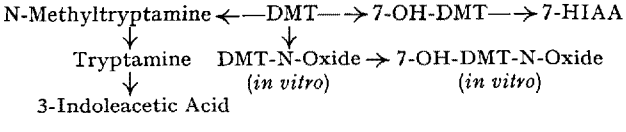
spots also gave color reactions with reagents claimed to be specific for 7-hydroxy indoles (such as acid diazo p-nitroaniline or sulfanilic acid)[7]. Since authentic 7-hydroxy-DMT and its N-oxide have not been described, we prepared these compounds enzymatically and by the model hydroxylating system[8]. When DMT-N-oxide was incubated with the microsomal enzyme system, only one compound was formed which gave the above-mentioned color reactions for 7-hydroxy indoles and had the same *R*f values as one of the two 7-hydroxy indoles formed from DMT.

These observations suggest that one of the hydroxylated metabolites of DMT is most probably 7-hydroxy-DMT-N-oxide. The remaining spot did not have the same *R*f as 7-hydroxytryptamine (prepared enzymatically from tryptamine), nor did it give a reaction for secondary amines, indicating that it is a tertiary amine, presumably 7-hydroxy-DMT. Additional evidence for the idendity of the latter compound was obtained by comparing it with 7-hydroxy-DMT formed in the model hydroxylating system, using DMT as a substrate. Both had the same *R* f values and color reactions. There was no evidence for hydroxylation on the 5-position by the microsomal enzyme system.

In the *in vivo* studies, rats[9] were given 10 mg DMT intraperitoneally and the urine was collected for 48 h. The urine was adjusted to pH 8·0, extracted with benzene and the benzene extract re-extracted with 1 N-HCl. The acid extract contained compounds having the same *R*f values and color reactions as DMT, N-methyl-tryptamine, 7-hydroxy-DMT (prepared enzymatically) and tryptamine. The residual urine was adjusted to pH 9·5 and extracted with n-butanol. After adding two volumes of n-heptane, the extract was shaken with acid. The acid extract contained compounds having the same *R*f values and color reactions as: 7-hydroxy-DMT (enzymatic), indole acetic acid, and an unidentified compound giving the reactions for 7-hydroxy indoles. When the acidified urine was extracted with n-butanol and the n-butanol phase re-extracted with dilute ammonia after adding n-heptane, two compounds were found having the same *R*f values, color reactions, and fluorescent characteristics as 3-indoleacetic acid and enzymatically produced 7-hydroxy-indole-acetic acid (7-HIAA).

From the results described above, the following scheme for the metabolism of DMT may be provisionally drawn:



Whether or not any of the metabolites of DMT play a role in the production of the psychotic phenomena must remain a question for future study.

Note added in proof. – In the meantime, Dr. JEPSON[9] called to our attention that the blue Ehrlich test and the immediate red color with acid diazo reagents which have been described and held as specific for 7-hydroxyindoles by ICHIHARA *et al.*[7] is not specific for 7-hydroxyindoles since 6-hydroxyindoles also give these tests, although in a slightly different color. We then prepared 6-hydroxy-dimetyltryptamine by total synthesis. This compound proved to be identical in every respect with the hydroxylated derivative of DMT produced by the microsomal enzyme system, by UDENFRIEND's model hydroxylating system, as well as with hydroxy-DMT isolated from urine. Thus, the hydroxyl group in the above studied indole derivatives is most probably in the 6- rather than the 7-position.

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Clinical Neuropharmacology Research Center and Laboratory of Clinical Sciences, NIMH, NIH, Washington (D.C.) and Bethesda (Md.), November 12, 1958.

Zusammenfassung

Der intermediäre Stoffwechsel des psychotropen N,N-Dimethyltryptamins wurde untersucht. N-Methyltryptamin, als Produkt der Demethylierung, und 6-Hydroxy-N,N-Dimethyltryptamin, als Produkt der Hydroxylierung, wurden nachgewiesen und papierchromatographisch charakterisiert. An weiteren Stoffwechselprodukten wurden nachgewiesen: N,N-Dimethyltryptamin-N-oxyd und sein 6-Hydroxy-Derivat (*in vitro*), sowie Tryptamin, Indol-3-essigsäure und 6-Hydroxyindol-3-essigsäure (*in vivo*).

Increased Excretion of 5-Hydroxy-indole-acetic Acid after the Administration of 3-Indole-acetic Acid (Heteroauxine)

It has been demonstrated recently that 5-hydroxy-indole-acetic acid (5-HIAA)[1] may be formed from certain exogenous precursors. SZARA[2] has observed an increased excretion of 5-HIAA after the application of dimethyltryptamine, ANDERSON, ZIEGLER, and DOEDEN[3] after consumption of a large amount of bananas. WAALKES *et al.*[4] have shown that besides other substances bananas contain large amounts of serotonine.

3-indole-acetic acid (IAA) is ingested in small amounts with some foods and is formed also as a tryptophan metabolite by the action of the intestinal bacterial flora. It is interesting therefore to follow the fate of IAA in the organism and its influence on the excretion of 5-HIAA. The fate of IAA in the animal was studied by ERSPAMER[5]. This author found that about one third of the administered IAA was excreted unchanged or in a conjugated form. He believes that the other part is probably metabolised after breaking up the indole ring.

In our own metabolic studies we have also investigated the fate of IAA in the human body.

In our experiment, eleven volunteers were given IAA, in the form of a 5% solution of the acid carbonate in tea[6], which was sweetened with saccharine or sucrose. The dosage of the applied IAA is shown on the Table.

[8] S. UDENFRIEND, C. T. CLARK, J. AXELROD, and B. B. BRODIE, J. biol. Chem. *208*, 731 (1954).

[9] J. J. JEPSON, S. UDENFRIEND, and XY. ZALZMANN, Fed. Proc. *81*, 754 (1959). (Personal Communication).

[1] We thank Sandoz, Ltd., Basle and Professor V. ERSPAMER, Institute of Pharmacology, University of Parma (Italy) for the generous gifts of 5-hydroxy-indole-acetic acid.

[2] ST. SZÁRA, Exper. *12*, 444 (1956).

[3] J. A. ANDERSON, M. R. ZIEGLER, and D. DOEDEN, Science *127*, 236 (1958).

[4] T. P. WAALKES, A. SJOERDSMA, C. R. CREVELING, H. WEISSBACH, and S. UDENFRIEND, Science *127*, 648 (1958).

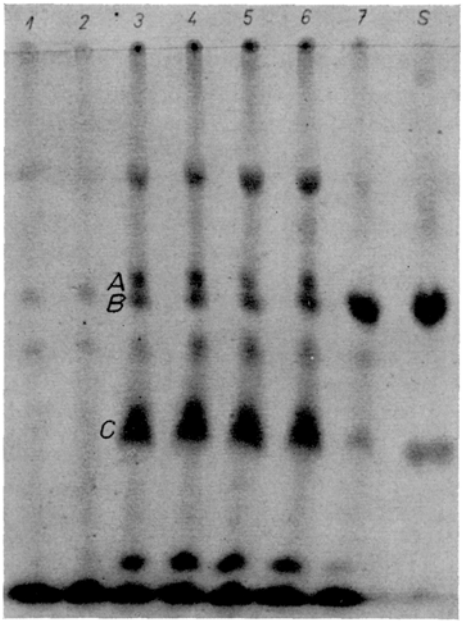
[5] V. ERSPAMER, J. Physiol. *127*, 118 (1955).

[6] I. A. MIRSKY, and D. DIENGOTT, Proc. Soc. exp. Biol. Med. *93*, 109 (1956).

Excretion of 5-HIAA/h 1–8 h, after the administration of IAA
(the values of 5-HIAA are calculated for hourly averages)

Experimental subject No.	Ingested IAA in g	5-HIAA/h in the urine (μg)			Percentual increase of 5-HIAA	
		before the administration	after the administration of IAA			
			1–4 h	4–8 h	1–4 h	4–8 h
1	6·85	190	727	399	289	112
2	8·00	239	286	308	19	29
3	4·90	205	475	246	132	20
4	10·50	253	476	771	88	205
5	6·30	191	416	490	117	156
6	3·00	148	290	—	96	—
7	3·00	377	657	683	74	81
8	3·00	81	297	333	267	311
9	3·00	104	335	345	222	232
10	3·00	150	345	226	129	51
11	3·00	210	412	351	96	67

4-h samples of urine were analysed, one being taken before the administration of IAA and two after it (e.g. urine was gathered for one 4-h period before the administration and two 4-h periods after it). This urine was kept in a frozen state before the analysis. 5-HIAA was measured in all cases by means of the colorimetric method[7] which we modified by increasing the number of chloroform extractions (three instead of two). In order to check the colorimetric values and to disclose the possible presence of other metabolites, we used paper chromatography on Whatman No. 3 paper and repeated it three times, in the solvent system propanol-ammonia (23%)-water (200 + 10 + 20), that is a modification of JEPSON's solvent system[8]. Spots were detected by means of dimethyl-aminobenzaldehyde[8] and diazotized sulphanilic acid[9]. The size of the samples of urine used for chromatography depended on the hourly diuresis; the extraction was made according to UDENFRIEND *et al.*[7].



Chromatogram of the urine extract after the detection with *p*-dimethyl-aminobenzaldehyde (experimental subject No. 9)

(*1,2* controls; *3,4* the first 4-h samples after the application of IAA; *5,6* the second 4-h period samples; *7* 5-HIAA added to urine; *S*-standards for 5-HIAA and IAA)

The content of 5-HIAA in the urine increased markedly in the very first hours, as is shown in the Table.

Spot *A* belongs to an unknown substance which does not occur in normal urine. During the detection it reacts immediately, taking on an intense bluegreen colour which partly fades after a while. According to its chromatographic qualities and colour reactions during detection, we presume it to be 7-hydroxy-indole-acetic acid[10]. Spot *C* corresponds to IAA, spot *B* to 5-HIAA; besides that there are further substances probably indole compounds which we have not identified. Some of them are new, others are increased.

We have demonstrated a marked increase of the excretion of 5-HIAA in the urine after the administration of large oral doses of IAA. We believe that the ingested IAA is hydroxylated in the organism in position 5. This is supported by the substrate non-specifity of tryptophane oxidase[11]. The experiments of ICHIHARA *et al.*[9] also support it. These authors demonstrated *in vitro* the oxidation of indole acids in position 5 and 7. They did not work with IAA, however. Our work points out a new possible way for the occurrence of 5-HIAA in the human organism.

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Medical Clinic of Postgraduate Medical Institute and the Department of Experimental Therapeutics, Institute of Human Nutrition, Prahá (Czechoslovakia), November 10, 1958.

Zusammenfassung

Nach peroraler Verabreichung von IAA wird eine vermehrte Ausscheidung von 5-HIAA festgestellt. Eine Hydroxylierung der aufgenommenen IAA wird vermutet. Damit wird ein neuer Weg der Entstehung der 5-HIAA beim Menschen aufgezeigt.

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