THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
Vol. 155, No. 2

Printed in U.S.A.

METABOLIC FATE OF β-(3,4-DIMETHOXYPHENYL)-ETHYLAMINE IN MAN[1]

K. D. CHARALAMPOUS[2] AND L. WAYNE TANSEY

Department of Psychiatry, Baylor University College of Medicine, and Houston State Psychiatric Institute, Houston, Texas

Accepted for publication September 14, 1966

ABSTRACT

CHARALAMPOUS, K. D. AND L. WAYNE TANSEY: Metabolic fate of β-(3,4-dimethoxyphenyl)ethylamine in man. J. Pharmac. exp. Ther. **155:** 318–329, 1967. The metabolism of C^{14}-labeled β-(3,4-dimethoxyphenyl)ethylamine (DMPEA), reported to be present in the urine of several patients with mental disorders, was studied in man after oral administration. The cumulative excretion of radioactivity in the urine and the concentration of the radioactive material in plasma and cerebrospinal fluid were determined. Unchanged DMPEA, 3,4-dimethoxyphenylacetic acid and 4-hydroxy-3-methoxyphenylacetic acid were identified and their concentration determined in the collected urine. The rate of deamination of ingested DMPEA was twice that of mescaline. Aspects of the metabolic fate of mescaline and DMPEA were compared with each other and with those of some of their urinary metabolites.

Several investigators (Friedhoff and Van Winkle, 1962; Takesada *et al.*, 1963; Sen and McGeer, 1964; Kuehl *et al.*, 1964; Bourdillon *et al.*, 1965) have reported the presence of β-(3,4-dimethoxyphenyl)ethylamine (DMPEA) in the urine of schizophrenics. Others (Perry, 1963; Perry *et al.*, 1964; Faurbye and Pind, 1964; Nishimura and Gjessing, 1965) could not replicate these results, and Studnitz and Nyman (1965) succeeded in identifying the substance in the urine of schizophrenic and nonschizophrenic subjects only after unrestricted diet. The relative importance of these findings could be judged—first, by knowing the fate of exogenous DMPEA given to human subjects; second, by developing analytic techniques based on the utilization of C^{14}-labeled DMPEA; and third, by investigating the effect of this compound in man.

In this paper we report on the metabolic fate of DMPEA in man and describe, in part, a clinical investigation of its behavioral activity and that of its main urinary metabolite. These findings are compared with those observed after C^{14}-mescaline administration.

Received for publication June 10, 1966.
[1] This investigation was supported in part by U.S. Public Health Service Research Grant MH-10777, from the National Institute of Mental Health.
[2] Present address: Department of Psychiatry, Neurology and Behavioral Sciences, The University of Oklahoma Medical Center, 800 N. E. 13th Street, Oklahoma City, Oklahoma 73104.

METHODS. MATERIALS. β-(3,4-Dimethoxyphenyl)ethylamine-8-C^{14} hydrochloride was obtained from New England Nuclear Corporation. DMPEA was purchased from Aldrich Chemical Company. This free amine was dissolved in ether and the solution was saturated with dry hydrogen chloride gas. The amine hydrochloride precipitated was recrystallized from ethanol–acetone (m.p., 156–157°C). Chromatography in solvent systems I and II, described below, produced only one spot. Radioactive N-acetyl-β-(3,4-dimethoxyphenyl)-ethylamine was prepared by acetylation of DMPEA with acetic anhydride. The compound melted at 99–100°C and gave only one spot in solvent system I (described below). 4-Hydroxy-3-methoxyphenylacetic acid (HMPA) was obtained from Regis Chemical Company. Ethyl acetate and the chromatography solvents were Baker analyzed reagents and were not purified further. The liquid scintillation fluors, 2,5-diphenyloxazole (PPO) and *p*-bis[2-(5-phenyloxazolyl)]benzene (POPOP), were purchased from Nuclear-Chicago Corporation.

COLLECTION OF SAMPLES. Three adult, healthy, male volunteers took by mouth 200 μc of β-(3,4-dimethoxyphenyl)ethylamine-8-C^{14} hydrochloride with 600 mg of nonradioactive carrier dissolved in 50 ml of distilled water. The solution was taken after a 12-hr fast. Each subject was catheterized with a Foley catheter, and the bladder was emptied immediately before the administration of the solution. After ingestion of the amine, the bladder was emptied at ½, 1, 2, 3, 4, 8, 12 and 24 hr. The catheter was removed, and the subject was in-

structed to collect his 24-hr urinary output for the following four days. The urine samples were immediately frozen and were kept at −15°C until used. Samples of blood were collected at ½, 1, 2, 4 and 6 hr. Cerebrospinal fluid samples were collected in only two subjects. From the first subject, samples were collected at ½-hr intervals from ½ hr to 3½ hr after ingestion. In the second subject, samples were collected at ½-hr intervals from 3½ hr to 6 hr after ingestion. The samples of blood and cerebrospinal fluid were refrigerated at 4°C until used.

One adult, male volunteer was given by mouth 5 μc of 3,4-dimethoxyphenylacetic acid-8-C^{14} with 500 mg of nonradioactive carrier dissolved in 50 ml of distilled water. Samples of urine were collected at ½, 1, 2, 3, 4, 6, 8, 10, 12, 24 and 48 hr. The samples collected were stored as previously described.

QUANTIFICATION OF RADIOACTIVITY. The radioactivity of the samples was determined on a Nuclear-Chicago liquid scintillation spectrometer model 725. The scintillation fluid was Diatol (toluene, 500 ml; dioxane, 500 ml; methanol, 300 ml; PPO, 6.0 g; POPOP, 0.150 g; and naphthalene, 100 g) or Liquifluor (naphthalene, 70 g; PPO, 4.16 g; POPOP, 0.052 g; and toluene, 1000 ml). All radioactive determinations were corrected to 100% efficiency by applying the Baillie channels ratio method (Baillie, 1960).

Urine. A 0.1-ml aliquot of urine was dissolved in 15 ml of Diatol and was counted for 1 min.

Cerebrospinal fluid. A 0.1-ml aliquot of cerebrospinal fluid was dissolved in 15 ml of Diatol and was counted for 100 min after standing 24 hr in the counting chamber.

Whole blood. An aliquot of 0.5 ml of whole blood was spotted on a strip of chromatographic paper and was dried in an oven at 100°C overnight. The strip was folded and was burned in an oxygen flask according to the method of Kalberer and Rutschman (1961). The carbon dioxide thus liberated was absorbed in 10 ml of 12% ethanolamine in methyl Cellosolve. Four milliliters of this solution were dissolved in 3 ml of methanol and 15 ml of Liquifluor. The samples were allowed to stand for 4 hr and then the radioactivity in the samples was quantified by counting for 10 min in the scintillator.

Plasma. The radioactivity in the plasma samples was quantified in the same manner as the radioactivity in the whole-blood samples above.

PAPER CHROMATOGRAPHY. All paper chromatograms were developed by the descending technique. Whatman no. 1 chromatography paper was used for strip chromatograms, while Whatman 3MM paper was used for two-dimensional and preparative chromatograms.

The solvent systems employed were: I, *n*-butanol–acetic acid–water, 4:1:1; II, *n*-butanol–*i*-propanol–concentrated ammonia–water, 3:1:1:1; and III, methanol–*n*-butanol–benzene–water, 2:1:1:1. The two-dimensional chromatograms were developed for 8 hr in solvent system I for the first direction and then for 8 hr in solvent system II for the second direction.

Kodak "no screen" X-ray film was used to locate radioactive areas on the chromatograms. In some instances these areas were removed from the chromatogram, cut into small pieces and placed in 15 ml of Diatol, and then the radioactivity was quantified in a liquid scintillation spectrometer.

MELTING POINTS OF COMPOUNDS. These were determined on a Fisher-Johns melting point apparatus. All values were uncorrected.

HYDROLYSIS OF URINE. An aliquot of 360 ml of a 12-hr urine sample was mixed with 100 ml of concentrated hydrochloric acid. The mixture was heated on a steam bath for 3 hr. A 0.3-ml sample of the mixture was spotted on Whatman 3MM chromatography paper and was developed in solvent system I and then in solvent system II. A radioautogram was made of this chromatogram.

RESULTS. *Urinary excretion of radioactivity.* Most of the radioactive DMPEA was excreted from the body in 24 hr. An average of three subjects showed that 89% of the radioactivity was recovered during this period. The recovery of radioactivity rose to 91% by the 5th day. The graph of the urinary excretion (fig. 1) shows that one-half of the radioactivity administered was excreted in 2½ hr. In order to study further differences in the elimination of the various metabolites at different times, a 0.3-ml aliquot of each individual urine sample was spotted on Whatman 3MM chromatography paper. The two-dimensional chromatogram was developed with solvent systems I and II. A radioautogram was made of this chromatogram. The film was exposed for 1 month. A typical radioautogram from one subject is shown in figure 2. The radioactive areas were cut and counted directly in Diatol. The percentage of each metabolite was obtained in the individual urine sample. These determinations are tabulated in table 1. Upon hydrolysis of the urine with hydrochloric acid, some of the spots were not observed; figure 3 indicates that these compounds were conjugates. Unknown no. 6, which constituted a large percentage of each sample, disappeared after hydrolysis.

Extraction of urine. The urine, which was collected over a 12-hr period, was poured into a 1-liter separatory funnel. Concentrated hydro-

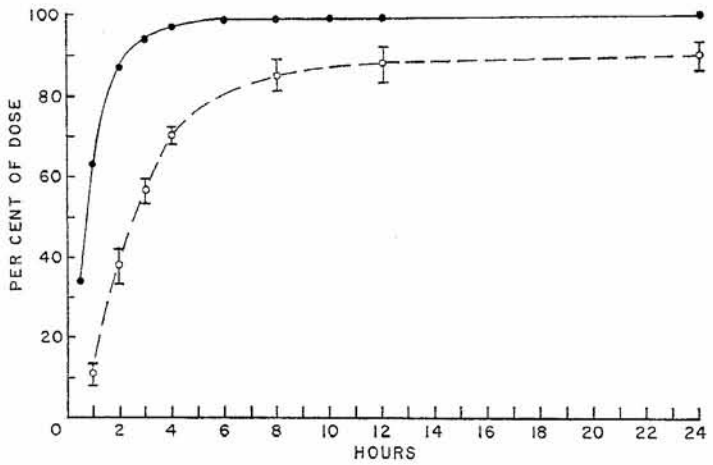

FIG. 1. Cumulative excretion of radioactivity in urine after oral administration of C^{14}-labeled: ●——●, DMPA, one subject; ○- - -○, DMPEA, three subjects. The vertical lines indicate minimum and maximum of radioactivity recovered.

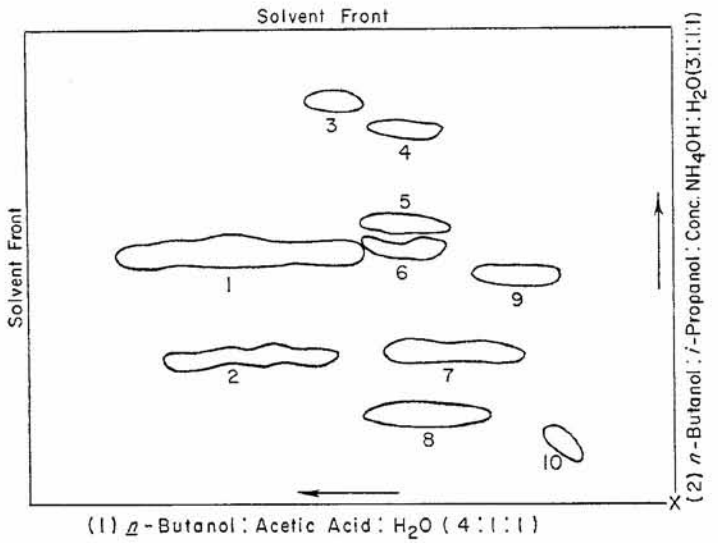

FIG. 2. Radioautogram of a two-dimensional chromatogram of the urine. The numbers refer to compounds listed in table 1.

chloric acid was added until a pH of 2 was obtained. The acidified solution was extracted with six 100-ml portions of ethyl acetate. The aqueous fraction was checked several times to be sure that the solution remained acidic. The ethyl acetate fraction, which contained the organic extractable acids and organic extractable neutral compounds, was extracted twice with 50 ml of 20% sodium hydroxide. The sodium hydroxide removed the acidic compounds from the ethyl acetate and left the extractable neutral compounds in the organic phase. The ethyl acetate portion, which contained only extractable neutral compounds, constituted approximately 0.7% of the total radioactivity in the urine.

The aqueous sodium hydroxide solution was adjusted to pH 2 with concentrated hydrochloric acid and was extracted four times with 50-ml portions of ethyl acetate. The ethyl acetate portion contained all of the organic extractable acidic compounds. In order to separate phenolic compounds from the more acidic ones, the solution

TABLE 1

Metabolites of DMPEA in urine as percent of radioactivity in each sample

Metabolites	Time of Urine Collection						
	0–½ hr	½–1 hr	1–2 hr	2–3 hr	3–4 hr	4–8 hr	8–12 hr
1. DMPA	77.9	83.1	72.9	65.7	63.1	34.7	58.6
2. HMPA	0	0.8	0.4	0.4	1.6	2.6	2.4
3. DMPEA	9.8	4.3	2.2	0.7	0.2	0.8	0.4
4. Unknown	5.2	3.4	5.7	10.1	12.3	24.3	9.1
5. Unknown	0	0.2	0.6	0.7	1.4	2.6	0
6. Unknown	6.1	5.9	16.4	19.7	14.3	23.7	21.5
7. Unknown	0	0.4	0.8	1.3	2.3	3.8	3.0
8. Unknown	0	0.2	0.3	0.6	1.2	2.9	3.6
9. Unknown	0.9	1.2	0.5	0.5	1.6	1.7	0
10. Unknown	0	0	0.1	0.1	0.1	0.1	0
Total radioactivity in sample (μc)	0.01	24.55	42.57	42.89	26.36	26.35	14.70

The figures of the table were based on determinations of the urine from one subject.

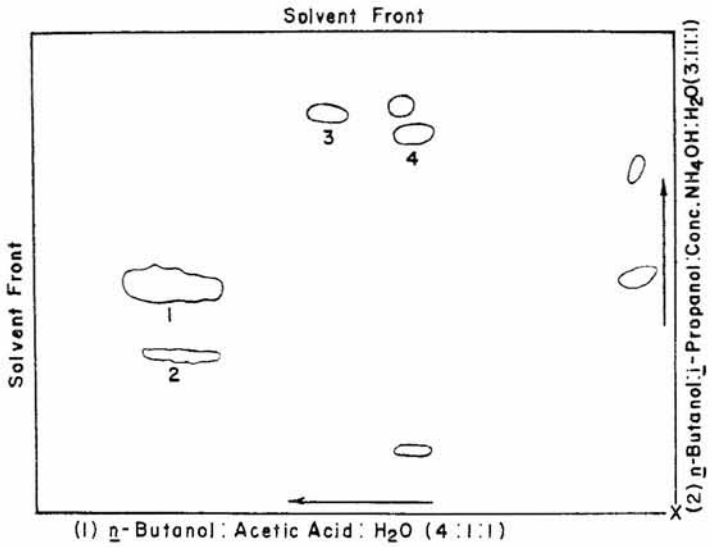

FIG. 3. Radioautogram of a two-dimensional chromatogram of acid hydrolyzed urine. The numbers refer to compounds listed in table 1.

was extracted three times with 50 ml of a saturated sodium bicarbonate solution. The aqueous bicarbonate fraction contained the strongly acidic compounds, while the phenolic compounds remained in the organic fraction. The phenolic extract was found to contain no radioactivity. The bicarbonate fraction was acidified with hydrochloric acid and was extracted with three 50-ml portions of ethyl acetate. This extract of strongly acidic compounds contained approximately 87% of the total radioactivity in the urine.

The acidified urine was made basic with sodium hydroxide and was extracted six times with 100-ml portions of ethyl acetate. The extractable organic bases were obtained. This fraction constituted approximately 0.1% of the total radioactivity. Figure 4 illustrates the extraction procedure.

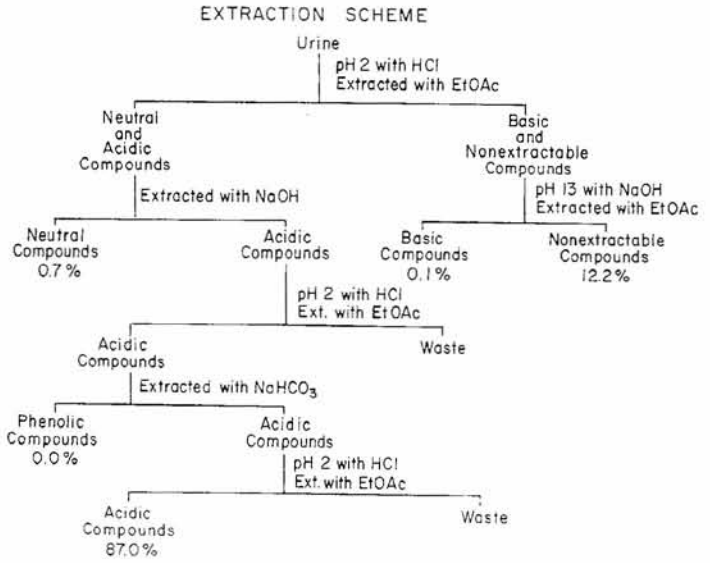

FIG. 4. Extraction scheme for urine.

The basic, neutral and acidic fractions were dried with anhydrous sodium sulfate and were reduced in volume on a rotatory evaporator to near dryness. A small volume of ethyl acetate was added, and each solution was applied as a line on Whatman 3MM chromatography paper. The basic and neutral fractions were developed for 8 hr in solvent system I. The acidic fraction was developed for 8 hr in solvent system II. After development, the chromatograms were exposed to Kodak "no screen" X-ray film in order to view the radioactive areas.

3,4-Dimethoxyphenylacetic acid (DMPA). The acidic extract from the urine gave two radioactive areas after chromatography in solvent system II. The bands occurred at R_f values of 0.29 and 0.48. The band at 0.48 was by far the larger. This band gave no color when sprayed with 2,6-dichloroquinonechlorimide or diazotized sulfanilic acid. The band was cut from the chromatogram and was moistened with concentrated hydrochloric acid. The acid metabolite was then eluted from the paper with ethyl acetate. The solvent was evaporated and the residue was recrystallized from *n*-heptane.

Standard DMPA was compared with the acid metabolite. The metabolite had an R_f value of 0.48 in solvent system II. The standard had an R_f value of 0.47 in the same solvent system. The R_f values in other solvent systems are given in table 2. The standard acid and the metabolite both had a melting point of 97.5–98.5°C. A mixture with equal amounts of each melted at 98.0–98.5°C.

In order to obtain further evidence that the acid metabolite and the standard were the same, the two were mixed and recrystallized. A 100-mg portion of standard DMPA was added to a small amount of the radioactive metabolite, and they were dissolved in hot *n*-heptane. Upon cooling, a precipitate formed which was filtered, dried and weighed. A small amount of weighed material was counted in the liquid scintillation counter. The specific activity remained constant during four recrystallizations.

A derivative was made of the acid metabolite and the reference compound (Wild, 1962). The acid metabolite (20 mg, 0.0001 mol) was dissolved in 5 ml of diethyl ether. A solution of piperazine hexahydrate (10 mg, 0.00005 mol) in ethyl alcohol was added to the ether solution. Immediately the crystals of the salt precipitated. The derivative was filtered and was recrystallized from ethyl alcohol (fig. 5). The sample of the compound prepared weighed 19 mg and melted at 160.0–160.5°C. The same procedure was followed with standard 3, DMPA. This derivative melted at 160.5–161.0°C. A mixture of equal parts of each derivative melted at 160.0–160.5°C.

A potassium bromide pellet was made with the extracted acid and the standard acid. The

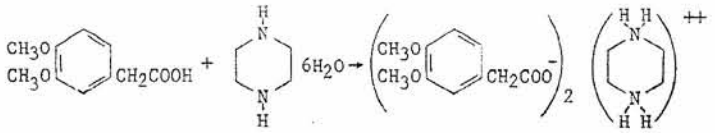

FIG. 5. Synthesis of derivative.

infrared spectra were almost identical. A sharp peak at 3500 cm^{-1}, which did not appear to be water, was the most significant difference. The metabolite was sublimed, the residue was made into a potassium bromide pellet and the spectral analysis was repeated. The spectra obtained were identical (fig. 6).

HMPA. The radioactive area shown by radioautography at R_f 0.29 was eluted from the chromatogram. The area was moistened with concentrated hydrochloric acid, and the compound was extracted with acetone. The solvent was evaporated to a small volume, and the solution which remained was spotted on Whatman 3MM chromatography paper. The chromatogram was developed in solvent system II, and the purified metabolite was eluted from the paper as described previously. The metabolite was spotted on Whatman no. 1 chromatography paper and was developed in solvent system II. Standard HMPA and standard HMPA together with extracted metabolite were chromatographed in a similar fashion. The chromatograms, when sprayed with diazotized sulfanilic acid, each gave one red spot. The respective R_f values were 0.27, 0.28 and 0.29. Duplicate chromatograms when sprayed with 2,6-dichloroquinonechlorimide gave a blue spot. The spray reagent, Brentamine, fast red B salt-diazotized 4-nitro-*o*-anisidine, gave a purple spot. A radioautogram of the chromatogram of the metabolite and of the chromatogram of the standard plus the metabolite gave one radioactive spot on each chromatogram. This spot corresponded to the red spot of diazotized sulfanilic acid. Two other solvent systems were employed to compare the metabolite with the standard. The R_f values and the systems used are given in table 3. A fluorescent spectrum was determined in 0.1 N HCl for the metabolite and the standard on an Aminco-Bowman spectrophotofluorometer. An activation wavelength of 280 mμ gave a fluorescence at 315 mμ for both samples.

DMPEA. The basic extract from the urine chromatographed in solvent system I gave three radioactive areas. The bands occurred at R_f values 0.70, 0.81 and 0.91. The spot with an R_f value of 0.70 gave a purple color when sprayed with ninhydrin. This ninhydrin-positive spot turned pink when it was sprayed with Ehrlich's reagent, *p*-dimethylaminobenzaldehyde (Friedhoff and Van Winkle, 1963). The other spots did not give a color when sprayed with ninhydrin. The band at R_f 0.70 was eluted from the paper chromatogram with ethyl alcohol. The solvent

TABLE 2

Characteristics of DMPA and of a compound isolated from urine

Procedures	Metabolite	DMPA	Mixture
n-Butanol–acetic acid–water, 4:1:1 (solvent system I)	R_f 0.87	R_f 0.86	R_f 0.86
n-Butanol–*i*-propanol–ammonia–water, 3:1:1:1 (solvent system II)	R_f 0.48	R_f 0.47	R_f 0.48
Methanol–*n*-butanol–benzene–water, 2:1:1:1 (solvent system III)	R_f 0.72	R_f 0.72	R_f 0.71
2,6-Dichloroquinonechlorimide	Negative	Negative	
Diazotized sulfanilic acid	Negative	Negative	
Melting point	97.5–98.5°C	97.5–98.5°C	98.0–98.5°C
Piperazine derivative	160.0–160.5°C	160.5–161.0°C	160.0–160.5°C
Co-crystallization	Constant specific activity		
Infrared spectroscopy	Identical spectra		

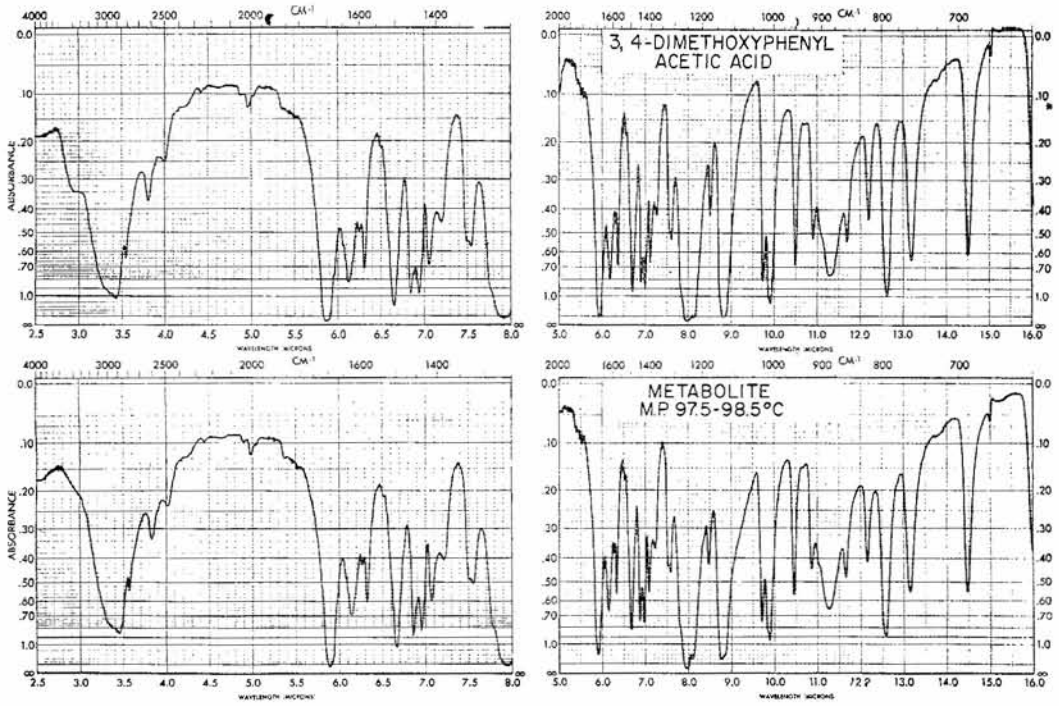

FIG. 6. Infrared spectrum of DMPA and a metabolite isolated from the urine.

TABLE 3

Characteristics of HMPA and of a compound isolated from urine

Procedures	Metabolite	HMPA	Mixture
n-Butanol–acetic acid–water, 4:1:1 (solvent system I)	R_f 0.82	R_f 0.82	R_f 0.83
n-Butanol–*i*-propanol–ammonia–water, 3:1:1:1 (solvent system II)	R_f 0.27	R_f 0.28	R_f 0.29
Methanol–*n*-butanol–benzene–water, 2:1:1:1 (solvent system III)	R_f 0.66	R_f 0.66	R_f 0.67
2,6-Dichloroquinonechlorimide	Blue	Blue	
Diazotized sulfanilic acid	Red	Red	
Brentamine (fast red B salt)	Purple	Purple	
Spectrophotofluorometry			
E_{max}	280 mμ	280 mμ	
F_{max}	315 mμ	315 mμ	

was evaporated to a small volume, and the solution was spotted as a line on another piece of chromatography paper. The chromatogram was developed in solvent system I, and the purified metabolite was eluted from the paper with alcohol.

The radioactive metabolite, radioactive DMPEA standard and the metabolite plus the standard were spotted on separate strips of Whatman no. 1 chromatography paper. The chromatograms were developed in solvent system I. They were sprayed with ninhydrin and each chromatogram gave only one purple spot. The respective R_f values were 0.64, 0.64 and 0.62. A radioautogram revealed only one radioactive spot on each chromatogram. This radioactive spot corresponded with the ninhydrin-positive spot in each case. This same procedure was repeated with solvent system II as well as solvent system III. Again one ninhydrin-positive

TABLE 4

Characteristics of DMPEA and of a compound isolated from urine

Procedures	Metabolite	DMPEA	Mixture
n-Butanol–acetic acid–water, 4:1:1 (solvent system I)	R_f 0.64	R_f 0.64	R_f 0.62
n-Butanol–*i*-propanol–ammonia–water, 3:1:1:1 (solvent system II)	R_f 0.87	R_f 0.89	R_f 0.87
Methanol–*n*-butanol–benzene–water, 2:1:1:1 (solvent system III)	R_f 0.79	R_f 0.80	R_f 0.81
1-Fluoro-2,4-dinitrobenzene derivative	R_f 0.93	R_f 0.93	R_f 0.94
Ninhydrin	Purple	Purple	
Ninhydrin followed by Ehrlich's reagent	Pink	Pink	
Diazotized sulfanilic acid	Negative	Negative	
Spectrophotofluorometry			
E_{max}	235 mμ	235 mμ	
F_{max}	315 mμ	315 mμ	

spot was observed on each chromatogram. This ninhydrin-positive spot was also radioactive. The R_f values obtained from the chromatograms are listed in table 4.

A derivative was made from 1-fluoro-2,4-dinitrobenzene and the metabolite. Five drops of 10% sodium carbonate were added to approximately 10 μg of the metabolite in a test tube. To this 5 drops of 0.2% 1-fluoro-2,4-dinitrobenzene in ethanol were added. A standard was treated similarly, and the two products were chromatographed separately. In addition the derivatives of the metabolite and the standard together were chromatographed. The three chromatograms were developed in solvent system II. Two yellow spots were observed on each chromatogram. The first yellow spot was apparently due to the reagents. However, the second yellow spot was radioactive. The R_f values for the second spot were 0.93 for the derivative of the metabolite, 0.93 for the derivative of the standard and 0.94 for the two derivatives mixed.

A fluorescent spectrum was determined in 0.1 N HCl for the metabolite and for the standard on an Aminco-Bowman spectrophotofluorometer. An activation wavelength of 235 mμ gave a fluorescence at 315 mμ for both samples.

Radioactivity in blood and cerebrospinal fluid. Samples of whole blood and plasma from the three different subjects were counted. The radioactivity of the plasma samples plotted against time is shown in figure 7. The whole-blood samples gave lower counts than the corresponding plasma samples. Samples of cerebrospinal fluid from two subjects were counted, and the results were plotted against time (fig. 8).

Administration of 3,4-dimethoxyphenylacetic acid-8-C^{14}. DMPA was excreted quite rapidly (fig. 1). One-half of the radioactivity was excreted in 45 min. An aliquot of each urine sample was spotted on Whatman 3MM chromatography paper and was developed two-dimensionally with solvent systems I and II. A radioautogram of the ½-hr sample revealed two spots. All of the other chromatograms had only one radioactive spot. The radioactivity administered was low, which may explain the presence of no more than two compounds. The two compounds observed were identified by chromatography as DMPA and HMPA. The HMPA appeared only in the ½-hr sample.

Discussion. Man absorbs and metabolizes DMPEA quite rapidly (fig. 9). Of the radioactive material given by mouth 89% was recovered in the first 24 hr. The recovery rose to 91% by the 5th day after ingestion. Half of the radioactivity ingested was excreted in the urine in approximately 2½ hr.

From a total of 10 compounds found consistently in unhydrolyzed urine, three were identified. Chromatography of the urine after acid hydrolysis gave only eight spots and four of them corresponded to those from unhydrolyzed urine. Three of these were identified. On analyzing the radioactive materials found in the urine collected between ½ and 1 hr, 83.1% was DMPA, 0.8% another acid, HMPA, and only 4.3% DMPEA. These accounted for 88.2% of the

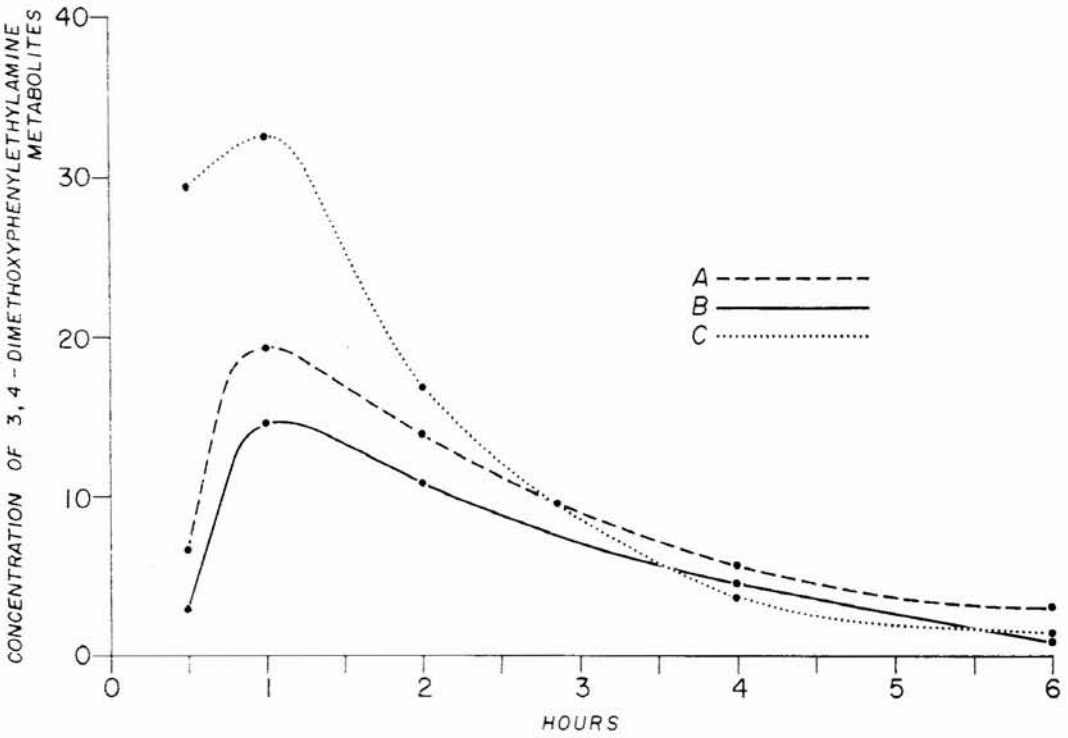

FIG. 7. Concentration of DMPEA metabolites in plasma. Each line represents a different subject. The concentration is expressed as micrograms per milliliter. Subject A received a dose of 5.41 mg/kg, subject B a dose of 8.76 mg/kg and subject C a dose of 10.74 mg/kg.

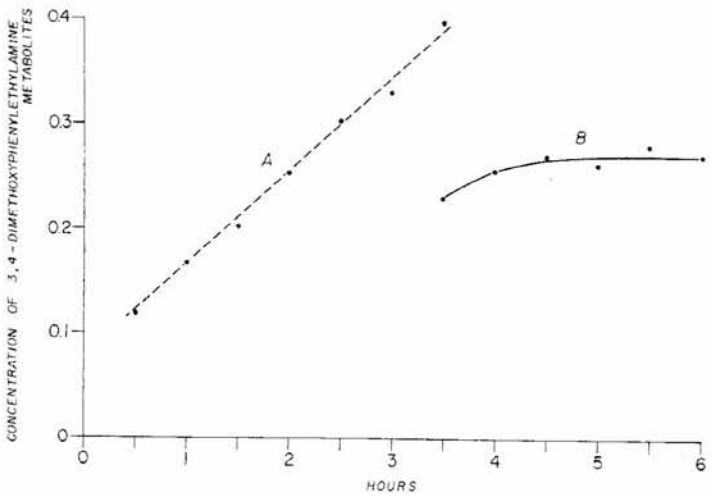

FIG. 8. Concentration of DMPEA metabolites in cerebrospinal fluid, each line representing a different subject. The concentration is expressed as micrograms per milliliter; subject A received a dose of 5.41 mg/kg and subject B received a dose of 8.76 mg/kg.

radioactivity recovered. In the urine collected between 8 and 12 hr, the radioactive material identified as DMPA was 58.6%, the HMPA was increased to 2.4%, while unchanged DMPEA was only 0.4%. In this sample, unknown no. 6, which was a conjugated product since it disappeared after acid hydrolysis, constituted 21.5% of the radioactivity. Table 1 indicates that the

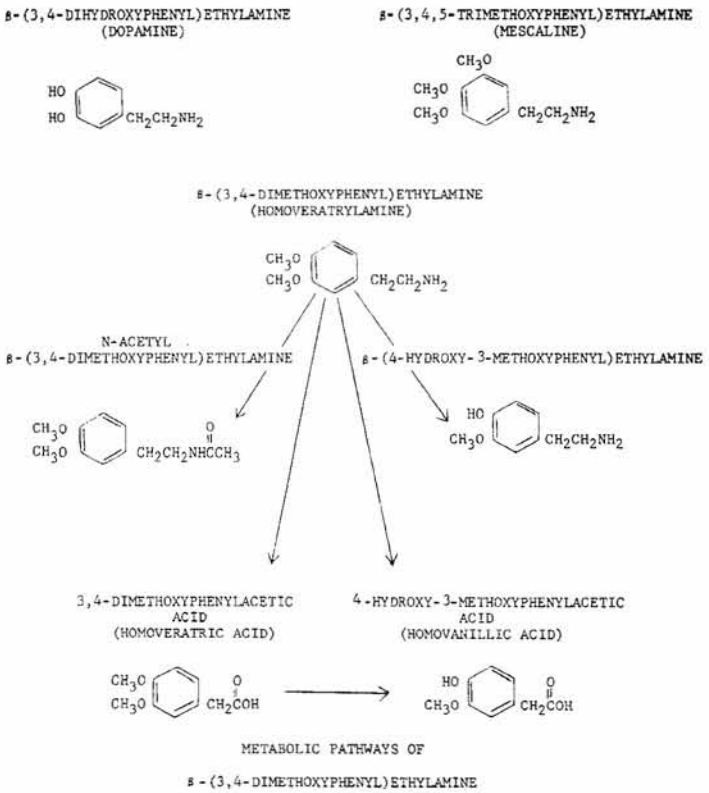

FIG. 9. Metabolic pathways of DMPEA.

concentration of the various metabolites changed with time, with deamination becoming less prevalent and conjugation more. Although table 1 was based on determinations of the urine from one subject, inspection of radioautograms from two other subjects showed the figures of this table to be typical. A radioautogram from one of these subjects, from pooled urine collected in the first 12 hr, gave the following percentages of radioactivity for each metabolite: DMPA, 90.6; HMPA, 1.4; DMPEA, 0.2; unknown no. 4, 1.2; unknown no. 5, 2.5; unknown no. 6, 4.0; unknown no. 7, 0.3; unknown no. 8, 0.4; unknown no. 9, 0.9; and unknown no. 10, 0.6.

Comparing these findings with those seen after β-(3,4,5-trimethoxyphenyl)ethylamine-mescaline administration (Charalampous *et al.*, 1966), one sees that the elimination of radioactivity with DMPEA was at least as fast as that with labeled mescaline. After mescaline administration, 55 to 60% of the radioactivity eliminated was found to be unchanged mescaline. The deaminated products were in the range of 27 to 30% and N-acetylated compounds around 5%.

These findings contrast strikingly with the metabolism of DMPEA. With DMPEA the unchanged amine was only 2.2% of the radioactivity eliminated in the urine between 1 and 2 hr and amounted to only 0.4% of the radioactivity eliminated between 8 and 12 hr. In the same samples DMPA was 72.9% in the samples collected between 1 and 2 hr and decreased to 58.6% in the sample collected between 8 and 12 hr. Thus, the rate of deamination of ingested DMPEA was twice that of mescaline. Despite efforts to identify N-acetylated products in the urine after C^{14}-DMPEA administration, none were found. Although their presence cannot be ruled out as yet, one could surmise that N-acetylation, if it occurs at all, is a minor metabolic process for ingested DMPEA. A probable intermediate metabolite between the parent amine DMPEA and HMPA, *i.e.*, β-(4-hydroxy-3-methoxyphenyl)ethylamine, has not been as yet identified.

The concentration of labeled compounds in plasma after oral administration of C^{14}-labeled DMPEA was of similar order to that after the

administration of C^{14}-mescaline. Similarly the concentration of the radioactivity in the whole blood was approximately half that of the plasma. This indicated that practically all radioactivity was found in the plasma. The concentration of radioactivity in the cerebrospinal fluid was again similar to that found after C^{14}-mescaline administration. More extended sampling is needed, however, to establish the shape of the disappearance curves.

In order to test the activity of DMPEA in man, several healthy volunteer subjects, inmates of the Texas Department of Correction, received the compound. Details of this study will be published elsewhere. The starting dose was 1.0 mg/kg and the highest for some subjects was 10.8 mg/kg. The observers and subjects reported no effects. Inferences for activity of DMPEA in man based on the subhuman studies of Noteboom (1934), of Smythies and Sykes (1966) and several others (Epstein *et al.*, 1932; Michaux and Verly, 1963; Ernst, 1965; Bergen, 1965; Nyomarkay *et al.*, 1965; Beck and Pfeiffer, 1966; Barbeau *et al.*, 1966) cannot be supported by our findings.

From our studies of mescaline (Charalampous *et al.*, 1964) it has been shown that 3,4,5-trimethoxyphenylacetic acid is inactive in man. This also appears to be the case with DMPA. None of our subjects experienced any effects after DMPA administration in doses as high as 12.0 mg/kg. The rapid deamination of DMPEA, one may theorize, prevents it from having any effects in man.

The administration of an amine oxidase inhibitor actively inhibiting the deamination of DMPEA in man could help clarify this point. We have given pargyline to three subjects under carefully controlled conditions. These subjects were challenged with progressively larger amounts of DMPEA. Dosages as high as 7.5 mg/kg produced no significant effects. More studies, however, are needed, including other monoamine oxidase inhibitors. In one of these subjects, extraction procedures on the 24-hr urine collected after C^{14}-labeled DMPEA administration, which followed monoamine oxidase inhibition, have shown a marked change in the ratio of the acidic to basic metabolites. This could facilitate the identification of the remaining unknown metabolites currently in progress.

CONCLUSIONS. Three normal subjects were given C^{14}-labeled DMPEA by mouth. Eighty-nine percent of the radioactivity was recovered from the urine in the first 24 hr. The half-life was approximately 2½ hr. The three main metabollites in the urine were isolated and identified. These were DMPEA, DMPA and HMPA.

The concentration of radioactivity was determined in both whole blood and plasma and the cerebrospinal fluid. The concentration curves resemble those following C^{14}-mescaline administration.

DMPEA was found inactive in man for either behavioral or physiologic effects. The doses given were as high as 10.8 mg/kg. DMPA was also found to be inactive in doses as high as 12.0 mg/kg.

ACKNOWLEDGMENT. We wish to thank the Texas Department of Corrections, Dr. George Beto, Director, for making this study possible.

REFERENCES

BAILLIE, L. A.: Determination of liquid scintillation counting efficiency by pulse height shift. Int. J. appl. Radiat. Isotopes **8:** 1–7, 1960.

BARBEAU, A., TÉTREAULT, L., OLIVA, L., MORAZAIN, L. AND CARDIN, L.: Pharmacology of akinesia—Investigations on 3,4-dimethoxyphenylethylamine. Nature, Lond. **209:** 719–721, 1966.

BECK, R. A. AND PFEIFFER, C. C.: Effect of mescaline-like compounds on the CNS of rabbits as measured by quantitative electroencephalography. Fedn Proc. Fedn Am. Socs exp. Biol. **25:** 503, 1966.

BERGEN, J. R.: Possible relationship of a plasma factor to schizophrenia. Trans. N.Y. Acad. Sci. (Ser. II) **28:** 40–46, 1965.

BOURDILLON, R. E., CLARKE, C. A., RIDGES, A. P., SHEPPARD, P. M., HARPER, P. AND LESLIE, S.A.: 'Pink spot' in the urine of schizophrenics. Nature, Lond. **208:** 453–455, 1965.

CHARALAMPOUS, K. D., ORENGO, A., WALKER, K. E. AND KINROSS-WRIGHT, J.: Metabolic fate of β-(3,4,5-trimethoxyphenyl)-ethylamine (mescaline) in humans: Isolation and identification of 3,4,5-trimethoxyphenylacetic acid. J. Pharmac. exp. Ther. **145:** 242–246, 1964.

CHARALAMPOUS, K. D., WALKER, K. E. AND KINROSS-WRIGHT, J.: Metabolic fate of mescaline in man. Psychopharmacologia **9:** 48–63, 1966.

EPSTEIN, D., GUNN, J. A. AND VIRDEN, C. J.: The action of some amines related to adrenaline. J. Physiol., Lond. **76:** 224–246, 1932.

ERNST, A. M.: Relation between the structure of certain methoxyphenylethylamine-derivatives and the occurrence of a hypokinetic rigid syndrome. Psychopharmacologia **7:** 383–390, 1965.

FAURBYE, A. AND PIND, K.: Investigation on the occurrence of the dopamine metabolite 3,4-dimethoxyphenylethylamine in the urine of schizophrenics. Acta Psychiat. scand. **40:** 240–243, 1964.

FRIEDHOFF, A. J. AND VAN WINKLE, E.: Isolation and characterization of a compound from the

urine of schizophrenics. Nature, Lond. **194:** 867–868, 1962.

FRIEDHOFF, A. J. AND VAN WINKLE, E.: A method for the detection of β-phenylethylamines and β-phenylethylamino acids. J. Chromat. **11:** 272–274, 1963.

KALBERER, F. AND RUTSCHMANN, J.: Eine Schnellmethode zur Bestimmung von Tritium, Radiokohlenstoff und Radioschwefel in beliebigem organischem Probenmaterial mittels des Flüssigkeitsscintillations-Zählers. Helv. chim. Acta **44:** 1956–1966, 1961.

KUEHL, F. A., JR., HICHENS, M., ORMOND, R. E., MEISINGER, M. A. P., GALE, P. H., CIRILLON, V. J. AND BRINK, N. G.: Para-O-methylation of dopamine in schizophrenic and normal individuals. Nature, Lond. **203:** 154–155, 1964.

MICHAUX, R. AND VERLY, W. G.: Action cataleptsigene des ethers methylique des mono- et polyphenolamines. Life Sci. **3:** 175–183, 1963.

NISHIMURA, T. AND GJESSING, L. R.: Failure to detect 3,4-dimethoxyphenylethylamine and bufotenine in the urine from a case of periodic catatonia. Nature, Lond. **206:** 963–964, 1965.

NOTEBOOM, L.: Experimental catatonia by means of derivatives of mescaline and adrenaline. Proc. K. ned. Akad. Wet. **37:** 562–574, 1934.

NYOMARKAY, I., BORSY, J. AND CSIZMADIA, Z.: Mescaline and its dimethoxy analogue: Effects on nervous system. Acta physiol. hung. **26:** suppl., 20–21, 1965.

PERRY, T. L.: *N*-methylmetanephrine: Excretion by juvenile psychotics. Science, N.Y. **139:** 587–589, 1963.

PERRY, T. L., HANSEN, S. AND MACINTYRE, L.: Failure to detect 3,4-dimethoxyphylethylamine in the urine of schizophrenics. Nature, Lond. **202:** 519–520, 1964.

SEN, M. P. AND MCGEER, P. L.: 4-Methoxyphenylethylamine and 3,4-dimethoxyphenylethylamine in human urine. Biochem. biophys. Res. Commun. **14:** 227–232, 1964.

SMYTHIES, J. R. AND SYKES, E. A.: Structure-activity relationship studies on mescaline: The effect of dimethoxyphenylethylamine and N:N-dimethyl mescaline on the conditioned avoidance response in the rat. Psychopharmacologia **8:** 324–330, 1966.

STUDNITZ, W. VON AND NYMAN, G. E.: Excretion of 3,4-dimethoxyphenylethylamine in schizophrenia. Acta psychiat. scand. **41:** 117–121, 1965.

TAKESADA, M., KAKIMOTO, Y., SANO, I. AND KANEKO, Z.: 3,4-dimethoxyphenylethylamine and other amines in the urine of schizophrenic patients. Nature, Lond. **199:** 203–204, 1963.

WILD, F.: Characterisation of Organic Compounds, p. 152, Cambridge University Press, London, 1962.