Psychopharmacologia (Berl.) 9, 48—63 (1966)

# Metabolic Fate of Mescaline in Man*

K. D. CHARALAMPOUS, K. E. WALKER, and JOHN KINROSS-WRIGHT

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

Received July 23, 1965

It was in 1888 that L. LEWIN rediscovered the small cactus *Lophophora williamsii*, and drew attention to its hallucinogenic properties. Eight years later A. HEFFTER isolated three alkaloids from this cactus, including mescaline; and in 1918 E. SPÄTH synthesized mescaline, establishing its chemical formula as 3,4,5-trimethoxyphenylethylamine. Yet its metabolism in man has been hitherto essentially unknown. An overview of the literature of mescaline can be obtained in the reviews of DOWNING (1962); FISCHER (1958); GIARMAN and FREEDMAN (1965); JACOBSEN (1963) and LAJTHA (1958). This data, derived mostly from subhuman species, must be supplemented with studies in man. The psychotomimetic state is largely subjective and can be only comprehended by communication from the subject. When one wishes to study behavioral parameters and correlate these with individual drug metabolism, subhuman species are unreliable.

We here report further studies on human subjects given $C^{14}$-mescaline by mouth. These include urinary excretion of mescaline, the blood and cerebrospinal fluid concentration of its labeled metabolites and the isolation and identification of several urinary metabolites. Chromatographic evidence is also presented for the presence of mescaline and three metabolites in the cerebrospinal fluid.

## Methods

### *Compounds*

Mescaline hydrochloride-2′-$C^{14}$ was obtained from New England Nuclear Corporation (Boston 18, Mass.) and mescaline hydrochloride from Sigma Chemical Company (St. Louis 18, Missouri). Both labeled and unlabeled N-acetylmescaline were prepared in this laboratory by acetylation of mescaline with acetic anhydride. The acetylated products were subjected to crystallization from hot *n*-heptane (m.p. 89°—90° C). A carbon, hydrogen, nitrogen analysis was performed on the unlabeled material with the following results: C, 61.82%; H, 7.6%; N, 5.20%

* This investigation was supported in part by Public Health Service Research Grant, MH-10777, from the National Institute of Mental Health.

(Theoretical: C, 61.64%; H, 7.65%; N, 5.55%). The melting point of the labeled material was the same as that of the unlabeled product. Radioautograms of chromatograms of the labeled N-acetylmescaline demonstrated only one spot. β-(3,5-dimethoxy-4-hydroxyphenyl)-ethylamine was kindly donated by Dr. A. PLETSCHER of F. Hoffman LaRoche and Co. Ltd.

*Collection of samples*

The subjects were twelve adult, normal male volunteers. Following a 12-hour fasting period, each subject received by mouth a single dose of 500 mg of mescaline hydrochloride containing 250 μC of mescaline hydrochloride-2′-$C^{14}$ dissolved in 50 ml of water. Each subject was catheterized with a Foley catheter and the bladder emptied immediately before the administration of the drug. Following the ingestion of the drug, the bladder was emptied at hourly intervals up to 12 hours. On the 24th hour the bladder was emptied again, then the catheter was withdrawn and the subject was instructed to collect his 24-hour urinary output for the following six days. The urine specimens were immediately frozen and were kept at — 15° until used. Blood specimens were collected at each hour through the 12th hour. Spinal fluid specimens were also collected from three subjects every 30 min for $9^1/_2$ hours after drug administration. The first two received a single drug administration. The last received two administrations several months apart. The first specimen from each subject was collected at the same hour as the last specimen from the previous subject (Fig. 4). Blood and spinal fluid specimens were immediately refrigerated at 4° until used.

*Radioactivity analysis*

A Nuclear-Chicago Liquid Scintillation Spectrometer Model 725 was used to determine the radioactivity of the samples and a model C 100 B Actigraph II connected with a Model 1620 B Analytical Count Ratemeter was employed for radioactivity scanning of some chromatograms. The scintillation fluid, diatol, had the following composition: Toluene, 500 ml; dioxane, 500 ml; methanol, 300 ml; PPO, 6.0 g: POPOP, 0.150 g; and naphthalene, 100 g.

*Urine.* 0.1 ml of urine was combined with 15 ml of diatol for counting.

*Plasma.* 0.1 ml of plasma was added to 1.0 ml of Hyamine and then was heated to dissolve the protein. Fifteen ml of diatol was added for counting.

*Whole blood.* 0.1 ml of whole blood was placed in a counting vial and was heated on the hot plate to destroy catalase. A few drops of 30% $H_2O_2$ was then added to decolorize, followed by the addition of 1.0 ml

of Hyamine. Heat was then applied to dissolve the protein. Fifteen ml of diatol was then added for counting.

*Cerebrospinal fluid.* 0.2 ml of cerebrospinal fluid was added to a counting vial. Fifteen ml of diatol was added and the sample was counted after standing 24 hours in the counting chamber.

*Combustion.* Paper containing the radioactive area was combusted in oxygen flasks according to the method of KALBERER and RUTSCHMANN (1961). The $CO_2$ produced was absorbed in 10 ml of 12% ethanolamine in methyl cellosolve. Four ml of this solution was then counted with 15 ml of diatol.

All radioactivity determinations were corrected to 100% efficiency by applying the Baillie channels ratio method (1960).

### *Paper chromatography*

All chromatograms were run by the descending technique. For two-dimensional chromatography, Whatmann 3 MM paper was used with the following solvents: (I) *n*-Butanol: acetic acid: $H_2O$, (4:1:1) in the first direction and (II) *n*-Butanol: iso-propanol; conc$NH_4OH$:$H_2O$, (3:1:1:1) in the second direction.

Radioactive areas on chromatograms were located by exposing the chromatogram to Kodak No-screen x-ray film for a suitable period. When it was desirable to estimate the radioactivity contained in a given area of the chromatogram, the area was cut out, chopped into small pieces and counted in 15 ml of diatol.

### *Preparation of Dowex 50 W-X4*

The resin was prepared by washing consecutively with five volumes of 1 N HCl, 50% aqueous ethanol which contained 5% concentrated ammonium hydroxide, 1 N HCl and distilled water. The column used was 2 cm in diameter and contained 80 ml resin, wet volume.

### *Melting point determinations*

Melting point determinations were determined using a Fisher-Johns Melting Point apparatus. All values are uncorrected.

## Results

### *Urinary excretion of radioactivity*

The excretion of radioactivity was determined in all subjects. The percentage of radioactivity eliminated during the first 48 hours in ten subjects was averaged (Fig. 1). An average of 87% of the radioactivity administered was excreted in the first 24 hours. Only small amounts were eliminated during the following six days. The biological half-life of

the radioactivity ingested as mescaline was approximately six hours. An aliquot of the 24 hours urinary collection of one subject was spotted on Whatmann 3 MM paper and developed in solvent system I in the first direction, and solvent system II in the second direction. A radioautogram of this chromatogram is shown in Fig. 2. In order to determine differences in the elimination of the various metabolites at different

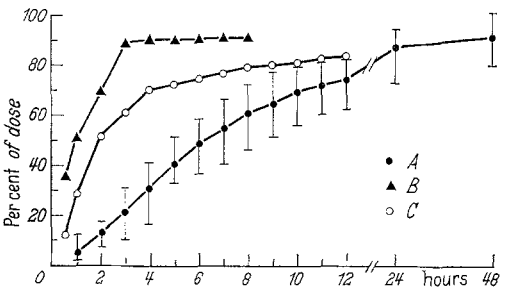



Fig. 1. Cumulative excretion of radioactivity in urine after oral administration of $C^{14}$-labeled: *A* mescaline (average of ten subjects); *B* 3,4,5-trimethoxyphenylacetic acid (one subject); *C* N-acetylmescaline (one subject). The vertical lines indicate minimum and maximum of radioactivity recovered

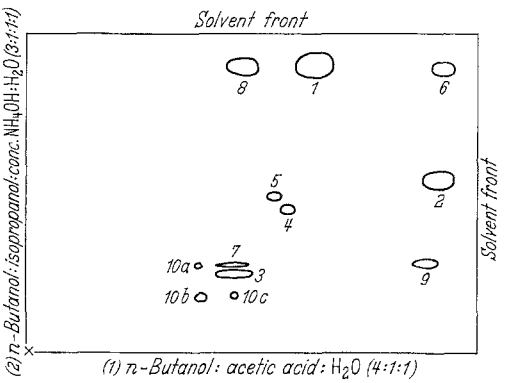



Fig. 2. Radioautogram of a two-dimensional chromatogram from an aliquot of the first 24-hour urine collected after oral $C^{14}$-mescaline administration. The numbers refer to compounds listed in Table 1

times, aliquots from the hourly collections of one subject were chromatographed as described for the 24-hours urine chromatogram. The percentage composition in the hourly urine for each metabolite was determined by cutting from the chromatogram each area of radioactivity—as determined by radioautography—and by counting directly in diatol. The radioactive areas from a duplicate chromatogram were cut out, combusted, and then counted. The values obtained were substantially the same as in direct counting. These determinations are tabulated in Table 1.

Table 1. *Metabolites of mescaline in urine as percent of radioactivity in each sample*

These determinations were made by running a two-dimensional paper chromatogram and locating the radioactive areas by radioautography. The radioactive areas were cut out from the chromatograms and counted directly in diatol. CW-1 is a conjugate of N-acetyl-β-(3,4-dimethoxy-5-hydroxyphenyl) ethylamine. Upon acidic hydrolysis, CW-2 produces an anime of the same $R_f$ value as mescaline. TMPA stands for 3,4,5-trimethoxyphenylacetic acid. HMPA is an O-demethylated phenylacetic acid and was isolated from the acid extract. The spots labeled BW are included in the table as HMPA since hydrolysis of the BW fraction produces HMPA only. Only those substances are included in the table which appeared consistently or have been identified

| | Time of urine collection (hours) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0—1 | 1—2 | 2—3 | 3—4 | 4—5 | 5—6 | 6—7 | 7—8 | 8—9 | 9—10 | 10—11 | 11—12 |
| 1. Mescaline | 81.4 | 55.7 | 54.7 | 49.8 | 53.7 | 59.0 | 58.5 | 63.6 | 52.0 | 50.3 | 43.0 | 50.5 |
| 2. TMPA | 13.2 | 33.9 | 31.9 | 36.6 | 34.4 | 26.4 | 27.9 | 21.6 | 30.0 | 37.5 | 38.5 | 36.9 |
| 3. CW-1 | 1.3 | 1.8 | 3.6 | 4.0 | 3.8 | 4.1 | 4.0 | 5.0 | 5.0 | 6.0 | 5.9 | 5.8 |
| 4. Unknown-1 | 0.2 | 0.7 | 0.6 | 0.7 | 1.1 | 0.6 | 0.8 | 1.3 | 0.8 | 1.1 | 2.5 | 1.2 |
| 5. Unknown-2 | 0 | 0 | 0.2 | 0.6 | 0 | 0.9 | 0.7 | 1.8 | 2.3 | 1.6 | 4.7 | 2.8 |
| 6. N-acetylmescaline | 0 | 0 | 0 | 0 | 0 | 0.3 | 0.3 | 0 | 0 | 0 | 0 | 0 |
| 7. CW-2 | 0.5 | 0.9 | 1.2 | 1.2 | 1.0 | 1.2 | 0.9 | 1.9 | 2.5 | 1.2 | 2.5 | 1.2 |
| 8. Unknown-3 | 2.1 | 3.1 | 3.8 | 1.7 | 1.2 | 2.9 | 2.6 | 2.8 | 3.4 | 0 | 0 | 0 |
| 9. HMPA | 0.1 | 0.9 | 0.8 | 1.0 | 1.0 | 0.7 | 0.7 | 1.0 | 0.9 | 0.5 | 1.2 | 0 |
| 10. a, b, c BW | | | | | | | | | | | | |

*Fractionation of urine*

The 24-hour urinary output of one subject was passed through a Dowex 50 W-X4 ($H^+$) column. The column was then washed with distilled water until the elution of radioactivity ceased. This wash effluent was combined with the effluent from the passage of the urine and is designated as *effluent A*. The column was then washed with 50% aqueous ethanol containing 5% concentrated ammonia. This is *effluent B*.

*Mescaline*

The ethanol was evaporated from effluent B and the aqueous residue was made acidic with HCl. This acidic solution was extracted with ethyl acetate in order to remove any extractable neutral components from the amines. The remaining aqueous solution was made strongly basic with KOH and the amines were quantitatively extracted with benzene. This extract represented approximately 60% of the excreted radioactivity. The benzene was dried with anhydrous $Na_2SO_4$ and was concentrated.

Table 2. *Melting points of amine hydrochlorides extracted from urine, of the standards, and of the α-naphthylthiourea derivatives*

| | M. P. °C |
|---|---|
| Authentic mescaline HCl | 184.0°—187.0° |
| Extracted amine HCl | 184.5°—185.8° |
| mixed 1:1 | 185.8°—187.0° |
| Mescaline α-naphthylthiourea | 156.1°—156.8° |
| Extracted amine α-naphthylthiourea | 155.6°—156.1° |
| mixed 1:1 | 155.5°—156.3° |

For the precipitation of the amines as their hydrochlorides, dry HCl was passed through the benzene solution. The precipitate was collected without leaving any labeled material in the benzene; its melting point was the same as that of authentic mescaline hydrochloride. α-Naphthylthiourea derivatives were also prepared, and their melting point data is presented in Table 2. Radioautograms of paper chromatograms of the amines showed only one labeled substance present with $R_f$ values in agreement with the mescaline standard (see Table 3).

*N-acetylmescaline*

The neutral extract from effluent B contained a labeled substance. This was identified as N-acetyl-mescaline (NAM). Authentic labeled NAM was mixed with this neutral metabolite so that one half the total redioactivity of the mixture was contributed by each. Radioautograms of strip chromatograms of this mixture demonstrated only one spot (see

Table 3. *Chromatography of two urinary metabolites*

| Solvent systems | $R_f$ values | | | |
|---|---|---|---|---|
| | Authentic labeled N-acetylmescaline + Neutral metabolite | Authentic N-acetylmescaline | Authentic Mescaline HCl | Excreted Amine HCl |
| *n*-butanol:acetic acid: $H_2O$ (4:1:5, lower phase) | 0.81 | 0.80 | — | — |
| chloroform:acetic acid: $H_2O$ (2:1:1, upper phase) | 0.69 | 0.69 | — | — |
| *n*-heptane:acetone:$H_2O$ (4:1:1, lower phase) | 0.88 | 0.88 | — | — |
| *n*-heptane:*n*-butanol:$H_2O$ (4:1:1, lower phase) | 0.85 | 0.85 | — | — |
| *n*-butanol:acetic acid: $H_2O$ (4:1:1) | 0.85 | 0.85 | 0.67 | 0.67 |
| *n*-butanol:isopropanol: conc $NH_4OH$:$H_2O$ (3:1:1:1) | 0.92 | 0.92 | 0.84 | 0.86 |
| isopropanol:formic acid: $H_2O$ (8:1:1) | — | — | 0.75 | 0.75 |

Table 4. *Cocrystallization of the neutral metabolite and authentic N-acetylmescaline*
Cold authentic N-acetylmescaline was added to the labeled neutral metabolite. This was then recrystallized from hot *n*-heptane. After each crystallization, the precipitate was dried to a constant weight and about 5 mg of this precipitate was weighed directly in a counting vial; 15 ml of diatol was added and the radioactivity was measured in a scintillation counter

| Solution | Precipitate (dpm/mg) | M.P. of Precipitate |
|---|---|---|
| First crystallization | 154 | |
| Second crystallization | 155 | |
| Third crystallization | 154 | 89°—90° C |

Table 3). Authentic nonlabeled NAM combined with the neutral metabolite and recrystallized from hot *n*-heptane gave constant specific activity in the precipitate (see Table 4). NAM represents less than 0.1% of the excreted radioactivity.

*Free acid fraction*

Effluent A from the column was extracted with ethyl acetate from basic solution first to remove neutrals, then it was made acidic and was extracted until only a trace of radioactivity remained in the extract. This acid extract contained the unconjugated acids and constituted about 30% of the excreted radioactivity. Radioautograms of chromatograms of this extract revealed it to consist of 3,4,5-trimethoxyphenylacetic acid (TMPA) and another acid (HMPA) in much smaller quantity.

The identification of TMPA has been published elsewhere (CHARALAMPOUS et al., 1964). HMPA together with its conjugates accounted for about 1% of the excreted radioactivity. Scanning chromatograms with a 2 $\pi$ scanner failed to reveal the prescence of HMPA in this extract.

A small amount of HMPA was separated from TMPA by preparative chromatography using Whatmann 3 MM paper and solvent system II. Studies of the distribution of radioactivity between ethyl acetate and an aqueous solution of pH 1, pH 8.5—9, and pH 12, indicated the presence of a carboxylic acid group, probably an O-demethylated carboxylic acid. In order to determine if this acid could be produced from TMPA as well as from the corresponding phenolic amine, $C^{14}$-labeled TMPA was given to a human subject. The radioactive TMPA was prepared as follows. The acid extract from the urine of a subject who had received a labeled dose of mescaline was applied as a line on ethyl acetate-washed Whatmann 3 MM paper and developed in solvent system II. Solvent system II produced excellent separation of TMPA from HMPA. A radioautogram of this chromatogram was made. The area of the chromatogram corresponding to TMPA was cut out, and after acidification with HCl, was eluted from the paper with ethyl acetate. Cold TMPA was added to the ethyl acetate extract and then the extract was taken to dryness at room temperature. The residue was extracted with boiling *n*-heptane. Upon cooling, the TMPA precipitated. This precipitate was collected, dried and orally administered to a human subject. This dose represented 7,402,992 dpm in 123.8 mg TMPA. The urine from this subject was extracted for carboxylic acids with ethyl acetate until only a trace of radioactivity remained in the extract. The extract was concentrated and applied to paper for development in solvent system II. The radioautogram of this chromatogram revealed the presence of TMPA and a substance of the same $R_f$ value (0.33) as the minor carboxylic acid (HMPA), derived from the mescaline administration. No attempt was made to quantify the amount but it was easily detectable by radioautography. Thus, HMPA could be derived from TMPA as well as from the corresponding O-demethylated amine.

*Unextracted substances*

The remaining mescaline metabolites, representing 10% of excreted radioactivity, was contained in that fraction which was unextractable into organic phase at any pH and was designated as W.

The neutralized W fraction was passed through a bed of charcoal and then washed with distilled water until no residue was visible after evaporation of 100 ml of the eluant water. The eluant water did not contain significant radioactivity, but it did contain much solid material. The radioactive compounds were eluted with aqueous ammoniacal

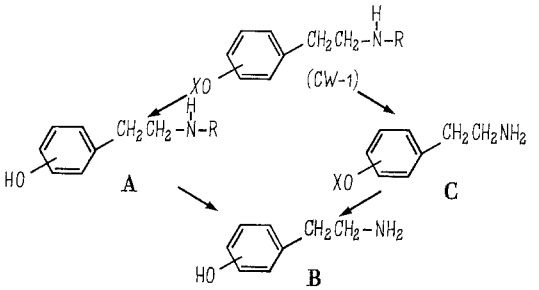
ethanol. The ethanol eluant was evaporated to dryness and then was dissolved in a small amount of water and chromatographed on a Dowex 50 W-X4 ($H^+$) column. Elution with distilled water consistently produced three fractions containing radioactivity: AW 26%, BW 6%, CW 68%, eluted in that order. Each fraction was concentrated to a small volume by lyophilization then further analysis was made.

Two-dimensional chromatography and radioautography demonstrated AW to consist of several components. These were not characterized.

The BW fraction consisted of three substances, which upon acidic hydrolysis produced only one product, a carboxylic acid. This hydrolysis product was shown to be the same as the minor carboxylic acid HMPA isolated with the original acid extract and constituted approximately 0.6% of the total radioactivity excreted.

The CW fraction was shown by two-dimensional chromatography to consist primarily of two substances: CW-1 (70%) and CW-2 (30%). CW-1 was separated from CW-2 by applying as a line on water-washed Whatmann 3 MM paper and by developing in solvent system II. The separated CW-1 and CW-2 were eluted from the paper with water. Acidic hydrolysis of CW-2 produced a substance of the same two-dimensional $R_f$ value as mescaline. The information tabulated in Fig. 5 was developed for CW-1. CW-1 produced only one radioactive area with chromatography in eleven solvent systems. It was unextractable at any pH into ethyl acetate. Upon acidic hydrolysis and two-dimensional chromatography of the hydrolysate, three radioactive spots were present which were different in $R_f$ value from the original compound (CW-1) and also were different from mescaline, N-acetylmescaline, TMPA and HMPA. These spots were designated as A, B, and C. Substance A was extractable from the aqueous solution with ethyl acetate under acidic conditions and 5% sodium bicarbonate solution, but was not extractable from 5% sodium hydroxide. This indicated the presence of a phenolic group and the absence of an amine or carboxylic acid group. Substance A was negative to ninhydrin and produced a blue color with Gibb's reagent which supports the above conclusion. C on the other hand, gave a positive ninhydrin reaction and was negative to Gibb's reagent. B was extractable only in the pH range of 8.5, produced a blue color with Gibb's reagent, and was positive to ninhydrin, which indicated a phenolic amine. The longer the hydrolysis of CW-1 the less the substance A and C and the more of substance B that was found. B, therefore, was the end product of hydrolysis, while A and C are intermediates. Substance C was small even with a short hydrolysis period. The foregoing data supports the following scheme for the hydrolysis of CW-1 and the nature of the substances present after hydrolysis, where R = a hydrolyzable electron

withdrawing group, i.e. an acetyl group, and X = a hydrolyzable polyhydroxylic or ionic group, i.e. a glucuronide or sulfuric ester group:




Scheme

Table 5. *Characterization data for N-acetyl-β-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine*

| Substance | $R_f$ values Solvent I | Solvent II | Ninhydrin | Gibbs | Extractability into ethyl acetate at aqueous pH 1 | 8.5—9 | 12 | Color with diazotized sulfanilic acid |
|---|---|---|---|---|---|---|---|---|
| CW-1 | 0.52 | 0.31 | (−) | (−) | (−) | (−) | (−) | |
| A | 0.86 | 0.81 | (−) | Blue | (+) | (+) | (−) | Dark rust+ |
| B | 0.44 | 0.63 | (+) | Blue | (−) | (+) | (−) | Yellow+ |
| C | 0.16 | 0.25 | (+) | (−) | (−) | (−) | (−) | |

Standards

| | | | |
|---|---|---|---|
| I. | β-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine | Blue* | |
| II. | β-(3,4-dihydroxy-5-methoxyphenyl)-ethylamine | Yellow-Blue* | |
| III. | β-(3,5-dimethoxy-4-hydroxyphenyl)-ethylamine | Green-Blue+* | Rose+ |
| IV. | β-(3,5-dihydroxy-4-methoxyphenyl)-ethylamine | Yellow* | |
| V. | β-(3,4,5-trihydroxyphenyl)-ethylamine | Yellow* | |

The $R_f$ values for Standard III in solvent I and II are 0.48 and 0.65 respectively.
* Reported by NEFF et al. (1963).
+ Determined in this laboratory.

The previous data indicated B as a phenolic amine. Only five phenolic amines can be postulated by O-demethylation of the mescaline molecule. These are presented in Table 5. Substance I is the only one of these amines that gives a blue color with Gibb's reagent which, of course, is also true for substance B. The only other phenolic amine in the list whose Gibb's color reaction might possibly be mistaken for the blue color of B is the blue-green reaction of III. Substance B gives an intense yellow color with diazotized sulfanilic acid. whereas III produces a rose color,

thus eliminating III as a possibility for the identity of B. Having established the probable identity of B as β-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine, we found upon administering N-acetylmescaline to man, that the major metabolite excreted is a substance with the same $R_f$ value as CW-1 and that hydrolysis of this major excretion product of N-acetylmescaline produces the same substances as those produced by hydrolysis of CW-1. It then becomes clear that A is probably N-acetyl-β-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine and that CW-1 is a conjugate of this. This metabolite (CW-1) represents approximately 5% of the radioactivity excreted after $C^{14}$-mescaline administration. None of the unconjugated phenolic amide was detected in the urine collected after either mescaline administration or after N-acetyl-mescaline administration.

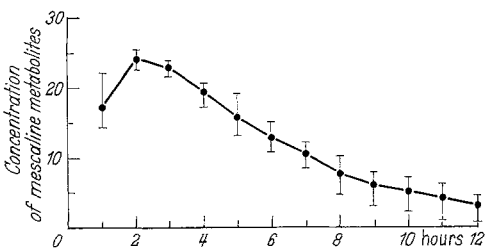



Fig. 3. Concentration of labeled compounds in plasma after oral administration of $C^{14}$-mescaline; average of three subjects. The concentration of metabolites is expressed as μmoles per ml of plasma per mg of the dose per kg of body weight times $10^{-4}$. The concentration of radioactivity of whole blood was approximately half that of the plasma, indicating that practically all radioactivity is found in the plasma

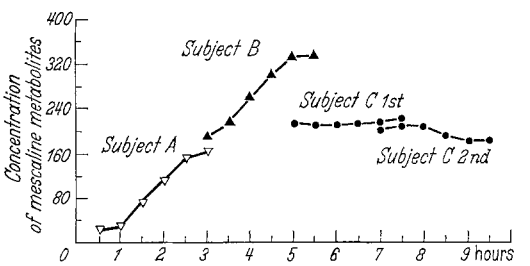



Fig. 4. Concentration of radioactivity in the cerebrospinal fluid in three subjects at various times after oral $C^{14}$-mescaline administration expressed as μmoles per ml of cerebrospinal fluid per mg of dose per kg of body weight times $10^{-6}$

### *Radioactivity in blood and cerebrospinal fluid*

The radioactivity present in each specimen was counted and converted to moles per milliliter. The concentration curves for the blood and cerebrospinal fluid are respectively illustrated in Figs. 3 and 4.

*Metabolites in cerebrospinal fluid*

The specimens withdrawn at $4^1/_2$, 5, and $5^1/_2$ hours after mescaline administration were combined and lyophylized to dryness. The residue was then washed several times with ethanol. This extract contained too many solids, relative to the radioactivity concentration, for chromatography. Because of this, the ethanol extract was taken to dryness and the residue was dissolved in water. The solution was first made strongly acidic and extracted with ethyl acetate. Then it was made strongly basic and extracted again with ethyl acetate. The two extracts were combined, concentrated, and subjected to two-dimensional chromatography—solvent system I, first direction; solvent system II, second direction. The radioautogram—exposure time 120 days—of this chromatogram revealed as the major radioactive substance a spot of the same $R_f$ value as mescaline. Three other spots were present in a much smaller amount. According to $R_f$ value, they were identified as 3,4,5-trimethoxyphenylacetic acid (TMPA), N-acetylmescaline (NAM) and N-acetyl-$\beta$-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine (NAHM).

The mescaline found in the cerebrospinal fluid represented at least 50% of the radioactivity measured. Although TMPA, NAM and NAHM are present in the cerebrospinal fluid in much smaller amount than mescaline, both NAM and NAHM are present in about equal amounts and each of these constitutes a larger percentage of radioactivity than TMPA.

*Administration of N-acetylmescaline to human subjects*

In the course of our present studies we have administered N-acetylmescaline to several human subjects starting with a small dose and gradually increasing the amount. In addition we have included in this evalution a "double blind" design where four subjects received the following: one a dose of 306 mg of N-acetylmescaline (4.08 mg per kg); one, 450 mg (5.25 mg per kg); one, 675 mg (9.0 mg per kg); and one, 756 mg (10.4 mg per kg). None of these subjects experienced any behavioral or physiological effects with the exception of the last one, who one hour after the administration of N-acetylmescaline experienced a mild degree of drowsiness.

## Discussion

Man appears to absorb and metabolize mescaline quite rapidly according to our findings. Eighty-seven percent of radioactivity administered by mouth is excreted in the first 24 hours, and the figure rises to an average of 92% by the end of the second 24 hours. The rapid elimination of radioactivity makes the utilization of labeled mescaline for metabolic studies in man feasible.

From a total of 12 compounds found consistently in unhydrolyzed urine after mescaline administration we have identified four and have partially characterized five more. These nine compounds constitute 96% to 98% of the radioactivity excreted.

The relationship of mode of metabolism to time has been studied. It appears that significant differences in excretion of mescaline, TMPA, N-acetylmescaline and unknown-3 occur. Mescaline, from a high percentage of 81.4 in the urine collected during the first hour, drops to a significantly lower level thereafter. In contrast, TMPA constitutes only 13.2% in the first hour but rises to a higher and comparatively constant level afterwards. Unknown-3 is found in variable amounts up to the 9th hour and none thereafter. N-acetylmescaline constitutes a very small amount and was found only in the urine collected between the 5th and 7th hours.

In an attempt to explore structure activity relations of the various metabolites of mescaline we have given TMPA to human subjects in dosages as high as 12.1 mg per kg. We have found and reported previously (CHARALAMPOUS et al., 1964) that TMPA has no physiological or psychological effects in man, in agreement with the findings of SLOTTA and MÜLLER (1936). From our present studies it also appears that N-acetylmescaline is essentially inactive and that N-acetylation is another detoxication pathway for ingested mescaline.

WEISSBACH et al. (1962), and SMITH and WORTIS (1962) report that N-acetylation plays a significant role in amine metabolism, particularly if there is suppression of monoamine oxidase activity. The high degree of deamination taking place with mescaline may delegate this mechanism to a secondary place. This is generally supported by the fact that we were able to demonstrate only trace amounts of N-acetylmescaline excreted after the administration of mescaline. However, in addition to N-acetylmescaline, we have identified another N-acetylated metabolite, N-acetyl-$\beta$-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine. This N-acetylated metabolite constitutes 5% of the excreted radioactivity. When administering 64 mg of labeled N-acetyl-mescaline to a human subject 68% of the excreted radioactivity was conjugated N-acetyl-$\beta$-(3,4-dimethoxy-5-hydroxyphenyl)-ethylamine and only a trace amount of N-acetylmescaline. From this, it is seen that a good possibility exists that N-acetylation of mescaline preceeds O-demethylation. Also, while TMPA constitutes about 30% of the excreted metabolites of mescaline and N-acetylation produces no more than 5%, the cerebrospinal fluid contains N-acetylmescaline and N-acetyl-$\beta$-(3,4-dimethoxy-5-hydroxyphenyl) ethylamine (unconjugated), each in greater amounts than TMPA. This may suggest that N-acetylation in the central nervous system is quantitatively more important than deamination.

The consensus of many observers including ourselves (CHARALAMPOUS and KINROSS-WRIGHT, 1963) is that the physiological and psychological effects after oral mescaline administration appear in 30 min, peak at four hours, and last from 12—14 hours. From Fig. 3 we see that the peak of these manifestations follows by two hours the peak of radioactivity concentration in the blood. Lack of correlation between behavioral activity and concentration of mescaline or its metabolites in the blood has been inferred before through the investigations of MOKRASCH and STEVENSON (1959) who gave mescaline intravenously to human subjects; and also from investigations of BLOCK et al. (1952), who gave $C^{14}$-mescaline to mice. BLOCK and his coinvestigators found the highest concentration of radioactivity in the blood just before the first-hour post intraperitoneal injection, while the sleeping phase began on the third hour. According to him, this sleeping phase corresponds to the hallucinatory phase in man. Looking now at Block's findings in the mouse brain, we see again that the peak of radioactivity is just before the first hour and practically no radioactivity is found after the fourth hour. NEFF et al. (1964), on the other hand, after giving $C^{14}$-mescaline intravenously to cats found that the brain tissue attained a maximum concentration of mescaline between one-half to two hours post injection, and that the level diminished gradually during the six hour period studied. However, the period of maximum concentration in the brain corresponded approximately to the period of "subjectively-rated" maximum intoxication with mescaline. They found the central nervous system disturbances in the cat reached a peak in one-half to one hour after intravenous administration. At $^1/_2$ hour, examination of the cerebrospinal fluid showed the concentration of radioactivity to parallel that in the brain, but at 1 hour the concentration in the brain tissue had increased, and that in the cerebrospinal fluid had decreased-at a rate half as fast as that of the brain. This decrease in the cerebrospinal fluid continues and parallels the concentration of radioactivity in the plasma. In Fig. 4 we see that radioactivity concentration in the cerebrospinal fluid of man remains at significant levels at least through $9^1/_2$ hours after drug administration and that the peak appears to correspond to the peak of behavioral manifestations. These findings we feel do not justify the often heard statement (RINKEL, 1965) that labeled mescaline disappears from the brain by the time the first mental effects occur.

**Summary**

Human subjects given $C^{14}$-mescaline by mouth excrete an average of 87% of the dose during the first 24 hours and an average of 92% during the first 48 hours. Average half-life of mescaline in six hours.

The composition of the urine in respect to the various metabolites of mescaline from hour to hour has been determined. The concentration

of mescaline and its metabolites in the plasma and the cerebrospinal fluid at the various times has also been determined.

Mescaline, 3,4,5-trimethoxyphenylacetic acid, N-acetyl-$\beta$-(3,4-dimethoxy-5-hydroxyphenyl) ethylamine and N-acetyl-mescaline have been identified in human urine after mescaline administration in the following amounts: mescaline 55—60%, 3,4,5-trimethoxyphenylacetic acid 27—30%, N-acetyl-$\beta$-(3,4,dimethoxy-5-hydroxyphenyl) ethylamine 5% and N-acetylmescaline less than 0.1%. Five other metabolites have been partially characterized.

Chromatographic evidence is presented for the presence of mescaline, 3,4,5-trimethoxyphenylacetic acid, N-acetylmescaline and N-acetyl-$\beta$-(3,4-dimethoxy-5-hydroxyphenyl) ethylamine in the cerebrospinal fluid in man after oral mescaline administration.

## References


Baillie, L. A.: Determination of liquid scintillation counting efficiency by pulse height shift. Int. J. appl. Radiat. **8**, 1—7 (1960).

Block, W., K. Block u. B. Patzig: Zur Physiologie des $^{14}$C-radioaktiven Mescalins im Tierversuch. III. Mitteilung. Mescalineinbau in Leberprotein. Hoppe-Seylers Z. physiol. Chem. **291**, 119—218 (1952).

— Zur Physiologie des $^{14}$C-radioaktiven Mescalins im Tierversuch. IV. Mitt. Vergleichende Untersuchungen mit $^{14}$C-Mescalin und $^{14}$C-$\beta$-phenyl-äthylamin. Z. Naturforsch. **8b**, 440, 444 (1953).

Charalampous, K. D., and J. Kinross-Wright: Hallucinogens, tools for research or therapeutic agents. Tex. St. J. Med. **59**, 848—852 (1963).

— A. Orengo, K. E. Walker, and J. Kinross-Wright: Metabolic fate of $\beta$-(3,4,5-trimethoxyphenyl)-ethylamine (Mescaline) in humans: isolation and identification of 3,4,5-trimethoxy-phenylacetic acid. J. Pharmacol. exp. Ther. **145**, 242—246 (1964).

Downing, D. F.: The chemistry of psychotomimetic substances. Quart. Rev. (Lond.) **16**, 133—162 (1962).

Fischer, R.: Pharmacology and metabolism of mescaline. Rev. canad. Biol. **17**, 389—409 (1958).

Giarman, N. J., and D. X. Freedman: Biochemical aspects of the actions of psychotomimetic drugs. Pharmacol. Rev. **17**, 1—25 (1965).

Jacobsen, E.: The clinical pharmacology of the hallucinogens. Clin. Pharmacol. Ther. **4**, 480—503 (1963).

Kalberer, F., u. U. J. Rutschmann: Eine Schnellmethode zur Bestimmung von Tritium, Radiokohlenstoff und Radioschwefel in beliebigem organischem Probematerial mittels des Flüssigkeits-scintillations-Zählers. Helv. chim. Acta **44**, 1956—1966 (1961).

Lajtha, A.: Biochemistry of hallucinogens. In: Psychopharmacology, pharmacologic effects on behavior. Edited by Pennes, H. H. New York: Hoeber 1958.

Mokrasch, L. C., and I. Stevenson: The metabolism of mescaline with a note on correlations between metabolism and psychological effects. J. nerv. ment. Dis. **129**, 177—183 (1959).

Neff, N., G. V. Rossi, G. D. Chase, and J. L. Rabinowitz: Distribution and metabolism of mescaline-C$^{14}$ in the cat brain. J. Pharmacol. exp. Ther. **144**, 1—7 (1964).

RINKEL, M.: The presidential address: the LSD movement in America. Paper read at Soc. Biol. Psychiat., New York City, April—May, 1965.

SLOTTA, K. H., u. J. MÜLLER: Über den Abbau des Mescalins und mescalinähnlicher Stoffe im Organismus. Hoppe-Seylers Z. physiol. Chem. **238**, 14—22 (1936).

SMITH, A. A., and S. B. WORTIS: Formation and metabolism of N-acetylnormetanephrine in the rat. Biochim. biophys. Acta (Amst.) **60**, 420—422 (1962).

WEISSBACH, H., B. G. REDFIELD, and J. AXELROD: The enzymic acetylation of serotonin and other naturally occurring amines. Biochim. biophys. Acta (Amst.) **54**, 190—192 (1962).

Dr. K. D. CHARALAMPOUS
1300 Moursund Avenue
Houston, Texas 77025 (U.S.A.)