Technetium-99m HM-PAO Stereoisomers as Potential Agents for Imaging Regional Cerebral Blood Flow: Human Volunteer Studies

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A total of nine normal volunteer subjects were studied with three forms of [^{99m}Tc]hexamethylpropyleneamineoxime (HM-PAO), a potential cerebral blood flow imaging agent. One, the d,l isomer, showed 4.1% uptake in the brain which remained constant over 8 hr. There was good differentiation between uptake in gray and white matter on tomographic slices. We propose that this agent may allow regional cerebral blood flow imaging to be performed on a routine basis.

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The clinical value of measuring regional cerebral blood flow (rCBF) has long been recognized. Local variations in flow are associated with many brain diseases such as stroke, tumors, epilepsy and dementia, and any technique which provides an image of blood flow is of particular interest. Radionuclide tracers have been used for this purpose for many years, but it is only recently that the potential for three-dimensional imaging of rCBF on a routine diagnostic basis has come close to being realized.

Xenon-133 is the most widely used tracer (*1–4*), but because the technique involves measuring the temporal variation in activity, tomography is possible only with purpose-built equipment (*5*) and image quality is relatively poor. Equilibrium imaging, by continuous administration of a diffusible radiotracer with a radioactive half-life shorter than the rate of washout from the brain, allows ample time for tomography to be performed. Intracarotid infusion of krypton-81m has been used, (*6*), but the technique is invasive and the radiopharmaceutical not readily available. In centers with a positron facility, inhalation of $C^{15}O_2$ can be used (*7*) but it is an expensive procedure.

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Perhaps the most convenient radiopharmaceutical for imaging rCBF is one that is trapped in its first passage through the brain. Technetium-99m- (^{99m}Tc) labeled microspheres have been used (*8*), but the resulting capillary occlusion may result in an unacceptably high degree of risk. The positron emitter nitrogen-13, in the form of intravenously injected $^{13}NH_3$, may also be a suitable tracer, it being trapped in the glutamate pool (*9*). Recently *N*-isopropyl-*p*- (^{123}I) iodoamphetamine (IMP) has been introduced; this gamma-ray emitting radiopharmaceutical is almost completely removed on its first pass through the brain (*10*). Iodine-123 is a cyclotron-produced radionuclide with a half-life of 13 hr and, as a result, the material is not readily available and the cost of an IMP investigation is very high. A material which behaves like IMP but could be labeled with ^{99m}Tc would represent a major advance towards the goal of routine imaging of rCBF.

Volkert and colleagues (*11*) have described the use of propyleneamineoxime complexed with ^{99m}Tc for the possible measurement of rCBF. However, the washout of material from the brain was too rapid for tomographic imaging. Results on a derivative of this material, hexamethylpropyleneamineoxime (HM-PAO) which have been reported (*12*), show much better retention in the brain.

Recently, two diastereoisomers have been isolated

from HM-PAO designated the "meso" and "d,l" forms (*13*). This paper will report the findings of an investigation into the behavior of the three forms of HM-PAO in human volunteers: the separated meso, d,l isomers, and the original mixture used in previous clinical studies (*11,14*).

MATERIALS AND METHODS

The three materials were supplied commercially* in vials containing the following freeze-dried constituents: 1.0 mg HM-PAO (mixture), 12.5μg stannous tartrate; 0.5 mg meso HM-PAO, 7.6μg stannous chloride dihydrate, 4.5 mg sodium chloride; and 0.5 mg d,l HM-PAO, 7.6μg stannous chloride dihydrate, 4.5 mg sodium chloride.

Vials were reconstituted with 6.5 ml of sodium pertechnetate and 30 mCi ^{99m}Tc. Radiochemical purity was checked in each case using chromatography on ITLC SG strips with methylethylketone (MEK) and saline as solvent. Technetium-99m HM-PAO exists as a lipophilic complex which runs with the solvent front in MEK but remains at the origin in saline. This converts with time to a secondary complex which remains at the origin in both systems. Free pertechnetate is found at the solvent front in both systems (*13*). The lipophilic and secondary complexes are separated by reverse phase high performance liquid chromatography and only the lipophilic complex crosses the blood-brain barrier in experimental animals. To contain the maximum amount of lipophilic complex, preparations were injected within 15 min of reconstitution. At the time of injection all preparations were estimated to contain between 85 and 95% lipophilic species and <5% free pertechnetate.

Initial radiation dose estimates were made from studies using laboratory rats and were based on the MIRD 11 scheme (*15*).

Acute single-dose toxicity studies were also performed in rats. No mortalities or obvious signs of toxicity were observed at 875 times the maximum human equivalent dose.

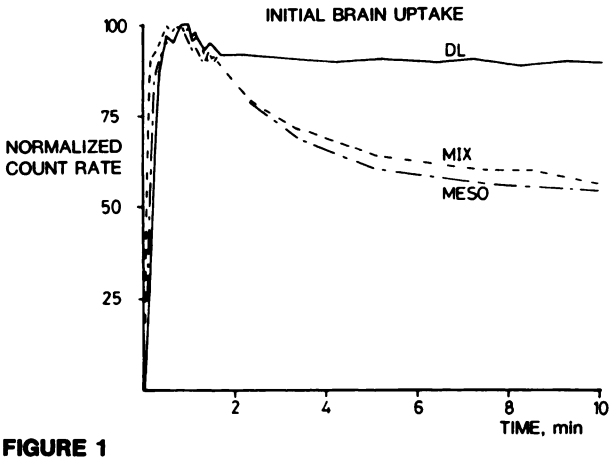

FIGURE 1
Time-activity curves from ROI drawn over anterior view of brain for first 10 min p.i. These are typical results for one observer

Studies were performed on a total of nine volunteers, seven male and two female, ranging in age between 25 and 42 yr. All volunteers were in good health and had no recent history of illness. Permission for the study was obtained from the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen. Blood samples were taken for biochemical analysis immediately prior to administering the material and again at 24 hr postinjection (p.i.). Vital signs were recorded 15 min prior to injection and 40 min p.i.

Two volunteers received the mixture and two the meso isomer; in all four studies 7 mCi of ^{99m}Tc-labeled material was administered. Five volunteers, three male and two female, were given the d,l isomer, in two cases 7 mCi was used and in three 15 mCi. In all cases, the material was administered intravenously with the subject lying beneath the gamma camera. A dynamic study of 100 6-sec image frames of the anterior view of the head was carried out.

The amount of injected dose present in each organ was measured by taking a series of views covering the whole body, both anterior and posterior, immediately after the dynamic study. Data were collected on a DEC Gamma 11 data processing system. Using regions of interest (ROIs), the total number of counts over the whole body at this time was measured. The geometric mean of anterior and posterior counts was used to make some correction for the effect of attenuation (*16*). As no excretion of material from the body will have occurred, this number of counts gives a measure of total injected activity. The fraction of activity in a particular organ was then measured by comparing the geometric mean of the total counts in the ROI drawn around the anterior and posterior views of the organ with the above estimate of total body counts.

These whole-body measurements were made at ~20 min, 2 hr 20 min, 4 hr 45 min, 8 hr, and 24 hr p.i. In all cases, the activity in an ROI was referred to the 20-min whole-body value. Seven-milliliter blood samples were taken at 2, 5, 10, 15, 20, 30, 40, 60, 120, 240, 480 min, and at 24 hr p.i. Activity in whole blood and in separated plasma and red-cells was measured and expressed as a percentage of the injected dose.

TABLE 1
Percentage of Injected Material in Brain

Time	Mixture	Meso	d,1
20 min	1.8, 2.1	1.7, 2.0	3.6, 5.7, 3.7, 3.9, 4.2
20 hr	1.9, 2.0	1.4, 1.8	3.4, 5.4, 3.9, 3.8, 4.2
4 hr	1.7, 2.1	1.6, 2.0	3.5, 5.5, 3.7, 3.7, 4.2
8 hr	1.6, 2.0	1.5, 1.9	3.4, 5.5, 3.7, 3.7, 4.1
24 hr	1.6, 1.9	1.6, 1.9	3.2, 4.7, 3.8, 3.3, 3.8

Total urine output was collected at intervals of 0–4, 4–8, 8–12, 12–24 hr, and in six cases also at 24–48 hr. Urine output was measured as a percentage of total injected activity.

Tomographic images were taken at ~1 hr, 4 hr, and 8 hr after injection using a rotating gamma camera or a multidetector, single slice imager†. Reconstruction, on both machines, was by filtered back projection. Acquisition time was 30 min for the rotating camera and 4 min per slice for the section scanner.

RESULTS

No adverse reactions were observed, body and blood chemistry were unchanged, and there were no significant alterations in vital signs.

Figure 1 shows the time-activity curve for each of the three materials produced from the initial 10-min dynamic study. Activity over the brain reached a maximum at about 1 min p.i., and this was followed by some loss of material which plateaued by about 10 min p.i. for mixture and meso and by 2 min p.i. for d,1. The retention of the mixture was 53 and 58% of the maximum for the two volunteers, for the meso it was 50 and 54%, and the d,1 had an average of 88.1% (s.d. 3.4%).

The absolute amount of injected activity retained in the brain (Table 1) showed, once again, that the d,1 isomer had the best performance with an average uptake of 4.1% (range 3.4 to 5.7%) over the first 8 hr compared with 1.9% for the mixture and 1.7% for the meso. In all cases the level of uptake remained approximately constant for the first 8 hr.

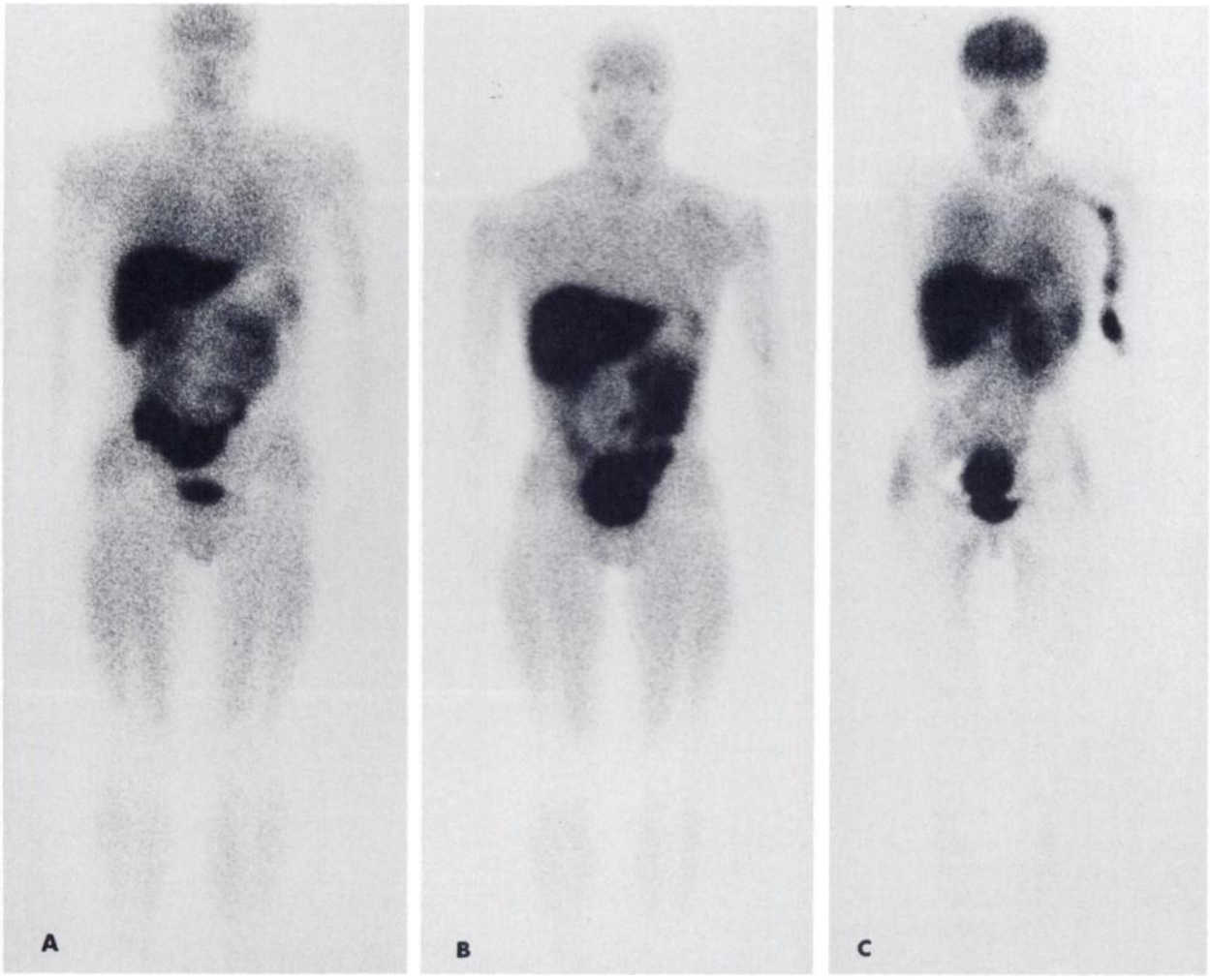

FIGURE 2
A: Anterior view whole-body scan of [^{99m}Tc]HM-PAO mixture taken 4 hr p.i. Uptake is seen in brain, skeletal muscle, and lung. Excretion is hepatobiliary; kidneys, bladder, liver, and small intestine are all visible. B: Anterior view whole-body scan of meso isomer 4 hr p.i. Distribution of material is similar to mixture but with lower lung uptake and obvious concentration of material in lacrimal glands. Brain uptake is only slightly higher than in soft tissue. C: Anterior view whole-body scan of d,1 isomer 4 hr p.i. High brain uptake is clearly seen as is uptake in myocardium, subcutaneous fat of buttocks and medial aspect of thighs. Retention of material in left brachiocephalic vein is also apparent. Some trapping of material in vein into which material is injected is common feature of these materials. There is large urinary bladder but relatively small amount of material in intestine

TABLE 2A
Percentage of Injected Activity Present at 20-min Postinjection*

Item	Brain	Liver	Kidneys	Intestine	Bladder + urine
Mixture	1.8, 2.1	13.3, 12.7	1.5, 2.4	0, 0	1.6, 1.2
Meso	1.7, 2.0	16.7, 16.5	1.6, 1.0	0, 0	0.6, 0.7
d,l	3.6, 5.7,	9.2, 10.8,	4.1, 4.0,	0, 0,	2.6, 2.1,
	3.7, 3.9,	9.7, 10.3,	3.3, 2.5,	0, 0,	1.7, 2.5,
	4.2	11.1	3.6	0	2.5

* Bladder activity is measured from images while urine activity is calculated from samples.

TABLE 2B
Percentage of Injected Activity Present at 24-hr Postinjection

Item	Brain	Liver	Kidneys	Intestine	Bladder + urine
Mixture	1.6, 1.9	7.1, 8.5	1.9, 1.7	14.3, 23.7	32.3, 34.7
Meso	1.6, 1.9	9.7, 9.5	0, 0	30.4, 22.7	25.4, 26.0
d,l	3.2, 4.7	5.9, 6.0,	0, 1.7,	14.5, 17.7,	47, 34.4,
	3.8, 3.3,	5.9, 6.8,	1.7, 2.4,	20.4, 16.4,	32, 29.2,
	3.8	6.4	3.1	22.2	34.3

TABLE 3
Percentage of Injected Material in Whole Blood

Time	Mixture	Meso	d,l
2 min	18.1, 16.8	8.2, 27.2	—, 27.2, 26.7, 26.4, 15.1
5 min	15.9, 13.3	7.6, 15.2	15.9, 19.1, 17.9, 18.2, 12.2
10 min	12.6, 11.8	7.5, 12.3	15.1, 17.1, 15.4, 16.1, 11.5
15 min	11.3, 10.4	7.0, 10.2	—, 16.0, 15.1, 15.8, 11.1
20 min	10.6, 10.2	6.7, 10.0	14.3, 15.4, 14.6, 14.9, 10.4
30 min	9.5, 9.5	6.4, 8.6	11.3, 14.3, 13.6, 14.1, 9.8
40 min	9.0, 9.0	5.7, 8.0	13.4, 13.7, 13.3, 13.2, 9.6
1 hr	8.1, 8.4	5.7, 7.7	12.2, 13.1, 12.2, 13.1, 9.3
2 hr	7.6, 7.5	5.6, 7.9	11.1, 11.6, 11.5, 11, 8.3
4 hr	6.2, 6.1	4.5, 6.6	8.4, 9.4, 9.7, 9.4, 6.8
8 hr	4.7, 6.1	3.3, 5.6	7.5, 7.5, 7.6, 7.4, 5.5
24 hr	3.0, 3.0	2.4, 3.4	4.5, 4.6, 4.6, 4.8, 3.4

Images of the whole-body distribution at 4 hr p.i. are shown in Fig. 2 and the quantitative uptake in various organs is demonstrated in Table 2.

All materials have both hepatobiliary and urinary excretion. At 20 min p.i. the d,l shows the lowest liver uptake, an average of 10.2%, the mixture 13% and the meso 16.6%. In contrast, the d,l isomer has the highest renal uptake, 3.5% compared with <2% for the mixture and meso. Total urinary excretion over 24 hr was 35.4% for the d,l, 32.7% for the mixture and 25.7% for the meso. Both the mixture and d,l showed about 2% retention in renal parenchyma at 24 hr p.i.

Soft-tissue distribution is predominantly in skeletal muscle but in two of the d,l studies there was significant lung uptake (8 and 12%). As can be seen in Fig. 2C there is accumulation of d,l in subcutaneous fat and about 2% concentrates in the myocardium although precise quantification of uptake is difficult.

Slight uptake in the thyroid, nasal and oral mucosa and, in some cases, the lacrimal glands (Fig. 2B) were also seen.

TABLE 4
Ratio of Activity in Red Blood Cells to that in Whole Blood

Time (hr)	Mixture	Meso	d,l
1*	0.28	0.37	0.35
2	0.34	0.37	0.44
4	0.40	0.45	0.52
8	0.43	0.46	0.55
24	0.51	0.62	0.60

* 1-hr Reading is average value over first hour.

Blood clearance of the three materials is shown in Table 3. There were discrepancies between the clearance curves for the two meso studies but good agreement between the five d,l studies and between the two mixture studies. The ratio of uptake in red blood cells to that in whole blood remained constant over the first hour, being lowest for the mixture, but showed an increase over the following 23 hr (Table 4).

Tomographic studies performed at 1 hr p.i. (Fig. 3)

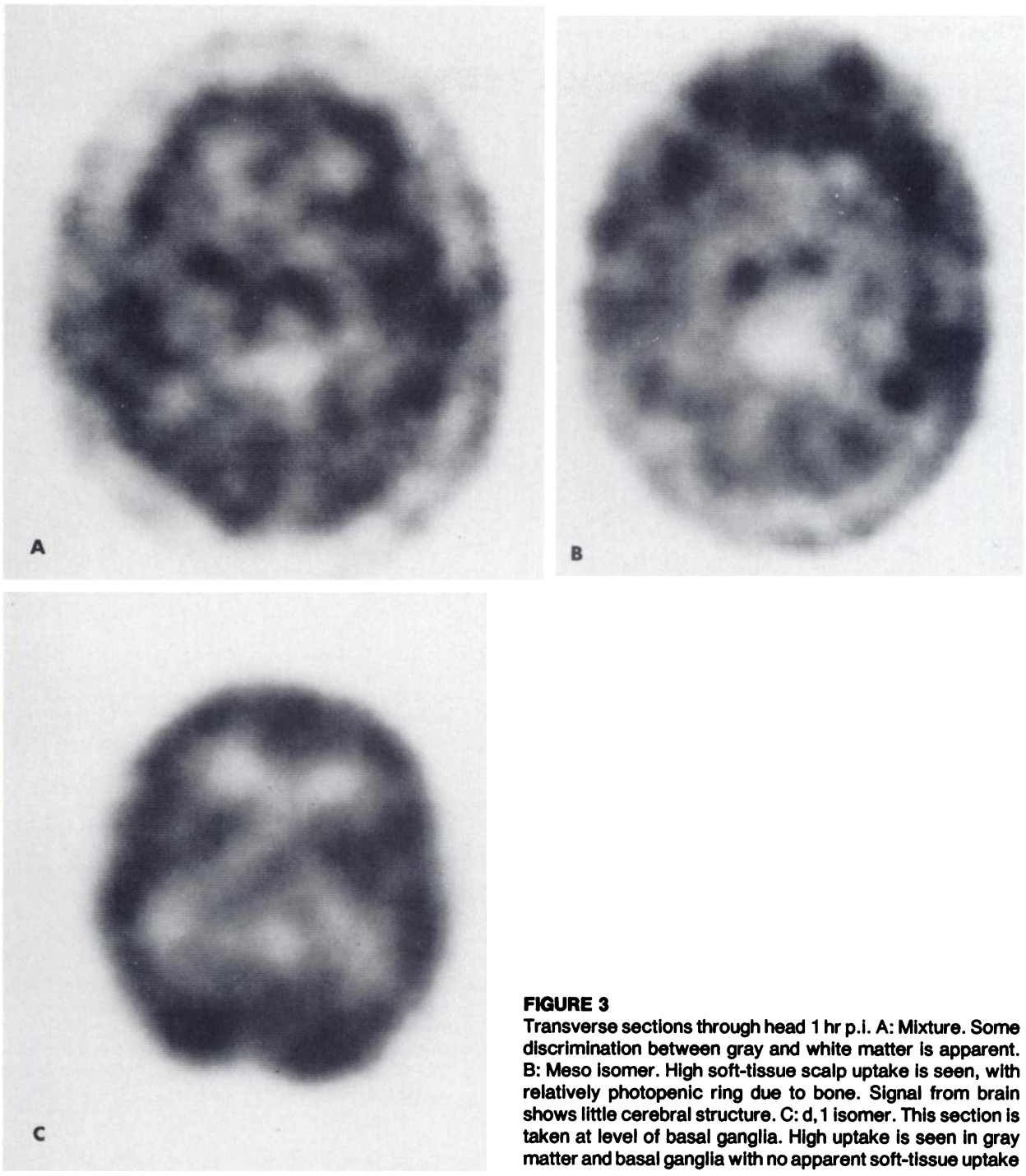

FIGURE 3
Transverse sections through head 1 hr p.i. A: Mixture. Some discrimination between gray and white matter is apparent. B: Meso isomer. High soft-tissue scalp uptake is seen, with relatively photopenic ring due to bone. Signal from brain shows little cerebral structure. C: d,1 isomer. This section is taken at level of basal ganglia. High uptake is seen in gray matter and basal ganglia with no apparent soft-tissue uptake

show that both the d,1 and mixture concentrate in gray matter, although the superior quality of the d,1 image is evident, while the meso does not appear to have any selective concentration.

Radiation dosimetry was calculated for the d,1 isomer using the MIRD 11 scheme (*15*). The results are shown in Table 5. They do not differ significantly from the original estimates made using laboratory rats.

DISCUSSION

The potential value of an agent which can be used for three-dimensional imaging of rCBF on a routine basis has been clearly demonstrated with IMP. Hill et al. (*17*) have shown that infarcts can be imaged the day on which the symptoms first appeared. This is superior to [^{99m}Tc]pertechnetate studies in which ischemic infarcts may not be seen for 7 to 10 days after the event although hemorrhagic infarcts may be seen much earlier (*18*). X-ray computed tomography will not usually show changes until 24 to 48 hr after an ischemic infarct and one recent study has indicated that 20% may never be seen (*19*).

TABLE 5
Calculated Radiation Dose to Subjects Following Administration of d,l [^{99m}Tc]HM-PAO*

Organ	Radiation dose (rad/mCi)
Brain	0.023
Liver	0.042
Kidneys	0.097
Urinary bladder wall	0.091
Ovaries	0.042
Testes	0.009
Small intestine wall	0.068
Upper large intestine wall	0.11
Lower large intestine wall	0.065
Whole body	0.016

* It has been assumed that urinary bladder is voided every 4 hr after injection.

Gemmell et al. (*20*) have indicated that IMP may demonstrate distinctive uptake patterns for different types of dementia.

The precise role that a rCBF agent would play still needs to be demonstrated, but there is clearly potential for its value in these and other clinical conditions such as migraine, epilepsy, and tumors.

To realize its full potential, a rCBF imaging agent must be simple to use, inexpensive and readily available. The latter two criteria are not met by IMP, while the d,l isomer of [^{99m}Tc]HM-PAO appears to satisfy all criteria. Its uptake in the brain of 4.1% is comparable to IMP when measured with our technique and the shorter half-life of ^{99m}Tc allows higher levels of activity to be given.

It is envisaged that clinical studies will use between 15 and 20 mCi of activity, which, in terms of radiation dosimetry, compares favorably with the 5 mCi used with IMP.

The high uptake noted in the lungs of two d,l volunteers will modify the radiation dosimetry slightly. The reason for lung uptake is unknown at present, but appears to be related to smoking. Of the five volunteers for the d,l study only these two were smokers. One volunteer for the mixture had been a smoker for about 12 mo but had no abnormal lung uptake. This lung uptake appears to be at the expense of body tissue uptake, brain, and other organ uptake being similar to that of the nonsmoking volunteers. Uptake in lacrimal glands will also modify the dosimetry slightly. The level of uptake is so low as to make quantitation difficult, but it is <1% of the injected activity, seen in two out of the five d,l studies, in both meso, and in one mixture study.

At this stage it is impossible to say whether the uptake of [^{99m}Tc]HM-PAO truly reflects rCBF. Some preliminary studies by Lassen et al. (*21*) show that the distribution of the d,l isomer of [^{99m}Tc]HM-PAO and ^{133}Xe in tomographic slices is very similar. This suggests that the cerebral distribution of [^{99m}Tc]HM-PAO may be blood flow dominated. Further work is necessary to investigate the precise information provided by this material.

FOOTNOTES

* Amersham International, Buckhamshire, England.

† University of Aberdeen, Foresterhill, Aberdeen (The Aberdeen Section Scanner Mark II).

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