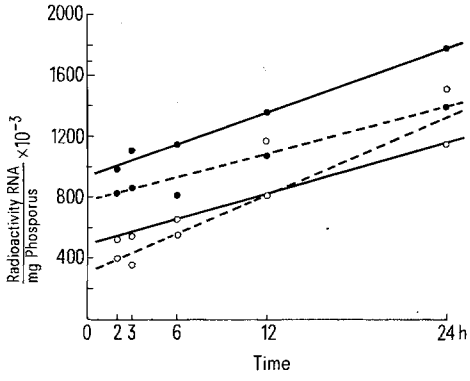
concentration of RNA particularly in the nerve cells, in an analogous manner to other neurotropic drugs[9,10]. At present, it is not possible to conceive whether LSD attains this effect by physico-chemical processes, by processes involving enzymes or acceptors[2]; one could also speculate whether, in 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 neurosecreation by the nerve cells[13].



Radioactivity of the RNA-fractions after incorporation of ^{3}H-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 ^{3}H-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 mercaptoaethanol[19], 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 attained[20].

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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[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-^{14}C. While this work was in progress Shah and Himwich[2] 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 gland[3]. 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 constrat 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.

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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-^{14}C

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-^{14}C 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
TMPA	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 TMPA 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-^{14}C hydrocloride in saline. The animals were decapitated and the brains were removed and homogenized with 5 volumes of methanol. After centrifugation of the homogenate at $1{,}000 \times g$ 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. Rf values. The unchanged mescaline and metabolites were identified by comparison with reference compounds for Rf 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 $75{,}000 \times g$ 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-^{14}C 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 homogenised in 5 volumes of ice cold 0.25 *M* sucrose. Differential centrifugation of the homogenate separated the cell debris, mitochondrial ($15{,}000 \times g$, 30 min), microsomal ($100{,}000 \times g$, 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, TMPA and TMPE (Table II); the major metabolite was N-acetylmescaline. SHAH[4] was able to demonstrate the oxidative deamination of mescaline to TMPA with rat brain homogenate; the presence of 'mescaline oxidase' in the brain is therefore implied. TMPA was found in cat brains after intravenous administration of mescaline-^{14}C; no other metabolites were reported[1]. In the solvent systems they used, the Rf values of TMPE were too close to either that of TMPA 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 fluid[5], suggesting its existence in the human brain. The identification of this metabolite was also made in the mouse brain[2] and, in the present study, in the rat brain. Our results correspond well with that reported in the human CSF[5] 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-^{14}C 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-Acetilmescalina fue identificada con otros metabolitos en el cerebro de las ratas después de recibir ^{14}C-mescalina. La formación de este metabolito acetilado

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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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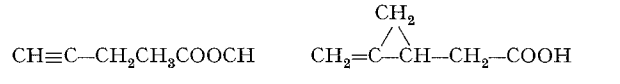
[7] The authors wish to thank Miss Sandra Clark for her technical assistance.

Elevation of Hepatic Tyrosine 2-Oxoglutarate Aminotransferase in Rats by 4-Pentynoic Acid, a Hypoglycemic Agent

Hardeland[1] reported that quinolinic acid and quinaldic acid, two tryptophan metabolites that are hypoglycemic, elevate tyrosine aminotransferase (TAT) in rat liver. He suggested that the enzyme change was related to the hypoglycemia caused by the two agents and possibly resulted from secretion of an anti-hypoglycemic hormone like glucagon[2]. Because of that suggestion, we are reporting studies on a structurally dissimilar hypoglycemic agent, 4-pentynoic acid, which according to unpublished studies in these laboratories has pharmacologic properties much like those of hypoglycin. Hypoglycin elevates plasma urea concentrations[4] by a mechanism thought to involve increased deamination of amino acids that serve as gluconeogenic precursors[5], providing another basis for expecting hypoglycin-like compounds to elevate amino acid catabolizing enzymes such as TAT.



4-pentynoic acid

methylenecyclopropylacetic acid (active metabolite of hypoglycin[3])

Materials and methods. Male 150 g Wistar rats were used in groups of 5. The rats received food and water ad libitum prior to the experiment but only water during the experimental period. All rats were killed at the same time (during midday), having received injections at specified times earlier, to minimize effects of diurnal variation in enzymes and metabolites[6]. 4-Pentynoic acid was synthesized in the Lilly Research Laboratories. Livers from decapitated rats were rapidly removed and frozen on dry ice. Hepatic TAT was measured spectrophotometrically[7], corticosterone was measured fluorometrically[8], and glucose and glycogen (after isolation and hydrolysis[9]) were measured using Worthington glucostat reagents[10].

Results and discussion. Figure 1 shows that the activity of hepatic TAT was elevated within 1 h after injection of 4-pentynoic acid. Highest enzyme activity, nearly 4 times the control level, was reached at 3 h. Enzyme activity had begun to return toward control values at 5 h.

Other changes induced by 4-pentynoic acid are shown in Figure 2. Plasma glucose levels were decreased, the maximum effect being at 1 h. Hepatic glycogen levels were decreased along a similar time course. Plasma corticosterone levels were markedly increased initially but had returned nearly to control levels by 5 h.

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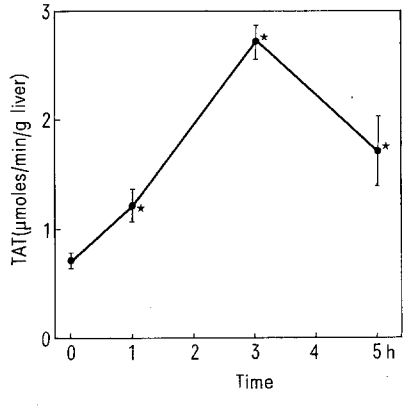

Fig. 1. Elevation of TAT by 4-pentynoic acid. 4-Pentynoic acid was injected s.c. at 10 mg/kg. Mean values with standard errors are shown for 5 rats per group. Values marked with an asterisk were significantly different ($P < 0.025$) from zero time.

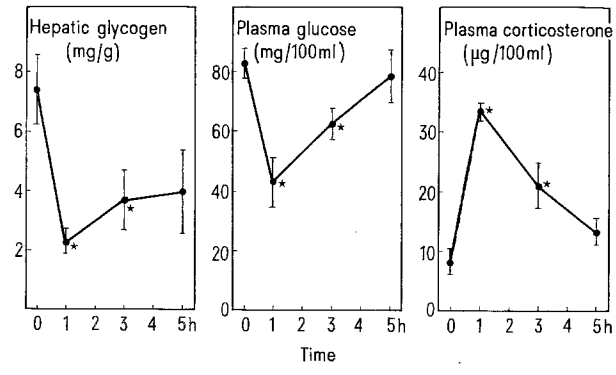

Fig. 2. Time course of changes in plasma glucose, hepatic glycogen, and plasma corticosterone levels caused by 4-pentynoic acid in rats. 4-Pentynoic acid was injected s.c. at 10 mg/kg. Mean values with standard errors are shown for 5 rats per group. Values marked with an asterisk were significantly different ($P < 0.05$) from zero time.