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).

Histologically no massive fat infiltration could be observed in the cirrhotic livers. The liver cells showed a distinct hydropic degeneration, while the ultimate picture was in general that of a finely nodular diffuse cirrhosis. The extent of the necrosis varied with the individual resistance of the animal and the dose administered.

As these results are in good agreement with the data obtained by HOLSTI[3] in the rabbit, the conclusion is certainly justified that a more general significance should actually be attached to this induction process.

A. STOLK

Histological Laboratory, Free University, Amsterdam (Netherlands), July 18, 1960.

Zusammenfassung

Es stellte sich heraus, dass tägliche Instillation von 3 ml einer 0,5%igen wässerigen Suspension des Natriumsalzes der 3-Monohydroxycholansäure in den Magen von *Iguana iguana* innerhalb dreier Monate Leberzyrrhose hervorrief.

Changes in the Topographical Distribution of Glycogen in the Brain of *Iguana iguana* in Dependence on the Environmental Temperature

Until recently it was assumed that the glycogen of the brain is a rather inert substance, which in the various metabolic processes shows no changes of any importance. Recently it was stated, however, that some functional conditions possess their metabolic analogue in changes in concentration of the glycogen in the brain tissue[1–4]. As the pertaining results were obtained with mammals, we decided to perform corresponding experiments with a lower vertebrate, namely the reptile *Iguana iguana*.

In three groups of *Iguana* males and females, the concentration of the glycogen in the brain tissue was determined in the parts of the brain and that at an environmental temperature of 19.7 ± 0.3°C, 29.6 ± 0.3°C, and 36.8 ± 0.3°C. The animals were killed by decapitation and the concentration of glycogen was determined in the cortex cerebri, the diencephalon, the mesencephalon, the cerebellum, and the medulla oblongata by the method of KERR[5], with the exception that the reduction compounds were determined according to NELSON's method[6]. See in this connection also the publication of JAKOUBEK and SVORAD[7].

From the results given in the Table, it follows that the drop in environmental temperature results not only in a decrease in concentration of the glycogen in the brain tissue, but even in an increase. Between the values for the male and the female sex, no significant differences were observed. Consequently, in the thermoregulation processes accompanying the decrease in environmental temperature, mainly glycogen quantities of tissue other than that of the brain are used. The drop in environmental temperature appears not to inhibit the synthesis of glycogen in thebrain tissue.

In general, these results correspond with those obtained in the rat[4]. Consequently, the possibility is not precluded that the glycogen metabolism of the brain tissue proceeds in essentially the same way as in mammals. Just as in the rat[4], the topographical determination of the glycogen concentration in the brain tissue points to essential changes of this metabolite in the diencephalon at a decrease in temperature, which under these conditions escapes attention, however, in a rough determination of the glycogen in the whole brain[8].

A. STOLK

Histological Laboratory, Free University, Amsterdam (Netherlands), April 11, 1960.

Zusammenfassung

Es wird über experimentelle Untersuchungen der topographischen Verteilung von Glykogen im Gehirn von *Iguana iguana* in Abhängigkeit von der Umgebungstemperatur berichtet.

Parts of the brain	Sex	Glycogen Values		
		E T 19.7 ± 0.3°C	E T 29.6 ± 0.3°C	E T 36.8 ± 0.3°C
C c	M	86.0 ± 6.1	107.1 ± 5.5	85.5 ± 5.2
	F	84.8 ± 7.2	110.6 ± 7.0	87.9 ± 6.5
D	M	98.6 ± 8.6	170.2 ± 9.2	125.1 ± 7.2
	F	97.2 ± 6.0	165.0 ± 7.1	124.6 ± 9.0
M	M	117.5 ± 7.5	126.1 ± 8.4	113.3 ± 6.4
	F	120.7 ± 5.2	129.3 ± 7.5	115.7 ± 5.3
C	M	86.2 ± 5.1	97.8 ± 7.7	82.4 ± 6.9
	F	80.5 ± 8.3	103.7 ± 6.8	79.0 ± 5.1
M o	M	109.9 ± 9.7	140.2 ± 9.7	107.4 ± 7.8
	F	112.3 ± 9.3	143.8 ± 8.0	110.9 ± 10.7

Changes in glycogen concentration in the brain of *Iguana iguana* at various environmental temperature. The glycogen values are given in mg/100 g fresh brain tissue. For the determination of each values 15 animals were used.

M = male, F = female, C c = cortex cerebri, D = diencephalon, M = mesencephalon, C = cerebellum, M o = medulla oblongata, ET = environmental temperature.

[1] M. R. A. CHANCE, J. exp. Biol. *30*, 468 (1953).

[2] A. V. PALLADINE, in Troisième Congr. Internat. de Biochimie (Rapports), Bruxelles (1955).

[3] N. SHIMIZU and Z. KUBO, J. Neuropath. exp. Neurol. *16*, 40 (1957).

[4] D. SVORAD, Science *125*, 156 (1957); A. M. A. Arch. Neurol. Psychiat. *77*, 533 (1957); *Symposium on Hypothermia*, Belgrade (1957); Nature (London) *181*, 775 (1958); Naturwissenschaften *46*, 533 (1959).

[5] S. E. KERR, J. biol. Chem. *116*, 1 (1936).

[6] N. NELSON, J. biol. Chem. *153*, 375 (1944).

[7] B. JAKOUBEK and D. SVORAD, Pflügers Arch. ges. Physiol. *268*, 444 (1959).

[8] V. PAVLOVIČ, C. R. Soc. Biol., Paris *149*, 2216 (1955).