THE EFFECT OF 4-HYDROXYINDOLES ON THE METABOLISM OF 5-HYDROXYTRYPTAMINE (SEROTONIN)*

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Psilocybin has been shown to be the active component of the Mexican mushroom, *Psilocybe mexicana*.[1] It belongs to the category of hydroxylated indoles showing hallucinogenic and vasopressor properties. Chemically it is the O-phosphoryl ester of psilocin (4-hydroxy-N-dimethyltryptamine) (FIGURE 1). Psilocin in turn is the 4-hydroxy analogue of bufotenine (5-hydroxy-N-dimethyltryptamine). Thus psilocin and bufotenine are the dimethyl derivatives of 4-hydroxytryptamine (4HT) and 5-hydroxytryptamine (serotonin, 5HT) respectively.

Serotonin, the decarboxylation product of 5-hydroxytryptophan, is oxidized to its aldehyde by the action of monoamine oxidase (MAO) and then further metabolized to 5-hydroxyindoleacetic acid (5HIAA). In an analogous fashion 4-hydroxytryptophan, which may be the natural precursor of psilocybin, is metabolized to 4-hydroxyindoleacetic acid (4HIAA).[2] These pathways of metabolism are catalyzed by the same enzymes, that is, 4HT is a substrate for MAO. However, psilocybin resists oxidation by MAO *in vitro*.[3] This may be due to interference by the phosphate ester group, although the phosphate ester is readily hydrolyzed by alkaline phosphatase (unpublished data) and intestinal phosphatase,[4] giving rise to psilocin. Both psilocin and bufotenine are substrates for MAO, although poor ones. Because of the similarity in chemical structure and pathways of metabolism between the 4- and 5-hydroxyindoles it was of interest to study the effect of the 4-hydroxyindoles on the catabolism of 5HT.

Methods and Materials

The terminal product of serotonin breakdown, 5HIAA, was measured by a modification of the method of Udenfriend *et al.*,[5] making use of the nitrosonaphthol reaction.

Equimolar solutions of pure indoles (0.6 μmole) were reacted and compared (FIGURE 2). It was observed that only indoles with a free 5-OH group, for example 5HT, show an absorption maxima at 540 mμ. No interference with 5HIAA recoveries was observed when psilocybin, psilocin, or 4HT were added to control urines. There was also no interference with recoveries when 5HIAA was added to urines collected after treatment with psilocybin. Attempts were made to measure the 4OH-indoles, using both the Keller[6] and xanthydrol[7] reactions, but these were not deemed of sufficient specificity and sensitivity to separate and measure the 4-hydroxyindoles. In this respect our experience is different from that of Horita and Weber[4] who measured psilocin as the nitrosonaphthol color complex at 430 mμ.

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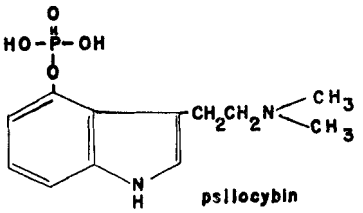
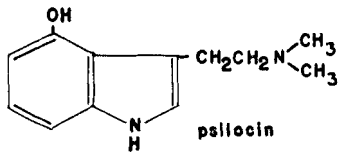
Urines were collected from seven patients receiving psilocybin intravenously. The excretion of 5HIAA[8] was measured before and after intravenous injection.

Male Sprague-Dawley rats, 3 per group, were injected intraperitoneally with 0.11 mM/kg. of psilocybin or psilocin. They were then placed in metabolism cages, and urines were collected for 6- or 24-hour periods.

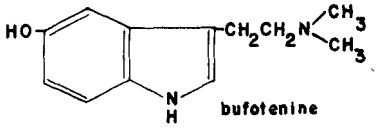
For the serotonin-load test rats received 2 mg./100 gm. 5HT intraperitoneally, simultaneously with the psilocybin or psilocin dose, and urines were collected in timed intervals. The test animals received intraperitoneal injections of psilocybin (3.2 mg./100 gm.) or psilocin (1.5 mg./100 gm.) as well as



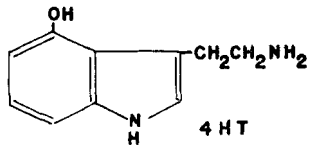
(O-phosphoryl-4-hydroxy-N-dimethyltryptamine)



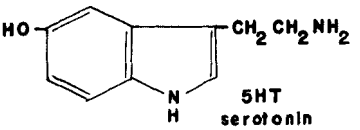
(4-hydroxy-N-dimethyltryptamine)



(5-hydroxy-N-dimethyltryptamine)



(4-hydroxytryptamine)



(5-hydroxytryptamine)

FIGURE 1.

the 5HT. The control animals were similarly injected with 5HT. In place of the 4-hydroxyindole solution, they received equivalent volumes of phosphate buffer, pH 7.4. The 0.2 M buffer was used as the solvent for all the indole solutions.

Male Sprague-Dawley rats or male albino guinea pigs were decapitated and the livers excised. These were then homogenized in 0.25 M sucrose and centrifuged at 18,000 g. for 2 hours. The supernatant and particulate fractions were separated and stored at $-15°$. After thawing, these were incubated with 5HT in 0.2 M phosphate buffer, pH 7.4, at 37° for 2 hours under a flow of 95 per cent oxygen–5 per cent CO_2. The 4-hydroxyindoles were added to the incubation mixtures in 10^{-3} M concentrations. Only 1 experiment was carried out on the particulate fraction prepared from guinea-pig liver, the fraction in which the

major portion of cell MAO is found. However, MAO activity is difficult to assess under these experimental conditions because most of the aldehyde formed is converted into the difficultly measurable pigment rather than further metab-

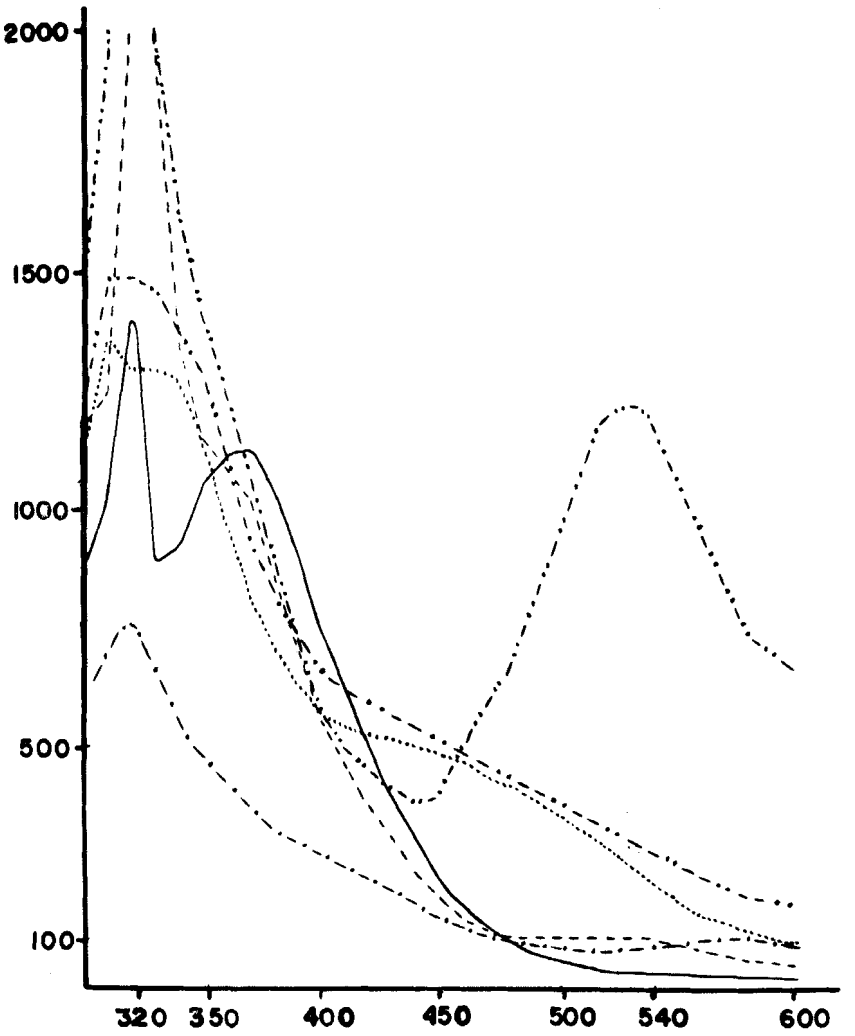


FIGURE 2. Absorption spectra of hydroxyindoles reacted with nitrosonaphthol reagent. Key: -·-·-·-·-, 5HT; - - -·- - -·- - -, 4HT; - - - - - - -, 5MeOT; ·····, psilocin; ———— psilocybin; and -·-·-·-·-, 6HT.

olized to 5HIAA. This does not allow for differentiation of 5HT metabolized along pathways other than MAO.

Results and Discussion

In urines collected from patients receiving psilocybin, no consistent change in excretion of endogenous 5HIAA could be discerned (TABLE 1). Similarly in

rats no change in endogenous 5HIAA excretion after intraperitoneal injection of psilocybin or psilocin was observed (TABLE 2). Because no effect on the endogenous metabolism of 5HT was apparent, animals were subjected to serotonin load tests.

If only a total 6- or 24-hour urine collection is assayed, no difference in the urinary 5HIAA response to a 5HT load may be discerned (TABLE 3). The results of two experiments indicate that the effect of both psilocybin and psilocin on 5HIAA excretion after a 5HT load varies with time. Thus psilocybin at first decreases the excretion of 5HIAA and later increases it. Psilocin has the reverse effect, first enhancing and then decreasing the excretion. Normally

TABLE 1
THE EFFECT OF INTRAVENOUS PSILOCYBIN ON URINARY 5HIAA IN HUMANS

Patient		Diagnosis	Psilocybin (mg.)	Mg. 5HIAA/6 hr.		Δ 5HIAA
Sex	Age			Basal	Postpsilocybin	
M	39	Anxiety neurosis	18	1.10	3.98	2.88
M	28	Alcoholism	12	0.77	0.94	0.17
F	26	Schizophrenia	18	0.53	0.76	0.23
F	43	Anxiety neurosis	18	1.15	0.99	−0.16
F	28	Severe hysteria	12	1.15	0.96	−0.19
F	21	Anxiety neurosis	12	0.44	0.17	−0.27
F	27	Anxiety neurosis	18	0.13	1.16	1.03

TABLE 2
THE EFFECT OF PSILOCYBIN AND PSILOCIN ON ENDOGENOUS URINARY 5HIAA IN RATS

Treatment	μg. 5HIAA/6 hr./100 gm.	μg. 5HIAA/24 hr./100 gm.
Control	9	18
Psilocybin*	5	20
Psilocin†	10	26

* Dosage, 3.2 mg. psilocybin/100 gm. intraperitoneally.
† Dosage, 2.3 mg. psilocin/100 gm. in 6-hr. experiment and 1.5 mg./100 gm. in 24-hr. experiment intraperitoneally.

the peak excretion of 5HIAA is observed in the 2 to 4 hour period after administration of a 5HT load (FIGURE 3), but treatment with psilocybin results in a delay in the appearance of this peak until the 4 to 6 hour period. Psilocin, on the other hand, accelerates the excretion of the major portion of exogenous 5HT recovered as urinary 5HIAA. The delay in excretion of 5HIAA after a 5HT load is probably due to the vasopressor effect of 5HT on the blood vessels of the kidney. Psilocybin also has a vasoconstrictor effect which is longer-lasting than that of 5HT. This effect has been correlated with a relatively lower rate of metabolism by the MAO pathway.[3] Because of its toxicity* the

* When psilocin in a solution, buffered to *p*H 7.4, was administered intraperitoneally at a dose of 2.3 mg./100 gm. along with 5HT, 2 mg./100 gm., 2 out of 3 rats so treated died within 24 hours, while the third developed marked necrosis at the site of injection. Two out of 3 animals, receiving psilocin only, developed similar ulceration. Administration of 5HT by itself does not have this effect.

dose of psilocin was reduced from 2.3 mg./100 gm. to one half, or less than one half, of the molar equivalent of the doses of 5HT or psilocybin administered. The immediate excretion of increased amounts of 5HIAA after psilocin may be due to an antagonistic effect of the latter on the pressor activity of 5HT. This would favor a more rapid circulation and metabolism of the amine and, therefore, earlier excretion of the metabolic product of 5HT breakdown. However, despite the increase in early excretion of 5HIAA, the total recovery of exogenous 5HT as urinary 5HIAA tends to be decreased by psilocin.

TABLE 3

THE EFFECT OF 4-HYDROXYINDOLES ON URINARY 5HIAA AFTER A 5HT LOAD

Treatment*	Dosage (mg./100 gm.)	μG. 5HIAA					
		Hrs. 0–2	Hrs. 2–4	Hrs. 4–6	Hrs. 6–24	Hrs. 0–6	Hrs. 0–24
Experiment 1							
5HT	2					204	
5HT + psilocybin	3.2					262	
5HT + psilocin	2.3					33	
Experiment 2							
5HT	2						973
5HT + psilocybin	3.2						803
5HT + psilocin	1.5						829
Experiment 3							
5HT	2	2	137	71	441	210	651
5HT + psilocybin	3.2	0.5	77.5	115	193	193	816
5HT + psilocin	1.1	66	186	122	374	374	495
Experiment 4†							
5HT	2	9	681	125	100	825	925
5HT + psilocybin	3.2	4	258	319	279	581	860
5HT + psilocin	1.5	260	306	162	210	728	938

* All indoles were dissolved in 0.2 *M* phosphate buffer, *p*H 7.4, and administered intraperitoneally.

† Two milliliters of 5 per cent glucose were administered by gastric tube to all rats 30 min. prior to indole injection. Drinking bottles also contained 5 per cent glucose instead of water.

In the studies carried out *in vitro* it was observed that only 4HT affects the metabolism of 5HT by guinea pig-liver mitochondria: it has a slight promoting effect on the amount lost during incubation (TABLE 4). In the supernatant fraction prepared from the same livers and incubated simultaneously, MAO activity was directly measured by 5HIAA formation, because no pigment production is observed. This pathway does not account for all the 5HT that disappears, as loss of 5HT occurs along metabolic pathways not as yet clearly defined.[9]

The results obtained with psilocybin confirm the observation made *in vivo* that this compound does not directly affect 5HT metabolism. Indeed, in liver supernatants of the two species, only psilocin had a marked inhibitory effect

on both 5HT disappearance and 5HIAA formation (TABLE 5). Although 4HT was inhibitory, this was of a lesser degree. A graphic presentation (FIGURE 4) of these data illustrates more succinctly that psilocybin affects neither total 5HT disappearance nor 5HIAA formation. Psilocin, on the other hand, greatly inhibits 5HT disappearance and 5HIAA formation. Inhibition of the latter appears to be slowly reversible with time.

These observations do not correlate with the results obtained *in vivo*. It

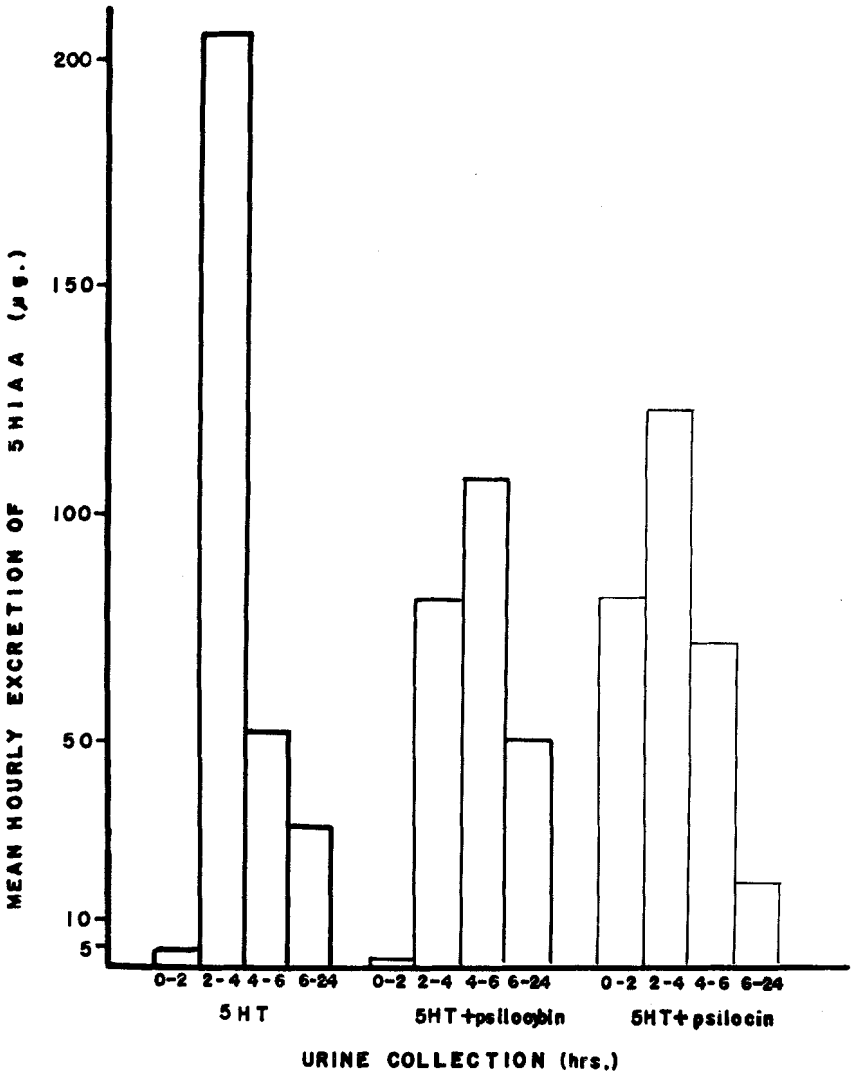


FIGURE 3. The effect of psilocybin and psilocin on urinary 5HIAA after a serotonin load. See the legend for TABLE 3 and the text.

will be recalled that psilocin enhances 5HIAA excretion in the first two-hour period after treatment. However, studies *in vitro* indicate that psilocin inhibits 5HIAA formation and that this effect is slowly reversible. On the other hand the over-all excretion of 5HIAA after a 5HT load and treatment with psilocin tends to be diminished.

TABLE 4
EFFECT OF 4-HYDROXYINDOLES ON 5HT METABOLISM BY GUINEA PIG-LIVER FRACTIONS

Treatment	Mitochondria		Supernatant	
	μG. total 5HT disappearing	μG. 5HIAA formed	μG. total 5HT disappearing	μG. 5HIAA formed = 5HT
Control (+5HT)*	1523	63	1197	631
+Psilocybin†	1523	64	1253	609
+Psilocin†	1523	63	584	463
+4HT†	1727	50	1075	479

* Mitochondrial preparations were incubated with 2.2×10^{-3} *M* 5HT, supernatants with 1.5×10^{-3} *M* 5HT.
† All 4-hydroxyindoles were added to a concentration of 10^{-3} *M*.

TABLE 5
THE EFFECT OF 4-HYDROXYINDOLES ON 5HT METABOLISM *IN VITRO*

Supernatant*	15 Min.		30 Min.		45 Min.		90 Min.†		120 Min.	
	μG. 5HT	μG. 5HIAA	μG. 5HT	μG. 5HIAA	μG. 5HT	μG. 5HIAA	μG. 5HT	μG. 5HIAA	μG. 5HT	μG. 5HIAA
Treatment‡										
Control	464	289	636	402	765	573	637	110	898	438
Psilocybin	458	288	563	438	683	550	632	93	940	421
Psilocin	111	58	196	111	264	158	177	12	438	319
4HT							417	53	806	319

* Supernatants prepared from livers of guinea pigs homogenized in 0.25 *M* sucrose (50 per cent w/v) and centrifuged at 18,000 g. for 2 hours at 2° C.
† Data obtained using supernatants prepared from rat livers.
‡ Ten^{-3} *M* 5HT in each incubate containing supernatant equivalent to 1 gm. wet weight of tissue $\pm 10^{-3}$ *M* 4-hydroxyindole.

From the data presented it can be concluded that psilocybin does not have a direct effect on 5HT metabolism. The delay in excretion of 5HIAA observed after administration of a 5HT load may be attributed to the prolonged vasopressor activity of psilocybin. On the other hand psilocin does decrease the rate of 5HT breakdown *in vitro*. This is not as clearly demonstrable *in vivo* because of what appears to be an "anti-5HT-pressor" effect of psilocin. Psilocybin does not cause any of the effects observed after treatment with psilocin. From this observation it may be inferred that if psilocybin and psilocin are in equilibrium *in vivo* the predominant direction of this equilibrium is toward psilocybin.

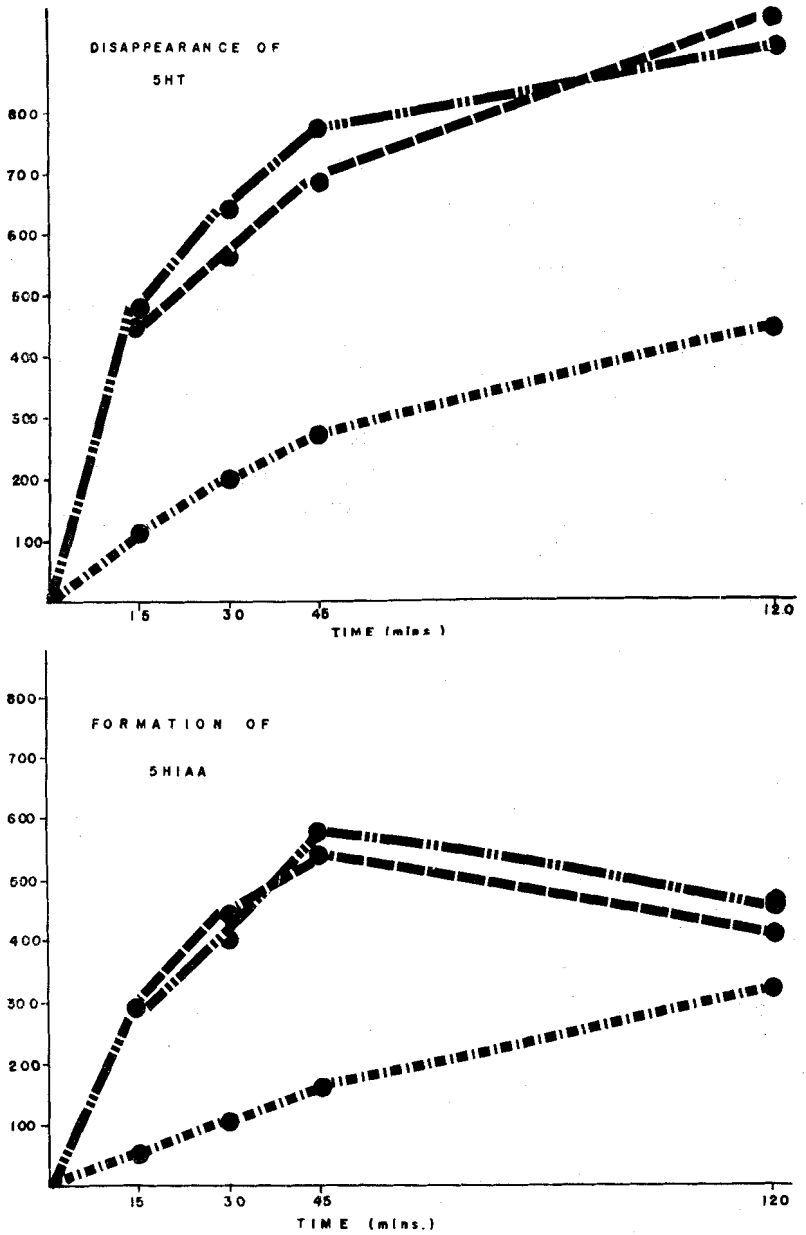


FIGURE 4. The effect of psilocybin and psilocin on 5HT metabolism *in vitro*. Key: ▬▬, control; ▬ ▬ ▬, $+10^{-3}$ *M* psilocybin; and ▬ ▮ ▬ ▮ ▬, $+10^{-3}$ *M* psilocin. See the legend for TABLE 5 and the text.

Acknowledgments

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Discussion of the Paper

Herbert Sprince (*Veterans Administration Hospital, Coatesville, Pa.*): My comment may seem peripheral to Wiseman-Distler's paper but I think it is important to consider, in general, the hydroxylation of tryptamines.

Alpha-hydroxytryptamine has been shown to be a monoamine oxidase inhibitor. Psilocybin and psilocin, 4-hydroxytryptamines, have been shown to be psychoactive.

Similarly serotonin, a 5-hydroxytryptamine, has been shown to be psychoactive or related to psychoactivity; and 6-hydroxytryptamine also is implicated in psychoactivity.

One wonders about 7-hydroxytryptamine and the general process of hydroxylation of the tryptamine nucleus with respect to psychotogenicity.

G. S. Duboff (*University of Michigan, Ann Arbor, Mich.*): The Wiseman-Distler paper, which seemed to evolve around the indole nucleus, prompts me to ask whether any of the investigators have considered the possibility of the formation of a hydrazine as a consequence of induction by the administration of these pharmacologic agents.

It is well known that for many years it has been considered that hydrazines are not produced by living substances, but recently there appeared to be fairly good evidence that living substances can produce hydrazines.[1]

In all procedures used in the studies today, a hydrazine would not be detected; although the methods presently used demonstrate indole-acetic acids and all the other metabolites. Such methods do not show clearly whether some very small amounts of hydrazine or other peculiar nitrogen-ring formation can produce psychomimetic and psychotropic effects. However, since these compounds are not being looked for and, apparently, are not being considered, it seems to me that the nitrogen in the indole, in the five-numbered pyrrole ring, could be subjected to certain rearrangements resulting, possibly, in the formation of a substituted hydrazine.

Axelrod: I am sure that if one looked hard enough one probably would find it.

Resnik (*Cleveland Clinic, Cleveland, Ohio*): With respect to Wiseman-Distler's measurement of the 5-hydroxyindoleacetic acid in the urine, I note that

there are spaces of 2 hours. Rats usually urinate at intervals that are somewhat haphazard due, probably, to a distribution problem. May I ask whether Wiseman-Distler took this into account in some way; also, may I inquire how many animals were used in those experiments. Could these variations, perhaps, be due solely to chance?

WISEMAN-DISTLER: No. This is a type of result that we have obtained over a great many experiments. Our animals usually weighed about 150 gm. We used 3 animals per group and, occasionally, gave them 5 per cent glucose to increase urination; we obtained no change in the results. This is the typical pattern that is seen after dosing an animal with serotonin.

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