lated to psilocin, a naturally occurring compound, which is nothing but its N,N-dimethyl derivative[2].

The present communication describes some pharmacological actions of 4-HT and 4-HTP, as compared with those of 5-hydroxytryptamine(5-HT) and 5-hydroxytryptophan(5-HTP). Cats were anaesthetized with chloralose and dogs with Pernocton.

4-Hydroxytryptamine and 5-hydroxytryptamine

Blood pressure. In the intact cat, 4-HT (100 µg/kg, i.v.) produced a rise of blood pressure which was 2–6 times less intense but more sustained than that elicited by the same dose of 5-HT. In the spinal cat, both 5-HT und 4-HT provoked hypertension but, whereas with 5-HT the pressure rise was proportional to the dose administered, this did not occur with 4-HT.

Respiration. In the dog, 5-HT(3-4µg/kg and more, i.v.) produced a transitory stimulation of respiration, eventually followed by a period of hypopnoea. 4-HT required a higher dosage than 5-HT and the result was always respiratory depression.

In the cat both 5-HT and 4-HT produced bronchospasm. The effect of 4-HT(100 µg/kg, i.v.) was 2–4 times less intense but lasted longer than that caused by 5-HT.

Dog urinary bladder in situ. The stimulant action of 4-HT was approximately 2 times less intense than that of 5-HT (minimum active dose: 1–2µg/kg, i.v.), but was more sustained.

Diuresis of hydrated rats. Unlike 5-HT, subcutaneous 4-HT showed only a poor antidiuretic effect. In fact, 6 mg/kg were less effective than 0·04 mg/kg 5-HT.

Nictitating membrane. 5-HT was 2–5 times more active than 4-HT in contracting the nictitating membrane of the cat, but whereas the 5-HT contraction was rapidly followed by relaxation, that caused by 4-HT lasted considerably longer.

4-Hydroxytryotophan and 5-hydroxytryptophan

Blood pressure. Intravenous 5-HTP, in doses of 2·5-5 mg/kg, elicited in the cat a moderate fall of blood pressure which lasted 15–20 min; the same dose of 4-HTP produced a more pronounced rise of blood pressure, lasting 30–50 min.

Bronchial musculature. Both 5-HTP and 4-HTP produced a spasm of the bronchial musculature when given i.v. in the cat. However, bronchospasm elicited by 4-HTP was considerably more intense and lasted longer than that produced by 5-HTP.

Dog urinary bladder in situ. The contraction produced by 2–4 mg/kg 4-HTP given i.v. was 3 to 4 times more intense and considerably more sustained than that caused by the same dose of 5-HTP.

Nictitating membrane. Single i.v. injections of 2–5mg/kg 5-HTP did not contract the membrane; the same doses of 4-HTP produced a contraction lasting 30–50 min.

Gastrointestinal tract. In the diarrhoea test in mice, the ED50 of 5-HTP by i.p. route was 25µg/mouse, the ED 100, 300 µg/mouse. For 4-HTP the ED50 could not be exactly established, but it was not less than 400 µg/mouse.

Diuresis of hydrated rats. Like 5-HTP, also 4-HTP caused in hydrated rats an evident antidiuretic effect. 4-HTP was approximately half as potent as 5-HTP, the minimum active i.p. dose being 20 mg/kg. At high dose levels, 100 mg/kg and more, 4-HTP produced renal lesions identical to those seen after 5-HTP.

Body temperature. In mice pretreated with 100 mg/kg iproniazid, 30–60 mg/kg 4-HTP, given i.p., produced, like 5-HTP, a remarkable increase of the rectal temperature (1·5° for 60 mg/kg 4-HTP) accompanied by tremors, lasting 60–90 min. No signs of excitement appeared, but the animals were rather depressed.

In rabbits, similarly pretreated with iproniazid, i.v. doses of 5 mg/kg 4-HTP produced a rise of rectal temperature (1·5–2°) similar to that caused by 15 mg/kg 5-HTP, lasting more than 4 h.

Intraspecific aggressive behaviour of mice. Like 5-HTP, also 4-HTP reduced the aggressive behaviour in male mice. At the i.p. dose of 120 mg/kg, 4-HTP abolished fighting in 50% of the treated couples. The same effect was obtained with 100 mg/kg 5-HTP.

Spontaneous electrical cortical activity of the «midpontine pretrigeminal cat». The slow intracarotid injection of 25–40 mg 4-HTP brought about a synchronization of the EEG, similar in every respect to that produced by 8–20mg 5-HTP or 1–2 mg thiopental.

It may be seen from the preceding data that 4-HT and 4-HTP display in the organism effects which are similar to those possessed by 5-HT and 5-HTP. Both amino acids act presumably after having been decarboxylated to the corresponding amines. However, whereas 5-HT and 5-HTP produce pharmacological effects which, with some important exceptions, are more intense than those caused by 4-HT and 4-HTP, the two 4-hydroxyindoles, and especially 4-HTP, cause more prolonged effects than the corresponding 5-hydroxyindoles. This may in part depend on the fact that 4-HT is a substrate for amine oxidase worse than 5-HT[3].

The failure of 4-HT, in sharp contrast to 4-HTP, to cause antidiuresis is puzzling, but it may depend upon the great lability of the phenolic hydroxy group of 4-HT towards oxidizing enzymes, which could prevent 4-HT from reaching the renal receptors in sufficient amounts or, more likely, for a sufficiently long time, to produce afferent glomerular vasoconstriction.

V. ERSPAMER, A. GLÄSSER, and P. MANTEGAZZINI

Laboratori Ricerche Farmitalia, Milano and Istituto di Farmacologia dell'Università di Parma (Italy), May 2, 1960.

Riassunto

5-HT e 5-HTP da una parte e 4-HT e 4-HTP dall'altra posseggono su tutti i numerosi reattivi saggiati effetti farmacologici similari i che in generale sono meno intensi ma più duraturi per i derivati 4-idrossiindolici.

[2] A. HOFMANN and F. TROXLER, Exper. *15*, 101 (1959).

[3] V. ERSPAMER, R. FERRINI, and A. GLÄSSER, to be published.

The Fate of 4-Hydroxytryptophan in the Rat Organism[1]

Among the many tryptophans so far studied in this Laboratory, DL-4-hydroxytryptophan (4-HTP) was one of the few which were found to be decarboxylated *in vitro* by mammalian decarboxylase[2]. It is probable that 4-HTP is the precursor amino acid of psilocin (N,N-dimethyl-4-hydroxytryptamine) and psilocybin (the O-phosphate ester of psilocin) and, hence, that 4-HTP is an amino acid occurring, together with 5-HTP, in nature. These consid-

A full report on the methods used in this study, as well as on the biochemical and pharmacological results will be published elsewhere, together with the discussion of the collected data.

[1] Supported by a grant from the Rockefeller Foundation, New York.

[2] V. ERSPAMER, A. GLÄSSER, C. PASINI, and G. STOPPANI, Nature, in press.

Urinary 4-Hydroxyindoles in Rats given 4-HTP

Dose and administration route of DL-4-HTP	4-Hydroxyindole content (in mg) of 24 h urine		
	4-HTP	4-HT (base)	4-HIAA (as 4-HTP)
80 mg (100 mg/kg) i. p.	13.4	6.3	25.0
72 mg (100 mg/kg) i. p.	9.6	3.0	25.5
33 mg (50 mg/kg) i. p.	3.6	1.6	10.8
14.5 mg (20 mg/kg) i. p.	2.0	1.0	4.2
6.8 mg (10 mg/kg) i. p.	0.7	0.6	1.2
39 mg (50 mg/kg) i. p.	4.7	3.7	8.2
40 mg (50 mg/kg) i. p.	3.8	2.2	5.4
16 mg (20 mg/kg) i. p.	1.8	1.0	2.0
8.3 mg (10 mg/kg) i. p.	1.1	0.9	1.1
7.8 mg (10 mg/kg) i. p.	0.5	0.5	0.7
180 mg (200 mg/kg) per os	6.7	7.7	45.5
86 mg (100 mg/kg) per os	4.6	3.8	20.6
44 mg (50 mg/kg) per os	2.0	2.1	7.8

erations prompted us to carry out a biochemical and pharmacological study of 4-HTP. This preliminary report describes the fate of the amino acid in the organism of the rat.

DL-4-HTP was given by intraperitoneal and oral routes at different dose levels. The semi-quantitative estimation of 4-HTP, 4-hydroxytryptamine(4-HT) and 4-hydroxyindoleacetic acid(4-HIAA) in urine and acetone tissue extracts was carried out by visual comparison of paper chromatograms obtained with different amounts of the above biological materials and paper chromatograms obtained with different amounts of pure DL-4-HTP and 4-HT creatinine sulphate. The values of 4-HIAA were provisionally expressed in terms of 4-HTP.

The solvent used in the ascending chromatography was the *n*-butanol : acetic acid : water mixture (4 : 1 : 5); the most commonly employed developing reagents were the Heinrich and Schuler's NNCD reagent (2-chloro-4-nitro-1-diazobenzene-α-naphtalene sulphuric acid), a stable diazonium salt[3], and the *p*-dimethylaminobenzaldehyde reagent. Urinary 4-HT was also determined by bioassay, using the rat uterus preparation.

Urine chromatograms showed the presence of unchanged 4-HTP and of at least eight 4-HTP metabolites. Five of them could be identified as 4-HT, 4-HIAA and the O-glucuronides of 4-HTP, 4-HT and 4-HIAA. Among the glucuronides, that of 4-HTP was present only in traces, that of 4-HT in considerable amounts. The identification of the glucuronides was carried out after their hydrolysis by β-glucuronidase.

The accompanying Table presents some quantitative data on the content of 4-HTP, 4-HT and 4-HIAA in the urine collected over a 24 h period.

In all examined rat tissues (gastrointestinal tract, liver, heart, testicles, brain, lung, kidney) unchanged 4-HTP and its main metabolites 4-HT and 4-HIAA could be easily detected by paperchromatography and, as for 4-HT, by bioassay.

1 h after intraperitoneal injection of 100 mg/kg DL-4-HTP to rats pretreated with iproniazid, brain contained 2·5 to 4 μg 4-HT base/g wet tissue, liver 60 to 100 μg, lung 4 to 6 μg, kidney 100 to 120 μg, and testicles 10 to 15 μg.

The following conclusions may be drawn from the above data:

(a) 4-HTP, like 5-HTP, is decarboxylated in the rat organism by several parenchymatous tissues, giving origin to 4-HT. This, in its turn, is attacked by amine oxidase, giving origin to 4-HIAA.

(b) liver and still more kidney decarboxylate 4-HTP with particular intensity.

(c) 4-HTP, again like 5-HTP, passes the blood brain barrier, penetrates into the nervous tissue and is there decarboxylated to 4-HT. Both unchanged 4-HTP and its metabolites 4-HT and 4-HIAA are detectable on chromatograms of brain extracts.

4-HT displays, on isolated organs and in the intact organism, many 5-HT-like actions. Obviously, these actions appear also when 4-HTP, the precursor amino acid of 4-HT, is given. However, in addition to peripheral effects, also central effects make their appearance after 4-HTP[4].

V. Erspamer, A. Glässer,
B. M. Nobili, and C. Pasini

Istituto di Farmacologia, Università di Parma (Italy), June 28, 1960.

Riassunto

Nell'organismo del ratto ha luogo una rapida ed intensa decarbossilazione del 4-idrossitriptofano a 4-idrossitriptamina. Oltre ad essere attaccata dalla monoaminossidasi, con formazione di acido 4-idrossiindolacetico, l'amina viene anche O-coniugata con acido glucuronico. Analogamente al 5-idrossitriptofano, anche il 4-idrossitriptofano valica la barriera ematoencefalica dando luogo a formazione di 4-idrossitriptamina nella compagine del tessuto nervoso.

[3] Sold by Hopkin & Williams Ltd., Chadwell Heath (Essex, England).

[4] V. Erspamer, A. Glässer, and P. Mantegazzini, Exper. *16*, 505 (1960).

Induction of Hepatic Cirrhosis in *Iguana iguana* by 3-Monohydroxycholanic Acid Treatment

Holsti[1] stated that a daily administration to the rabbit of a desiccated whole bile preparation rapidly causes a hepatic cirrhosis. On the strength of later findings, it was suggested that the effect was linked to the activity of bile acids[2], although several of the first tested and commoner variants exhibited only a feeble liverdamaging effect when subjected to the present method[2]. Recently, the same author succeeded in inducing in the rabbit hepatic cirrhosis by gastric instillation of 3-monohydroxycholanic acid or lithocholic acid[3].

It seemed desirable to us to perform a corresponding experiment in a lower vertebrate to establish whether a more general significance is to be attached to this induction process. As experimental material, we chose the reptile *Iguana iguana*.

A daily gastric instillation into 14 males and 17 females of 3 ml of a 0·5% water suspension of the sodium salt of 3-monohydroxycholanic acid (Light & Co., Ltd.) appeared to be highly effective and to induce hepatic cirrhosis within a period of three months. No cases of cirrhosis were observed in the untreated control animals of equal size and life time. By administration of higher concentrations of the sodium salt of 3-monohydroxycholanic acid, a more extensive liver damage was obtained and ultimately death of the animals.

[1] P. Holsti, Acta path. microbiol. Scand. Suppl. 113 (1956).
[2] P. Holsti, Naturwissenschaften *45*, 165 (1958).
[3] P. Holsti, Nature *186*, 250 (1960).