A Neutral Lipophilic Technetium-99m Complex for Regional Cerebral Blood Flow Imaging

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Technetium-99m-DMG-2MP (Chloro[bis{2,3-butanedionedioxime(1-)-0}{2,3-butanedionedioximato (2-)-N,N',N'',N''',N'''',N'''''} (2-methylpropyl borato (2-)) technetium]), also known as SQ 32097 is a member of a family of neutral lipophilic compounds generally known as boronic acid adducts of technetium dioxime complexes (BATOs). After i.v. administration, the concentration of [^{99m}Tc]DMG-2MP in various regions of the brain appears to be proportional to blood flow. In rats, 1.1% ID was in the brain at 5 min postinjection when the blood contained <3% ID. Over 24 hr excretion was 59% in the feces and 23% in the urine. The activity in monkey brain at 5 min was 2.8% ID and it cleared with a $t_{1/2}$ of 86 min. Autoradiographs of monkey brain sections showed excellent regional detail with a gray/white ratio of 3.6 at 10 min. The distribution of [^{99m}Tc]DMG-2MP in the monkey brain corresponds to the known cytoarchitectural pattern of cerebral glucose metabolism. The properties of [^{99m}Tc]DMG-2MP make it a potentially useful agent for cerebral perfusion imaging in man.

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The measurement of regional cerebral blood flow (rCBF) is useful for the neurologic assessment of stroke and seizure patients. Single-photon emission computed tomography (SPECT) using iodine-123-iodoamphetamine (^{123}I)IMP or technetium-99m- (^{99m}Tc) hexamethyl propyleneamine oxime (HMPAO) as the radiotracer is used for this measurement. A useful radiopharmaceutical for imaging rCBF must have (1) a high single-pass cerebral extraction and (2) be retained in the brain without redistribution during image acquisition.

Technetium-99m-labeled neutral lipophilic agents that cross the intact blood-brain barrier (BBB) are well suited for rCBF SPECT imaging in humans. In 1984, Volkert et al. showed that propylene amine oxime (PnAO), 3,3'-(1,3-propanediyldiimino)bis-(3-methyl-2-butanone)-dioxime forms a neutral lipid soluble complex with ^{99m}Tc (^{99m}Tc-PnAO), which crosses the intact BBB (*1,2*). This complex showed a single-pass extraction of 80% in the baboon at normal cerebral blood flow (*1*). However, the residence time within the brain is not long enough to allow single-headed rotating SPECT imaging. Subsequently, Nowotnik et al. prepared several derivatives of PnAO and found that the d,l isomer of ^{99m}Tc-HMPAO is well extracted and retained in the brain for sufficient time to allow rotating SPECT imaging (*3*).

The neutral, lipid soluble Tc(V) oxo complexes based on the 3,6-diazaoctane-1, 8-dithiol (N_2S_2) ligand system have shown significant uptake in the brain (*4,5,6*). Most of them, like PnAO, were rapidly cleared from brain, thus making them unsuitable for single-headed rotating gamma camera SPECT cerebral perfusion studies. One direction taken to improve upon the cerebral retention of ^{99m}Tc-N_2S_2 complexes was the addition of amine-containing substitutents to the ligand. One complex from this class, [^{99m}Tc]NEP-DADT, has been shown to have longer cerebral retention in man and primates (*7*). Recently, a diester N_2S_2 complex, [^{99m}Tc]L, L-ethyl cysteinate dimer (ECD), was shown to cross the BBB in several species with significant retention only in higher species (*8–9*).

We have developed a new class of neutral, lipophilic complexes generally known as boronic acid adducts of technetium dioxime complexes (BATOs) (*10*). They are easily prepared by template synthesis from vicinal dioximes. Details on their chemistry are described elsewhere (*11–12*). They are heptacoordinate complexes in which the technetium is bound to the six nitrogens of the three vicinal dioximes and an axial ligand. The dioximes are capped at one end via the oxygens to a tetrahedrally coordinated boron atom of a boronic acid moiety and at the other end the three oxygens are held by a two-proton bridge. A large number of neutral,

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lipophilic compounds can be prepared by varying the oxime, boronic acid and the axial ligand.

We present here the results from a biodistribution study of [^{99m}Tc]DMG-2MP (a BATO formed from dimethyl glyoxime and 2-methyl-1-propyl boronic acid) in rats, the blood and brain time-activity data in monkeys, planar imaging of a monkey brain, and autoradiographs of monkey brain sections. The monkey was chosen for imaging and autoradiographic studies because its brain size permits detailed analysis and because of its anatomic similarity to humans.

MATERIALS AND METHODS

Preparation of [^{99m}Tc]DMG-2MP and Quality Control

The [^{99m}Tc]DMG-2MP was prepared from a lyophilized kit by the addition of 1 ml pertechnetate containing 1–100 mCi ^{99m}Tc and heating at 100°C for 15 min, cooling to room temperature, and diluting with 1 ml of 0.9% saline. Each vial contains dimethylglyoxime (12.7 μmole) and 2-methylpropyl boronic acid (30 μmole) as active ingredients, anhydrous stannous chloride (0.26 μmole) as a reducing agent along with citric acid (45 μmole), diethylenetriaminepentaacetic acid (5 μmole), NaCl (1711 μmole) and gamma cyclodextrin (38.6 μmole) required to achieve a stable preparation with high radiochemical yield.

The radiochemical purity (RCP) of each preparation was measured either by reversed-phase high performance liquid chromatography (HPLC) or by paper chromatography. The HPLC system consisted of a Lichrosorb C-18 column (250 × 4.6 mm) eluted with 90% ACN in 0.1 *M* ammonium acetate buffer, pH 4.6, at a flow rate of 1.0 ml/min. An online NaI(Tl) detector in conjunction with a high voltage power supply (Tennelec #TC948, Oak Ridge, TN), a linear amplifier (Tennelec #TC211A), and a single-channel analyzer (Tennelec #441) was used to measure the radioactivity.

Two paper chromatography systems were used to separate the ^{99m}Tc-labeled DMG-2MP complexes from pertechnetate and other non-lipophilic constituents. One system (Gelman ITLC-SAF strip with 0.9% NaCl, Ann Arbor, MI) was used to separate the pertechnetate from labeled lipophilic complexes and the other (Whatman 31ET paper with acetone/0.9% NaCl (50;50 v/v), Clifton, NJ) was used to separate insoluble reduced hydrolyzed technetium. The RCP defined as the percent of total activity associated with the DMG-2MP complex is calculated as:

$$\%RCP = (100 - (\%\text{pertechnetate} + \%\text{reduced hydrolyzed Tc})).$$

Animal Studies

Biodistribution in Rats. The time-course of organ distribution and clearance of [^{99m}Tc]DMG-2MP was examined in rats at 0.08, 1, 2, 4, 8, 16 and 24 hr after injection. Seven groups of five male Sprague-Dawley rats weighing between 100 and 155 g were used. Each rat was anesthetized with nembutal sodium at an intraperitoneal dose of 50 mg/kg. Following anesthetization, the jugular vein was exposed via an incision and each rat was injected with 0.1 ml (50 μCi) of [^{99m}Tc] DMG-2MP. The penis of each rat in the first two groups was ligated immediately prior to injection to facilitate collection of urine from the bladder. Following injection, the incisions of rats in the remaining groups were closed and then the rats were placed in individual metabolic cages to facilitate collection of urine and feces.

An aliquot of blood was withdrawn just prior to killing. The urine was removed from the bladder and combined with urine, if any, collected from the metabolic cage. The organs, tissues, and fluids of interest were assayed against a 1% standard using an LKB 1282 Compugamma counter.

Imaging Studies and Time-Activity Curves of Monkey Brain

Five Cynomolgus monkeys of either sex weighing between 2 and 3.6 kg were used. The animals were anesthetized with nembutal sodium administered intravenously at a dose of 30 mg/kg. Catheters were placed in a femoral vein and a femoral artery. The venous catheter was used to administer the sample and maintenance doses of anesthetic, and the arterial catheter was used to withdraw blood.

All imaging studies were performed using an Elscint Apex 409 camera equipped with a HRES collimator. For each study, the animal was placed supine on the imaging table for a right lateral view of the head and neck. Dynamic imaging was started immediately after injection of ~2 mCi of [^{99m}Tc]DMG-2MP into the femoral vein. The acquisitions were for 60 min at one frame/min in a 128 × 128 matrix. At the end of the acquisition, the animal was killed and the brain excised and assayed for ^{99m}Tc activity against a 1%–2% standard.

The brain time-activity (T-A) curves in monkeys were constructed according to the following procedure. A fast study grouping (FSG) of the dynamic acquisition was performed. The composite image was then used to construct a whole brain region of interest (ROI). Using this ROI, a time-activity histogram of the brain was generated for the 60-min acquisition. Using the measured percent injected dose (%ID) in the brain at 60 min and the corresponding histogram datum, the rest of the data were converted to percent injected dose and then used to construct the T-A curves of the brain.

The brain T-A curves were analyzed by non-linear regression analysis using RS/1 software. The best fit of the brain T-A curves was achieved with a bi-exponential function containing both growth and clearance terms, of the form:

$$Y_{\text{Brain}} = A(e^{-\alpha t} - e^{-\beta t}),$$

where A is a constant in units of %ID and α and β, respectively, are the clearance and growth rate constants.

Blood Clearance of [^{99m}Tc]DMG-2MP in Monkeys

The blood activity data were obtained by collecting duplicate 10-μl aliquots of blood through the arterial catheter at 1, 2, 3, 4, 5, 10, 15, 30, 45, and 60 min postinjection. The samples were then assayed against a measured 0.01% ^{99m}Tc standard and the percent injected dose present in the blood pool was calculated using a blood volume of 75 ml/kg.

The average blood T-A data in monkeys were analyzed by non-linear regression analysis using RS/1 software. The data were best fitted to a bi-exponential function of the form:

$$Y_{\text{Blood}} = A\, e^{-\alpha t} + B\, e^{-\beta t},$$

where the coefficients A and B represent, respectively, the activities associated with the two clearance components and α and β the clearance rate constants.

Autoradiography

Five Cynomolgus monkeys weighing between 2 and 4.5 kg were used. The animals were anesthetized with nembutal sodium administered intravenously at a dose of 30 mg/kg. A dose of 31 to 53 mCi of [^{99m}Tc]DMG-2MP in 1 to 2 ml was administered to each animal via the femoral vein. Three animals were killed at 10 min and two at 60 min postinjection. Immediately after killing, the brains were excised and assayed for radioactivity against a 1%–2% standard using the gamma camera.

The entire brain was then slowly frozen over dry ice (to prevent cracking due to uneven cooling) and then embedded in a 5% solution of carboxymethyl cellulose (CMC) using a dry ice/acetone bath.

Brain tissue sections for autoradiography were obtained with a SLEE model TS 260 sledge microtome. The CMC-embedded brain was prepared for microtome stage mounting by cutting 1-cm thick coronal, sagittal, or transverse sections using a band saw. Two or three sections were mounted on the stage of a cryomicrotome, which was maintained at −18°C, and then trimmed until the top surface was uniform. Scotch 810 (3M) tape was placed on the top of the tissues before each 30- or 40-micron slice was cut to maintain specimen integrity during and after slicing.

A set of eight standards were prepared from 1:1 serial dilutions of $^{99m}TcO_4$–starting at an activity concentration of 15 μCi/ml. Five microliter aliquots of each of the standards were spotted in duplicate rows on 5 × 20 cm thin-layer chromatography (TLC) sheets (Baker-flex, silica gel IB-F).

Two or three tissue slices and a standard strip were transferred to a pre-chilled (−18°C) x-ray film cassette containing Kodak XAR-5 film so that the tissues and the TLC material were in contact with the emulsion. The film was exposed for ~20 hr at −18°C, then the tissue slices and standard strip were separated from the film. The film was allowed to come to room temperature and was developed in an AFP 14XL film processor.

Small pieces (~75 mg) of pure gray and pure white matter were dissected from the frozen sections of the brain after the sections had been taken for autoradiography and were placed in separate preweighed tubes and the radioactivity measured. The activity in these portions was expressed as CPM/g-tissue.

The autoradiograms were evaluated with an MCID Image Analysis System (MCID: Imaging Research, Ontario, Canada). Three to six autoradiograms from each animal were digitized. The digitization system is capable of 256 gray levels and has a resolution of 512 × 480 pixels. The digitized images were each evaluated for relative optical density (ROD) of gray and white tissue from frontal, parietal, and occipital regions. Relative optical density is defined as:

$$ROD = \log_{10}(256/\text{pixel level}).$$

About 360 measurements were required to evaluate the entire autoradiogram. For brains sectioned in the transverse and sagittal planes gray to white ratios of ROD were determined for frontal, parietal and occipital cortex areas. A weighted mean gray to white ratio, using the pixel area of respective regions for weighting, was also determined for each autoradiogram. These three regions were not represented in brain sections cut in the coronal plane.

Pseudocolor enhancements of the images were generated by computer assignment of colors to tissue pixel gray levels. Regions of cytoarchitectonic interest were digitized at higher magnification and pseudocolor enhancements were made.

Dosimetry

The human radiation dosimetry of [^{99m}Tc]DMG-2MP was estimated based on the rat in-vivo distribution data. The FT-A data were used to determine the cumulative activities in various source organs. Absorbed doses in target organs were calculated using the published "S" factors (*13,14*).

RESULTS

Figure 1 shows a typical HPLC scan of [^{99m}Tc]DMG-2MP prepared from a lyophilized kit. The major peak with a retention time of 3.8 min is the [^{99m}Tc]DMG-2MP, and it is coincident with an authentic sample of [^{99m}Tc]DMG-2MP. The RCP of the preparation is usually >90% and the complex is stable for over 6 hr. Paper chromatography also gives an RCP of >90%. The results of RCP measurements (mean ± s.d.) on eight kit preparations by both methods are 92.7 ± 0.7 by HPLC and 93.1 ± 0.4 by paper chromatography.

The results of the tissue distribution study in rats are given in Table 1. The blood activity levels were calcu-

FIGURE 1
A typical HPLC chromatogram of [^{99m}Tc]DMG-2MP using a Lichrosorb C-18 column and eluted with 90% ACN-10% ammonium acetate buffer, pH 4.6 at a flow rate of 1 ml/min. The percent activity in the main peak at ~3.8 min is >90%.

TABLE 1

Average Distribution of Radioactivity in Rat Tissues After Intravenous Administration of ^{99m}Tc-SQ 32097—Organ Uptake (% ID)

	0.83 hr		1 hr		2 hr		4 hr		8 hr		16 hr		24 hr	
Tissue	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.	mean	s.d.
Whole blood†	2.53	0.14	1.76	0.11	1.81	0.21	1.48	0.14	1.15	0.10	1.14	0.10	1.04	0.19
RBCs	1.09	0.25	1.07	0.21	1.09	0.12	1.11	0.10	0.96	0.11	1.01	0.09	0.87	0.16
Plasma	1.44	0.24	.68	0.26	0.72	0.09	0.37	0.10	0.19	0.06	0.13	0.09	0.17	0.13
Heart	1.23	0.04	0.22	0.04	0.20	0.02	0.12	0.01	0.06	0.01	0.06	0.01	0.04	0.01
Lungs	1.25	0.07	0.72	0.07	0.66	0.10	0.40	0.04	0.15	0.01	0.12	0.02	0.10	0.02
Brain	1.08	0.13	0.55	0.07	0.57	0.08	0.39	0.05	0.29	0.03	0.28	0.02	0.22	0.04
Liver	25.63	1.56	11.82	0.91	11.14	0.39	8.16	0.67	4.83	0.74	3.46	0.55	3.07	0.67
Stomach	1.49	0.26	1.04	0.20	1.35	0.75	0.90	0.26	0.34	0.16	0.50	0.57	0.94	1.41
Sm. intestine	11.35	0.56	33.05	2.89	39.82	3.63	49.24	2.93	4.82	1.14	2.62	2.30	1.29	0.25
Up. lg. intestine	0.86	0.10	0.85	0.07	1.00	0.14	2.38	1.61	29.34	5.58	19.17	17.82	2.53	1.29
Lo. lg. intestine	1.16	0.11	0.86	0.12	1.20	0.23	1.20	0.60	17.10	5.20	2.30	1.48	1.56	0.75
Bladder	0.06	0.02	0.18	0.10	0.27	0.11	0.12	0.12	0.01	0.00	0.01	0.00	0.01	0.00
Testes	0.34	0.02	0.30	0.03	0.25	0.02	0.22	0.02	0.11	0.02	0.09	0.01	0.07	0.01
Spleen	0.64	0.09	0.32	0.02	0.32	0.05	0.20	0.02	0.12	0.03	0.07	0.01	0.05	0.01
Pancreas	0.98	0.09	0.29	0.03	0.21	0.02	0.15	0.04	0.05	0.01	0.03	0.00	0.03	0.01
Kidneys	3.61	0.20	1.24	0.05	1.32	0.22	1.25	0.12	1.88	0.08	1.96	0.17	1.48	0.26
Adrenals	0.17	0.01	0.05	0.02	0.04	0.01	0.02	0.01	0.01	0.00	0.00	0.00	0.01	0.00
Thymus	0.24	0.04	0.16	0.03	0.13	0.03	0.06	0.01	0.03	0.01	0.02	0.00	0.02	0.01
Thyroid	0.12	0.02	0.05	0.01	0.05	0.01	0.02	0.01	0.01	0.00	0.01	0.00	0.01	0.00
Eyes	0.05	0.01	0.02	0.00	0.02	0.01	0.02	0.00	0.01	0.00	0.01	0.00	0.01	0.00
Harderians	0.22	0.02	0.22	0.03	0.23	0.03	0.19	0.02	0.09	0.01	0.07	0.00	0.04	0.01
Salivarys	1.25	0.19	0.59	0.05	0.55	0.06	0.35	0.04	0.19	0.03	0.12	0.01	0.09	0.02
Skin	0.13	0.06	0.13	0.03	0.15	0.05	0.08	0.01	0.06	0.02	0.03	0.01	0.03	0.01
Muscle	0.31	0.11	0.25	0.03	0.26	0.03	0.16	0.02	0.07	0.02	0.06	0.01	0.04	0.01
Fat	0.09	0.04	0.12	0.04	0.12	0.04	0.09	0.04	0.04	0.01	0.02	0.01	0.01	0.01
Bone‡	4.32	0.36	2.89	0.13	2.84	0.34	1.67	0.12	0.77	0.09	0.58	0.06	0.43	0.10
Femur	0.34	0.01	0.21	0.03	0.22	0.02	0.13	0.01	0.07	0.01	0.05	0.00	0.04	0.01
Feces	0.00	0.00	0.00	0.00	0.01	0.02	0.03	0.05	9.89	10.12	43.61	20.19	59.28	2.49
Urine	0.64	0.33	5.93	1.25	9.44	1.27	14.97	1.99	19.96	3.39	18.32	3.50	22.59	2.82
Carcass	44.24	1.60	39.09	3.15	28.66	1.12	17.65	1.98	9.33	1.36	5.89	0.48	5.40	0.67

* Values shown are mean and s.d. for 5 rats.

† The total % of the dose in blood was calculated by assuming that blood comprised 6.5% of the total body weight.

‡ The % dose in the bone was calculated assuming that the skeleton comprised 10% of the total body weight.

lated based on a blood volume of 6.5% body weight. The clearance of [^{99m}Tc]DMG-2MP from blood was rapid; at 5 min after injection <3% of the administered dose was present in blood. The highest recorded activity in the brain was 1.1% ID at 5 min postinjection. By 1 hr the %ID in brain had fallen to ~0.6%. At 5 min postinjection, the liver contains its peak activity of 25.6% ID. The hepatobiliary system is the major route of excretion of the administered radioactivity.

The radiation dosimetry estimations based on data in Table 1 are shown in Table 2. The estimations show that the upper large intestine, small intestine, and lower large intestine are the target organs and will receive 200, 180, and 120 mrad/mCi [^{99m}Tc]DMG-2MP injected, respectively. The liver, kidneys, and ovaries each receive ~50 mrad/mCi. Thus, based on these rat data and a maximum dose of 5,000 mrad to any single organ, it is estimated that a dose of up to 25 mCi [^{99m}Tc]DMG-2MP may be administered per study to humans.

The blood clearance in monkeys of [^{99m}Tc]DMG-2MP (average %ID in total blood versus time in min) along with the fit-function is shown in Figure 2. Only 11% of the injected activity is cleared through the slow component with $T_{½}$ of ~58 min and the remainder through the fast component with $T_{½}$ of 1.0 min. Thus, the blood clearance is fast; with more than 87% of the activity cleared from the blood by 5 min postinjection.

The average brain T-A curve in monkeys, with the fit-function is shown in Figure 3. The time to attain the maximum activity in the brain (T_{max}) is estimated from the relation:

$$T_{max} = In(b/a)/(b - a).$$

From this equation, the maximum activity in the brain is estimated to occur at 4.9 min after administration of [^{99m}Tc]DMG-2MP.

A planar image of a monkey brain obtained 5 min after i.v. administration of 30 mCi [^{99m}Tc]DMG-2MP is shown in Figure 4. Autoradiographs of coronal, transverse, and sagittal sections of monkey brain 10 min

TABLE 2
Estimations of Human Absorbed Dose Following Intravenous Administration of [^{99m}Tc]DMG-2MP Based on the Rat Biodistribution Data in Table 1

Organ	Dose (mrad/mCi Inj.)
Heart wall	10.0
Lungs	8.0
Brain	3.0
Liver	44.0
Stomach	28.0
Sm. intestine	180.0
Upper lg. intestine	200.0
Lower lg. intestine	120.0
Bladder	16.0
Testes	26.0
Spleen	17.0
Pancreas	25.0
Kidneys	46.0
Adrenals	19.0
Thyroid	19.0
Bone	11.0
Red marrow	22.0
Whole body	14.0
Eyes	19.0
Ovaries	48.0

after injection of [^{99m}Tc]DMG-2MP are shown in Figure 5; they show excellent regional detail. A standard curve showing the linearity of relative optical density versus the activity of the standards is shown in Figure 6.

Gray/white matter ROD ratios for frontal, parietal, and occipital cortex as well as weighted averages for all three cortices are shown in Table 3. These values were obtained from two brains excised 10 min after administration of [^{99m}Tc]DMG-2MP; one sectioned in the sagittal plane and the other in the transverse plane. In addition, another brain sectioned in the transverse plane was excised 60 min after administration of the compound. Regional differences in gray/white ROD ratios were clearly demonstrated among the three cortical regions analyzed at both time points and both

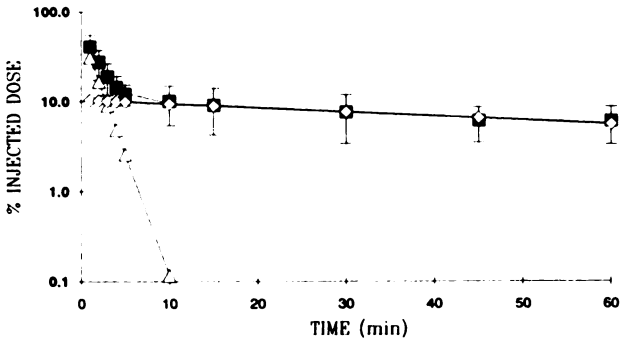

FIGURE 2
Blood clearance of [^{99m}Tc]DMG-2MP in monkeys; average activity (% ID) in total blood with s.d. (n = 5) versus time (min) and the fit function: $63.4e^{-0.682t} + 10.9\ e^{-0.0125t}$.

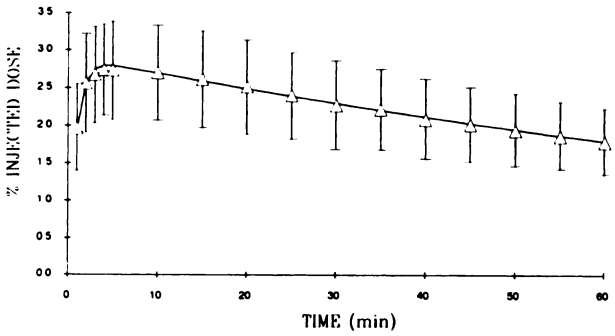

FIGURE 3
Time/Activity of [^{99m}Tc]DMG-2MP in monkey brain; average activity (% ID) in brain with s.d. (n = 5) versus time (min) and the fit function: $2.92^{*}\ (e^{-0.0081t} - e^{-1.114t})$.

planes of section. The difference between the 10-min sagittal values and the 10-min transverse values can be attributed to inter-animal variation possibly due to differences in depth of anesthesia.

A coronal autoradiograph approximately at the AP 12.5 plane, obtained 60 min after i.v. injection of [^{99m}Tc]DMG-2MP, along with the corresponding coronal surface of a fresh brain is shown in Figure 7. The [^{99m}Tc]DMG-2MP autoradiograph shows excellent regional detail.

Pseudocolor-enhanced images of transverse and coronal autoradiographs along with magnified sections of visual cortex and hippocampal regions are shown in Figure 8. These images reveal high uptakes in layer IV of parietal/somatosensory and occipital/visual cortices, as well as the molecular layer of the hippocampal formation.

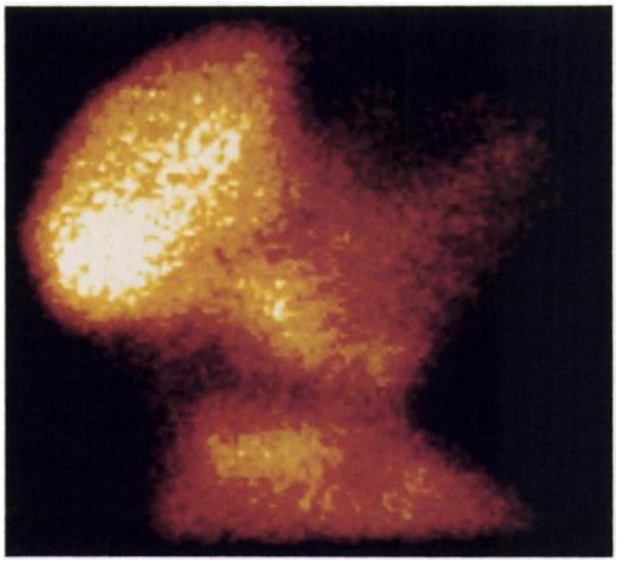

FIGURE 4
Planar image of a monkey brain after i.v. administration of 30 mCi [^{99m}Tc]DMG-2MP.

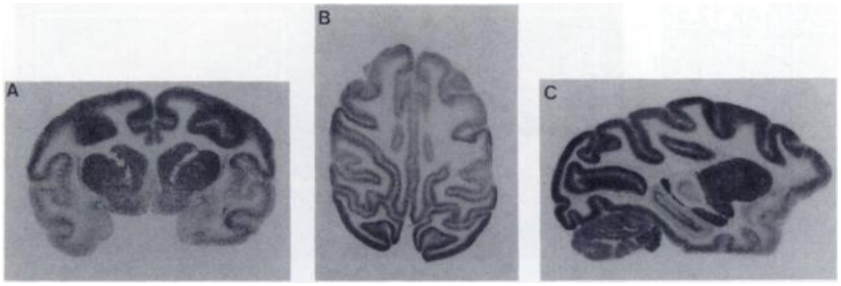

FIGURE 5
Autoradiographs of (A) coronal, (B) transverse, and (C) sagittal sections of monkey brain 10 min after i.v. administration of [^{99m}Tc]DMG-2MP.

DISCUSSION

The radiochemical purity of [^{99m}Tc]DMG-2MP prepared from lyophilized kits is >90% and is stable for at least 6 hr. Both HPLC and PC methods gave equivalent results. The PC method is easy, fast and convenient and could be used in any nuclear medicine department.

The clearance of [^{99m}Tc]DMG-2MP from blood is rapid in both rats and monkeys; in rats only 3% ID and in monkeys ~13% remain at 5 min postinjection. Planar imaging in monkeys shows an average brain uptake of 2.8% ID 5 min postinjection. These findings suggest good extraction of [^{99m}Tc]DMG-2MP by the monkey brain. The clearance half-time of 86 min is more than sufficient to obtain SPECT images of the brain.

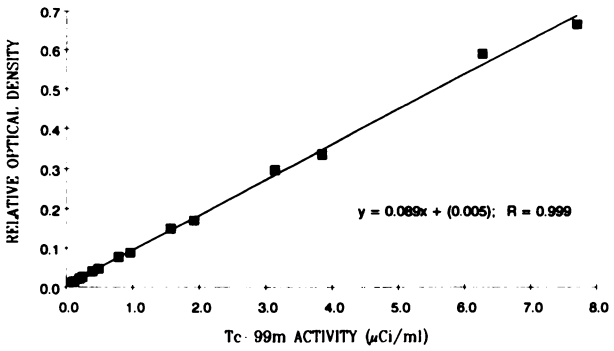

FIGURE 6
Standard curve showing the linearity of ROD versus activity (Ci) in standards. The line representing the linear regression on the data is also shown.

The whole brain autoradiographs show that the distribution of radioactivity in the brain is heterogeneous. This is expected for a flow agent. The average regional cortical gray/white ratios that we have determined to be between 3.0 and 3.6 at 10 min postinjection, with occipital values ranging up to 5, are comparable to cortical values reported for ^{99m}TcECD (*15*). A gray/white ratio of 1.7 ± 0.1 at 60 min postinjection from ROD measurements of a transverse autoradiograph is identical to that obtained from activity measurements of dissected gray and white tissue samples 1.7 ± 0.2 (n = 6). The drop in value of this ratio from 3.6 at 10 min to 1.7 at 60 min suggests differential washout of the tracer from gray and white tissues, which is expected because of the different regional blood flows in the brain.

A dark band of radioactivity in occipital visual and somatosensory cortices appears to correspond to layer IV. The high degree of electrical activity in the sensory systems of anesthetized animals may be attributable to a high level of spontaneous activity as well as thalamic spindling induced by nembutal.

The molecular layer of the hippocampal formation is another dense neuropil that is heavily labeled. It is visible in the medial portion of the temporal lobe just below the lateral geniculate nucleus in the coronal

TABLE 3
Gray/White Determinations from RODs of Transverse and Sagittal Autoradiographs

ARG description and number		Gray/white ratio			Weighted mean	Cumulative mean ± s.d. (n)
		frontal	parietal	occipital		
10-min sagittal	1	2.08	2.95	2.67	2.86	
	2	2.54	2.92	3.33	2.91	
	3	2.89	3.13	3.48	3.11	2.96 ± 0.13 (9)
10-min transverse	1	3.22	3.98	4.06	3.69	
	2	3.25	4.18	4.51	3.83	
	3	3.2	3.35	4.1	3.34	
	4	2.93	3.47	4.03	3.39	
	5	3.74	3.88	4.13	3.75	
	6	3.18	3.5	5.01	3.54	3.59 ± 0.20 (18)
60-min transverse	1	1.41	1.68	1.7	1.59	
	2	1.57	1.78	1.7	1.73	
	3	1.62	1.78	1.68	1.71	1.68 ± 0.07 (9)

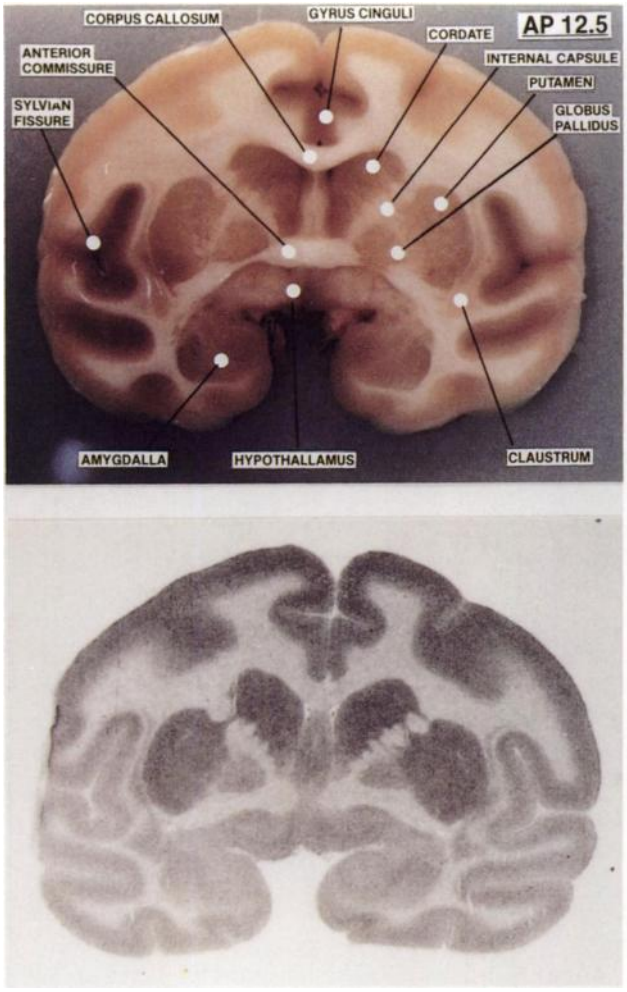

FIGURE 7
Autoradiograph of a coronal section approximately at the AP 12.5 plane and a corresponding coronal surface of a fresh brain. The ARG was obtained at 60 min after injection of [^{99m}Tc]DMG-2MP. The regional details in the autoradiograph match closely with the image of the fresh brain slice.

autoradiogram (Figure 8). The rich neuropil formed by the synapses of numerous axon terminals onto soma-dendrite regions of stellate cells in layer IV and pyramidal and granule cells of the hippocampal formation is characterized by intense electrophysiologic activity. This activity is associated with a high metabolic rate of glucose and high levels of cytochrome oxidase activity (*16*) and would, therefore, require high blood flows.

Thus, the distribution of [^{99m}Tc]DMG-2MP in the monkey brain autoradiographs corresponds to the known cytoarchitectonic pattern of cerebral glucose metabolism. In light of the tight coupling between flow and metabolism in the brain during normal physiologic states, this finding satisfies an additional condition for demonstrating that [^{99m}Tc]DMG-2MP images cerebral blood flow.

The rapid blood clearance, significant brain uptake coupled with a long clearance half-time in brain (86 min) of [^{99m}Tc]DMG-2MP and its ability to show mic-

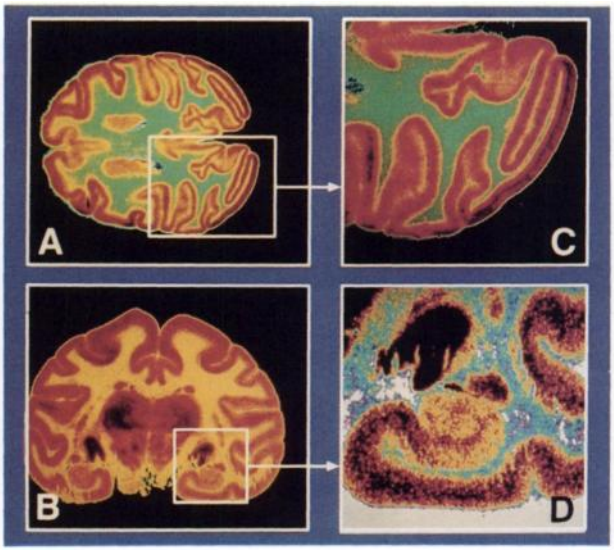

FIGURE 8
Pseudocolor enhanced images of the transverse (A) and coronal (B) autoradiographs. The visual cortex from the transverse ARG and the hippocampal region from the coronal ARG were digitized at higher magnification and pseudocolor images were constructed (C&D). The details at the cytoarchitechtonic level are clearly evident in all these images.

roregional details on the monkey brain autoradiographs suggest that [^{99m}Tc]DMG-2MP will be a useful cerebral perfusion imaging agent in humans.

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