RNA in Planarians (*Dugesia lugubris*) Treated with LSD

According to recent works, nucleic acids RNA and DNA react chemically with neurotropic substances, particularly with LSD[1-3]. Among the different forms of RNA, the one present in the cellular membranes might be particularly exposed to the action of the drugs[4-8]. If this RNA takes part in the permeability process of the cellular membrane[3], its interaction with the drug could affect the permeability process. In a general way, an investigation on the possible relationship between the chemical action of a drug and the RNA contents and metabolism within the cells seems to be of interest, because it is known that various chemical activities or physical stimulations can modify RNA composition[9,10].

In the present work, the quantitative changes are analyzed of the RNA present in the cells of the cephalic or retrocephalic body parts of either normal or LSD-treated fresh-water planarians. Informations are also reported on the incorporation, under these conditions, of ^{3}H-uridine into RNA.

Fresh-water planarians are very reactive to the action of psychodrugs. For instance, the regenerative powers, normally highly developed in these animals, are blocked by amphetamine[11] and restored by a contemporary administration of riboflavine or its derivatives[12]. On the other hand, LSD only reduces the planarian regenerative powers, but induces in a few days a complete atrophy of the male and female gonads, both in whole animals and those regenerated after beheading[13].

Experiments. In our experiments, 600 specimens of the planarian *Dugesia lugubris*, of a uniform length and age, were used. These specimens were distributed into 4 groups: 2 of 180 animals each (groups I A and I B), and 2 of 120 animals each (groups II A and II B). The animals of groups I A and II A were kept for 8 days in a spring water solution of LSD-tartrate (mg 0.02/ml), and on the last day a sufficient quantity of tritiated uridine to obtain a radioactivity of 20 μC/ml was added. The animals of groups I B and II B, kept in spring water alone, served as a control to the other groups and were similarly treated on the 8th day with the same quantity of tritiated uridine.

At the end of the experiment, 20 planarians were taken at a time and transversely cut in 2 pieces by a cutting plane anterior to the pharynx. The anterior and posterior pieces, separately, were immediately homogenized, in the cold, in a micropotter with 1–1.2 ml distilled water. Five aliquots of 0.2 ml were taken from each solution and were kept at −25 °C until used. The aliquots taken from groups I A and I B were analysed for total phosphorus and for radioactivity incorporated in the RNA. The samples from groups II A and II B were quantitatively analysed for RNA and total phosphorus. The RNA contents were analyzed by a slightly modified version of the methods of CORNING and FREED[14], SCOTT et al.[15]. The total phophorus analysis was done by the method of FERRARI[16], modified[17] for the organic materials. The incorporation of ^{3}H-uridine into RNA has been determined as radioactivity according to the LIEBERMAN et al. method[18]. Our results, statistically analyzed, are described in Tables I and II and in the Figure.

According to the data of Table I, the amount of RNA in cephalic pieces, containing the cerebral plexus or 'brain' very rich in nerve cells, was significantly greater in planarians treated with LSD than in control ones. On the contrary, in retrocephalic pieces, containing only a small number of nerve cells mainly condensed in the slender posterior nerve cords, the amount of RNA is about the same in the treated and control planarians. From such results, always confirmed in our repeated experiments, it seems reasonable to suppose that LSD increases the

Table I. RNA concentration in the cephalic or retrocephalic pieces of the normal or LSD-treated specimens of the planarian, *Dugesia lugubris*

Cephalic pieces of planarians treated with 0.02 mg/ml LSD for 8 days	1880 ± 43	$t = 8.405$
Cephalic pieces of planarians kept in spring water alone	1130 ± 78	$P < 1\%$
Retrocephalic pieces of planarians treated with 0.02 mg/ml LSD for 8 days	1301 ± 71	$t = 0.650$
Retrocephalic pieces of planarians kept in spring water alone	1367 ± 63	$P > 50\%$

The average values of RNA concentration, obtained from 6 determination using pools of 20 animals each, are given γ RNA/mg of phosphorus.

Table II. Radioactivity of the RNA fractions after incorporation of ^{3}H-uridine, at different time

Time (h)	LSD (0.02 mg/ml) Cephalic pieces	LSD (0.02 mg/ml) Retrocephalic pieces	Controls Cephalic pieces	Controls Retrocephalic pieces
2	980 ± 20[a]	520 ± 36	830 ± 48	405 ± 24
3	1100 ± 27	545 ± 17	863 ± 86	358 ± 21
6	1144 ± 115	655 ± 25	815 ± 40	553 ± 24
12	1364 ± 44	1171 ± 43	1074 ± 34	816 ± 25
24	1770 ± 322	1116 ± 103	1398 ± 78	1524 ± 149

The average values are expressed as H_3 Uridine incorporated (dpm per mg total Phosphorus $\times 10^{-3}$). [a] Standard deviation.

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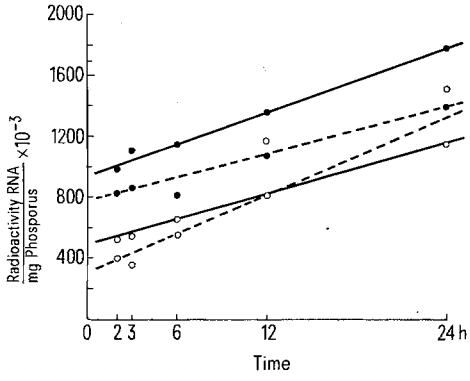
concentration of RNA particularly in the nerve cells, in an analogous manner to other neurotropic drugs[9,10]. At present, it is not possible to conceive whether LSD attains this effect by physico-chemical processes, by processes involving enzymes or acceptors[2]; one could also speculate whether, in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