Technetium-99m d,l-HM-PAO: A New Radiopharmaceutical for SPECT Imaging of Regional Cerebral Blood Perfusion

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Following investigation of a large number of new ligands based upon propylene amine oxime (PnAO) the d,l-diastereoisomer of hexamethyl propyleneamine oxime (HM-PAO) was selected as the preferred ligand for ^{99m}Tc as a tracer for cerebral perfusion imaging. The neutral, lipophilic ^{99m}Tc complex of d,l-HM-PAO was formed in high yield by stannous reduction of ^{99}Mo/^{99m}Tc generator eluate using a kit formulation of the ligand. Two minutes following i.v. administration of this complex in rats, 2.25% of the injected dose appears in the brain. Little washout of the tracer is observed up to 24 hr postinjection. By qualitative autoradiographic comparison with iodoantipyrine this new radiopharmaceutical displays blood flow dependent brain uptake with little redistribution of the tracer over time. The lipophilic ^{99m}Tc complex converts slowly in vitro to a secondary complex. This conversion process may account for the ability of [^{99m}Tc]d,l-HM-PAO to be retained within the brain without redistribution.

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The discoveries of new radiopharmaceuticals such as selenium-75-labeled di-(piperidinoethyl)selenide (PIPSE) and di-(morpholinoethyl)selenide (MOSE) (*1*), iodine-123- (^{123}I) labeled p-iodo-N-isopropylamphetamine (IMP) (*2*) and N,N-dimethyl-N′-(2-hydroxy-5-iodo-3-methylbenzyl)-1,3-propanediamine (HIPDM) (*3*), and thallium-201 (^{201}Tl) diethyldithiocarbamate (DDC) (*4*) have generated wide interest in possibilities for routine imaging of regional cerebral blood flow (rCBF) using rotating head single photon emission computed tomography (SPECT). In particular, [^{123}I]IMP has attracted much attention (*5,6*), and has been the agent of choice for SPECT studies of rCBF (*7*). However, none of these radiopharmaceuticals is suitable for routine rCBF studies. Selenium-75 and ^{201}Tl display poor physical characteristics, while ^{123}I suffers from high production costs, and limited availability.

As technetium-99m (^{99m}Tc) has none of these disadvantages, several investigators have sought to develop new rCBF tracers based upon this radionuclide (*8–11*). The main biologic requirements for such a radiopharmaceutical are the abilities to cross the intact blood-brain barrier (BBB) and to distribute in the brain proportionally to blood flow. Once in the brain, the tracer should retain a fixed regional distribution for a time sufficient to permit image acquisition. For a rotating gamma camera system, this is typically 20–30 min.

To cross the BBB, the technetium complex has to be relatively small (<500 daltons), lipophilic, and possess a net charge of zero. While the new tracers have the required chemical characteristics, and are capable of crossing the BBB, all display rapid efflux from brain tissue (*8–11*). Recently, a diamine dithiol (DADT) derivative with an amine side chain was reported to provide a ^{99m}Tc complex with improved brain retention (*12*).

Following the discovery that the ^{99m}Tc complex of propyleneamine oxime (PnAO) (Fig. 1) is neutral and lipophilic (*11*) and demonstrates transient flow-related brain uptake in rats (*13*), dogs (*14*), and humans (*15*), a large number of derivatives of PnAO were synthesized at the Amersham International Laboratories (*16*). The aim of this work was to obtain a ligand which not only

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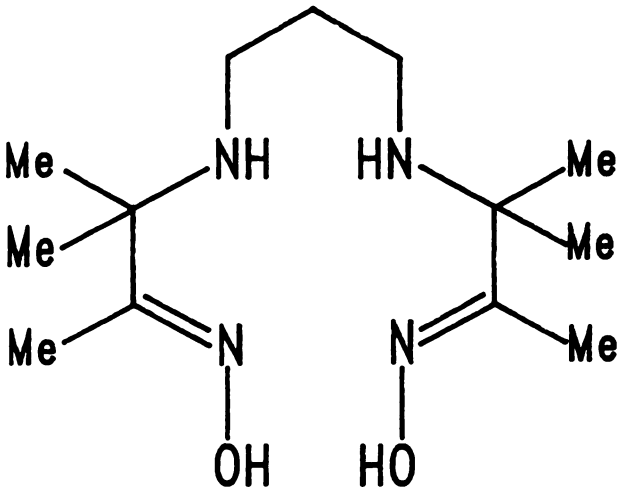

FIGURE 1
Structure of PnAO.

transported ^{99m}Tc across the BBB, but allowed the radiotracer to be retained with a fixed distribution for a time sufficient to permit SPECT imaging. From that study, the ligand which combined the best overall features of high brain uptake, fixed regional distribution within the brain, and ease of radiopharmaceutical preparation was hexamethyl propyleneamine oxime (HM-PAO). The potential of HM-PAO shown in laboratory animals (*17*) was confirmed by the initial clinical findings (*18,19*).

HM-PAO exists in two diastereoisomeric forms, d,l- and meso- (Fig. 2). While the original clinical studies with HM-PAO were conducted with a mixture of these two isomeric forms it was subsequently shown in rats (*20*) and humans (*21*) that one of the diastereoisomers, d,l-HM-PAO, provides a ^{99m}Tc complex with superior brain uptake and retention compared with the complex from the stereoisomeric mixture. We now report on further studies which examine the biologic and chemical characteristics of [^{99m}Tc]d,l-HM-PAO.

MATERIALS AND METHODS

Chemistry

1. Synthesis of d,l-HM-PAO. Organic compounds were characterized by melting point,* ^{1}H NMR,† and IR‡ spectroscopy, and elemental analysis§. In addition, the stereoconfiguration of d,l-HM-PAO was confirmed by an x-ray crystal structure analysis¶.

(a). Preparation of 4,8-diaza-3,6,6,9-tetra-methylundecane-3,8-diene-2,10-dione bisoxime. 2,3-Butanedione monoxime** (11.66 g, 115.4 mmol) was dissolved in benzene (50 ml) containing acetic acid (75 ml), and the solution was brought to reflux in an apparatus fitted with a Dean and Stark trap, and under a nitrogen atmosphere. To this was added a solution of 2,2-dimethyl-1,3-propanediamine** (5.0 g, 5.88 ml, 49 mmol) in benzene (100 ml) over a period of 5 hr. The resulting yellow brown solution was refluxed for a further 16 hr under nitrogen, then allowed to cool to room temperature. The precipitated solid was removed under suction, and washed with a little cold (−40° C) acetonitrile, giving the product as a white powder. Drying under high vacuum for 2 hr gave the product: 7.9 g (60%). Recrystallization from benzene gave crystals for analysis. (m.p. 132–134° C): IR (KBr) 1,640 cm^{-1} (C=N, C=NOH) ^{1}H NMR (d_6-DMSO) δ 3.2 (4H, s, CH_2N), 1.9 (12H, s, MeC), 1.0 (6H, s, CMe_2). Anal. calc. for $C_{13}H_{24}N_4O_2$: C, 58.2: H, 9.0; N, 20.9; Found: C, 58.1; 8.9; N, 20.9.

(b). Preparation of (RR,SS)-4,8-diaza-3,6,6,9-tetra-methylundecane-2,10-dione bisoxime (d,l-HM-PAO). The above bisimine (75 g, 287 mmol) was slurried in 95% aqueous ethanol (690 ml) at 0° C. Sodium borohydride (10.9 g, 287 mmol) was added in portions over 30 min, and the mixture stirred at 0° C for 2 hr. Water (230 ml) was added and the mixture was stirred well for a further 2 hr. The ethanol was removed and more water (140 ml) was added. The pH was adjusted to 11, and the resulting precipitate was removed by filtration, washed with a little water, dried giving the impure HM-PAO: 36 g (47%). Double recrystallization from acetonitrile provided the diastereoisomeric mixture of HM-PAO free from major impurities: 25 g (33%), (m.p. 121–125° C). Fractional crystallization from ethyl acetate provided the d,l-diastereoisomer (m.p. 128–130° C): IR (KBr) 3,310 cm^{-1} (OH), 3,219-3,100

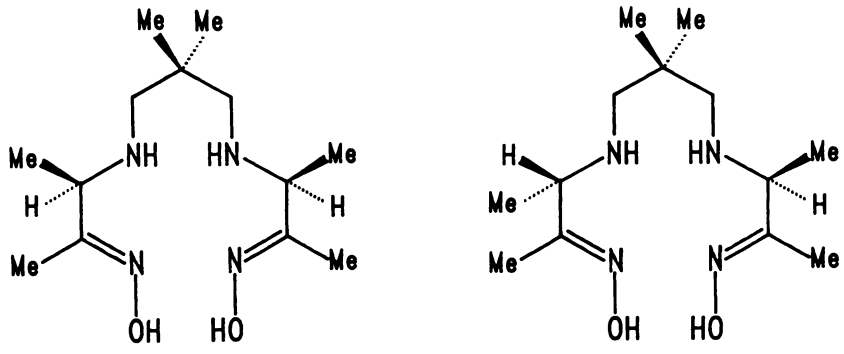

FIGURE 2
Diasteriosomers of HM-PAO.

(OH,NH): ^{1}H NMR (d_6-DMSO) δ 10.3 (2H, s, OH), 3.3 (2H,s,NH), 3.12 (2H,q,CH), 2.12(4H,q,CH_2), 1.64 (6H,s,ME), 1.07 (6H,d,Me), 0.78 (6H,s,Me). Anal. calc. for $C_{13}H_{28}N_4O_2$: C, 57.3; H, 10.4; N, 20.6; Found: C, 57.5; H, 10.2; N, 20.6%.

Stereoisomeric purity was confirmed using normal phase high performance liquid chromatography (HPLC), employing uv detection of the HM-PAO diastereoisomers.

2. Technetium complexation. Sodium pertechnetate-^{99m}Tc was obtained from a commercial ^{99}Mo/^{99m}Tc generator.[††] The ^{99m}Tc-99m complex of d,l-HM-PAO was formed using a freeze dried formulation (Ceretec[††]) of 0.5 mg of d,l-HM-PAO, 7.6 μg of stannous chloride dihydrate, and 4.5 mg of sodium chloride, in a sealed 10-ml glass vial under an atmosphere of nitrogen. Five milliliters of sodium pertechnetate (^{99m}Tc, 2–7 mCi/ml) was injected into the vial. The vial was shaken to dissolve the solid contents. Complex radiochemical purity (RCP) was assayed by thin layer chromatography.

The influences of generator eluate age, time since previous generator elution, and radioactive concentration on RCP were studied. Eluate was taken from generators which had been eluted 2, 24, and 70 hr previously. These eluates were used to reconstitute vials of the freeze-dried formulation at 0.5, 2, and 4 hr postgenerator elution. These vials were analyzed for percentage primary and secondary complexes, reduced hydrolyzed technetium and pertechnetate at 2, 30, and 60 min postreconstitution. A single vial was reconstituted with 256 mCi of generator eluate, and the contents analyzed at 2, 10, and 30 min postreconstitution.

3. Analysis of ^{99m}Tc complexes. The distribution of radioactivity on chromatograms was quantitatively analyzed using a 256 channel gas flow energy-proportional counter interfaced to an HP 9816[‡‡] computer system.

(a). Thin layer chromatography. A combination of three chromatographic systems was used for a complete characterization of the radiochemical composition of the solutions prepared in 2, above.

Ten microliter test samples were applied 2.5 cm from the base of three chromatographic strips, two were ITLC/SG[§§] (2 × 20 cm) and the third was Whatman No. 1 (2 × 30 cm). Immediately after the application of the sample, the chromatograms were developed by ascending chromatography in tanks containing fresh solvent to a depth of 1 cm. One ITLC/SG strip was developed in 2-butanone (MEK) and the other in 0.9% saline. The Whatman No. 1 strip was developed in 50% aqueous acetonitrile. After development, the strips were dried, and the radioactivity distribution was determined.

(b). Electrophoresis. Ten microliter test samples of the ^{99m}Tc complex of d,l-HM-PAO were applied to Whatman 541 strips, saturated with 50 m*M* pH 7.4 phosphate buffer, in an electrophoresis bath[§§]. 300V was applied across 20 cm of the strip for 1 hr. The strips were dried, and the distribution of radioactivity on the strip was determined.

(c). High performance liquid chromatography. The complex was analyzed using a divinylbenzene-styrene copolymer column[¶¶] fitted to a radioactivity detector. At a flow rate of 2 ml/min, 20 m*M* phosphate buffer pH 7.4 was pumped through the column. When the test sample was injected onto the column, a 0–25% linear solvent gradient of tetrahydrofuran (THF) was commenced (25% THF in 6 min). The radioactivity detector was fitted to a ratemeter and microcomputer programmed for peak integration.

Biology

1. Biodistribution of ^{99m}Tc complexes. Biodistribution studies were performed in male Sprague Dawley rats (130–170 g). Under light ether anesthesia each animal was administered 100 μl of the test preparation through the lateral tail vein. The RCP of all preparations was checked prior to injection, and at all times, the purity of the ^{99m}Tc complex was not less than 85%. At killing, samples of blood, muscle, bone, skin, and fat were collected in preweighed containers. Other organs were removed intact, as listed in Table 3. All organs and tissues were assayed for radioactivity in a twin crystal automatic gamma counter. The accumulated activity in each organ or tissue was calculated as a percentage of the total dose. For blood, bone, muscle, skin, and fat the calculation was based upon the measured activity and weight of the sample, and body composition data (blood, bone, muscle, skin, and fat are 5.8, 5.0, 43.0, 18.0, and 7.0%, respectively).

2. Whole-body autoradiography. Carbon-14- (^{14}C) labeled d,l-HM-PAO was prepared from [2,2-dimethyl-C^{14}]-2,2-dimethyl-1,3-propanediamine and butanedione monoxime by the method outlined for the synthesis of the inactive ligand. The specific activity was 105 mCi/mmol. The material was stored as a freeze-dried powder at −20° C, and when required, this was dissolved in sterile water to give a radioactive concentration of 370 μCi/ml.

Four Sprague Dawley rats (95–105 g) were injected with [^{14}C]d,l-HM-PAO (37 μCi) and four rats with [^{99m}Tc]d,l-HM-PAO (36 mCi in 600 μl) through a lateral tail vein under light ether anesthesia. At 2 min and at 1 hr postinjection two animals from each group were killed. Twenty micrometer whole-body sagittal sections were prepared by standard procedures and exposed to a fast x-ray film[***] for ^{99m}Tc, or a slower, fine grain film[†††], for ^{14}C.

After exposure for an appropriate period, films were developed and, in each case, films of the midline section and a lateral section were used as negatives to produce contact prints. On the latter, regions with high radioactive concentration appear black, and low activity regions appear white.

3. Rat brain autoradiography. Four male Sprague Dawley rats (250–400 g) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and their right jugular vein cannulated. The animals were killed at specific times following the i.v. injection of 1 ml of [^{99m}Tc]HM-PAO (40–50 mCi of ^{99m}Tc) over 20 sec. Killing times were 5 sec following peak brain uptake (determined by a collimated NaI (Tl) crystal detector placed over the head and interfaced to a multichannel analyzer), and 1, 5, 30, and 60 min postinjection. After killing, the brains were removed and frozen in liquid nitrogen. Twenty micrometer serial coronal sections were taken through the cerebral hemispheres and cerebellum at −24°C in a refrigerated microtome. The sections were mounted on plastic cover strips, dried, and exposed to x-ray film.[‡‡‡]

For studies comparing the cerebral distribution of [^{99m}Tc] d,l HM-PAO and ^{14}C-labeled iodoantipyrine ([^{14}C]IAP[‡‡]), the above procedure was used. One animal was injected with a 0.75-ml solution containing 43 mCi of [^{99m}Tc]d,l-HM-PAO and 62 μCi of [^{14}C]IAP. Following killing at 5 sec postpeak, and slice preparation as described above, slices were placed on fast x-ray film[‡‡‡] for 24 hr to provide the ^{99m}Tc autoradiograph. Three days later, when ^{99m}Tc had decayed to background levels, the slices were exposed to a slow film[§§§] for 10

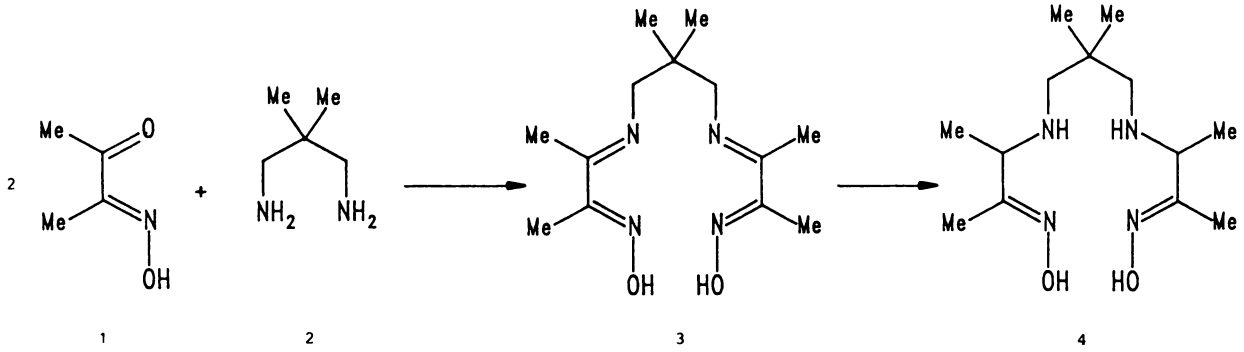

FIGURE 3
Synthesis of HM-PAO.

days to provide the ^{14}C autoradiograph. Each section was stained with 2% cresyl violet and then fixed in 70% ethanol. The cresyl violet stain provided a clear differentiation of grey and white matter. Images were stored in a microcomputer, using a system described previously (*22*). Images shown in Figure 8 were obtained from the monitor display.

4. Calculation of radiation doses. Using the standard MIRD 11 format (*23*), radiation doses to humans receiving intravenous [^{99m}Tc]d,l-HM-PAO were estimated on the basis of the rat biodistribution data. Account was taken of a number of urinary bladder voiding patterns.

5. Toxicology. Toxicology studies have been performed in male and female rats and rabbits following single and repeated intravenous injections. For single dose studies, animals received 17 vials/kg (equivalent to 1,200 vials/70 kg); for repeat dose studies, animals received 14 consecutive daily doses of 14.3 vials/kg (the equivalent to 1,000 vials/70 kg).

RESULTS

Chemistry

The route of synthesis of HM-PAO is shown in Figure 3. Condensation of the propanediamine (Fig. 3, no. 2) with two molecular equivalents of the keto-oxime (Fig. 3, no. 1) provides the bisimine (Fig. 3, no. 3) in 60% yield. Reduction of the two imine groups with sodium borohydride provides HM-PAO (Fig. 3, no. 4) as an equal mixture of the two diastereoisomers, meso- and d,l, as demonstrated by HPLC analysis (Fig. 4). Repeated crystallization from ethyl acetate permits the separation of the two diastereoisomers (Fig. 4), but results in considerable reduction in the overall yield of the ligand. An x-ray crystal structure analysis demonstrated that the stereoisomer with longest retention on HPLC analysis was d,l-HM-PAO (Fig. 5).

The freeze-dried formulation of d,l-HM-PAO allows the ^{99m}Tc complex to be prepared simply by adding generator eluate to the vial. Thin layer chromatography permits the quantitative determination of radioactive components following complex formation. In Table 1 are listed the R_f values of the observed radioactive components on the three chromatographic systems used. Addition of [^{99m}Tc]pertechnetate to this freeze-dried formulation provides the primary lipophilic com-

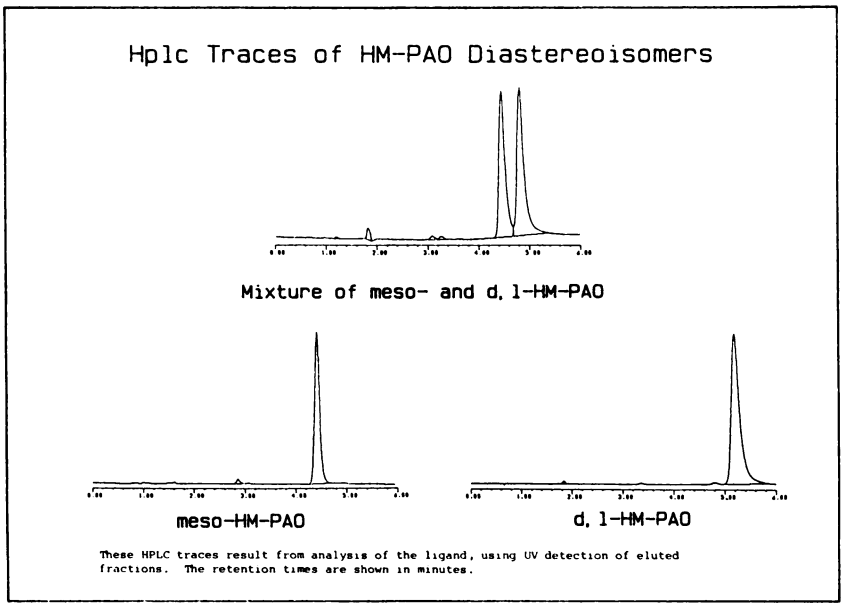

FIGURE 4
HPLC analysis of HM-PAO diastereoisomers.

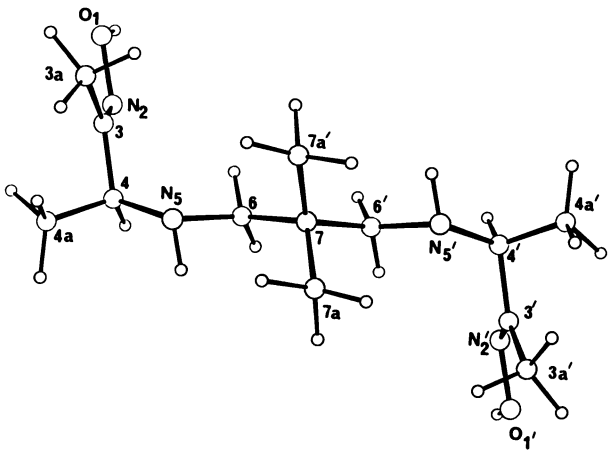

FIGURE 5
Structure of d,l-HM-PAO from x-ray crystal analysis.

plex of d,l-HM-PAO in >90% yield immediately after complex formation. Other observed components are a less lipophilic ^{99m}Tc complex of d,l-HM-PAO (termed the secondary complex), and very small amounts of reduced hydrolyzed technetium and pertechnetate.

Combined use of the three chromatographic systems allows the full quantitative assessment of the radiochemical composition of the mixture using the following relationships:

% reduced, hydrolyzed Tc = % activity remaining at the origin in the Whatman No. 1/50% aq. acetonitrile system;
% pertechnetate = % activity at the solvent front in the ITLC/saline system;
% secondary complex = % activity at R_f 0-0.3 in the ITLC/MEK system minus % reduced hydrolyzed Tc;
% primary complex = % activity at R_f 0.3-1 in the ITLC/MEK system minus % pertechnetate.

Table 2 lists the observed proportion of each radioactive component over a period following the addition of [^{99m}Tc]pertechnetate to the freeze-dried formulation.

TABLE 1
R_f Values of the ^{99m}Tc Components on TLC

Component	System 1*	2†	3‡
[^{99m}Tc]d,l-HM-PAO primary	0.9–1.0	0	0.9–1.0
[^{99m}Tc]d,l-HM-PAO secondary	0	0	0.9–1.0
^{99m}Tc reduced, hydrolyzed	0	0	0
[^{99m}Tc]pertechnetate	0.9–1.0	0.9–1.0	0.9–1.0

* System 1: ITLC/SG—MEK.
† System 2: ITLC/SG—saline.
‡ System 3: Whatman No. 1—50% aqueous acetonitrile.

There is a slow conversion of the primary complex to a secondary complex. In biologic studies this conversion limits the time postpreparation in which the material can be used. In all cases, the radiopharmaceutical was used with proportion of the primary ^{99m}Tc complex never < 85%.

The rate of loss of primary complex is increased notably when using generator eluate of age >2 hr, or employing a high radioactive concentration. In these cases, higher pertechnetate levels are the primary cause of the reduced proportion of primary complex.

The time since previous elution of the generator has little effect on the rate of loss of primary complex with the commercial ^{99}Mo/^{99m}Tc generator†† used in this study. However, evaluation of a number of commercial generators revealed that, in some cases, the rate of loss of primary complex increased when using eluate from a generator, not previously eluted within 24 hr (Tyrrell DA et al. unpublished data).

HPLC analysis also demonstrates the formation of two ^{99m}Tc complexes of d,l-HM-PAO, as shown in Figure 6. The analysis shown was performed 30 min after complex formation.

We have demonstrated previously (*24*) that HPLC retention on this reverse phase system can provide an estimate of the lipophilicity of ^{99m}Tc complexes. Traditionally, lipophilicity is determined by the concentration ratio (P) of a compound partitioned between two immiscible solvents (usually n-octanol and water). This method is only suitable for pure compounds, but a calibrated HPLC system can provide estimates of log P from the retention times of components in a mixture. The estimated log P value for the primary complex is 1.2 and the value for the secondary complex is <0. By electrophoresis, both the primary and secondary complexes of d,l-HM-PAO remain at the point of application, suggesting that both are neutral.

Biology

The biodistribution of the primary complex of d,l-HM-PAO is shown in Table 3. The primary complex displays 2.25% (% i.d. in whole organ) brain uptake soon after injection. Retention of activity is high, with 84% of the initial activity (decay corrected) remaining in the brain 1 hr postinjection, and 73% at 24 hr postinjection. Background activity clears by both the hepatobiliary and urinary pathways.

An initial period of rapid blood clearance to 12% of injected dose is followed by a phase of very slow clearance. Examination of the distribution of this radioactivity in blood reveals that >80% of that activity is in the red blood cells.

Whole-body autoradiography (Fig. 7) provides a qualitative assessment of regional distribution of the primary ^{99m}Tc complex of d,l-HM-PAO. Uptake of the tracer within the brain is clearly visible. However, the

TABLE 2
Determination of RCP, Examining the Influences of Generator Eluate Age, Time Since Previous Elution, and Radioactive Concentration

Generator eluate/kit reconstitution details			RCP at times after reconstitution (%)											
			2 min				30 min				60 min			
Time since previous elution of generator (hr)	Age of generator eluate on kit reconstitution (min)	Total activity added to the vial (mCi in 5 ml)	No. 1*	No. 2†	TcO_4^-	RHT‡	No. 1	No. 2	TcO_4^-	RHT	No. 1	No. 2	TcO_4^-	RHT
	5	27.3	96	0	0	4	92	3	1	5	86	9	1	4
2	240	28.1	95	1	1	4	89	5	4	2	78	8	12	2
	5	31.3	93	2	1	5	88	9	0	3	84	11	0	4
24	120	33.5	95	3	1	2	92	5	1	2	87	7	4	2
	240	27.8	95	1	1	3	88	4	5	3	77	10	11	2
	5	32.2	95	3	0	2	93	4	0	3	88	10	0	2
70	120	30.0	96	2	0	2	93	3	1	3	88	7	2	2
	240	30.0	95	3	0	1	93	4	1	2	83	8	9	1
			2 min				10 min				30 min			
			No. 1	No. 2	TcO_4^-	RHT	No. 1	No. 2	TcO_4^-	RHT	No. 1	No. 2	TcO_4^-	RHT
18	30	256	96	3	1	1	94	3	2	1	76	7	17	1

*No. 1 = Primary complex of [^{99m}Tc]d,l-HM-PAO.
†No. 2 = Secondary complex of [^{99m}Tc]d,l HM-PAO.
‡RHT = Reduced, hydrolyzed technetium.

uncomplexed ligand does not cross the BBB; the autoradiographs following administration of ^{14}C-labeled d,l-HM-PAO demonstrate the absence of the free ligand in the brain.

Results of studies to determine the regional distribution of the primary [^{99m}Tc]d,l-HM-PAO complex within the rat brain are shown in Figure 8. Qualitatively, the regional distribution of [^{99m}Tc]d,l-HM-PAO at 5 sec postpeak brain uptake is similar to that observed for ^{14}C-labeled iodoantipyrine (IAP), a reference tracer for rCBF (*25*). The prominent uptake of both tracers in regions rich in gray matter is highlighted by comparison of the autoradiographs with the cresyl violet stained section. Figure 9 displays the results of temporal measurement of the regional distribution of [^{99m}Tc]d,l-HM-PAO in the rat brain. Over the period of study (1 hr) there appears to be little redistribution of the tracer as the image of the blood flow distribution pattern remains unaltered.

Results of organ radiation dose calculations are summarized in Table 4. The highest doses are received by the urinary bladder and upper large intestines.

In all toxicity testing, no treatment-related effects were evident. Observations were made of body weights, clinical signs, food and water consumption, hematology, blood chemistry, ophthalmology, and urinalysis.

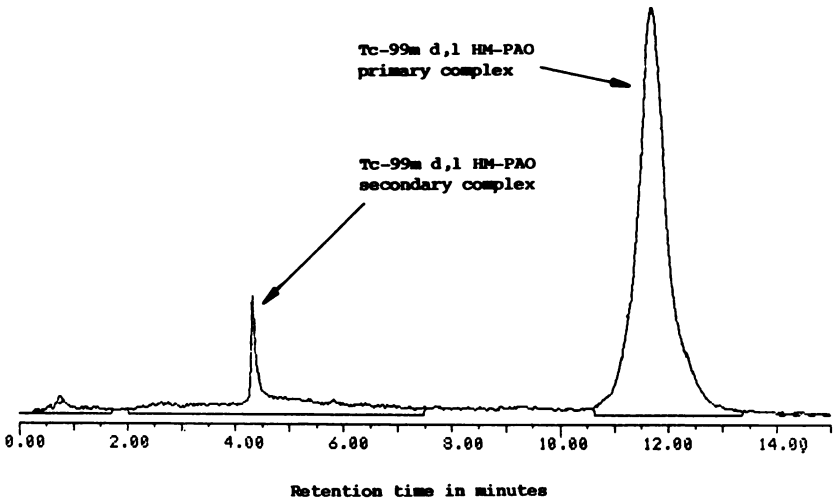

FIGURE 6
HPLC analysis of [^{99m}Tc]d,l-HM-PAO.

TABLE 3

Biodistribution of the Primary ^{99m}Tc Complex of d,l HM-PAO in Rats (Mean ± s.d. of Six Animals, % Injected Dose in Whole Organ or Tissue)

	Time postinjection										
Organ/tissue	30 sec	1 min	2 min	5 min	20 min	1 hr	2 hr	4 hr	7 hr	24 hr	48 hr
Bone	4.09 ± 0.28	4.14 ± 0.37	4.18 ± 0.35	3.77 ± 0.41	3.71 ± 0.26	2.90 ± 0.32	2.63 ± 0.34	2.29 ± 0.26	2.04 ± 0.82	1.17 ± 0.82	0.73 ± 0.26
Muscle	31.44 ± 7.58	26.73 ± 8.02	26.49 ± 1.9	26.94 ± 6.24	20.65 ± 2.89	19.49 ± 6.38	22.19 ± 4.32	15.36 ± 4.98	15.24 ± 3.40	10.56 ± 3.29	10.77 ± 2.32
Blood	12.40 ± 1.58	12.83 ± 1.52	12.25 ± 1.28	11.25 ± 0.57	9.97 ± 0.79	8.74 ± 0.95	7.98 ± 0.83	6.91 ± 0.46	6.51 ± 0.79	3.95 ± 0.34	2.79 ± 0.29
Kidney	0.65 ± 0.62	6.34 ± 0.62	5.18 ± 0.74	5.74 ± 0.44	5.84 ± 0.47	5.81 ± 0.49	4.91 ± 0.21	4.75 ± 0.20	4.73 ± 0.20	3.88 ± 0.36	3.36 ± 0.53
Bladder urine	0.12 ± 0.06	0.11 ± 0.09	0.16 ± 0.09	0.89 ± 0.86	5.64 ± 0.97	13.46 ± 4.97	18.25 ± 1.77	24.13 ± 1.45	25.07 ± 6.24	30.90 ± 4.52	41.30 ± 5.08
Lung	4.17 ± 0.68	3.85 ± 0.54	3.97 ± 1.00	3.34 ± 0.41	3.29 ± 0.63	2.66 ± 0.10	2.22 ± 0.14	2.04 ± 0.34	1.79 ± 0.33	1.00 ± 0.10	0.64 ± 0.07
Liver	11.21 ± 1.74	12.01 ± 2.23	12.16 ± 2.03	12.28 ± 1.64	11.95 ± 1.87	10.03 ± 1.33	7.87 ± 1.39	5.65 ± 0.56	4.64 ± 0.33	2.73 ± 0.41	1.73 ± 0.18
Spleen	0.54 ± 0.10	0.47 ± 0.16	0.58 ± 0.25	0.54 ± 0.11	0.52 ± 0.06	0.54 ± 0.10	0.49 ± 0.09	0.32 ± 0.05	0.28 ± 0.04	0.17 ± 0.03	0.14 ± 0.05
Stomach	1.56 ± 0.57	1.97 ± 0.89	1.57 ± 0.86	1.39 ± 0.23	1.62 ± 0.63	0.87 ± 0.34	0.77 ± 0.06	0.66 ± 0.10	0.82 ± 0.16	0.33 ± 0.07	0.25 ± 0.05
Stomach contents	0.38 ± 0.26	0.45 ± 0.27	0.32 ± 0.19	0.55 ± 0.22	1.05 ± 0.29	0.21 ± 0.09	0.23 ± 0.12	0.14 ± 0.06	0.19 ± 0.04	0.45 ± 0.95	0.08 ± 0.06
Small intestines	6.49 ± 1.39	6.56 ± 0.85	6.70 ± 1.16	6.48 ± 1.30	6.63 ± 1.03	4.81 ± 1.19	4.93 ± 0.89	3.55 ± 0.60	2.70 ± 0.39	1.16 ± 0.25	0.51 ± 0.09
SI contents	2.92 ± 0.53	2.99 ± 0.89	1.92 ± 0.55	4.16 ± 1.56	8.85 ± 1.42	14.96 ± 1.73	14.88 ± 0.97	5.22 ± 1.06	2.43 ± 0.59	0.71 ± 0.10	0.31 ± 0.08
Large intestines	2.05 ± 0.48	2.57 ± 1.12	2.77 ± 1.46	2.45 ± 0.75	1.88 ± 0.48	1.56 ± 0.48	1.23 ± 0.18	1.95 ± 0.76	2.19 ± 1.64	0.67 ± 0.15	0.40 ± 0.08
LI contents	0.61 ± 0.12	0.66 ± 0.06	0.69 ± 0.54	0.73 ± 0.40	0.67 ± 0.31	0.99 ± 0.18	1.61 ± 0.79	14.04 ± 1.58	14.87 ± 5.14	1.93 ± 0.78	0.55 ± 0.19
Heart	1.17 ± 0.16	1.10 ± 0.14	0.95 ± 0.07	0.92 ± 0.15	0.73 ± 0.07	0.66 ± 0.04	0.60 ± 0.06	0.56 ± 0.05	0.46 ± 0.04	0.30 ± 0.02	0.23 ± 0.03
Thyroid	0.18 ± 0.03	0.19 ± 0.04	0.17 ± 0.05	0.15 ± 0.03	0.15 ± 0.03	0.11 ± 0.04	0.09 ± 0.04	0.13 ± 0.02	0.19 ± 0.02	0.11 ± 0.01	0.10 ± 0.03
Brain	2.22 ± 0.20	2.22 ± 0.37	2.25 ± 0.51	1.92 ± 0.17	2.24 ± 0.21	1.88 ± 0.19	1.79 ± 0.19	1.79 ± 0.25	1.79 ± 0.34	1.64 ± 0.18	1.30 ± 0.23
Eyes	0.13 ± 0.03	0.10 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.06 ± 0.01	0.07 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.05
Salivaries	0.89 ± 0.32	0.93 ± 0.21	0.81 ± 0.28	0.67 ± 0.14	0.72 ± 0.21	0.55 ± 0.21	0.46 ± 0.23	0.51 ± 0.15	0.39 ± 0.10	0.19 ± 0.06	0.19 ± 0.10
Pituitary	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03
Pancreas	0.85 ± 0.24	0.68 ± 0.24	0.79 ± 0.30	0.65 ± 0.17	0.53 ± 0.18	0.43 ± 0.05	0.38 ± 0.06	0.35 ± 0.07	0.29 ± 0.07	0.22 ± 0.07	0.14 ± 0.03
Adrenals	0.21 ± 0.04	0.21 ± 0.06	0.22 ± 0.05	0.21 ± 0.09	0.15 ± 0.06	0.15 ± 0.04	0.13 ± 0.03	0.09 ± 0.02	0.08 ± 0.01	0.07 ± 0.02	0.08 ± 0.04
Gonads	0.54 ± 0.15	0.63 ± 0.16	0.62 ± 0.16	0.58 ± 0.16	0.63 ± 0.13	0.44 ± 0.06	0.39 ± 0.04	0.40 ± 0.08	0.40 ± 0.09	0.28 ± 0.07	0.20 ± 0.06
Skin	10.73 ± 2.33	10.42 ± 1.56	11.59 ± 1.25	12.59 ± 0.94	10.49 ± 1.57	9.83 ± 1.27	8.51 ± 1.34	8.37 ± 0.46	6.50 ± 0.66	6.89 ± 1.13	5.64 ± 0.71
Fat	4.31 ± 1.59	3.84 ± 1.28	4.43 ± 2.24	4.19 ± 1.16	2.44 ± 0.32	2.32 ± 0.27	1.78 ± 0.48	1.66 ± 0.42	1.46 ± 0.29	0.89 ± 0.31	1.36 ± 0.45
Feces	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.03	0.02 ± 0.02	0.12 ± 0.16	0.18 ± 0.39	6.82 ± 5.51	31.22 ± 1.84	31.61 ± 4.06

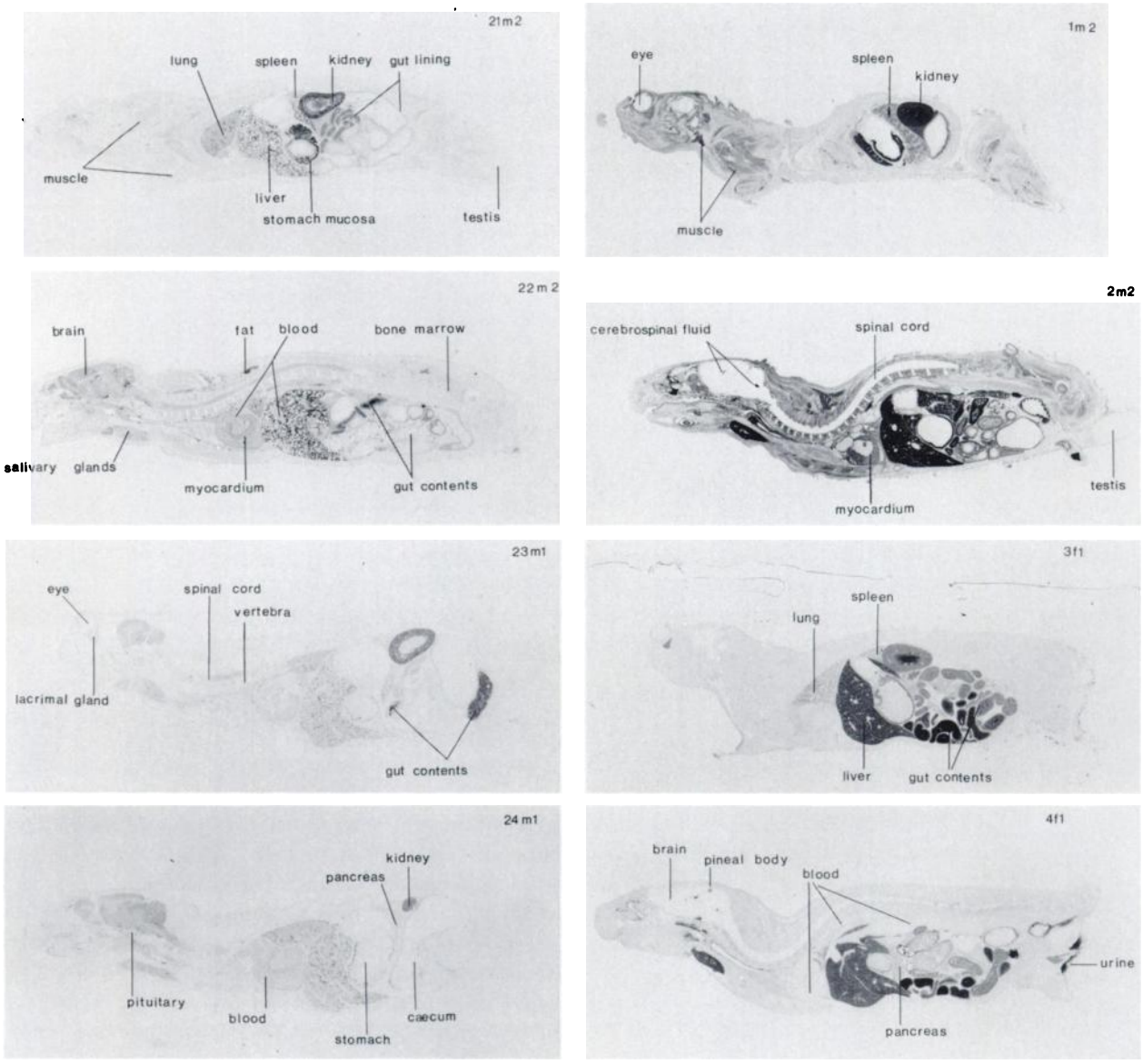

FIGURE 7
Whole-body autoradiographs of [^{99m}Tc]d,l-HM-PAO and [^{14}C]d,l-HM-PAO. Left column: [^{99m}Tc]d,l-HM-PAO. Right column: [^{14}C]d,l-HM-PAO. Top row: lateral section, 2 min. Second row: midline section, 2 min. Third row: lateral section, 60 min. Bottom row: midline section, 60 min.

At termination complete necropsy and histopathology revealed no abnormalities.

DISCUSSION

The ligand d,l-HM-PAO readily forms a neutral, lipophilic complex with ^{99m}Tc from a freeze-dried 'kit' to provide a new radiopharmaceutical for imaging cerebral perfusion. The quality (RCP) of the radiopharmaceutical is dependent upon several factors concerning generator eluate age, time since previous elution of the generator, and radioactive concentration. It is therefore recommended to (a) use eluate from a ^{99m}Tc generator which was previously eluted within 24 hr; (b) use generator eluate within 2 hr of elution; (c) add no more than 30 mCi of ^{99m}Tc to the vial; and (d) use the radiopharmaceutical within 30 min of reconstitution.

High brain uptake was demonstrated in rats, and up to 48 hr postinjection little washout occured. A qualitative assessment of regional distribution of the tracer within rat brain indicates a blood flow-dependent uptake with minimal redistribution of the tracer up to at least 1 hr postinjection. The background activity levels clear, principally by the urinary system, but also through the hepatobiliary system. In humans, the higher proportion of cardiac output to the brain gives rise to higher brain uptake (*21*).

The rat biodistribution data were used to estimate

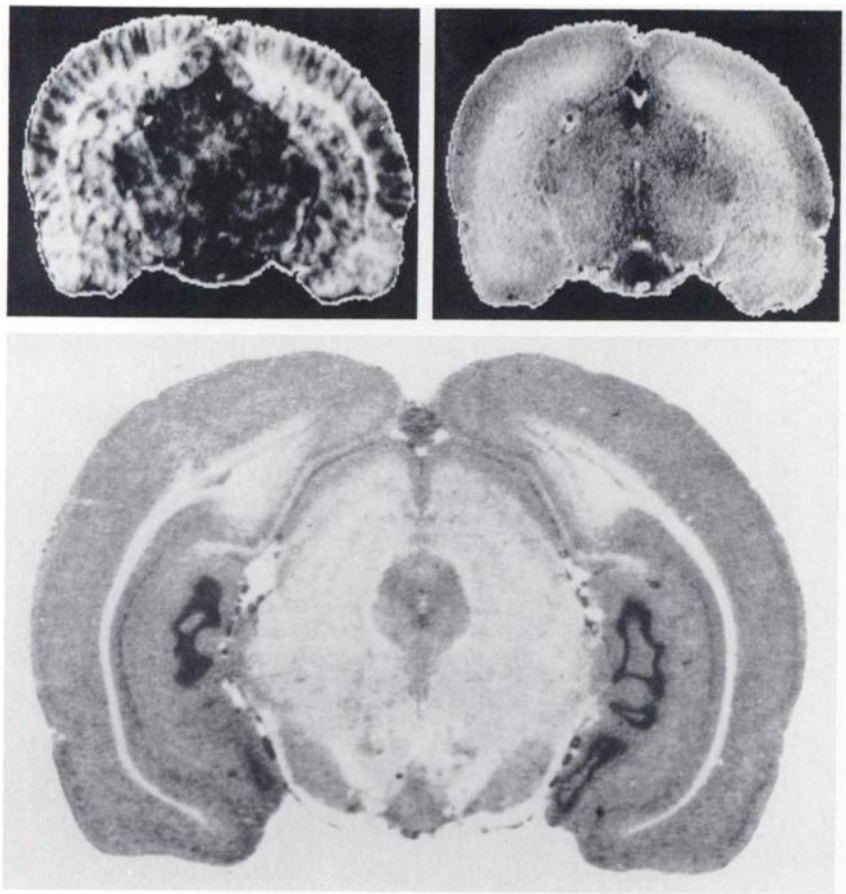

FIGURE 8
Comparison of [^{99m}Tc]d,l-HM-PAO and [^{14}C]iodoantipyrine cerebral distribution. (Rat brain autoradiographs with stained sections).

the radiation dosimetry in humans. These estimates indicate that no problems due to radiation exposure can be anticipated at dose levels of up to 15 mCi in humans, even with delayed bladder voiding. These data compare favorably with the corresponding dose estimates for [^{123}I]IMP, even discounting the contaminants of ^{124}I and ^{125}I in the iodinated radiopharmaceutical (*26*). Similarly, the acute toxicity study employing the freeze-dried formulation as test article would indicate that no toxicological effects can be expected at normal human dose levels.

The structure of the primary technetium complex of d,l-HM-PAO has been determined (*27*) and shown to be very similar to the complex obtained with PnAO (*28*). While both ligands can transport technetium across the BBB [^{99m}Tc]PnAO behaves like a freely diffusable tracer, whereas [^{99m}Tc]d,l-HM-PAO is retained in the brain and displays a fixed regional distribution within that organ. This is essential for SPECT imaging using the rotating head gamma camera. It has been proposed that retention in the brain of [^{99m}Tc]d,l-HM-PAO results from in vivo conversion of the primary complex to the more hydrophilic species (*29*). It has been shown previously (*30*) that neutral compounds with log P values in the range of 0.9–3.5 can cross the intact BBB. The log P value for the primary complex falls within this range, but the value for the secondary complex is below the range. Studies to confirm the mechanism of retention are underway.

A high proportion of the radioactivity remaining in the blood appears to be trapped within red blood cells; it is possible that the mechanism for entrapment within these cells is similar to that of brain retention. The high blood levels (10–12% of injected dose) of this radiopharmaceutical should not adversely affect SPECT image quality. In humans, the volume of blood within the brain is 31 ml, or 0.6% of total blood volume (*31*). Technetium-99m-d,l-HM-PAO displays 4.1% i.d. uptake in the brain in humans, with blood levels at 1 hr postinjection averaging 12.0% of injected dose (*21*). Thus, the contribution of blood activity to total counts from the brain is only 1.7% (1 hr postinjection).

The ability of the primary complex to cross the BBB is highlighted further by the whole-body autoradiographs comparing the biodistribution of [^{99m}Tc]d,l-HM-PAO, and the ^{14}C-labeled ligand. While uptake of the complex is clearly visible, the ligand itself is not transported across the BBB. Hence, d,l-HM-PAO only accumulated in the brain when complexed to ^{99m}Tc as the primary complex. These findings are similar to those observed for [^{99m}Tc]PnAO and ^{14}C-labeled PnAO (*32*). Thus, the primary ^{99m}Tc complex of d,l-HM-PAO can be classified as a "technetium essential" radiopharmaceutical (*33*).

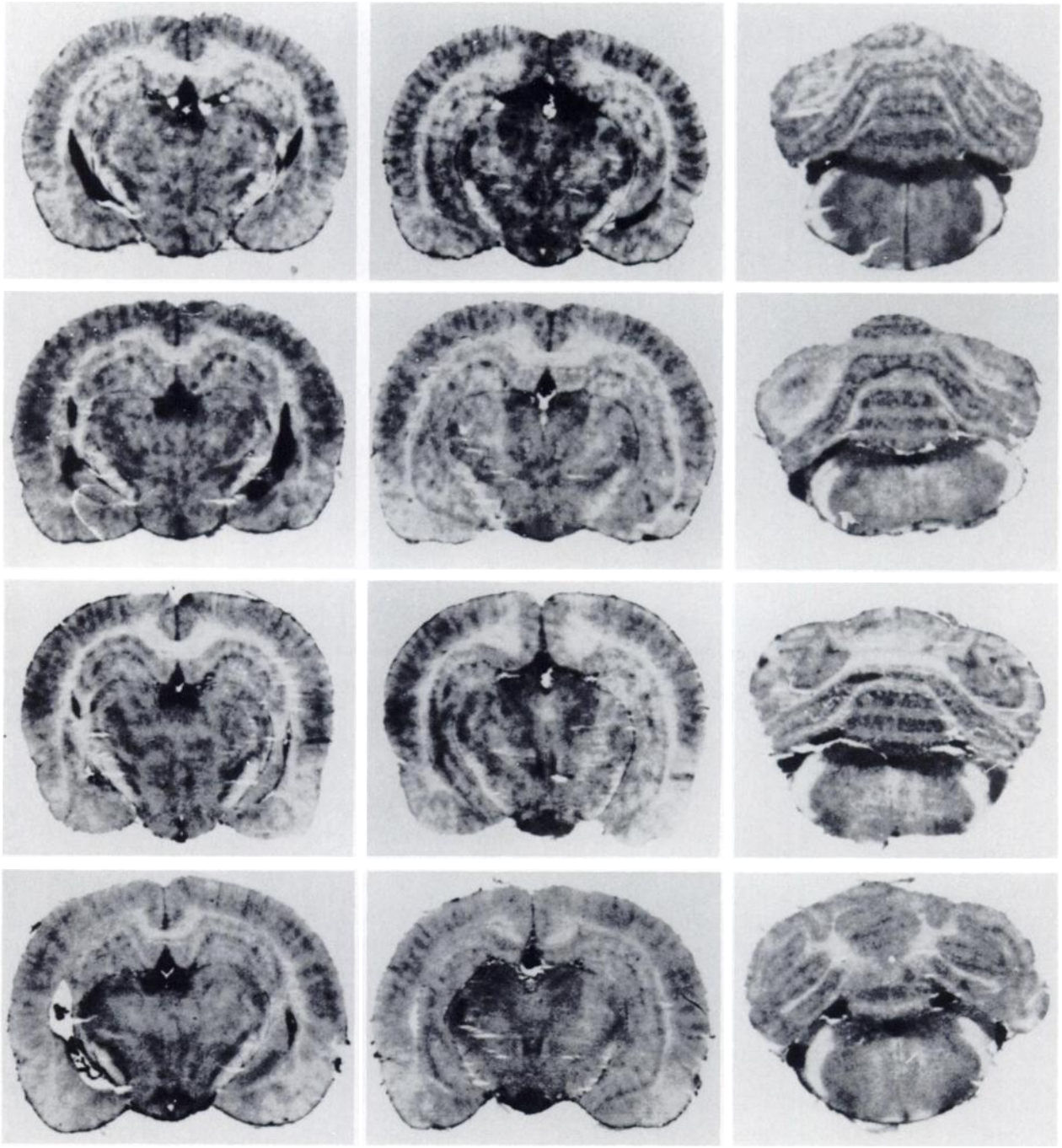

FIGURE 9
Regional distribution of d,l HM-PAO in the rat brain as a function of time. From left to right: thalamus, midbrain, and cerebellum. From top to bottom: 1 min, 5 min, 30 min, and 60 min.

Technetium-99m d,l-HM-PAO displays a regional distribution in the brain which, from the qualitative autoradiographic comparison with IAP, appears to be blood flow dependent. The 5-sec [^{99m}Tc]d,l-HM-PAO autoradiograph displays far better substructural detail than the corresponding IAP autoradiograph. The lower quality image provided by IAP could be attributed to some diffusion of this tracer within the brain after sacrifice, but served to highlight the excellent structural detail provided by [^{99m}Tc]d,l-HM-PAO. Recent studies in rabbits (*34*) and dogs (*35*), which quantitatively compare the regional distribution of [^{99m}Tc]d,l HM-PAO in the brain with labeled microspheres, confirm that [^{99m}Tc]d,l-HM-PAO has a blood flow-related brain distribution.

Extensive clinical studies have now been conducted at a large number of centers (*21,36–42*) and have confirmed the promise shown by the preliminary data

TABLE 4
Technetium-99m d,l-HM-PAO Summary of Organ Radiation Doses

Organ	Absorbed radiation dose					
	rads/mCi			mGy/MBq		
	2-hourly voids	4-hourly voids	No voids	2-hourly voids	4-hourly voids	No voids
Bone (total)	0.014	0.014	0.016	0.004	0.004	0.004
Red bone marrow	0.024	0.024	0.027	0.007	0.007	0.007
Testes	0.008	0.009	0.016	0.002	0.002	0.004
Ovaries	0.040	0.042	0.052	0.011	0.011	0.014
Lungs	0.009	0.009	0.009	0.002	0.002	0.002
Thyroid	0.005	0.005	0.005	0.001	0.001	0.001
Total body	0.015	0.016	0.019	0.004	0.004	0.005
Breast	0.015	0.016	0.019	0.004	0.004	0.005
Liver	0.042	0.042	0.042	0.011	0.011	0.011
Small intest. wall	0.100	0.100	0.105	0.027	0.027	0.028
Upper large intest. wall	0.180	0.180	0.183	0.049	0.049	0.050
Lower large intest. wall	0.078	0.080	0.091	0.021	0.022	0.025
Kidneys	0.097	0.097	0.098	0.026	0.026	0.027
Urinary bladder wall	0.052	0.091	0.328	0.014	0.025	0.089
Brain	0.015	0.015	0.015	0.004	0.004	0.004

presented here. Technetium-99m d,l-HM-PAO has provided high quality SPECT images in patients with a variety of cerebrovascular and neurological disorders.

NOTES

* Gallenkamp.
† R24B, Perkin-Elmer, Norwalk, CT.
‡ 684, Perkin Elmer, Norwalk, CT.
§ Elemental Micro-Analysis Ltd.
¶ North London Polytechnic.
** Aldrich Chemical Co., Milwaukee, WI.
†† Amersham International plc, Buckinghamshire, England.
‡‡ Hewlett Packard Co., Andover, MA.
§§ Gelman Sciences, Inc., Ann Arbor, MI.
¶¶ PRP-1, Hamilton Co., Reno, NV.
*** Osray M3, Agfa-Gevaert Rex, Inc., White Plains, NY.
††† Industrex A, Eastman Kodak Co., Rochester, NY.
‡‡‡ MRF 31 CT film, DuPont Co., No. Billerica, MA.
§§§ SP-5 Kodak film, Eastman Kodak Co., Rochester, NY.

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