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# *IN VIVO* HUMAN PHARMACODYNAMICS OF THE PSYCHODYSLEPTIC 4-Br-2,5-DIMETHOXYPHENYLISOPROPYLAMINE LABELLED WITH $^{82}$Br OR $^{77}$Br

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**Summary**—The psychodysleptic compound 4-Br-2,5-dimethoxyphenylisopropylamine (4-Br-DPIA) was synthesized with $^{82}$Br or $^{77}$Br and administered to five human subjects in three oral and three intravenous experiments. *In vivo* organ concentrations were measured by gamma ray scintigraphy with computerized area integration, and by whole-body counting. Urine was analyzed by solvent separation, and thin layer chromatography of dansylated metabolites. The urine contained less than 5% radiobromine precipitable by acidic $AgNO_3$, demonstrating that the Br label remained organically bound. Upon intravenous administration, radioactivity appeared immediately in the lungs by first-pass extraction, was rapidly released and reached maximum concentrations in the liver at 0·5–1·5 hr, plasma at 2–3 hr and brain at 3–6 hr. Orally the pattern was the same except that the initial lung concentration did not occur. This sequence of maximum organ concentrations suggests that a metabolite of 4-Br-DPIA is the centrally active compound which concentrates in the brain. Solvent separation of urine showed 81·8% of metabolites to be aqueous soluble and 12·5% free base; slower whole-body excretion correlated with higher levels of free base in the urine. The methods used demonstrate a new approach to the study of *in vivo* distribution and kinetics of drugs which can be labelled with gamma-emitting radioisotopes.

The techniques of external gamma ray counting and imaging, which have been developed in the field of nuclear medicine, have only rarely been applied to pharmacodynamic investigations. The power of these techniques is that they yield continuous data on organ concentration of a suitably labelled material, in an animal or human subject, without disturbing normal body function during the course of the experiment. One reason for the lack of such application is that most molecules of pharmacological interest do not contain atoms for which there is a suitable gamma-emitting radionuclide.

We report here the study of a pharmacologically active compound, 4-bromo-2,5-dimethoxyphenylisopropylamine (4-Br-DPIA†, Figure 1A) labelled with $^{82}$Br or $^{77}$Br, administered to human subjects. The radioactivity was followed *in vivo* with the Anger Mark II Whole-body Scanner and Whole-body Counter, plasma clearance was determined, and urinary products were analyzed. The results provide a clear picture of the kinetics of organ accumulation and clearance, and some tentative conclusions as to the metabolic fate of this compound.

A preliminary pharmacological study showed that 4-Br-DPIA produces mild psychodysleptic symptoms in humans at doses of 0·2–2·0 mg (SHULGIN, SARGENT and NARANJO, 1971). It is of additional interest because the ring substitution pattern, namely 2,4,5, is the same as that of 6-hydroxydopamine (Fig. 1B), a similarity which was the original basis for the suggestion that compounds of this type may form an etiologic basis of schizophrenia (SHULGIN, SARGENT and NARANJO, 1969).

## METHODS

### *Radioisotopes*

Of the four potentially useful radioisotopes of bromine we chose to use $^{77}$Br and $^{82}$Br. The $^{77}$Br ($T_{1/2}$ = 57 hr) was produced at the Lawrence Berkeley Laboratory 88-in. variable

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† This compound has also been referred to as 4-bromo-2,5-dimethoxyamphetamine and DOB.

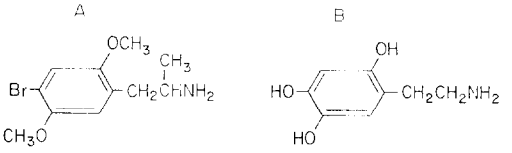


Fig. 1. A: 4-Br-DPIA; B: 6-Hydroxydopamine.

gradient cyclotron by bombardment of a powdered arsenic metal target with 25 MeV alpha particles, by the reaction $^{75}As$ ($\alpha$,2n) $^{77}Br$. With irradiations of 10 $\mu$A-hr, approximately 2–4 mCi of carrier-free $^{77}Br$ were produced, but some difficulty was encountered in the synthesis of 4-Br-DPIA with sufficiently high specific activity, so only one experiment is reported using this isotope. $NH_4{}^{82}Br$ ($T_{1/2}$ = 35 hr) was obtained from New England Nuclear (Boston, Mass.), at a specific activity of 4 mCi/mg.

*Chemical synthesis*

The radioactive 4-Br-DPIA was synthesized by direct bromination with elemental $^{77}Br_2$ or $^{82}Br_2$ of 2,5-dimethoxyphenylisopropylamine (DPIA), (Fox Chemical Co., Los Angeles, Calif.) in acid solution. The solution was made alkaline, the product extracted into $CH_2Cl_2$ and washed with $NH_4OH$ solution to leave unreacted bromine in the aqueous phase. The $CH_2Cl_2$ solution was extracted into 0·1 M HCl solution, and further purified by ion exchange. It was then streaked onto silica gel t.l.c. plates (Brinkman 500 $\mu$m, 20 cm × 20 cm) and chromatographed in $H_2O$–methanol–$NH_4OH$ (20:2:1) to separate 4-Br-DPIA from unreacted DPIA. These two compounds had $R_f$ values of 0·18 and 0·27 respectively, and were identified by spots of pure reference compounds, under 365 nm u.v. light. The area containing the 4-Br-DPIA was scraped off, neutralized with HCl and eluted with 0·9% physiological saline and passed twice through successive sterile 0·22 $\mu$m millipore filters. The purity of the prepared compound was further checked by gas chromatography; the concentration was determined by u.v. spectroscopy and the quantity of radioactivity by an Argonne-type whole-body counter (SARGENT, 1962), calibrated by IAEA standard reference sources. Gamma ray pulse height spectroscopy assured radioactive purity. The maximum specific activities obtained were 1·3 mCi/mg for 4-$^{82}$Br-DPIA and 0·21 mCi/mg for 4-$^{77}$Br-DPIA.

Although 4-Br-DPIA contains an optically active centre at the alpha carbon atom, the compound as synthesized is a racemic mixture. Through the courtesy of Dr. David Nichols of the University of Iowa, we were able to obtain the separately synthesized *R* and *S* DPIA precursors (NICHOLS, BAREKNECHT, RUSTERHOLZ, BENNINGTON and MORIN, 1973) and performed one experiment with the resulting *R*-4-Br-DPIA.

*Human subjects and dosage*

The minimal detectable oral human dose of 4-Br-DPIA has been reported to be 200 $\mu$g (SHULGIN *et al.*, 1971). The doses of radioactivity used were such that, with the specific activity available, the oral doses were less than 200 $\mu$g and the intravenous doses were less than 25 $\mu$g. No psychodysleptic or other pharmacologic effects were noted at these dose levels. This resulted in radioactivity doses of 20–30 $\mu$Ci and radiation doses to the subjects initially estimated at 9 mrad whole body and 23 mrad to the liver. Four of the authors served as the subjects: subject A received one oral dose of racemic 4-$^{82}$Br-DPIA; subject B received one intravenous (designated $B_{i.v.}$) and one oral dose (designated $B_o$) of racemic 4-$^{82}$Br-DPIA; subject C received one intravenous dose of racemic 4-$^{82}$Br-DPIA (designated $C_{rac}$) and one intravenous dose of the *R* isomer (designated $C_R$); subject D received one oral dose of racemic 4-$^{77}$Br-DPIA.

*Radioactivity measurements*

Plasma measurements were made with an automatic well counter (Nuclear Chicago) utilizing appropriately diluted standards. Whole-body retention was measured with the whole-body counter, utilizing the 1-m arc geometry. The entire urine specimens were also counted in the whole-body counter, at a distance of one meter for the high activity speci-

mens and on top of the crystal for low activity, again with appropriate standards. The various urine extracts described below were also counted in this manner. All data were corrected for radioactive decay; statistical counting errors were not more than 3% for the lowest counting rates encountered, and generally were lower.

The Anger Mark II Whole-body Scanner (ANGER, 1972) was used to obtain scintiphotos of *in vivo* localization of the radioactivity and to measure the quantity in any desired area of the body at selected times after administration. This machine consists of 64, 2·5 × 2·5 cm NaI (Tl) crystals in a single collimator block, in a staggered array. When the subject is moved over them on a variable speed bed, all parts of the body are viewed by the crystal array during the preset scanning time. The position of each scintillation event is recorded on-line on the high speed magnetic disc of the Hewlett–Packard 5047A computer (BUDINGER, 1973). These data are then framed into 64 × 64 unit digital matrices, using six such frames to cover the entire body, and recorded on magnetic tape. This information may then be manipulated at any future time in a variety of programmes available in the computer. In this study it was presented as a 64 × 64 unit picture on an oscilloscope screen of dots representing accumulated radioactivity. By means of a light pen an "area of interest" was drawn over the screen and the computer then integrated the total number of events in that area. Reference to the anatomical location is made by a shadowgram (Figs. 2 and 3) obtained by scanning the subject while he is in the beam of 60 keV gamma rays from a $^{241}$Am source above the bed. By this method it was possible to outline any non-regular area of the body and obtain the amount of radioactivity contained therein as a function of the time after administration. Areas were drawn for the brain, lung, liver, stomach and thigh, the last to represent general tissue distribution. These areas are shown in the shadowgrams. The areas were chosen to include, as nearly as possible, an entire organ without overlapping adjacent areas. To avoid interference by radioactivity in the liver, only the left lung was used. Due to the high energy of the gamma rays from $^{82}$Br, there was some penetration of the collimators and corresponding "shine through" of activity from one area into adjacent areas. The area for the thigh was drawn equal to that of the brain, to serve as a normalized reference area for the brain. Counting statistics varied considerably according to the size of the area, activity in the area, time after administration, etc. Except where error limits are shown in the figures, statistical errors of counting were less than the size of the symbol used for each point.

*Urine analysis*

Urine samples were collected at 1, 3, 6, 12 and 24 hr after administration of the 4-Br-DPIA. Urines were first counted for radioactivity *in toto*, then aliquots were solvent extracted for metabolite analysis by radioactivity distribution and thin-layer chromatography.

*Fractionation of urine.* A 20-ml aliquot of each urine collection was acidified with HCl, and extracted with an equal volume of methylene chloride. Following centrifugation, the phases were separated and the organic phase was counted as the "acid and neutral" fraction. The aqueous phase was brought to pH 11 with concentrated KOH, again extracted with an equal volume of methylene chloride, centrifuged to effect a clear separation between the phases, and separated. The resulting organic phase constituted the "free base" fraction. After careful removal of all residual traces of methylene chloride, the aqueous fraction was acidified with 2·5 ml concentrated HCl, and heated in an 80°C water bath for 30 min to cleave any conjugates. After cooling, the pH was adjusted to about 9·5 with KOH, followed by the addition of 5 ml of an ammonium hydroxide:ammonium chloride buffer (pH 9·5). This solution was then extracted with 20 ml of methylene chloride and centrifuged to separate the phases. The organic phase constituted the "conjugated base" fraction, and the residual aqueous phase was the "residual aqueous" fraction.

Samples of both this "residual aqueous" fraction, as well as the parent urine before extraction, were fractionated into ionic bromide and organic-bound bromide by acidification with nitric acid followed by treatment with excess silver nitrate. The precipitated solids were removed by centrifugation and washed with dilute nitric acid. The remaining

insolubles were assumed to represent free bromide ion in the original sample, the supernatant and washings organic bromide.

*Chromatographic analysis.* A 30-ml sample of each urine was acidified with HCl to pH 1·0 and hydrolyzed for 20 min in a boiling water bath. After hydrolysis, the urine was adjusted to pH 6·2 with NaOH and separated by ion-exchange chromatography on an Amberlite-IRC-50 column into a basic, or amine fraction and a non-basic, presumably non-amine fraction. Another 30-ml sample of urine was chromatographed on Amberlite-IRC-50 without preceding hydrolysis. After ion-exchange chromatography, fractions were counted for total radioactivity. While the non-amine fraction was not further analyzed the amines in the second fraction were dansylated according to the method of SEILER (1970), modified by BRAUN (1974). The dansyl derivatives were dissolved in ethyl acetate and separated by two-dimensional thin layer chromatography on silica-gel plates prepared by spreading Silica gel Type H in 0·25 mm thickness on 20 cm × 20 cm plates. Solvent systems used were ethyl acetate/cyclohexane (75:50) as solvent I and benzene/methanol/cyclohexane (85:15:10) as solvent II. In some experiments the more polar solvent system benzene/triethylamine/methanol (100:20:30) was used as solvent III.

Detection of dansylated amines was performed immediately after chromatography by observation of their intense fluorescense under ultraviolet light of 365 nm. The localization of the radioactive spots on t.l.c. plates was performed after chromatography with a Radioscanner Varian Aerograph-Berthold, Type 6000, LB 2722 and LB 2031.

*Blood samples*

An attempt was made to place an indwelling catheter in the anticubital vein in each study, so that frequent and accurately timed blood samples could be withdrawn. This was especially desirable in the case of intravenous administration, to obtain data on the initial clearance of the compound. Unfortunately, placement of the catheter was successful in only one of the intravenous studies, $B_{i.v.}$, but in both of the oral studies, A and D. For subject $C_{r.c}$ and $C_R$, and $B_{o.}$ serial venipuncture was employed. Two ml samples of whole blood and of plasma were counted as described earlier. Whole blood samples were not obtained from subject $B_{i.v.}$. Results were calculated in terms of fraction of the injected dose present in the entire blood or plasma volume, assuming a blood volume of 75 ml/kg body weight, and a plasma volume of 42 ml/kg.

## RESULTS

Less than 5% of the $^{82}Br$ appeared in the residual aqueous fraction as bromide ion precipitable by $AgNO_3$; the remainder of the $^{82}Br$ was in the supernatant. Thus it can be said with certainty that the radioactivity was not removed as an ion and that therefore all of the results reported below reflect organically bound bromine, in all probability still on the aromatic ring.

The general sequence of organ concentration can be seen in the sequential scintiphotos shown in Figures 2 and 3. Figure 2 is representative, in terms of distribution, of the three oral administration experiments. Figure 2 shows the single experiment using $^{77}Br$, which has somewhat better resolution of detail because the lower energy gamma rays of this isotope are more easily collimated. The radioactivity, initially in the stomach, appeared first in the liver as it disappeared from the stomach. Later, about 3 hr after administration, activity began to concentrate in the brain, and there also appeared to be some in the area of the lungs. There was also a general distribution in other tissues, seen for example in the thighs. It is to be noted that the brain is well outlined in the 44-min scan at about 4 hr, being clearly distinguished from the facial structures. At 24 hr, (not shown) the relative distribution had not changed appreciably.

When administered intravenously, as shown in Figure 3, the radioactivity rather surprisingly first concentrated in the lungs. The first scan was started immediately after injection and the lung area was seen at approximately 30 sec after injection. To check the possibility that the compound may have caused macroaggregation of plasma proteins which were then trapped in the lungs, a sample of the administered dose was added to fresh human

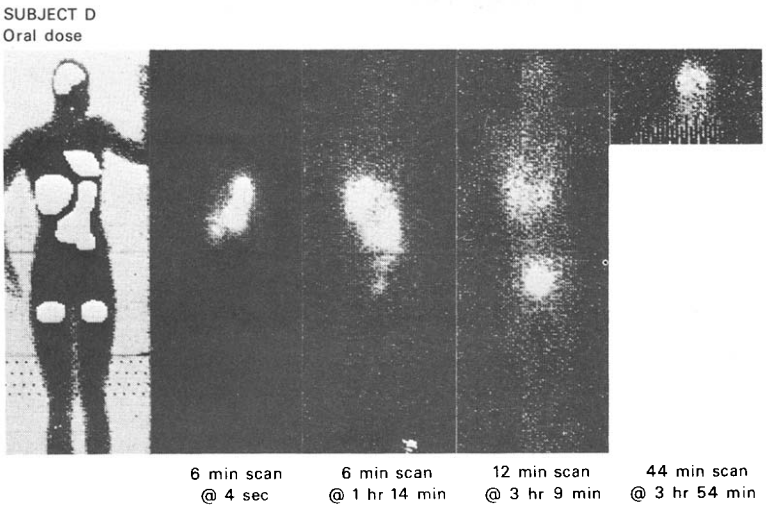


Fig. 2. Gamma ray scintiscans of 4-$^{77}$Br-DPIA after oral administration to subject D; composite photos of HP 5407A computer CRT displays. The first picture at left is the 60 keV body outline scan, with areas of interest drawn by light pen shown as white patches. All views are anterior. "6 min scan at 4 sec" refers to a scan begun 4 sec after administration of the dose, requiring 6 min to scan from feet to head. The scan of the head only was at 44 min foot-to-head speed but was terminated as soon as localization in the head was shown. Accumulation of radioactivity in the bladder can be seen in some of the scans; data from this area was not included in any of the calculations.

plasma. The plasma was electrophoresed and none of the radioactivity remained with the protein bands on the developed film, showing that the compound was not strongly bound to proteins. Another sample of this plasma was passed through a 0·22 $\mu$m millipore filter, and all of the radioactivity passed through the filter. Since the lungs only trap particles of 10 $\mu$m and larger, such trapping could not account for the concentration of radioactivity in the lungs. The activity in the lungs then diminished, and subsequently was seen in the liver; thereafter activity appeared to concentrate in the brain. Thus, the appearance in the various organs seems to differ in the two routes of administration only in the very early concentration seen in the lung upon intravenous administration.

The HP 5047A scintigraphic data computer was used to analyze the accumulated scan data. Figure 4 shows the relative activity in each organ on a logarithmic scale, as a function of time after administration for each of the experiments. The activity in each organ is expressed as a fraction of the initial total body activity. In the paired experiments on single

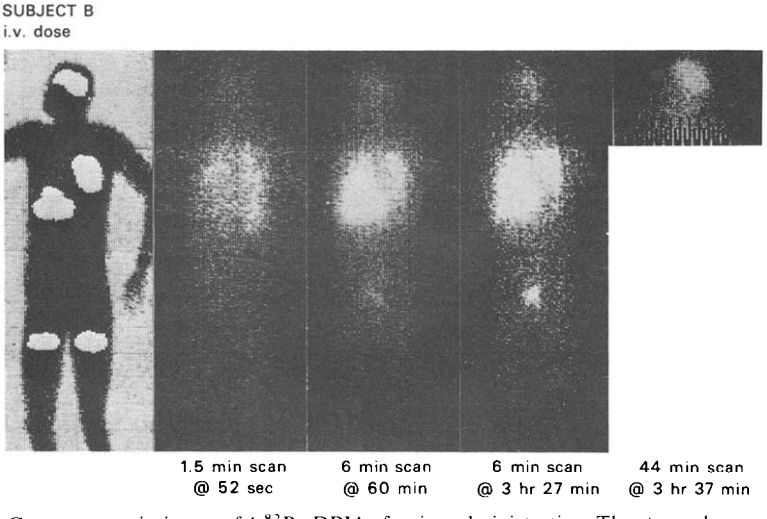


Fig. 3. Gamma ray scintiscans of 4-$^{82}$Br-DPIA after i.v. administration. The stomach was not outlined for the i.v. doses.

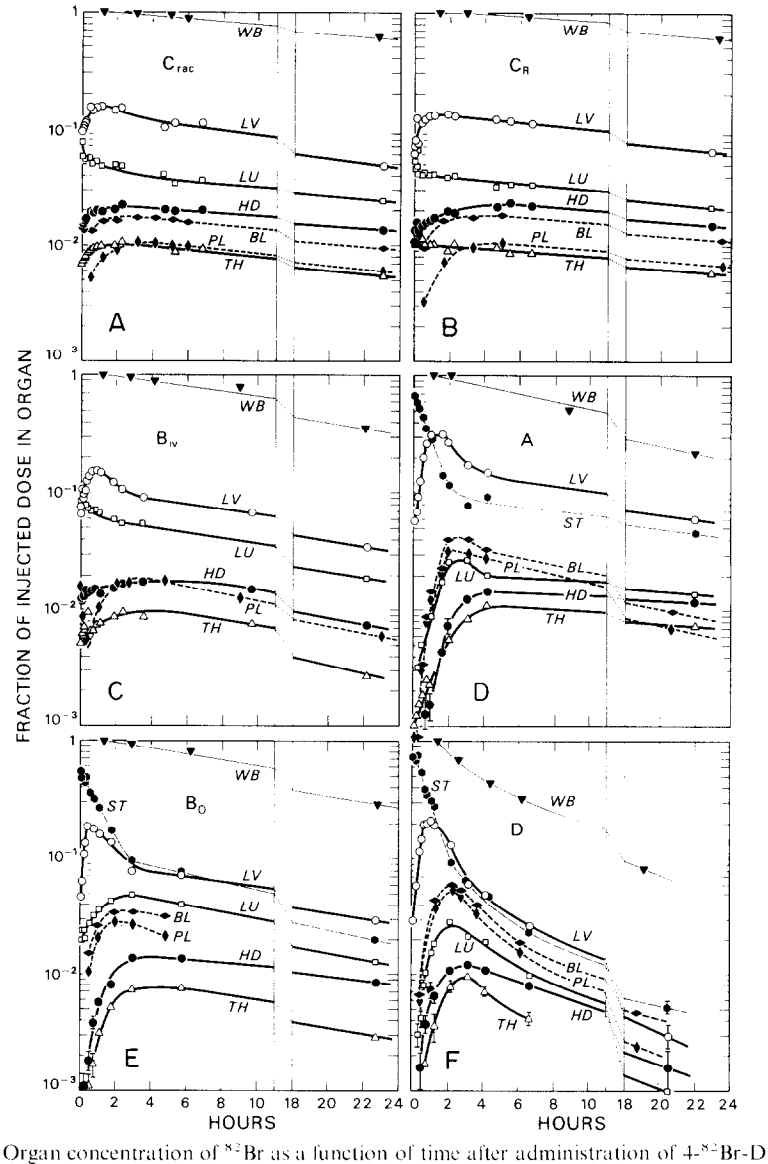


Fig. 4. Organ concentration of $^{82}$Br as a function of time after administration of 4-$^{82}$Br-DPIA in subjects A, B and C, and 4-$^{77}$Br-DPIA in subject D. The ordinate is fraction of the administered dose, measured and calculated as follows: whole body (WB) by the whole body counter, 1·0 = count before first urine; lung (LU), liver (LV), head (HD), stomach (ST) and thighs (TH) by computer summation of whole body scanner counts within areas of interest, 1·0 = mean of total body counts by the scanner before first urine; plasma (PL) and whole blood (BL) by scintillation well counter, 1·0 = total injected dose by appropriately diluted standards in well counter. Subjects A and D: oral dose; $B_{i.v.}$: intravenous dose; $B_o$: oral dose; $C_{rac}$: intravenous dose of racemic 4-$^{82}$Br-DPIA; $C_R$: intravenous dose of the *R* isomer of 4-$^{82}$Br-DPIA.

subjects, Figure 4A,B and 4C,E, the same areas of interest were used, so that the curves can be directly compared. Whole-body retention and blood levels are also shown in these figures.

A number of features may be noted from inspection of these curves:

(1) In all subjects, regardless of route of administration, the activity in the liver reached a maximum between 0·5 and 1·5 hr, the maximum value ranging from 13 to 32% of the administered dose.

(2) When administered intravenously, the lung showed a high concentration at the earliest measured time, in one case within 30 sec, and subsequently the activity was fairly rapidly released. The initial maximum in the left lung was from 6 to 9% of the total dose, and if both lungs were to be included (assuming equal lung volumes) it would be 12–18%, approximately the same as the maximum in the liver. When given orally, the maximum

concentration in the lung occurred at about 3 hr in all three subjects, well after the time of the peak in the liver. In Figure 4C and E, representing two routes of administration in one subject, the lung curves are widely disparate from 0 to 3 hr but are essentially identical thereafter.

(3) In all subjects, regardless of route of administration, the activity in the head did not reach a maximum until 3–6 hr, after which it declined at approximately the same rate as in the whole body.

(4) Activity in the thigh, representing general tissue distribution, behaved very much like that in the head, except that the concentration in the head was from 1·3 to 2 times higher.

(5) The plasma activity curves (Fig. 4) show a maximum at 2–3 hr. In the cases of oral dose, the peak occurred before the maximum activity was reached in the head; for the intravenous doses the plasma and head maxima were not as clearly defined. At the time of the maximum, plasma radioactivity following oral administration was approximately twice that following intravenous administration. The plasma appeared to contain almost as much of the total activity in circulation as the whole blood after oral administration. In subject C, the only subject for whom we had both whole blood and plasma data after intravenous administration, the plasma contained a considerably smaller proportion compared to whole blood than it did after oral administration, suggesting a significant binding by red cells. For subject $B_{i.v.}$, for whom we had plasma samples at 3, 5 and 8 min, the early and very rapid clearance of the initial dose from plasma corresponds to the uptake by lung.

For subject C, who received the racemic mixture in one dose and the *R*-isomer in the other, the liver and lung counts may be slightly lower in the case of the *R*-isomer, although this difference is probably insignificant. In all subjects, after 3–5 hr, the activity in all organs declined at essentially the same rate as that in the whole body.

*Whole-body counting*

The biological half-life ($T_{1/2\,biol}$) of the radiobromine, in whatever chemical form it was present in the body, was measured in each subject with the whole-body counter; the results are shown in Figure 5. The whole-body retention of subject C could be fit by a straight line, representing a single exponential function. The remaining subjects all had retention curves which were the sum of two exponentials. The two-component curves were graphically resolved by back-extrapolation of the final slope (longer $T_{1/2}$) to 2 hr (time of the first urine), yielding the intercept $\sigma_1$ as shown in Figure 5. The extrapolated line was subtracted

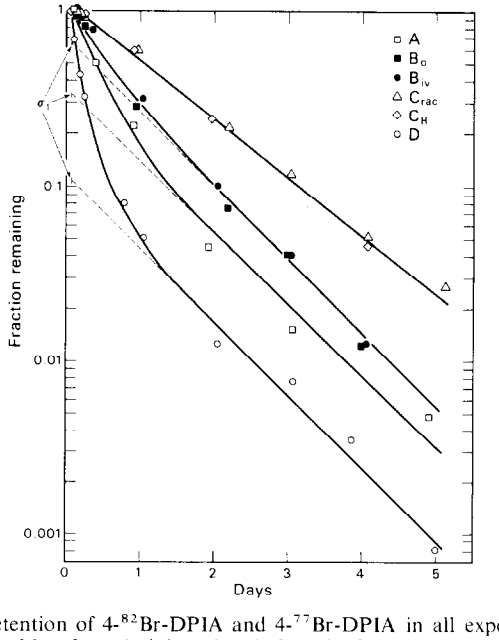


Fig. 5. Whole-body retention of 4-$^{82}$Br-DPIA and 4-$^{77}$Br-DPIA in all experiments. The initial count at approximately 2 hr after administration, before the first urine was taken = 1·0. Subjects as in Figure 4.

Table 1. Whole-body biological half-lives and intercepts, and urine fraction distributions

| Subject | Route | Whole-body biological half-life ($T_{1/2}$) and intercepts ($\sigma_1$, $\sigma_2$) | | | | Percent 12 hr cumulative radioactivity distribution in urine fractions | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Solvent separation | | | | Ion exchange separation | |
| | | $T_{1/2}$ (hr) | $\sigma_2$ | $T_{1/2}$ (hr) | $\sigma_1$ | Residual aqueous | Free base | Conjug. base | Neutral | Basic | Non-basic |
| A | Oral | 5·4 | 0·67 | 17 | 0·33 | 86·7 | 5·3 | 5·6 | 3·3 | 23·4 | 76·6 |
| B | I.V. | 6 | 0·35 | 17 | 0·65 | 86·6 | 7·9 | 3·2 | 2·2 | 27·2 | 72·8 |
| | Oral | 6 | 0·35 | 17 | 0·65 | 90·4 | 5·3 | 2·8 | 1·6 | 34·5 | 65·5 |
| C | I.V. (rac) | | | 22 | 1·0 | 60·1 | 32·2 | 4·1 | 3·6 | 37·1 | 62·9 |
| | I.V. (*R*) | | | 22 | 1·0 | 74·9 | 15·6 | 4·6 | 1·9 | 41·3 | 58·7 |
| D | Oral | 3·2 | 0·89 | 17 | 0·11 | 92·4 | 3·9 | 3·0 | 0·6 | 30·4 | 69·6 |
| Mean | | 5·1 | | 18·7 | | 81·8 | 13·8 | 3·4 | 2·2 | 32·3 | 67·7 |

from the data curve yielding the second slope (shorter $T_{1/2}$) and its intercept $\sigma_2$. The values obtained for the $T_{1/2}$'s and $\sigma_1$ and $_2$ are shown in Table 1. The longer $T_{1/2}$ averaged 18·7 hr and the shorter 5·1 hr, while the intercept $\sigma_1$ varied considerably. (The intercept $\sigma_2$ naturally varies as $1 - \sigma_1$).

*Urine analysis*

Table 1 shows the percentage of the total administered radioactivity found in each of the cumulative 12-hr urine extracts. Most of the excreted products were in the residual aqueous fraction, with a variable additional amount excreted as free base. Examination of the table reveals that the amount excreted as free base may bear a relationship to the $\sigma_1$ intercept of the whole-body retention curves.

Two-dimensional thin layer chromatographs of the dansylated urine extract were obtained from the periods of peak excretion, 2–4 hr. In solvent systems which were nonpolar, two spots of radioactivity were noted in addition to activity remaining at the origin. One of these was unchanged 4-Br-DPIA, based on chromatography of the original compound as a reference, $R_f = 0{\cdot}78$ in solvent I, $R_f = 0{\cdot}52$ in solvent II. The second spot had $R_f = 0{\cdot}69$ in solvent I and $R_f = 0{\cdot}70$ in solvent II. To resolve the material remaining at the origin, chromatography was also performed with the more polar solvent III in the second dimension, which yielded two additional spots with $R_f = 0{\cdot}32$ and $R_f = 0{\cdot}40$.

The results of the separation of the urine samples by ion exchange chromatography into basic and non-basic fractions are shown in Table 1. In all subjects about one-third of the radioactivity was excreted in the basic fraction, which undoubtedly represents free amines, and about two-thirds in a non-basic form.

## DISCUSSION

In this study, the combined techniques of nuclear medicine and pharmacology have demonstrated the kinetics of organ distribution of a compound and its primary metabolites *in vivo* in human subjects. From the results we may draw some conclusions as to the distribution and fate of 4-Br-DPIA in the body.

Of primary importance is the observation that the $^{82}$Br is not biologically removed from the benzene ring, thus assuring that location and measurement of radioactivity represents that of the original compound or of its principle metabolites. The stability of the bromine at the para-position lends additional support to the concept that increased resistance to metabolic attack at this position is associated with increased potency (SHULGIN *et al.*, 1969).

The uptake by the lung within 30 sec after intravenous administration is another striking finding. Uptake within such a short period implies a first pass removal on passage through the lung. 4-Br-DPIA chemically resembles amphetamine in that the side chain contains an α-methyl group. AXELROD (1954) found in a study of tissue concentrations of (+)-amphetamine in dogs that the lung had one of the highest organ concentrations at one hour. Only the kidney in Axelrod's study had a concentration higher than lung, and it is possible that the kidneys were not distinguishable from liver in our study. There is also some structural similarity between 4-Br-DPIA and dopamine and norepinephrine; nore-

pinephrine is known to be rapidly and selectively removed from circulation by lung tissue (GILLIS and IWASAWA, 1972). EICHELBAUM, HENGSTMANN and DENGLER (1970) found that chlorphentermine was found in highest concentration in the lung in the rat, rabbit and pig.

While it is not axiomatic that drugs are found in highest concentration in their target organ, we found here that 4-Br-DPIA or a metabolite does indeed concentrate in brain, the organ where this compound exerts its primary effect. The maximum concentration of radioactivity in the brain was 3·0% of the injected dose. Since the minimum active psychodysleptic dose is 200 μg, this represents a concentration of 6 μg in 1500 g of brain tissue or 0·004 μg/g. This may be compared to a minimum brain level for perturbation of rat performance by (+)-amphetamine of 0·38 μg/g found by MAICKEL, COX, MILLER, SEGAL and RUSSELL (1969).

The sequence of maxima of radioactivity after oral administration was in the order of liver, blood, followed by brain, then thigh and lung together. After intravenous administration the sequence was lung, liver, blood, followed by brain and thigh. One possible interpretation of this is that the liver metabolizes the original compound, and that it is a metabolite which then is carried to the brain and other tissues via the plasma and is responsible for the CNS action.

The simplest interpretation of the two-component whole-body excretion curves is that the pool of metabolites which passes through the kidney contains chiefly two compounds or groups of compounds and that these are excreted by the kidney at different rates. Using this model, one has a $T_{1/2}$ of 17–22 hr and constitutes a fraction $\sigma_1$ of the metabolite pool, and the other has a $T_{1/2}$ 3·2–6 hr and is a fraction $\sigma_2$ of the pool. As seen in Table 1, higher values of $\sigma_1$ are associated with increased amount of radioactivity in the free base extract of the urine. Thus, it seems probable that the 17–22 $T_{1/2}$ is the excretion half-time of the free base metabolites and correspondingly that the 3·2–6 hr $T_{1/2}$ is the half-time of the residual aqueous metabolites. This is in accord with the general rule that water-soluble metabolites are more readily cleared by the kidney than lipid-soluble ones. It appears that the various subjects had differing abilities to form and excrete water-soluble metabolites, the greater ability to do so being associated with more rapid excretion. In Figure 5 there is no evidence of a third component of longer $T_{1/2}$ at levels as low as 0·001, which indicates that there is no long-term residual component of drug metabolites in the body greater than 0·1% of the administered dose.

In the two experiments with subject C using the racemic and the *R* compounds, the only significant difference between the two was in the urinary excretion of radioactivity in the free base and residual urine fractions. Even with this considerable difference, the whole-body $T_{1/2}$ was the same, presumably because subject C already had such low levels of aqueous metabolites that large percentage differences in aqueous excretion of *R* and *S* isomers were not enough to affect the $T_{1/2}$. While time did not allow us to perform the experiment with the *S* isomer, the amounts which could be expected to be excreted in the free base and residual aqueous extracts can be calculated as follows. When we administered the *R* compound, 18·6% of the radioactivity appeared in the free base; the racemic compound yielded 32·2% in the free base. Because half of the racemic compound was *R*, half of 18·6%, or 9·3% of the free base radioactivity from the racemic compound was due to the *R* isomer, which means that the balance or 22·9% was from the *S*. Thus, if the same dose containing 100% *S* were given, we would expect 2 × 22·9 or 45·8% of the radioactivity to appear in the free base extract. By similar reasoning we would expect 45·2% of the radioactivity from pure *S* compound to appear in the residual aqueous extract.

In rats, the major pathway of amphetamine metabolism is via *p*-hydroxylation, while in man and rabbit it is by deamination (AXELROD, 1970). In the present study, *p*-hydroxylation cannot occur as it does with amphetamine because of the Br substitution. AXELROD (1955) found that the deaminating enzyme in rabbit liver microsomes was highly specific for the *laevo* isomer of amphetamine (now known to be of *R* configuration). Our finding that a greater proportion (74·9%) of the *R* isomer is excreted in the residual-aqueous fraction than would be expected from the *S* isomer (45·2%) indicates that deamination acts preferentially on the *R* isomer of 4-Br-DPIA in humans as well.

The balance of the 4-Br-DPIA appears to be metabolized by methods other than deamination. Examination of Table 1 reveals that the radioactivity found in the free base fraction by solvent separation averaged 12·5%, while by ion exchange preceded by acid hydrolysis an average of 32·5% was found in the basic eluate. Thus, hydrolysis yielded a greater amount of radioactivity attached to a basic moiety. In two of the urine samples, fractions were run through the ion exchange column without prior hydrolysis, with the result (not shown in Table 1) that the amount found in the basic eluate was reduced to 9·5%, compatible with that found by solvent separation. These results indicate that about 20% (32·2 minus 12·5) of the radioactivity in the urine is associated with an amine group neutralized by *N*-acetylation or *N*-conjugation. Other possible metabolic routes are β-hydroxylation and *O*-demethylation; such processes might produce the suggested active metabolite.

In view of the large body of work reported on the metabolism and mode of action of compounds related to amphetamine, especially with regard to mechanisms of psychosis, it is surprising that so little has been done with the methoxylated analogues which produce psychotomimetic effects with a single dose. To our knowledge this study is the first report of human *in vivo* distribution of a centrally active compound. Further elucidation of the mechanisms of action of these drugs which are so similar in structure to the catecholamine neurotransmitters might be expected to shed light on the etiology of endogenous psychosis.

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