Membrane Transport of Tc-99m-Labeled Radiopharmaceuticals. I. Brain Uptake by Passive Transport

Michael D. Loberg, Elizabeth H. Corder, Anna T. Fields, and Patrick S. Callery

University of Maryland, Baltimore, Maryland

The membrane transport properties of twelve Tc-99m complexes were studied by determining each complex's brain uptake index (BUI), extent of protein binding, and octanol-to-saline partition coefficient. The chelating agents used were classified as either N-substituted carbamoylmethyliminodiacetates, substituted oxines, N,N'-diesters of EDTA, or N-substituted derivatives of DTPA. The Tc-99m complexes were found to cross the blood–brain barrier in proportion to their lipophilicity. Of the four types of chelating agents tested, substituted oxines appear to be most suitable for the development of diffusible Tc-99m-labeled compounds for imaging nonexcretory organs.

J Nucl Med 20: 1181–1188, 1979

The in vivo localization of Tc-99m-labeled radiopharmaceuticals is based on the ability of specific organs to remove foreign substances from the blood. It could be anticipated that Tc-99m, for which there is no known metabolic requirement, would be most readily handled by one of the body's detoxification mechanisms. It is perhaps for this reason that Tc-99m radiopharmaceuticals have been most successful in the examination of the various elimination mechanisms of the liver, lungs, and kidneys. Attempts to design Tc-99m-labeled radiopharmaceuticals for imaging the nonexcretory organs have been less successful. Tc-99m-labeled fatty acid analogs containing iminodiacetate (IDA), ethylenediaminetetra-acetate (EDTA), diethylenetriamine penta-acetic acid (DTPA), or diethylenetriamine (DTA) (*1,2*) have been incapable of concentrating within the myocardial cell, and the DTA derivatives of tolbutamide have been unsuccessful in imaging the pancreatic islet cells (*3*).

The inability of these and other existing Tc-99m radiopharmaceuticals to image the nonexcretory organs may be attributed to the hydrophilicity associated with existing Tc-99m complexes (*4*). Their observed hydrophilicity would be expected to restrict their membrane transport enough to preclude their use in either imaging the nonexcretory organs or measuring regional blood perfusion. The development of diffusible tracers labeled with Tc-99m is essential to further expansion in the use of Tc-99m radiopharmaceuticals.

Diffusible radiotracers have been developed using other radionuclides, notably O-15 water (*5*), I-123- or I-131-tagged iodoantipyrine (*6–8*), and various radioactive inert gases (*9*). Sakurada et al. (*10*) demonstrated that [^{14}C]iodoantipyrine was more diffusible than was antipyrine itself and suggested that iodoantipyrine labeled with a suitable gamma emitter could be used to measure regional cerebral perfusion, even at high flow rates. Ducassou and coworkers (*8*), studying the cerebral distribution of [^{123}I]iodoantipyrine in human subjects, reported that this agent could be used in the detection of ischemic cerebral disease. In response to these findings, and to the reduction in the number of emission brain scans performed since the introduction of computerized tomography, Oldendorf (*11*) proposed the development of a new class of Tc-99m radiopharmaceuticals that would be sufficiently lipophilic to penetrate the intact blood–brain barrier (BBB). The clinical utility of a Tc-99m-labeled perfusion agent was further emphasized by the recent work of Gustafson et al. (*12*), who demonstrated the practicality of quantitative, transaxial

Received March 19, 1979; revision accepted May 21, 1979.

For reprints contact: Michael D. Loberg, E. R. Squibb & Sons, Georges Rd., New Brunswick, NJ 08903.

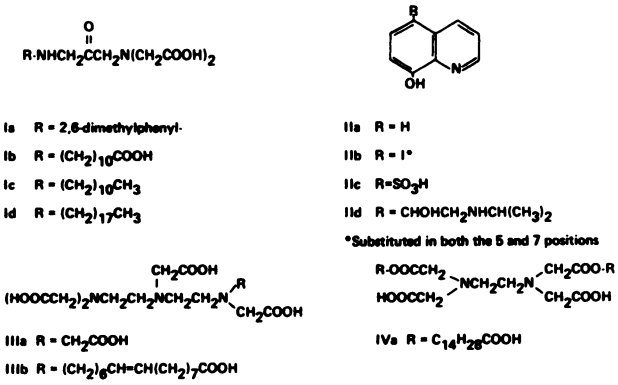

FIG. 1. Structures of various N-substituted carbamoylmethyliminodiacetates (I), substituted oxines (II), N-substituted congeners of DTPA (III), and N,N′-diesters of EDTA (IV).

imaging using single-photon gamma emitters.

This paper reports the synthesis of a series of Tc-99m-labeled radiochemicals of varying lipophilicity, containing either N-substituted carbamoylmethyliminodiacetate, substituted oxine, N,N′-diesters of EDTA, or N-substituted derivatives of DTPA (Fig. 1). All these were evaluated with regard to their purity, relative lipophilicity, protein binding, and ability to diffuse across the intact BBB. The relationship between lipophilicity and membrane transport is examined for Tc-99m complexes, and an explanation is offered as to why current Tc-99m radiopharmaceuticals have been unsuccessful in imaging nonexcretory organs. We report here the initial work toward the development of a diffusible tracer labeled with Tc-99m.

MATERIALS AND METHODS

The complexing agents listed in Fig. 1 were reacted with reduced Tc-99m 2,6-dimethylphenylcarbamoylmethyliminodiacetate (Ia) was synthesized by published methods (*13*). Compounds Ib, Ic, and Id were synthesized from the anhydride of nitrilotriacetic acid (NTA) and the appropriate amine (*14*). The preparation of undecylcarbamoylmethyliminodiacetic acid (Ic) is a typical example of this reaction scheme. A mixture of 50 ml of dry pyridine (molecular sieves) and NTA (26 mmole) was heated to 50°C in a three-neck, round-bottom flask equipped with a reflux condenser and $CaSO_4$ drying tube. After 10 min at 50°C, acetic anhydride (3.0 ml) was added and the reaction mixture heated to 100°C for 30 min, then cooled to 50°C in an ice bath. *n*-Undecylamine (26 mmole) was added, and the mixture heated again to 100°C for 30 min. The resulting solution was concentrated to dryness on a rotary evaporator and the residue suspended in 5 *N* NH_4OH (30 ml), decolorized with charcoal, and filtered. The filtrate was then acidified to pH 3 with concentrated HCl and refrigerated. The resulting precipitate was recrystallized from methanol. NTA, 11-aminoundecanoic, octadecylamine hydrochloride, and 8-hydroxyquinoline-5-sulfonic acid dihydrate (IIc), were purchased commercially*.

8-Hydroxyquinoline† (IIa) (recrystallized from medium-boiling petroleum ether) and 5,7-diiodo-8-hydroxyquinoline‡ (IIb) were obtained commercially. 5-(1-hydroxy-2-isopropylaminoethyl)-8-hydroxyquinoline (IId; quinterenol)‖ and diethylenetriaminehexadecenoic acid, tetraacetic acid (IIIb), and N,N′-di(14-carboxytetracecyl) ester of EDTA (IVa)§ (*1,15*) were donated. [^{14}C]nicotine, [^{14}C]iodoantipyrine, and diethylenetriamine penta-acetic acid (IIIa) were obtained commercially.

Preparation of technetium complexes. The Tc-99m complexes of Ia, Ib, Ic, and IId were prepared as previously described (*13*) using the stannous reduction method in aqueous solution. Tc-99m $(Id)_2$¶ and Tc-99m IIa were prepared by stannous reduction in absolute methanol (*16*). The preparation of the mixed ligand complex, Tc-99m Ibic, used a 1:7 molar ratio of Ic to Ib. The three complexes formed were separated by high-pressure liquid chromatography (HPLC) on a μBondapak C_{18} column by solvent programming from 16–70% CH_3CN/phosphate buffer (pH 6.8, 0.05 *M*), at a flow rate of 1 ml/min.

Tc-99m complexes of IIa and IIb were prepared by adding 100λ of stannous chloride solution (10^{-5}*M* in methanol) to 1 mmole of each ligand in 1 ml of $CHCl_3$. $^{99m}TcO_4^-$ was added and extracted with H_2O. The $CHCl_3$ layer was evaporated to dryness and reconstituted with CH_3OH. Tc-99m complexes of IIIb and IVa were prepared according to Eckelman (*15*). The radiochemical purity of each Tc-99m complex was studied by paper chromatography using CH_3OH, normal saline, and $CHCl_3$ as solvents, and by HPLC as previously described (*17*).

The brain uptake index (BUI) was determined for each Tc-99m complex and for [^{14}C]nicotine and [^{14}C]iodoantipyrine using the method developed by Oldendorf (*18*). A test solution contained approximately 10 μCi of the Tc-99m complex and 5 μCi of ^3H_2O in 0.2 ml of 0.05 *M* phosphate-buffered saline. In those instances in which some methanol was necessary to prepare a soluble Tc-99m complex, the percentage of methanol in the injectate was less than 5%. To examine the effect of methanol on the BUI, studies were performed using Tc-99m IIa and Tc-99m $(Ia)_2$ containing from 5 to 50% methanol.

The BUI was measured in male Wistar rats (weighing 300–350 g) which had been rendered unresponsive with intraperitoneal pentobarbital (45 mg/kg). The right common carotid artery was exposed and punctured with a 30-gauge needle, followed by injection of 0.2 ml of the radioactive test solution over a 1-sec interval. The needle remained in place during the 15 sec that elapsed before decapitation, since its small outside diameter allowed

unimpeded cerebral blood flow during this interval. In those experiments requiring a postinjection interval greater than 15 sec, the needle was advanced after injection until it excited the far artery wall—and was left in this position until decapitation.

Immediately after decapitation the brain was excised and the portion rostral to midbrain on the ipsilateral side was placed in a 3-ml syringe. Brain samples weighing 0.1–0.2 g were extruded through a 20-gauge needle into scintillation vials containing 1 ml of a tissue solubilizer** and 0.1 ml water. A standard of the test solution was then prepared and the Tc-99m radioactivities in the brain and standard were determined using an automatic gamma counter.

After solubilization overnight at room temperature, the tritium radioactivity in both brain and standard were determined using a liquid-scintillation counter. The tritium radioactivity was determined repeatedly over several days until constant values were obtained, indicating the absence of residual Tc-99m. The BUI was then calculated according to the equation

$$\text{BUI}(\%) = \frac{^{99m}\text{Tc} - \text{R(Br)}/^{99m}\text{Tc} - \text{R(Std)}}{^{3}\text{H}_2\text{O(Br)}/^{3}\text{H}_2\text{O(Std)}} \times 100$$

where R designates the complexing agent and (Br) and (Std) refer to aliquots of brain tissue and injectate, respectively.

The extent of protein binding was determined for each Tc-99m complex, [^{14}C]nicotine, and [^{14}C]iodoantipyrine using equilibrium dialysis under a nitrogen atmosphere. The degree of protein binding was determined as a function of time to ensure that equilibrium had been reached. All values reported are for a dialysis time of 16 hr.

The relative lipophilicity of each Tc-99m complex was assessed relative to its ability to partition between octanol and 0.05 *M* phosphate-buffered saline, pH 6.8. The octanol-to-buffered-saline partition coefficients were normalized to a constant weight of solvent.

The ability of Tc-99m IIa to penetrate the BBB was further studied by the bolus injection of 1 mCi of Tc-99m IIa into the left common carotid artery of a dog. The injection vehicle consisted of 1.0 ml of 0.05 M phosphate-buffered saline, pH 6.8, containing 25% by volume of ethanol. Sequential images of the brain were obtained up to 2 hr after injection, using a scintillation camera.

RESULTS

With the exception of IIIb and IVa, the chelating agents listed in Fig. 1 were found to form pure radiochemical complexes with reduced Tc-99m. Paper chromatography showed that at least 95% of the radioactivity was distributed in each case at the R_f value given in Table 1, but in many instances, the peaks were too broad to preclude the existence of multiple Tc-99m complexes.

TABLE 1. PAPER CHROMATOGRAPHY OF VARIOUS Tc COMPLEXES

Tc-99m complex	R_f values		
	CH_3OH	Saline	$CHCl_3$
Tc-(Ia)$_2$	0	1.0	0
Tc-(Ib)$_2$	0	0.63	0
Tc-(Ic)$_2$	0.91	0.66	0
Tc-(Id)$_2$	0.90	0	0
Tc-IIa	0.80	0	1.0
Tc-IIb	0	0	1.0
Tc-IIc	0	1.0	0
Tc-IId	0.76	0.91	0
Tc-IIIa	1.0	1.0	0
Tc-IIIb	0.83	1.0	0
Tc-IVa	1.0	0	0
TcO_4^-	0.32	0.75	0
Tc-colloid	0	0	0

TABLE 2. HPLC ANALYSIS OF VARIOUS Tc-99m COMPLEXES

Tc complex	HPLC solvent conditions*	HPLC retention time in min (% eluted)
Tc-(Ia)$_2$	16% CH_3CN/PO_4^{3-}	6.0 (99%)
Tc-(Ib)$_2$	16% CH_3CN/PO_4^{3-}	7.3 (98%)
Tc-Ibic	50% CH_3CN/PO_4^{3-}	8.2 (98%)
	16–70% CH_3CN/PO_4^{3-}†	9.0 (98%)
Tc-(Ic)$_2$	50% CH_3CN/PO_4^{3-}	8.4 (98%)
Tc-IIa	10% CH_3CN/0.05 *M* HAc	4.3 (90%)
Tc-IIb	85% THF/hexane	4.8 (96%)
Tc-IIc	10% CH_3CN/PO_4^{3-}	4.0 (98%)
Tc-IId	6% CH_3CN/0.05 *M* HAc	9.3 (90%)
Tc-IIIb	25% CH_3CN/PO_4^{3-}	12.5 (23%)
		8.1 (60%)
		3.8 (17%)
Tc-IVa	50% CH_3CN/PO_4^{3-}	7.8 (80%)
		4.8 (15%)
		2.5 (5%)

* Conditions: A μBondapak C_{18} column with flow rate 1 ml/min was used—except for Tc-IIb, for which a silica column, Zorbax 516, 4.6 mm (Dupont) was used.

† Solvent programmer Waters Model 660, program No. 10, 6 min.

Paper chromatographic systems defined in Table 1 were, however, adequate to define the upper limit of pertechnetate and technetium colloid in each radiochemical as contributing less than 2% of the total radioactivity. The HPLC systems described in Table 2 were found to be more useful in evaluating the radiochemical purity of the chelate fraction. The Tc-99m-labeled complexes chromatographed as a single radiochemical for all compounds, except Tc-99m IIIb and Tc-99m IVa, which were found to consist of three radiochemicals. Where

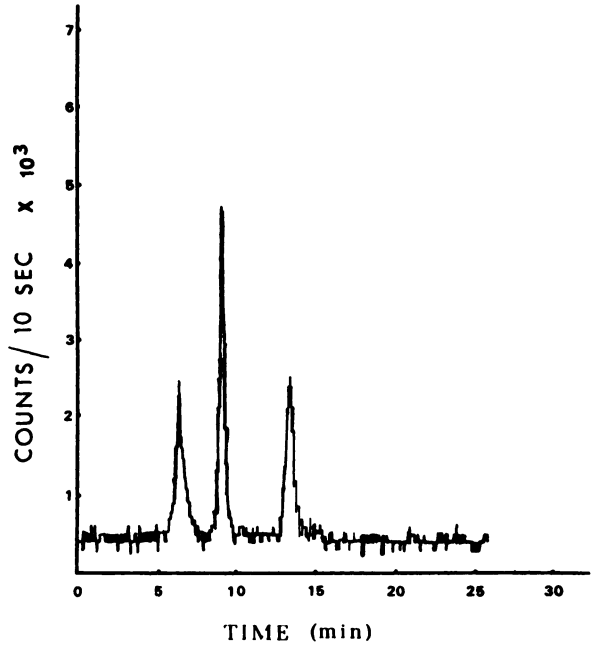

FIG. 2. High-pressure radiochromatogram of radioactive products resulting from reaction of $^{99m}TcO_4^-$ with 7:1 molar ratio of stannous complexes of Ib and Ic. First and third peaks correspond to pure Tc-99m $(Ib)_2$ and Tc-99m $(Ic)_2$, respectively. Second peak is presumed to be Tc-99m IbIc. $^{99m}TcO_4^-$ has retention time of 5 min.

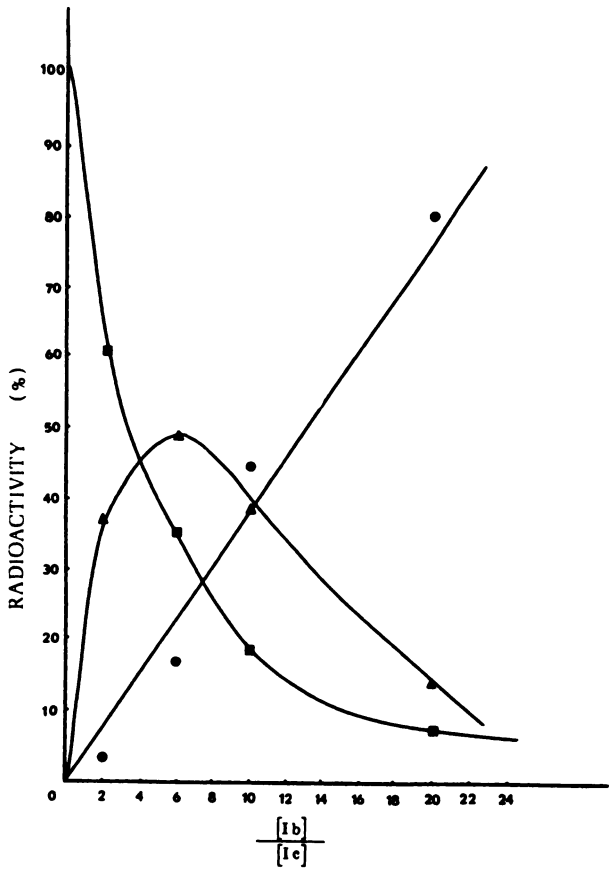

FIG. 3. Reaction yields of Tc-99m $(Ib)_2$, Tc-99m IbIc, and Tc-99m $(Ic)_2$ as function of (Ib)/(Ic).

possible, each radiopharmaceutical was analyzed using C_{18} reverse-phase chromatography, since the polar eluting solvents were suitable for further analytical studies. However, the highly lipophilic compounds Tc-99m IIa and Tc-99m IIb were too strongly retained to permit the use of reverse-phase chromatography. In these instances a silica-gel column was used.

The observed radiochemical purity was invariant up to 3 hr after preparation except in the case of Tc-99m IbIc. Figure 2 shows a HPLC radiochromatogram obtained by injecting pertechnetate into a lyophilized kit containing a 7:1 mixture of Ib and Ic. Chromatography of the reaction product on a reverse-phase column yielded three peaks with retention times of 7.2, 9.0, and 12 min. The first and third peaks have identical retention times to that of pure Tc-99m $(Ib)_2$ and Tc-99m $(Ic)_2$, respectively. The second peak, which had lipophilicity and retention times intermediate between the other two, is presumed to be the asymmetrical mixed-ligand product, Tc-99m IbIc. This product was routinely isolated by collection of the appropriate eluent from the HPLC, although it was relatively unstable, with only 15% of the initial Tc-99m IbIc remaining at 2 hr. The stability of Tc-99m IbIc was found to be at a maximum when the HPLC eluent was collected in an evacuated vial containing the lyophilized stannous complexes of Ib and Ic in the same 7 to 1 ratio used in preparing the initial radiochemicals. When the HPLC-purified Tc-99m IbIc was chromatographed after 5 hr of storage in the presence of both stannous Ib and Ic, the radiochemical profile was found to be 85% Tc-99m IbIc, 6% Tc-99m $(Ib)_2$, and 9% Tc-99m $(Ic)_2$.

The choice of an Ib to Ic molar ratio of 7 to 1 was based upon the data shown in Fig. 3. The radiochemical profile of the reaction mixture was studied as a function of the mole fraction of Ib in the reaction mixture. It was seen that the maximum quantity of Tc-99m IbIc (50%) was produced in a mole fraction Ib of 0.85 corresponding to a molar ratio of Ib:Ic = 7:1.

After determination of radiochemical purity and stability, each Tc-99m-labeled radiochemical was studied with regard to its octanol:saline partition coefficient, protein binding, and BUI. The results are shown in Table 3. In general, as the lipophilicity of the Tc-99m-labeled compounds increased, (evidenced by increasing partition coefficients) so too did their protein binding and BUI(%). Further, while no rigorous comparison was undertaken, it was apparent that the retention time observed on reverse-phase chromatography for each Tc-99m-labeled complex correlated at least qualitatively with its observed partition coefficient. This is consistent with the observation of other workers (*19*) studying organic compounds. Figure 4 is a graph of the BUI(%) for each compound plotted against its partition

TABLE 3. BRAIN UPTAKE INDEX, PARTITION COEFFICIENTS, AND PROTEIN BINDING OF SELECTED Tc-99m COMPLEXES

Tc-99m complex	Partition coefficient	% protein binding	%BUI*
Tc-$(Ia)_2$	0.02	51.3	4.8 (4.7–4.9)
Tc-$(Ib)_2$	0.01	80.9	6.2 (6.0–6.3)
Tc-$(Ic)_2$	4.0	87.6	22.2 (20.5–23.8)
Tc-$(Id)_2$	25.7	84.1	41.6 (30.9–52.2)
Tc-IIa	23.6	96.8	53.8 (48.8–58.7)
Tc-IIb	78.5	98.6	68.6 (65.5–74.6)
Tc-IIc	0.02	90.9	10.8 (8.2–12.9)
Tc-IId	0.05	64.2	13.9 (9.7–15.2)
Tc-IIIa	0.02	35.0	7.1 (6.7–7.7)
Tc-IIIb	0.2	93.5	9.0 (4.5–18.8)
Tc-IVa	6.7	93.9	42.4 (33.7–51.1)

* All values represent mean and range.

coefficient. Included on this graph for comparative purposes are the BUIs(%) for [^{14}C]nicotine and [^{14}C]iodoantipyrine. [^{14}C]Nicotine has been reported previously to have a BUI of 131% (20). Compounds with low partition coefficients exhibited BUIs of approximately 6%, a value that can be assumed to reflect the vascular space within the brain. Oldendorf reported a lower baseline level of 2% (*20*).

Figure 5 shows images of a dog's head obtained at 2 and 40 min after injection of 6 mCi of Tc-99m IIa into the left common carotid artery. The radioactivity was seen to undergo initial uptake into the brain followed by gradual redistribution. At later times, a homogeneous uptake pattern was found, similar to that of a diffusible tracer. The BUI uptake for Tc-99m IIa varied as a function of time after injection, indicating that the tritiated water was diffusing out of the brain at a faster rate than was Tc-99m IIa. Varying methanol concentrations

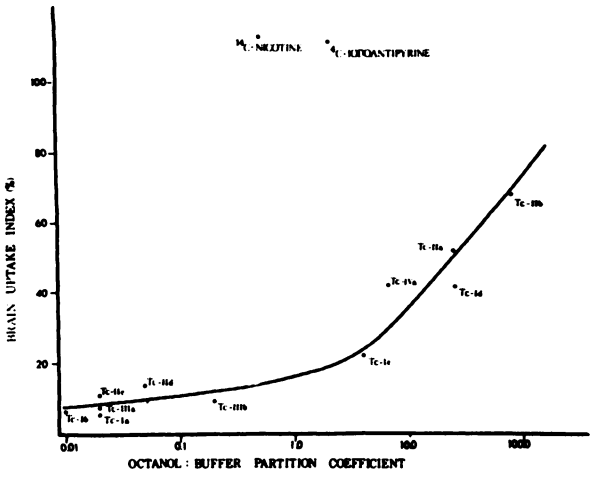

FIG. 4. Variation in BUI(%) as function of substrate lipophilicity for series of Tc-99m complexes.

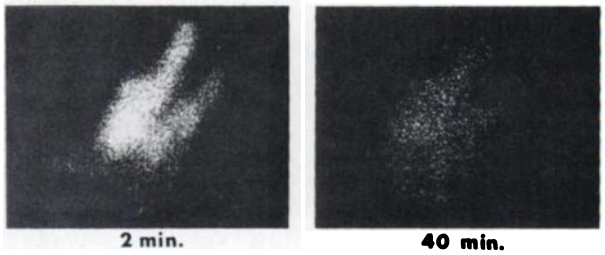

FIG. 5. Cerebral uptake of ^{99m}Tc oxine. Images of dog's head obtained after common carotid injection of 6 mCi of Tc-99m IIa. Images obtained over 10-sec interval using Pho Gamma III scintillation camera at intensity 500. Images at 2 and 40 min contain 25,000 and 12,000 counts, respectively.

from 5 to 50% altered the BUI obtained for Tc-99m IIa. At 5% methanol, the BUI was 30.32%, whereas at 50% it was 53.8%. The BUI of Tc-99m $(Ia)_2$ did not rise above background (4.8%) even when injected in 50% methanol.

DISCUSSION

In 1946, Krogh (*21*) observed that the BBB, which separates the plasma from the brain's extracellular fluid (ECF), had biological characteristics similar to those of a cellular membrane. Other investigators (*20–22*) have subsequently supported this thesis, and the BBB is now considered to have a selective permeability similar to that of the red cell's plasma membrane (*23*). It is generally accepted that the anatomic basis of the BBB lies in the continuous tight junctions between adjacent endothelial cells in the cerebral capillaries. For a substrate to gain access to the brain ECF, it is necessary for it to pass through the endothelial cell's luminal membrane, cytoplasm, and outer cell membrane. Thus, access into the brain ECF can be equated with membrane transport. Two general mechanisms for transport across the BBB have been demonstrated: passive diffusion based on substrate lipophilicity, and carrier-mediated transport based on specific binding between substrate and membrane-bound carriers.

This work demonstrates the ability of Tc-99m-labeled radiopharmaceuticals to cross the intact BBB in proportion to their partition coefficients. The graph of BUI plotted against partition coefficient shown in Fig. 4 has a sigmoidal shape in the first part in the range of partition coefficients from 0.01 to 78.5. The threshold for BBB transport occurred at a partition coefficient of approximately 0.5. The data for all of the Tc-99m-labeled chelates were found to fall on the same sigmoidal curve, irrespective of which of the four chelating agents shown in Fig. 1 was used in the preparation of the radiolabeled compound. This distribution further supports the thesis that all of these compounds cross the BBB by a passive diffusion process proportional only to radiopharmaceutical lipophilicity and independent of the

stoichiometry and charge distribution of the labeled compound.

Oldendorf's data relating the brain uptake of organic compounds to their olive-oil:water partition coefficients further supports this viewpoint. Oldendorf, in studying C-14-labeled, nonmetallic biochemicals, observed a similar sigmoidal response when brain uptake index was plotted against partition coefficient (*20*). In his work it was determined that the threshold for brain uptake occurred at an olive-oil:water partition coefficient of 0.3, which relates favorably with the octanol:buffered-saline partition coefficient of 0.5 reported in this work. The only significant difference observed between the compounds labeled with Tc-99m and C-14 was in the rate of increase in BUI(%) after the threshold values had been exceeded. The C-14 compounds exhibited a much sharper rise in brain uptake index than did the Tc-99m compounds.

This difference can be explained by the relative differences in their protein binding. When [^{14}C]nicotine and [^{14}C]iodoantipyrine were studied in our laboratory, their BUI values were found to lie considerably off the curve generated for Tc-99m compounds (Fig. 4). A possible explanation for this difference lies in the relatively low extent of protein binding of the C-14 compound against a protein binding of greater than 80% for all of the Tc-99m compounds that showed brain uptake. Whereas Oldendorf's technique of injecting a bolus into the common carotid artery served to minimize the effects of protein binding on BUI(%), it did not eliminate them completely. The dramatic decrease in brain uptake index caused by protein binding was examined for Tc-99m IIa by preincubating it with albumin before the carotid injection. Protein binding decreased the BUI(%) from an initial value of 53.8% down to 5%, which was considered indistinguishable from background. While Tc-99m IIb has sufficient lipophilicity for use as a diffusible tracer, its high degree of protein binding (96.8%) would preclude its use for this purpose subsequent to i.v. injection. The BUI for [^{14}C]iodoantipyrine was not decreased when it was preincubated with protein before injection. The partition coefficient (5.5) and extent of protein binding (17.4%) observed for [^{14}C]iodoantipyrine should serve as a model for the continued development of new lipophilic Tc-99m-labeled tracers.

The aminopolycarboxylate-containing derivatives—Fig. 1 compounds I, III, and IV—were able to cross the BBB only after substitution of long alkyl chains. The requirement of a long alkyl chain is apparently necessary in order to offset the negative charge associated with the chelate bond between reduced Tc-99m and aminopolycarboxylates (*24*). Since it is necessary to add very large, highly lipophilic substituents to the aminocarboxylates in order to obtain cellular transport, it appears that substituted aminopolycarboxylates have limited utility in serving as Tc-99m-labeled bifunctional radiopharmaceuticals for examination of nonexcretory organs.

Whereas the chemical structures of the Tc-99m complexes of compound I are known, those of Tc-99m complexes of III and IV have yet to be reported. The significance of such information can be appreciated by a comparison of the partition coefficients and the BUI(%) for Tc-99m IIIb and Tc-99m IVa. The latter was observed to have partition coefficient and corresponding brain uptake indices much higher than those of Tc-99m IIIb. This discrepancy would have been difficult to predict based on an examination of the chelating agents alone, and suggested the importance of the chemical structure in determining in vivo distribution. Similar results have been observed for the in vivo distribution of Tc-99m HIDA against that of [^{14}C]HIDA and [^{113}Sn]HIDA (*25*).

The radiochemical structure of Tc-99m-labeled derivatives of compound I have been determined using two different procedures (*24,26*), and in both instances these derivatives were shown to exist as *bis* structures with two ligands symmetrically located around a Tc-99m atom in the +3 oxidation state, with the entire complex carrying a charge of −1. Using this information, we obtained a mixed ligand complex, Tc-99m IbIc, in high radiochemical purity by HPLC. Its partition coefficient and BUI were intermediate between those of Tc-99m $(Ib)_2$ and Tc-99m $(Ic)_2$, consistent with its anticipated asymmetrical *bis* structure containing a methyl group and a carboxy group at the terminal portions of the molecule. While the anticipated 1:2:1 product ratio for *bis*-structured compounds was observed (*24*), it occurred at a Ib:Ic molar ratio of 7:1 instead of the 1:1 molar ratio reported by other workers studying HIDA analogs. This strongly suggests that either the formation constant for Tc-99m $(Ic)_2$ is larger than that for Tc-99m $(Ib)_2$, or that the relative product composition is governed not by thermodynamics but by relative reaction rates. In that case, reduced Tc-99m would have to react more rapidly with Ic than with Ib, and the relative reaction products, once formed, must be relatively stable with respect to ligand exchange reactions.

Tc-99m-labeled derivatives of II substituted with neutral substituents offer promise for the development of new, intracellular tracers. Tc-99m IIa and Tc-99m IIb are highly lipophilic and highly extracted by the brain, whereas the substitution of a negative charge (as in IIc) or substitution of a positive charge (as in IId) served to decrease greatly the lipophilicity and brain uptake. The observed lipophilicity of Tc-99m IIa is similar to that found for the cell-labeling agent, In-111 IIa, except that Tc-99m IIa was observed to be inert with respect to ligand substitution when incubated with a large molar excess of EDTA, whereas In-111 IIa was observed to undergo an immediate ligand exchange reaction to In-111 EDTA.

The synthesis of Tc-99m IIa has been reported previously. Anghileri et al. (*27*) prepared a colloidal Tc-99m

IIa complex using 0.5 mg of Sn-oxine in 0.1 ml of dimethylsulfoxide and 20 ml of saline. The brain uptake of the Tc-99m-labeled derivatives of II in our studies cannot be attributed to colloid formation, since colloidal Tc-99m exhibited a BUI(%) of 0.57%. Further, the BBB is incapable of either phagocytosis or pinocytosis and the absolute quantity of Tc-99m in the brain was seen to decrease with time. Mikulaj et al. (*28*) prepared a soluble $^{99m}Tc^{5+}$ complex with IIa that was extractable into chloroform and was sufficiently stable to preclude its back extraction into acid.

CONCLUSIONS

This work demonstrates that Tc-99m complexes can cross the blood-brain barrier in quantities proportional to their lipophilicity; it offers an explanation as to why Tc-99m-labeled derivatives of fatty acids using aminopolycarboxylates are incapable of imaging the myocardium. Furthermore, the necessity of substituting long lipophilic alkyl substituents onto the aminopolycarboxylates will diminish their usefulness in the scintigraphic determination of function in the nonexcretory organs. While the high protein binding of the current derivatives of II preclude their use as diffusible tracers, these compounds have demonstrated the ability to cross lipophilic barriers. The hindrance to the development of a diffusible Tc-99m-labeled tracer has now been defined as a physical-chemical problem of retaining lipophilicity while minimizing protein binding. Once this difficult step has been accomplished, Tc-99m-labeled radiopharmaceuticals should be available as substitutes for I-123- and I-131-tagged iodoantipyrine for the measurement of regional perfusion. This article demonstrates the general importance of lipophilicity in the membrane transport of Tc-99m compounds and suggests new mechanisms for developing radiopharmaceuticals specific for the nonexcretory organs.

FOOTNOTES

* Aldrich Chemical Co., Milwaukee, WI.
† Fisher Scientific Co., Pittsburgh, Pa.
‡ Eastman Kodak Co., Rochester, NY.
‖ Pfizer Inc., Groton, CT.
§ W. Eckelman, Washington Univ., St. Louis, MO.
¶ Bis structure formation is noted when applicable—see Refs. *26, 27*.
** NCS, Amersham/Searle Corp., Arlington Heights, IL.

REFERENCES

1. KARESH SM, ECKELMAN WC, REBA RC: The biological distribution of chemical analogs of fatty acid and long chain hydrocarbons containing a strong chelating agent. *J Pharm Sci* 66: 225-228, 1977
2. LOBERG MD, FIELDS AT, PORTER DW: Radiochemically pure Tc-99m labeled fatty acid analogs. *J Nucl Med* 19:717, 1978 (abst)
3. HEINDEL ND, RISCH VR, BURNS HD, et al: Syntheses and tissue distribution of ^{99m}Tc-sulfonylureas. *J Pharm Sci* 64: 687-689, 1975
4. MCAFEE JG, SUBRAMANIAN G: Radioactive agents for imaging. In *Clinical Scintillation Imaging*, Johnson PM, ed. New York, Grune & Stratton, 1975, pp 13-114
5. RAICHLE ME, EICHLING JO, GRUBB RL: Brain permeability of water. *Arch Neurol* 30:319-321, 1974
6. OLDENDORF WH, KITANO M: The symmetry of I^{131} 4-iodoantipyrine uptake by brain after intravenous injection. *Neurology* 15:994-999, 1965
7. USZLER JM, BENNETT LR, MENA I, et al: Human CNS perfusion scanning with ^{123}I-iodoantipyrine. *Radiology* 115:197-200, 1975
8. DUCASSOU D, BRENDEL A, MORETTI J-L, et al: Iodo 123 antipyrine and brain: a clinical investigation. In *Abstracts, World Federation of Nuclear Medicine and Biology*, Washington, DC, 1978, p 61
9. WELCH MJ: Choice and preparation of radioactive gases. In *Progress in Nuclear Medicine*, vol. 3. Baltimore, University Park Press, 1973, pp 37-48
10. SAKURADA O, KENNEDY C, JEHLE J, et al: Measurement of local cerebral blood flow with iodo[^{14}C] antipyrine. *Am J Physiol* 234:H59-H66, 1978
11. OLDENDORF WH: Need for new radiopharmaceuticals. *J Nucl Med* 19:1182, 1978
12. GUSTAFSON DE, BERGGREN MJ, SINGH M, et al: Computed transaxial imaging using single gamma emitters. *Radiology* 129:187-194, 1978
13. CALLERY PS, FAITH WC, LOBERG MD, et al: Tissue distribution of technetium-99m and carbon-14 labeled N-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid. *J Med Chem* 19:962-964, 1976
14. BURNS HD, SOWA DT, MARZILLI LG: Improved synthesis of N-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid and analogs. *J Pharm Sci* 67:1434, 1978
15. ECKELMAN WC, KARESH SM, REBA RC: New compounds: fatty acid and long chain hydrocarbon derivatives containing a strong chelating agent. *J Pharm Sci* 64:704-706, 1975
16. FIELDS AT, PORTER DW, CALLERY PS, et al: Synthesis and radiolabeling of technetium radiopharmaceuticals based on N-substituted iminodiacetic acid: Effect of radiolabeling conditions on radiochemical purity. *J Labeled Compounds Radiopharm* 15:387-399, 1978
17. LOBERG MD, FIELDS AT: Stability of ^{99m}Tc-labeled N-substituted iminodiacetic acids: ligand exchange reaction between ^{99m}Tc-HIDA and EDTA. *Int J Appl Radiat Isot* 28:687-692, 1977
18. OLDENDORF WH: Measurement of brain uptake of radiolabeled substances using a tritiated water internal standard. *Brain Res* 24:372-376, 1970
19. MIRRLEES MS, MOULTON SJ, MURPHY CT, et al: Direct measurement of octanol-water partition coefficients by high-pressure liquid chromatography. *J Med Chem* 19:615-619, 1976
20. OLDENDORF WH: Lipid solubility and drug penetration of the blood brain barrier. *Proc Soc Exp Biol Med* 147:813-816, 1974
21. KROGH A: The active and passive exchanges of inorganic ions through the surfaces of living cells and through living membranes generally. *Proc Roy Soc (B)* 133L140-200, 1946
22. CRONE C: The permeability of brain capillaries to non-electrolytes. *Acta Physiol Scand* 64:407-417, 1965
23. OLDENDORF WH: Brain uptake of radiolabeled amino acids, amines and hexoses after arterial injection. *Am J Physiol* 221: 1629-1639, 1971
24. BURNS HD, WORLEY P, WAGNER HN, JR, et al: Design of

technetium radiopharmaceuticals. In *The Chemistry of Radiopharmaceuticals*, Heindel ND, Burns HD, Honda T, et al, eds. New York, Masson, 1978, pp 269-289

25. RYAN J, COOPER M, LOBERG M, et al: Technetium-99m-labeled N-(2,6-dimethylphenylcarbamoylmethyl) iminodiacetic acid (Tc-99m HIDA): a new radiopharmaceutical for hepatobiliary imaging studies. *J Nucl Med* 18:997-1004, 1977
26. LOBERG MD, FIELDS AT: Chemical structure of technetium-99m labeled N-(2,6-dimethylphenylcarbamoylmethyl)iminodiacetic acid (Tc-99m HIDA). *Int J Appl Radiat Isot* 29:167-173, 1978
27. ANGHILERI LJ, FIRUSIAN N, HEIDBREDER M: ^{99m}Tc-Sn oxine complex: a radiocompound with possibilities for lymphographic studies. *Eur J Nucl Med* 2:109-111, 1977
28. MIKULAJ V, RAJEC P, FABEROVA V: Chem Zuesti: Chemical stabilization of daughter technetium-99m after the β-decay in chelate systems with molybdenyl-molybdenum-99. *Chem Zuesti* 28:37-46, 1974