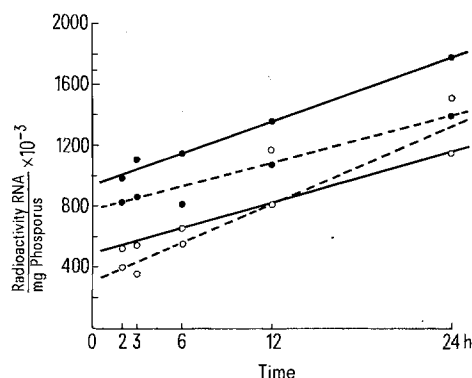


concentration of RNA particularly in the nerve cells, in an analogous manner to other neurotropic drugs^{9,10}. At present, it is not possible to conceive whether LSD attains this effect by physico-chemical processes, by processes involving enzymes or acceptors²; one could also speculate whether, in the presence of LSD, the RNA molecules are not stabilized in an inert (i.e. transitionally more stable) metabolic form. It is also possible that the increasing of RNA synthesis, induced by LSD, represents a compensatory mechanism, in reference to the modified releasing of a proteic neurosecretion by the nerve cells¹³.



Radioactivity of the RNA-fractions after incorporation of ³H-uridine refer to 1 mg of total phosphorus. —●—, cephalic pieces of planarians treated with LSD; —○—, retrocephalic pieces of planarians treated with LSD; ---●---, cephalic pieces of planarians kept in spring water; ---○---, retrocephalic pieces of planarians kept in spring water.

Table II and the Figure illustrate the radioactivity of the RNA fraction after incorporation of ³H-uridine. This radioactivity is significantly higher in the cephalic pieces with respect to the retrocephalic ones, and particularly in the cephalic pieces of the LSD-treated planarians with respect to the cephalic pieces of the untreated control animals. Some substances, as for instance mercaptoaethanol¹⁹, increase the incorporation of the RNA-precursor. According to our results, also LSD seems to have an analogous effect, specially in the cerebral plexus of planarians, where an increased synthesis of RNA is attained²⁰.

Riassunto. Se si mantengono Planarie (*Dugesia lugubris*) per otto giorni in una soluzione di LSD (0,02 mg/ml), si nota un aumento di RNA nella parte cefalica del soggetto rispetto ai corpi e anche rispetto alle teste dei controlli. Anche l'incorporazione di uridina triziata segue lo stesso andamento. Vengono proposte alcune ipotesi.

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¹⁹ A. GABRIEL, C.R. Acad. Sci., Paris 266, 406 (1968).

²⁰ Acknowledgments. We gratefully acknowledge the generous gift of LSD from Sandoz and the Institute of Zoology of the University of Bologna who supplied the planarians used in our experiments.

Acetylation of Mescaline in Rat Brains

NEFF et al.¹ identified 3,4,5-trimethoxyphenylacetic acid (TMPA) as the only metabolite in the cat brain following i.v. administration of mescaline-¹⁴C. While this work was in progress SHAH and HIMWICH² reported the presence of N-acetylmescaline and TMPA in mouse brain following an i.p. injection of mescaline.

Although acetylation in general is considered to take place in the liver, the acetylation of serotonin has been demonstrated to occur in the rat brain and beef pineal gland³. In the present study, N-acetylmescaline has been identified along with TMPA and 2-(3,4,5-trimethoxyphenylethanol (TMPE) in the brain of rats receiving

mescaline. In contrast to that reported by SHAH and HIMWICH, with mice, the formation of N-acetylmescaline from mescaline was observed in vitro with the soluble supernatant fraction obtained from the rat brain. This report describes the results of our finding.

¹ N. NEFF, G. V. ROSSI, G. D. CHASE and J. L. RABINOWITZ, J. Pharmac. exp. Ther. 144, 1 (1964).

² N. S. SHAH and H. E. HIMWICH, Neuropharmacology 10, 547 (1971).

³ H. WEISSBACH, B. G. REDFIELD and J. AXELROD, Biochim. biophys. Acta 43, 352 (1960).

Table I. Characteristics of mescaline and derivatives

Compound ^a	Solvent system ^b (Rf)				
	A	B	C	D	E
Mescaline	0.02	0	0	0.62	0.83
TMPA	0.28	0.39	0.17	0.85	0.56
N-Acetylmescaline	0.53	0.16	0.17	0.84	0.88
TMPE	0.72	0.63	0.35	0.87	0.88

^a TMPA: 3,4,5-trimethoxyphenylacetic acid; TMPE: 2-(3,4,5-trimethoxyphenyl)ethanol. ^b Solvent systems for Silica plates: A, 2.5% methanol in chloroform (developed twice); B, ethyl acetate; C, 5% methanol in benzene; D, *n*-butanol-acetic acid-water (4:1:1); E, isopropanol-ammonium hydroxide (4:1).

Table II. Percent of metabolites in rat brain extract after administration of mescaline-¹⁴C

Metabolites ^a	Percent of metabolites ^b		
	3.5 mg/kg	11.5 mg/kg	25 mg/kg
Mescaline	57.8	67.8	76.8 (76.3)
N-Acetylmescaline	33.2	25.9	19.7 (19.6)
TMPA	3.1	2.7	2.0 (2.4)
TMPE	5.9	3.6	1.5 (1.7)

Animals were sacrificed 30 min after injection of varying doses of the radioactive mescaline. The figures in parentheses represent the percent of metabolites at 20 min. ^a Abbreviation for the compounds is same as that shown in Table I. ^b Corrected to 100% recovery.

Table III. In vitro metabolism of mescaline-¹⁴C in subcellular fractions obtained from the rat brain

Metabolites ^a	Percent of metabolites in each fraction ^b					
	Soluble		Microsomal		Mitochondrial	
	with acetyl CoA	without acetyl CoA	with acetyl CoA	without acetyl CoA	with acetyl CoA	without acetyl CoA
Mescaline	89.6	92.9	93.4	93.0	92.5	91.8
N-Acetylmescaline	5.1	0.9	1.4	1.8	2.1	1.9
TPMA	0.8	1.2	0.9	0.8	1.0	1.2
TMPE	1.3	1.5	1.2	1.4	1.1	1.4
Other ^c	3.2	3.5	3.1	3.0	3.3	3.7

^a Abbreviation for the compounds is same as that shown in Table I. ^b Corrected to 100% recovery. ^c Two unidentified metabolites of about equal magnitude in radioactivity, one being located at the solvent front and the other between TPMA and mescaline.

Materials and methods. Male Sprague-Dawley rats (200–250 g) were injected i.v. via the tail vein with 3.5, 11.5, and 25 mg/kg of mescaline-8-¹⁴C hydrochloride in saline. The animals were decapitated and the brains were removed and homogenized with 5 volumes of methanol. After centrifugation of the homogenate at 1,000 × g for 10 min, aliquots of the supernatant were assayed by liquid scintillation spectrometry. The remaining brain homogenate was evaporated in vacuo to a small volume (approx. 0.2 ml) and chromatographed on Silica Gel C precoated plates using 2.5% methanol in chloroform. The percentage of each metabolite on the plates was calculated by plotting of the radioactivity vs. R_f values. The unchanged mescaline and metabolites were identified by comparison with reference compounds for R_f values in various solvent systems (Table I).

For the in vitro metabolic study, the rat brain was homogenized in 5 volumes of ice cold 0.01 M phosphate buffer, pH 7.4, centrifuged at 75,000 × g for 1 h, and the supernatant was used for incubation. A mixture containing 50 μmoles of Tris buffer, pH 8.0, 15 μmoles of EDTA, 0.4 μmoles of mescaline-8-¹⁴C hydrochloride (specific activity 4.6 μCi/μmole), 0.57 μmoles of acetyl CoA, and 0.3 ml of the supernatant in a final volume of 1.5 ml was incubated at 37°C for 2.5 h. The control contained no acetyl CoA. The mixture was diluted to 10 ml with water and then extracted twice with 20-ml portions of benzene (designated as neutral extract). The aqueous phase was made strongly acidic with HCl and again extracted twice with benzene (acidic extract). Finally, the aqueous phase was made strongly alkaline with NaOH, and extracted with benzene (basic extract). The extracts from the same pH were combined, concentrated, and chromatographed as above. The percent of distribution of metabolites in the incubation mixture was calculated by combining the amounts of each metabolite in all three extraction fractions.

For the in vitro study of subcellular metabolism of mescaline 2 rat brains were combined and homogenized in 5 volumes of ice cold 0.25 M sucrose. Differential centrifugation of the homogenate separated the cell debris, mitochondrial (15,000 × g, 30 min), microsomal (100,000 × g, 60 min) and the soluble fraction. The mitochondria and microsome were each suspended in 5 ml of 0.01 M phosphate buffer, pH 7.4, and aliquots (0.3 ml) of mitochondrial and microsomal fractions were incubated with mescaline with or without the addition of acetyl CoA as described above.

Results and discussion. In the present study, using the Solvent System A or B, it is possible to separate all compounds listed in Table I.

The results of our study showed the presence of three metabolites of mescaline in rat brains: N-acetylmescaline, TPMA and TMPE (Table II); the major metabolite was N-acetylmescaline. SHAH⁴ was able to demonstrate the oxidative deamination of mescaline to TPMA with rat brain homogenate; the presence of 'mescaline oxidase' in the brain is therefore implied. TPMA was found in cat brains after intravenous administration of mescaline-¹⁴C; no other metabolites were reported¹. In the solvent systems they used, the R_f values of TMPE were too close to either that of TPMA or that of mescaline. Thus a possibility still exists for the formation of TMPE in the cat brain. N-acetylmescaline was reported to be the major metabolite of mescaline in human cerebrospinal fluid⁵, suggesting its existence in the human brain. The identification of this metabolite was also made in the mouse brain² and, in the present study, in the rat brain. Our results correspond well with that reported in the human CSF⁵ in that N-acetylmescaline is the major metabolite of mescaline in the CNS.

Increasing the dose of mescaline resulted in a decrease in the amounts of all metabolites (Table II). With 25 mg/kg dose the distribution of mescaline and metabolites in the rat brain did not vary from 20 to 30 min postinjection.

In the presence of the co-factor acetyl CoA, N-acetylmescaline, as in the case of in vivo study, is the major metabolic product of mescaline (Table III 'soluble fraction'). The amount decreased, however, when the addition of acetyl CoA was omitted in the incubation. Also included in Table III are the data on the metabolism of mescaline-¹⁴C in other subcellular, particulate fractions obtained from the rat brain. All these results indicate the ability of rats to acetylate mescaline in the brain, and the acetylation appears to occur predominantly in the non-particulate fraction of the cell.

Resumen. N-Acetylmescalina fue identificada con otros metabolitos en el cerebro de las ratas después de recibir ¹⁴C-mescalina. La formación de este metabolito acetilado

⁴ N. S. SHAH, Arch. int. Pharmacodyn. 193, 357 (1971).

⁵ K. D. CHARALAMPOUS, K. E. WALKER and J. KINROSS-WRIGHT, Psychopharmacologia 9, 48 (1966).

se demostró incubando la mescalina radioactiva con la fracción soluble del cerebro de las ratas. Nuestros resultados indican que el cerebro de dichos animales

puede acetilar mescalina, y que la acetilación ocurre predominantemente en la fracción soluble.

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⁷ The authors wish to thank Miss SANDRA CLARK for her technical assistance.

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7 August 1972.*