electrophoresis of ^{67}Ga compounds shown

^{68}Ga activity as possible, the ethanolic solution was transferred to a new test tube and heated in a 70°C water bath for 5–10 min. The ethanol solution was then diluted with 2.5 ml saline and filtered through a 0.2-μm polytetrafluoroethylene filter. Yield 15–20 mCi. The gallium-67(^{67}Ga) complex was prepared in a similar manner, starting from Ga-67 in 0.1 *N* HCl.

Synthesis of [Ga(sal)$_2$TETA]I

To 0.65 g H_3[(sal)-$_3$TETA] in 150 ml hot ethanol was added 0.5 g Ga(acac)$_3$ in 50 ml hot ethanol and 0.4 g KI in 2 ml water. The mixture was heated at 70°C for 2 hr and the ethanol evaporated under reduced pressure. The resulting yellow oil was washed with 10 ml benzene and then dissolved in 25 ml hot ethanol. The hot solution was filtered and allowed to cool slowly, depositing pale yellow crystals, melting point >320°C. The fast atom bombardment (FAB) mass spectrum of the product (in glycerol matrix) shows the parent ion peak at m/e = 421 for $C_{20}H_{24}N_4O_2{}^{69}Ga$. Infrared spectrum (KBr) pellet): ν(C=N) 1625 cm^{-1}. [^{67}Ga(sal)$_2$TETA]$^+$ was prepared by substitution of H_3[(sal)$_3$TETA] for H_3[(5-MeO-sal)$_3$TAME] in the procedure described for [^{67}Ga(5-MeOsal)$_3$TAME].

Synthesis of ^{68}Ga transferrin

The ^{68}Ga generator eluent (30 mCi/2.5 ml 1*N* HCl) was evaporated to dryness and to the residue was added 0.025 g human transferrin‡ dissolved in 0.6 ml acetate buffer (0.5 *M*, pH 7.35). The tube was agitated and allowed to stand for 5 min at room temperature. The solution was purified by chromatography on a Sephadex G-10-120 gel column (0.7 × 7 cm) rapidly eluted with normal saline. One-milliliter fractions were collected. The two fractions containing >90% of the eluted activity were combined and filtered through a Millex-GV 0.2-μm filter§ before use. Yield 10 mCi.

Paper electrophoresis

The behaviors of [^{67}Ga(5-MeOsal)$_3$TAME], [^{67}Ga-(sal)$_2$TETA], and ^{67}Ga citrate upon paper electrophoresis were determined using pH 7.0 McIlvanie's phosphate-citrate buffer (*21*) diluted to 20% ethanol (to ensure solubility of the lipophilic complexes). The results shown in Fig. 2 were obtained simultaneously using a constant current of 9 mA for 6 hr (4–5 V/cm). The paper strips were dried for 10 min at 140°C before determining the distribution of activity. To measure the effect of electro-osmotic transport (*22,23*), a saturated solution of caffeine in 80% phosphate-citrate buffer/20% ethanol was mixed with an equal volume of radiolabeled Ga[(5-MeOsal)$_3$TAME] complex. Following paper electrophoresis of the mixture, the caffeine spot was visualized by illumination under a ultraviolet lamp (254 nm).

X-ray crystallography

A single-crystal x-ray structural determination was undertaken for Ga[(5-MeOsal)$_3$TAME]. Crystal data for $C_{29}H_{30}N_3O_6Ga$ at −160°C: a = 15.574(3), b = 15.574(3), c = 17.584(4) Å; γ = 120.00°; V = 3693.53 Å^3; Z = 6 in rhombohedral space group R3c; (R(F) = 1.78%, R_w(F) = 2.01% for 473 observed [$F_0 > 2.33\sigma(F_0)$] and absorption-corrected reflections using anisotropic thermal parameters for all nonhydrogen atoms. All hydrogens bound to carbon were refined isotropically. (Complete structural details are available in Molecular Structure Center Report No. 84901, which can be requested from the Chemistry Library, Indiana University, Bloomington, IN 47405, USA.)

Animal studies

The biodistribution of *n*[1-^{11}C]butanol was available from previous work (unpublished data, Welch MJ). The biodistributions of ^{68}Ga[(5-MeOsal)$_3$TAME] and ^{68}Ga transferrin were determined after injection into the femoral vein of Sprague-Dawley rats, which were killed

by decapitation at appropriate time intervals after injection. Total blood volume was taken to be 7% of the body weight.

The relative biodistributions of ^{68}Ga[(5-MeOsal)$_3$-TAME] and [^{125}I]iodoantipyrene (P = 21 ± 2) were determined following co-injection of the two tracers. An ethanolic solution of [^{125}I]iodoantipyrene was added to ^{68}Ga[(5-MeOsal)$_3$TAME] in ethanol, and the mixture diluted to 15% ethanol with saline. Following filtration through a 0.2 μm polytetrafluoroethylene filter, the mixture was injected into the rats by femoral vein. The rats were killed at 1 min after injection and the organs removed as rapidly as possible to minimize the effects of tracer redistribution.

Arterial blood clearance curves for ^{68}Ga[(5-MeOsal)$_3$TAME] and [^{125}I]iodoantipyrene were determined in two rats following bolus (0.15 ml) co-injection of the two tracers. The tracer mixture was injected into the right femoral vein, with blood samples collected in preweighed capillary tubes from a catheter inserted into the left femoral artery. Arterial blood samples were collected at 5-sec intervals beginning immediately upon injection. The capillary tubes were then reweighed and counted to determine ^{68}Ga and ^{125}I levels in the blood.

Open-chest dog studies

To determine the extraction (E) and clearance (k) of ^{68}Ga[(5-MeOsal)$_3$TAME] by the heart, three open-chest anesthetized dogs were studied. The animals (27–45 kg) were premedicated with morphine sulphate, anesthetized with thiopental (12.5 mg/kg) and α-chloralose (60 mg/kg), and ventilated with oxygen-enriched room air.

In each animal the heart was exposed by a left lateral thoracotomy, then elevated in a pericardial cradle. The left circumflex or left anterior descending coronary artery was isolated within a centimeter of its origin and cannulated with an 18-gauge cannula (Quick-cath). A distal major branch of the cannulated vessel was freed and ligated to produce a zone of ischemia. A cannula was inserted into the left atrial appendix for microsphere administration, and a reference cannula was inserted into the left femoral artery to provide samples for flow determination. To compare the extraction and clearance of ^{68}Ga complex with the diffusible tracer $H_2{}^{15}O$, a bolus of 2 mCi of $H_2{}^{15}O$ in 0.1 ml was injected intracoronarily by means of a perfusion pump, at a flow rate of 28 ml/min. In earlier studies the injection technique was demonstrated to alter distal arterial pressure by less than 5% (*10*). Positron activity was detected in the ischemic zone, and in a zone of normal perfusion within the distribution of the cannulated vessel, by means of beta probes positioned over the myocardium. The beta probes permit determination of positron activity within 4 mm of the epicardial surface, and they minimize contributions from circulating activity or gamma activity (*10*,*24*). Ten minutes after the injection of $H_2{}^{15}O$ ($T_{1/2}$ = 2.04 min), 2 mCi of ^{68}Ga[(5-MeOsal)$_3$TAME] was injected as a bolus (in a volume of 0.2 ml). After 30 sec 100 μCi of scandium-46(^{46}Sc) microspheres (15 μm) were injected via the left atrium, and an arterial reference withdrawal sample obtained. Ten minutes after the injection of tracer and characterization of the time-activity curves, Lissamine green dye was injected into the cannula to demarcate the zones of normal perfusion and of ischemia, KCl was given to induce ventricular fibrillation, the heart was sectioned, and ^{68}Ga and ^{46}Sc activity were measured, with a gamma well counter, in a total of 30 samples in each study. The counting was repeated after 24 hr (after ^{68}Ga decay), to yield net ^{46}Sc counts, and hence ^{68}Ga counts by subtraction. The results are expressed as microsphere flow/min and ^{68}Ga content (decay-corrected), both per gram wet weight.

Myocardial imaging with PET

As a preliminary assessment of the suitability of ^{68}Ga[(5-MeOsal)$_3$ TAME] as a myocardial imaging agent, three additional anesthetized, closed-chest dogs were studied using the PETT VI instrument (*25*). Images were obtained in the high-resolution mode (full width at half-maximum = 0.7 cm) with correction for attenuation using a transmission scan obtained with the animal in the same position. Images acquired for 400 or 800 sec after intravenous injection of 11 to 12 mCi ^{68}Ga[(5-MeOsal)$_3$TAME] were compared with images acquired for 40 sec after bolus i.v. injection of 40 to 50 mCi O-15 water. Correction for intravascular tracer was performed as previously described (*10*), using red blood cells labeled with carbon[^{15}O]monoxide. In each dog the interval between $H_2{}^{15}O$ and ^{68}Ga imaging was less than 15 min.

Two of the dogs imaged had a 50 to 70% coronary

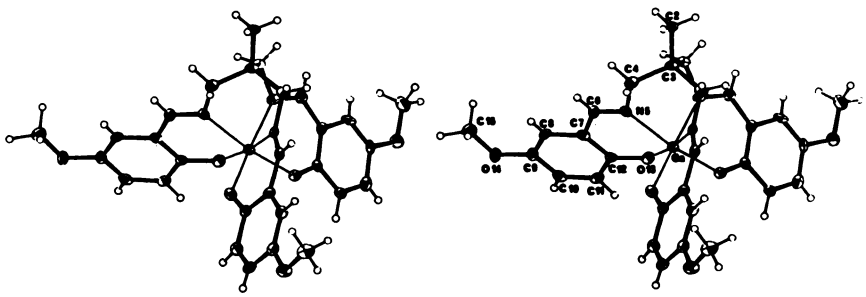


FIGURE 3
Stereoscopic ORTEP drawing showing structure of Ga[(5-MeOsal)$_3$-TAME]

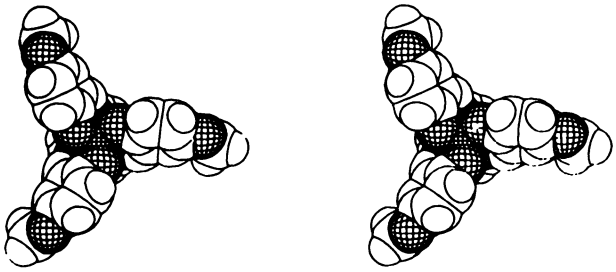


FIGURE 4
Stereoscopic space-filling ORTEP drawing of Ga[(5-MeOsal)$_3$TAME] viewed along threefold axis

artery stenosis that produced flow heterogeneities during pharmacologically-induced vasodilation with dipyridamole (1 mg/kg i.v.). The third dog was studied after 24 hr of reperfusion following lysis of an occlusive thrombus in the left anterior descending coronary artery.

RESULTS AND DISCUSSION

Synthesis and characterization of gallium complexes

Ga[(5-MeOsal)$_3$TAME] was prepared by reaction of Ga(acac)$_3$ with H$_3$[(5-MeOsal)$_3$TAME] in hot ethanol. The insoluble product was characterized and identified by infrared and mass spectroscopy and elemental analysis. Insolubility precludes chromatographic characterization of this complex at the macroscopic level. The reaction as described produced crystals suitable for an x-ray structural analysis, which was undertaken. The structural study confirmed the hexadentate nature of the ligand and demonstrated the absence of other coordinated ligands. As illustrated in Fig. 3, the (5-MeOsal)$_3$TAME ligand affords a neutral Ga(III) complex by bonding through the three imino-nitrogen lone pairs and the three deprotonated phenolic oxygen atoms, thus giving a nearly octahedral coordination geometry. The compound contains a crystallographic threefold axis passing through the Ga and two ethyl carbon atoms (C2, C3). Figure 4 shows a space-filling ORTEP drawing of the molecule viewed along the C_3 axis. The Ga-O and Ga-N bond lengths (1.941 ± 0.005 Å and 2.107 ± 0.005 Å, respectively) are within the range of values previously reported (*26–28*) for six coordinate gallium(III) complexes. Additional structural features are discussed elsewhere (*29*).

The ^{68}Ga and ^{67}Ga complexes of (5-MeOsal)$_3$-TAME were prepared in 50–70% yields. The complexes were found to be lipophilic with octanol/water partition coefficients P = 27 ± 2. The radiolabeled complex migrates as a single radioactive peak with R_f = 0.87 upon chromatography on Whatman 1 paper eluted (*30*) with H_2O/EtOH/NH_4OH (700:200:0.35 ml). Paper electrophoresis shows the ^{67}Ga complex to be uncharged (vide infra).

The tris(salicylaldimine) of triethylenetetramine, H$_3$-(sal)$_3$TETA, has been reported (*19*) to react with metal ions in aqueous solution, with hydrolysis of the bridging imino group to afford complexes of the [(sal)$_2$TETA] ligand. In addition, Bailar has shown (*31*) that $GaCl_3$ and $InCl_3$ react with triethylenetetramine (TETA) and two equivalents of salicylaldehyde to give [M(sal)$_2$TE-TA]$^+$ salts [M = Ga(III), In(III)], which were characterized by infrared spectroscopy and elemental analysis. To confirm that the bis(salicylaldimine) complex of Ga can be prepared directly from the tris(salicylaldimine) precursor, we prepared the iodide salt by reaction of Ga(acac)$_3$ with H$_3$[(sal)$_3$TETA]. The infrared spectrum of the product so obtained matches that reported in the literature. The identity of the cationic product was confirmed by the fast-atom-bombardment mass spectrum, which shows the parent ion peak due to [Ga(sal)$_2$-TETA].$^+$ The ^{67}Ga complex of this ligand was prepared for use as a cationic standard in the electrophoresis experiments.

To determine the charge on the radiolabeled Ga[(5-MeOsal)$_3$TAME] complexes in aqueous solution at neutral pH, the behavior of the ^{67}Ga complex upon paper electrophoresis was investigated. Figure 2 shows that ^{67}Ga citrate (top panel) is an anionic complex. The bottom panel of Fig. 2 shows that the ^{67}Ga complex formed with the salicylaldimine of triethylenetetramine

TABLE 1
Biodistribution of ^{68}Ga Complex of 1,1,1-Tris(5-Methoxysalicyliminomethyl)Ethane Following Intravenous Injection in Rats (Decay-Corrected)*

Item	% Injected dose per organ			
	1 min	5 min	30 min	60 min
Blood	12.4 ± 3.1	6.7 ± 0.6	3.3 ± 0.2	2.0 ± 0.4
Liver	19.8 ± 2.7	17.4 ± 2.8	10.5 ± 1.8	6.4 ± 0.8
Spleen	0.65 ± 0.26	0.31 ± 0.06	0.13 ± 0.02	0.09 ± 0.02
Kidneys	6.0 ± 0.7	3.6 ± 0.7	1.3 ± 0.2	0.81 ± 0.11
Lung	2.9 ± 1.1	1.3 ± 0.3	0.41 ± 0.06	0.29 ± 0.04
Heart	0.97 ± 0.20	0.66 ± 0.08	0.23 ± 0.03	0.14 ± 0.02
Brain	0.054 ± 0.027	0.021 ± 0.004	0.012 ± 0.004	0.009 ± 0.002

* Values shown represent mean of four to seven rats (230–400 g).

TABLE 2
Biodistribution of ^{68}Ga Transferrin Following Intravenous Injection in Rats (Decay-Corrected)*

Item	% Injected dose per organ			
	1 min	5 min	30 min	60 min
Blood	77 (72–81)	67 (62–71)	33 (31–35)	29 (27–30)
Liver	7.1 (6.7–7.9)	6.9 (6.4–7.9)	4.6 (4.1–5.4)	5.5 (4.6–6.0)
Spleen	0.28 (0.23–0.32)	0.40 (0.36–0.43)	0.31 (0.23–0.37)	0.32 (0.21–0.40)
Kidneys	1.6 (1.5–1.7)	1.7 (1.5 –1.9)	0.92 (0.82–1.05)	0.81 (0.76–0.88)
Lung	2.7 (2.0–3.9)	2.5 (1.7–3.5)	1.8 (1.0–3.0)	1.5 (1.0–2.3)
Heart	0.80 (0.71–0.88)	0.89 (0.77–0.98)	0.60 (0.59–0.60)	0.54 (0.48–0.60)
Brain	0.24 (0.20–0.27)	0.21 (0.19–0.23)	0.11 (0.10–0.12)	0.09 (0.08–0.10)

* Values shown represent mean of three rats (270–350 g) and range of values.

is cationic, consistent with the results obtained at macroscopic concentrations. The middle panel of Fig. 2 shows that ^{67}Ga[(5-MeO-sal)$_3$TAME] migrates very slightly towards the cathode. This slight migration from the origin can be attributed to electro-osmotic transport (*22,23,32,33*) and is not inconsistent with the presence of a neutral ^{67}Ga complex identical to that crystallographically characterized. This interpretation is supported by our observation that caffeine, which has been used at neutral pH to assess the rate of electro-osmosis through paper (*22,23*), migrates at the same rate as the radiolabeled Ga[(5-MeOsal)$_3$TAME]. (We have also considered the possibility that this cathodic migration could indicate that the crystallographically characterized complex is sufficiently basic so that in aqueous solution at physiological pH it exists to some extent in protonated form. However, we have observed that the octanol/water partition coefficient is invariant between pH 6.5 and 9.5, strongly suggesting that such a process does not occur to a significant extent).

Biological studies

The biodistribution of ^{68}Ga[(5-MeOsal)$_3$TAME] over 1 hr following intravenous injection in rats is shown in Table 1. The compound is rapidly cleared from the blood. Comparison with the biodistribution of ^{68}Ga transferrin (Table 2) or a ^{67}Ga chelate (citrate) (*34*), which undergoes rapid exchange with transferrin, clearly shows that ^{68}Ga[(5-MeOsal)$_3$TAME] does not simply undergo exchange to form the protein complex. Over this time period, the heart and lung uptake and washout of ^{68}Ga[(5-MeOsal)$_3$TAME] very closely resemble those of carbon-11- (^{11}C) labeled *n*-butanol (Table 3), which has been shown to be suitable for use in assessment of brain and myocardial blood flow by PET (*8,9,35*). However, despite its lipophilicity, the ^{68}Ga complex does not cross the blood-brain barrier, possibly because its molecule is too large (*12,13*).

The biodistributions of ^{68}Ga[(5-MeOsal)$_3$TAME] and [^{125}I]iodoantipyrene, a freely diffusible blood flow tracer (*36–41*), were directly compared in rats following bolus coinjection of the two tracers. Arterial blood clearance curves for the two were determined over a 80–100 sec period following bolus co-injection (Fig. 5). The compounds exhibit comparable clearances from the blood stream over this time period, dropping rapidly during the first 10 sec and then leveling off at about 10% of the maximum level measured 0–5 sec after injection.

The relative biodistributions of ^{68}Ga[(5-MeOsal)$_3$TAME] and [^{125}I]iodoantipyrene 1 min following bolus coinjection of the tracers is given in Table 4, expressed as organ-to-blood ratios. The liver, spleen, lung, and heart each show comparable uptakes of the two

TABLE 3
Biodistribution of ^{11}C-Labeled *n*-Butanol Following Intravenous Injection in Rats (Decay-Corrected)*

Item	% Injected dose per organ			
	2 min	5 min	30 min	60 min
Blood	11.9 ± 4.3	6.3 ± 0.4	4.9 ± 2.5	3.1 ± 1.2
Liver	15.9 ± 9.4	20.4 ± 9.5	7.6 ± 3.5	7.6 ± 2.9
Spleen	0.39 ± 0.26	0.45 ± 0.08	0.25 ± 0.04	0.30 ± 0.17
Kidneys	2.7 ± 1.1	1.8 ± 0.09	1.3 ± 0.34	0.71 ± 0.27
Lung	2.0 ± 0.98	2.3 ± 0.6	1.0 ± 0.2	0.6 ± 0.17
Heart	0.96 ± 0.5	0.62 ± 0.07	0.26 ± 0.08	0.17 ± 0.06
Brain	1.8 ± 1.7	0.5 ± 0.06	0.36 ± 0.13	0.22 ± 0.09

* Values shown represent mean of four to seven rats (170–230 g).

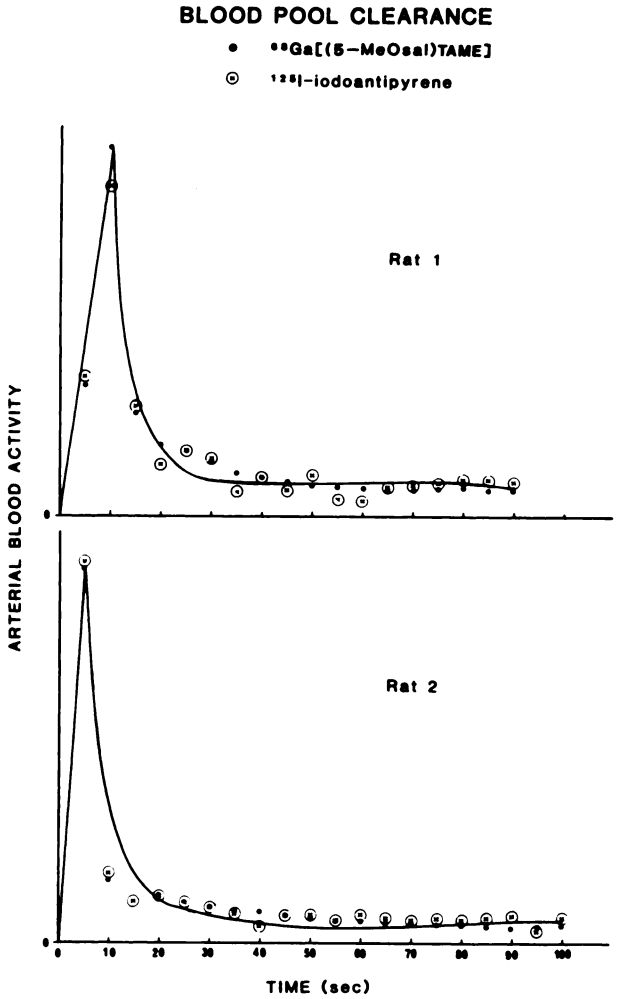


FIGURE 5
Blood clearance curves for $^{68}Ga[(5\text{-MeOsal})_3TAME]$ (•) and [^{125}I]iodoantipyrene (O) following bolus coinjection in two rats

tracers. There is significantly higher uptake of ^{68}Ga relative to ^{125}I in the kidneys, possibly indicating that the gallium preparation contains a few percent of ionic impurities. The two tracers also differ in that ^{68}Ga complex does not cross the blood-brain barrier whereas iodoantipyrene is taken up in the brain. Again, this difference can probably be attributed to the significantly lower molecular weight of iodoantipyrene, since the octanol/water partition coefficients of the two compounds are similar and lie well within the range of values exhibited by lipophilic ^{11}C compounds that cross the blood-brain barrier (*11,13,42,43*).

Open-chest dog studies

The purpose of the open-chest dog studies was to determine the relationship between tissue flow measured by microspheres and the single-pass extraction and subsequent clearance of $^{68}Ga[(5\text{-MeOsal})_3TAME]$. The study design also permitted comparison of the extraction and clearance of the ^{68}Ga complex with the extraction and clearance of $H_2{}^{15}O$ under the same experimental constraints. Earlier studies demonstrated that $H_2{}^{15}O$ is highly extracted by the myocardium over a wide range of flow, and clearance conforms to a monoexponential function (*10*).

The results of the open-chest dog experiments are given in Table 5. Mean transmural flow in the zone of ischemia was calculated in each animal. For the three dogs studied, values ranged from 0.34 ml/g-min to 0.57 ml/g-min in ischemic zones, and from 0.74 to 2.01 ml/g-min in normal zones. The extraction of $H_2{}^{15}O$ averaged 99.3% in normal zones and 103.6% in ischemic zones. The clearance (k,sec^{-1}) of water was reduced in ischemic zones and was monoexponential, with the mean correlation coefficient of the slope, 0.914 ± 0.058 (Fig. 6). However, in contrast to $H_2{}^{15}O$, which was highly extracted, the ^{68}Ga complex was extracted only partially, with a higher residual fraction in ischemic zones (47.5%) and a lower residual fraction in normal zones (30.1%).

$^{68}Ga[(5\text{-MeOsal})_3TAME]$ is only partially extracted in both normal and ischemic conditions, and the extraction fraction is not independent of flow. The rate of clearance of ^{68}Ga complex did not conform to the characteristics of a diffusible tracer. The rate constants (k) for periods from 15 sec to 10 min are given in Table 5. Continued clearance of ^{68}Ga tracer is demonstrated, but the rate of clearance diminishes with time.

Tissue analysis demonstrated a relationship between flow as measured by microspheres and residual ^{68}Ga content at 10 min (correlation coefficients average 0.86 ± 0.05 for the three studies). Continued clearance of ^{68}Ga complex was evident, however, and the rate of clearance was not significantly associated with flow but declined with time (see k values for 15–30 sec, 30 sec–3 min, 3 min–10 min, Table 5). Although net residual ^{68}Ga content at 10 min shows a relationship to flow, this may not hold at later time intervals on account of continued clearance from normal and from ischemic zones.

TABLE 4
Biodistribution of $^{68}Ga[5\text{-MeOsal}_3TAME]$ and [^{125}I] Iodoantipyrene, Expressed as Organ-to-Blood Ratios at 1 min After Bolus Venous Coinjection of the Two Tracers*

Organ	Ratio of (activity per gram of organ)/(activity per gram of blood) ^{68}Ga	^{125}I
Liver	2.8 ± 0.2	2.7 ± 0.2
Spleen	0.97 ± 0.14	0.92 ± 0.09
Kidney	4.5 ± 0.7	1.5 ± 0.2
Lung	1.4 ± 0.2	1.3 ± 0.1
Heart	1.6 ± 0.1	1.4 ± 0.1
Brain	0.036 ± 0.005	1.1 ± 0.1

* Mean of five rats (250–330 g).

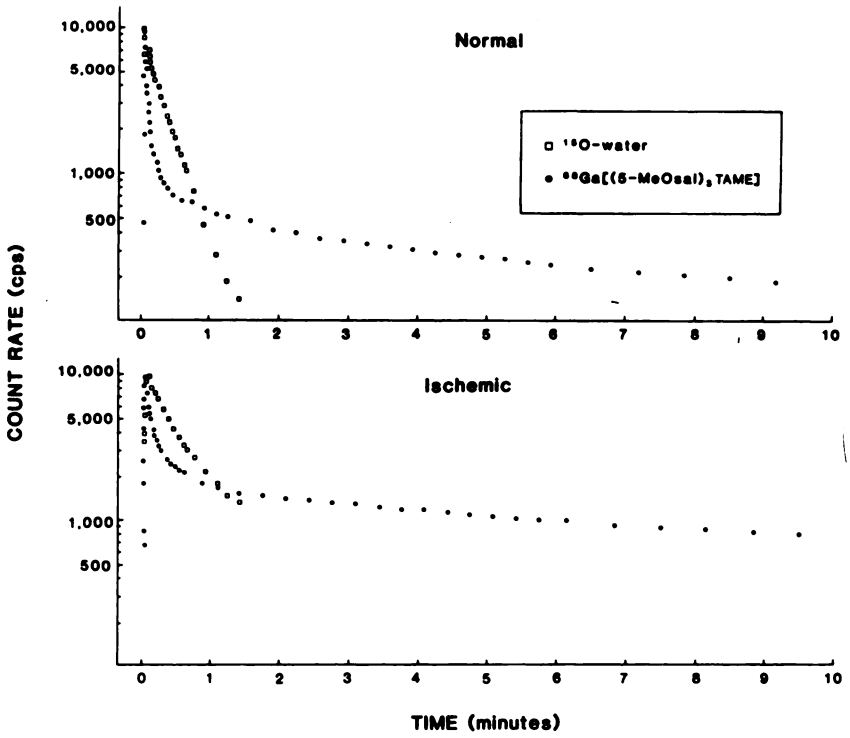


FIGURE 6
Regional time-activity curves (decay-corrected and normalized for a peak of 10,000 cps) for normal and ischemic myocardial regions following intracoronary injections of [^{15}O]water and ^{68}Ga[(5-MeOsal)$_3$TAME] in dog

Myocardial imaging with ^{68}Ga[(5-MeOsal)$_3$TAME]

The biodistribution studies suggested that ^{68}Ga-[(5-MeOsal)$_3$TAME] might be used to evaluate regional myocardial blood flow by PET. Figure 7 shows the PET images obtained in 400 and 800 sec for two adjacent slices of the infarcted heart of a dog injected with 11 mCi ^{68}Ga[(5-MeOsal)$_3$TAME]. For comparison, the images of the same two slices obtained with oxygen-15(^{15}O) water, a routinely used myocardial blood-flow tracer (*10*), are also shown. In all the images, blood-pool activity has been subtracted using carboxyhemoglobin labeled with carbon[^{15}O]monoxide.

Qualitatively the myocardial images for ^{68}Ga[(5-MeOsal)$_3$TAME] and ^{15}O water were quite similar for each of the three dogs studied. The suitability of ^{68}Ga[(5-MeOsal)$_3$TAME] for quantitation of myocardial blood flow can be assessed by comparison of its relative regional distribution in myocardium with the relative distribution of [^{15}O]water. Figure 8 shows that there is a linear correlation (r = 0.93 for 13 data points)

TABLE 5
Extraction and Clearance $H_2{}^{15}O$ and ^{68}Ga Complex in Open-Chest Dogs*

Item	Residual fraction† (%)	k (sec^{-1})‡	$t_{1/2}$ (min)§	Microsphere determined flow (ml/g-min)
Normal				
^{68}Ga 15–30 sec	30.1 ± 8.8	0.0243 ± 0.0004	0.51 ± 0.10	1.77 ± 0.54
30 sec–3 min		0.00513 ± 0.0010	2.48 ± 0.43	
3 min–10 min		0.001380 ± 0.00036	10.23 ± 4.8	
$H_2{}^{15}O$	99.3 ± 6.4	0.0410 ± 0.0109	0.34 ± 0.12	
Ischemia				
^{68}Ga 15–30 sec	47.5 ± 5.1	0.0252 ± 0.0042	0.45 ± 0.04	0.45 ± 0.06
30 sec–3 min		0.00552 ± 0.00079	2.18 ± 0.26	
3 min–10 min		0.001251 ± 0.00015	10.15 ± 1.84	
$H_2{}^{15}O$	103.6 ± 4.2	0.0283 ± 0.0045	0.43 ± 0.0789	

* Values represent mean of three dogs.
† Residual fraction = fraction of peak counts retained in myocardium (from myocardial time-activity curve).
‡ k = rate constant for rate of biological clearance of radioactivity.
§ $t_{1/2}$ = half-time for clearance of activity (min).

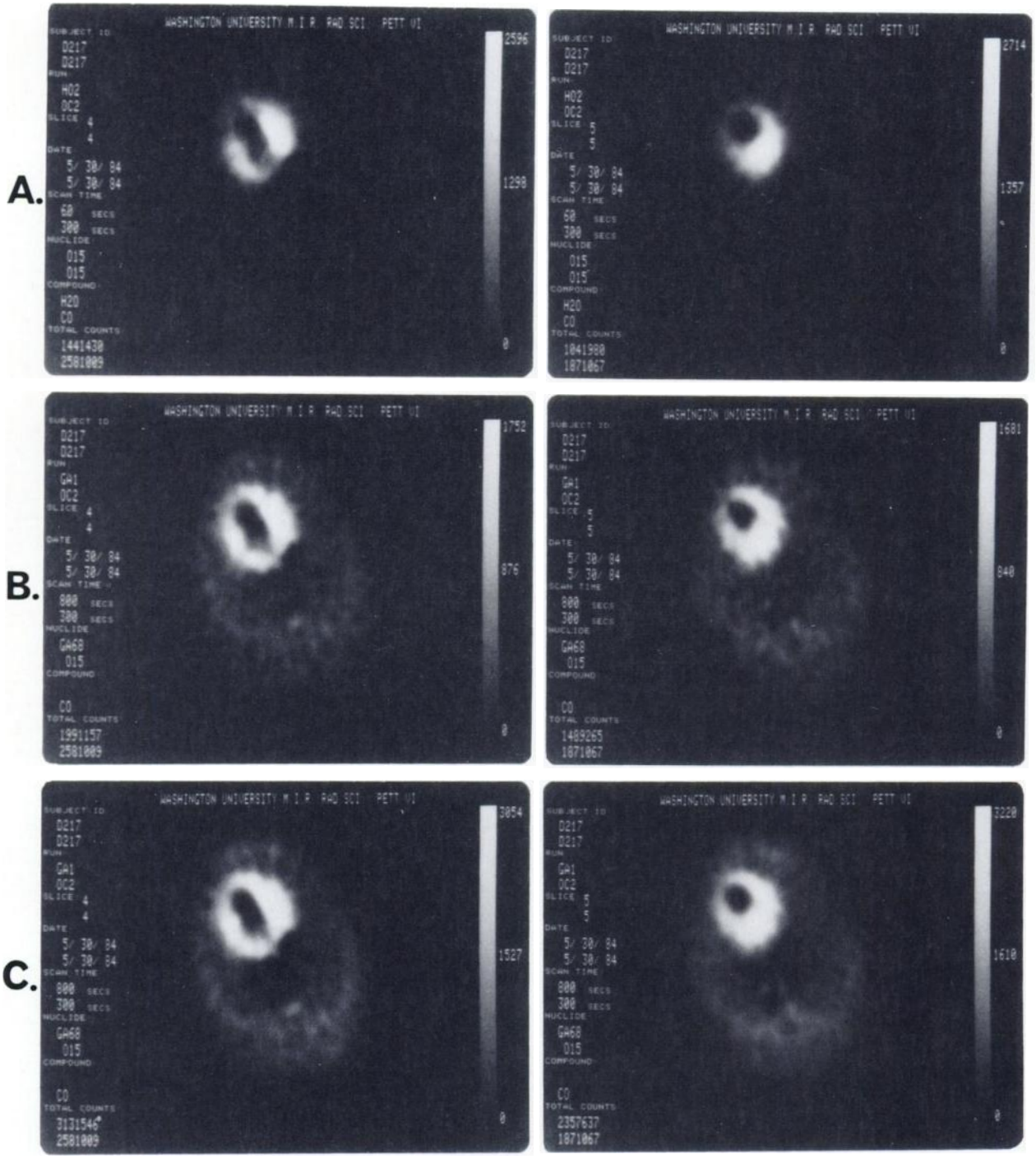


FIGURE 7
Two adjacent slices of PET myocardial images from infarcted dog. (A) [^{15}O]water images; (B) ^{68}Ga[(5-MeOsal)$_3$TAME] images acquired in 400 sec; (C) ^{68}Ga[(5-MeOsal)$_3$TAME] images acquired in 800 sec

between the ^{68}Ga distribution (infarcted dog, 400-sec image) and the regional myocardial blood flow measured by ^{15}O water. Relative ^{68}Ga activity was consistently higher than that of $H_2{}^{15}O$, thus tending to overestimate flow in the region of infarction. The images acquired in 800 sec qualitatively resemble the 400-sec images. However, the correlation with the relative distribution of [^{15}O]water is somewhat lower (r = 0.91 for 13 data points representing the same myocardial regions as above), consistent with continued redistribution of the tracer, as suggested by the results from the open-chest dogs. The PET myocardial images (not shown) from two dogs with coronary artery stenosis showed similar correlations (r = 0.90 and r = 0.87 for 13 and 11 data points, respectively) between the relative distributions of ^{68}Ga[(5-MeOsal)$_3$TAME] and [^{15}O]water, although

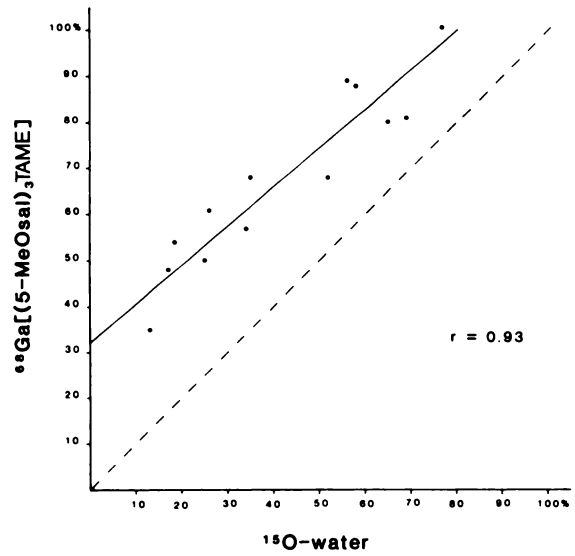


FIGURE 8
Relative distributions of two tracers over various myocardial regions, expressed as % of maximum region in given PET slice, using images in Figs. 7A and 7B

the ranges of relative flow were smaller than for the dog with myocardial infarction.

CONCLUSIONS

The synthesis and characterization of a neutral octahedral gallium tris(salicylaldimine) complex, Ga[(5-MeOsal)$_3$TAME], was described. The ^{68}Ga complex of this ligand is lipophilic, and electrophoresis experiments suggest that the solid-state structure may persist in aqueous solution (pH 7) at the no-carrier-added level. This ^{68}Ga complex does not undergo rapid exchange with transferrin when injected intravenously into rats, and has a biodistribution pattern showing that it has potential as a radiopharmaceutical for the evaluation of regional myocardial blood flow by positron emission tomography in institutions without a biomedical cyclotron. The PET myocardial images obtained from dogs injected with ^{68}Ga[(5-MeOsal)$_3$TAME] compare favorably, both qualitatively and quantitatively, with those obtained using [^{15}O]water. Although there is a correlation between ^{68}Ga[(5-MeOsal)$_3$TAME] uptake and myocardial blood flow, single-pass coronary extraction studies show that the complex behaves neither as a freely diffusible tracer nor as a microsphere analog. ^{68}Ga[(5-MeOsal)$_3$TAME] is therefore less suitable than [^{15}O]water or [^{11}C]butanol for evaluation of myocardial blood flow by PET, but can be used to qualitatively distinguish areas of low perfusion. The molecular weight of this compound is too large, however, for use as a brain blood-flow tracer (*13*), and we are currently pursuing the synthesis of complexes with lower molecular weight, which we hope will have potential for measuring cerebral blood flow.

FOOTNOTES

* New England Nuclear Corporation, Billerica, MA.
† Aldrich Chemical Company, Milwaukee, WI.
‡ Sigma Chemical Company, St. Louis, MO.
§ Millipore Corporation, Bedford, MA.

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REFERENCES

1. Finn RD, Molinski VJ, Hupf HB, et al: *Radionuclide Generators for Biomedical Applications*, NAS-NS-3202, Technical Information Center, U.S. Dept. of Energy, Oak Ridge, 1983
2. Knapp FF, Butler TA, eds: *Radionuclide Generators*, ACS Symposium Series 241, American Chemical Society, Washington, D.C., 1984
3. Lederer CM, Shirley VS: *Table of Isotopes, 7th ed.*, New York, John Wiley, 1978
4. Raichle ME: Positron emission tomography tracer techniques. In *Short-Lived Radionuclides in Chemistry and Biology*, Root JW, Krohn KA, eds. *Adv Chem Ser* 197, American Chemical Society, Washington, D.C., 1981, pp 419-436
5. Raichle ME: Positron emission tomography. *Ann Rev Neurosci* 6:249-267, 1983
6. Herscovitch P, Markham J, Raichle ME: Brain blood flow measured with intravenous $H_2{}^{15}O$. I. Theory and error analysis. *J Nucl Med* 24:782-789, 1983
7. Raichle ME, Martin WRW, Herscovitch P, et al: Brain blood flow measured with intravenous $H_2{}^{15}O$. II. Implementation and validation. *J Nucl Med* 24:790-798, 1983
8. Hack SN, Eichling JO, Bergmann SR, et al: External quantification of myocardial perfusion by exponential infusion of positron-emitting radionuclides. *J Clin Invest* 66:918-927, 1980
9. Hack SN, Bergmann SR, Fichling JO, et al: Quantification of regional myocardial perfusion by exponential infusion of ^{11}C-butanol. *IEEE Trans Biomed Eng* 30: 716-722, 1983
10. Bergmann SR, Fox KAA, Rand AL, et al: Quantification of regional myocardial blood flow in vivo with $H_2{}^{15}O$. *Circulation:* 70:724-733, 1984
11. Dischino DD, Welch MJ, Kilbourn MR, et al: Relationship between lipophilicity and brain extraction of C-11 labeled radiopharmaceuticals. *J Nucl Med* 24: 1030-1038, 1983
12. Moerlein SM, Dischino DD, Raymond KN, et al: Factors affecting the cerebral uptake of gallium complexes. *J Lab Comp* 19:1421-1422, 1982
13. Levin VA: Relationship of octanol/water partition coefficient and molecular weight to rat brain capillary permeability. *J Med Chem* 23:682-684, 1980

14. Harris WR, Recoraro VL: Thermodynamic binding constants for gallium transferrin. *Biochemistry* 22: 292-299, 1983
15. Holm RH, Everett GW, Chakrovorty A: Metal complexes of Schiff bases and β-ketoamines. In *Progress in Inorganic Chemistry 7*, Cotton FA, ed. Wiley, New York, 1966, pp 83-214
16. Morgan GT, Drew HDK: Researches on residual affinity and coordination. Part V. Gallium acetylacetone and its analogues. *J Chem Soc* 119:1058-1066, 1921
17. Carty AJ, Tuck DG, Bullock E: Nuclear magnetic resonance spectra and conductivities of some metal acetylacetonates. *Can J Chem* 43:2559-2565, 1965
18. Fleischer EB, Gembala AE, Levey A, et al: Conversion of aliphatic and alicyclic polyalcohols to the corresponding primary polyamines. *J Org Chem* 36:3042-3044, 1971
19. Das Sarma B, Bailar JC: The stereochemistry of metal chelates with polydentate ligands. Part I. *J Am Chem Soc* 77:5476-5480, 1955
20. Mustard JF, Packham MA: Factors influencing platelet function: adhesion, release, and aggregation. *Pharmacol Rev* 22:97-187, 1970
21. Sober HA, ed: *CRC Handbook of Biochemistry*, Cleveland, Chemical Rubber Company, 1968, pp J195-J198
22. Wunderly C: Paper electrophoresis. In *Electrophoresis*, Bier M, ed. New York, Academic Press, 1959, pp 187-188
23. Crestfield AM, Allen FW: Improved apparatus for zone electrophoresis. *Anal Chem* 27:422-423, 1955
24. Lerch RA, Bergmann SR, Ambos HD, et al: Effect of flow-independent reduction of metabolism on regional myocardial clearance of ^{11}C-palmitate. *Circulation* 65(4):731-738, 1982
25. Ter-Pogossian MM, Ficke DC, Hood JT, et al: PETT VI: A positron emission tomograph utilizing cesium fluoride scintillation detectors. *J Comp Assist Tomogr* 6:125-133, 1982
26. Kennard CHL: The crystal structure of (Hydrogen ethylenediaminetetraacetato)aquoferrate (III) and gallate (III). *Inorg Chim Acta* 1:347-354, 1967
27. Restivo R, Palenik GJ: A distorted octahedral gallium complex. *J Chem Soc Chem Commun* 867, 1969
28. Riley PE, Pecoraro VL, Carrano CJ, et al: Siderophilin metal coordination. 3. Crystal structures of the cobalt (III), gallium (III) and copper (II) complexes of ethylenebis[(o-hydroxyphenyl)glycine]. *Inorg Chem* 22: 3096-3103, 1983
29. Green MA, Welch MJ, Huffman JC: Synthesis and crystallographic characterization of a gallium salicylaldimine complex of radiopharmaceutical interest. *J Am Chem Soc* 106:3689-3691, 1984
30. Hnatowich DJ: A method for the preparation and quality control of ^{68}Ga radiopharmaceuticals. *J Nucl Med* 16: 764-768, 1975
31. Das Sarma B, Ray KR, Sievers RE, et al: The stereochemistry of metal chelates with multidentate ligands. *J Am Chem Soc* 86:14-16, 1964
32. Wunderly C: *Principles and Applications of Paper Electrophoresis*, New York, Elsevier, 1961, pp 15-17
33. Wieme RJ: Theory of electrophoresis. In *Chromatography, 3rd Ed*, Heftmann E, ed. New York, Van Nostrand Reinhold, 1975, pp 234-235
34. Moerlein SM, Welch MJ, Raymond KN, et al: Tricatecholamide analogs of enterobactin as gallium and indium binding radiopharmaceuticals. *J Nucl Med* 22: 710-719, 1981
35. Raichle ME, Martin WRW, Herscovitch P, et al: Measurement of cerebral blood flow with C-11 butanol and positron emission tomography. *J Nucl Med* 24:P63, 1983 (abstr)
36. Ducan C, Ment L, Lambrecht RM: Radiotracers for cerebral blood flow and emission tomography. In *Applications of Nuclear and Radiochemistry*, Lambrecht RM, Morcos N, eds. New York, Pergamon, 1982, pp 278-281
37. Flora JH, Phillips DF, Arcidiacono F, et al: Distribution of 4-iodoantipyrene after intravenous injection in the rat. *Circ Res* 11:252-256, 1962
38. Sapirstein LA: Regional blood flow by fractional distribution of indicators. *Am J Physiol* 193:161-168, 1958
39. Straub WH, Flanagan DF, Aaron R, et al: Fate of injected radioiodinated 4-iodoantipyrine in the dog and rat. *Proc Soc Exp Biol Med* 116:1119-1122, 1964
40. Forrester DW, Spence VA, Bell I, et al: The preparation and stability of radioiodinated antipyrine for use in local blood flow determinations. *Eur J Nucl Med* 5:145-146, 1980
41. Sakurada O, Kennedy C, Jehle J, et al: Measurement of local cerebral blood flow with iodo[^{14}C]antipyrine. *Am J Physiol* 234:H59-66, 1978
42. Raichle ME, Eichling JO, Straatmann MG, et al: Blood-brain barrier permeability of ^{11}C-labeled alcohols and ^{15}O-labeled water. *Am J Physiol* 230:543-552, 1976
43. Oldendorf WH: Lipid solubility and drug penetration of the blood brain barrier. *Proc Soc Exp Biol Med* 147: 813-816, 1974