4. The relationship between the rate of oxidation and the partial pressure of dissolved oxygen has been studied at several temperatures.

5. A discussion of the effect of temperature on the parameters of the rate equations for the oxidation of nitrite is presented.

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REFERENCES

Aleem, M. I. H. & Alexander, M. (1958). *J. Bact.* **76**, 510.
Butt, W. D. & Lees, H. (1960). *Biochem. J.* **76**, 425.
Dixon, M. & Webb, E. C. (1958). *Enzymes*, p. 126. London: Longman, Green and Co.
Eddy, B. P. (1958). *Leeuwenhoek ned. Tijdschr.* **24**, 81.
Goldberg, S. S. & Gainey, P. L. (1955). *Soil Sci.* **80**, 43.
Hattori, T. & Furusaka, C. (1960). *J. Biochem., Tokyo*, **48**, 831.
Laudelout, H. & van Tichelen, L. (1960). *J. Bact.* **79**, 39.
Lees, H. (1958). *Nature, Lond.*, **182**, 732.
Lees, H. & Simpson, J. R. (1957). *Biochem. J.* **65**, 297.
Meyerhof, O. (1916). *Pflüg. Arch. ges. Physiol.* **164**, 353.
Shin, M. B. (1941). *Industr. Engng Chem. (Anal.)*, **13**, 33.
Silver, W. S. (1960). *Trans. 7th int. Congr. Soil Science, Madison, Wis.*, vol. 2, p. 592.
Zavarzin, G. A. (1958). *Mikrobiologiya*, **27**, 401.

Biochem. J. (1962) **85**, 447

5-Hydroxytryptophol: a Metabolite of 5-Hydroxytryptamine in Rats

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McIsaac & Page (1959) and Weissbach, Lovenberg, Redfield & Udenfriend (1961) found 1′-*N*-acetyl-5-hydroxytryptamine as a metabolite of 5-hydroxytryptamine in rats. Keglević *et al.* (1959) found after administration of labelled 5-hydroxytryptamine several radioactive compounds in rat urine, but none of them corresponded to 1′-*N*-acetyl-5-hydroxytryptamine. Therefore it was thought that this compound, if formed, might be further metabolized or conjugated. On the other hand, Axelrod, Kopin & Mann (1959), Goldstein, Friedhoff, Pomerantz & Simmons (1960) and Smith & Wortis (1960) reported that in the metabolism of some amines intermediate alcohols could be formed. This possibility has been examined for 5-hydroxytryptamine and it will be shown that one of its major metabolites ('metabolite C' of Keglević *et al.* 1959) is a glucuronide of 5-hydroxy-3-(β-hydroxyethyl)indole (5-hydroxytryptophol).

EXPERIMENTAL

Materials. 5-Hydroxy[1′-^{14}C]tryptamine as the creatinine sulphate complex (2·26 μc/mg.) was prepared as described by Keglević-Brovet, Kveder & Iskrić (1957). Synthesis of the *N*-acetyl derivative was carried out according to Desaty, Hadžija, Iskrić, Keglević & Kveder (1962). Bacterial β-glucuronidase was purchased from Sigma Chemical Co., St Louis, Mo., U.S.A.

5-Hydroxytryptophol. This was prepared as described by Elderfield & Fischer (1958) for 6-methoxytryptophol. Oxalyl chloride (2·0 g.) in dry ether (10 ml.) was added dropwise with stirring to a solution of 1·95 g. of 5-benzyloxyindole in dry ether (10 ml.). 5-Benzyloxyindol-3-yloxalyl chloride was filtered off, washed with ether and added in small portions to a stirred suspension of $LiAlH_4$ (4 g.) in dry tetrahydrofuran (40 ml.). The mixture was refluxed for 1 hr. with stirring and kept at room temperature for an additional 3 hr. The complex and the excess of hydride were decomposed with water, and the solution was filtered and evaporated *in vacuo*, leaving 2·15 g. of semicrystalline mass. Three recrystallizations from benzene gave 1·53 g. (66%) of 5-benzyloxytryptophol, m.p. 93–95° (Found: C, 76·2; H, 6·4; N, 5·3. $C_{17}H_{17}NO_2$ requires C, 76·4; H, 6·4; N, 5·2%).

The above-mentioned product (534 mg.), dissolved in methanol (25 ml.), was treated at s.t.p. with 10% Pd–$BaSO_4$ (500 mg.). After 2 hr. the reduction was complete and the catalyst was removed by centrifuging. The solvent was evaporated *in vacuo*, the residue dissolved in ethyl acetate (2 ml.) and fractionally precipitated with hexane. 5-Hydroxytryptophol was obtained as white prisms (200 mg., 56·5%), m.p. 102–105°. After one recrystallization from ethyl acetate, m.p. was 105–107° (Found: C, 67·9; H, 6·5; N, 8·2. $C_{10}H_{11}NO_2$ requires C, 67·8; H, 6·3; N, 7·9%). The compound was stable and showed no tendency to decompose as indicated by Koo, Avakian & Martin (1959).

Animals. Adult male albino rats from our Institute stock (aged 3–4 months) maintained on a standard diet were used throughout. Injections were applied intraperitoneally, the dose of 5-hydroxy[1′-^{14}C]tryptamine creatinine sulphate being 2·9 mg./100 g. body wt. The animals were placed in the funnel type of metabolism cage, and were allowed water but no food.

Treatment of urine. Urine (24 hr. sample) was filtered through a glass-wool plug and treated with deactivated

charcoal according to the procedure of Dalgliesh (1955). The phenolic eluate was evaporated *in vacuo* and subjected to paper chromatography.

Tissue experiments. Slices of liver (approx. 1 g. wet wt.) were cut by means of a Stadie–Riggs microtome (Stadie & Riggs, 1944) and incubated with substrate in 10 ml. of Krebs–Ringer phosphate, pH 7·4 (Umbreit, Burris & Stauffer, 1949) for 2 hr. at 37° in an atmosphere of oxygen with shaking. At the end of the incubation period, the reaction mixture was decanted from the slices and concentrated *in vacuo* to about 2 ml. After the precipitation of salts and proteins with acetone (15 ml.), the filtered solution was evaporated *in vacuo* to about 2 ml. and subjected to paper chromatography.

Paper chromatography. Descending, one-dimensional, chromatography on Whatman no. 1 and 3MM paper (for preparative work) was used. Standard solvents (proportions by vol. and times of run) were: (1) butan-1-ol–acetic acid–water (4:1:5; 19 hr.); (2) propan-2-ol–aq. NH_3 soln.–water (10:1:1; 22 hr.); (3) methanol–benzene–butan-1-ol–water (4:2:2:2; 14 hr.). Radioactive spots were eluted with ethanol–water (1:1, v/v). In two-dimensional chromatography (frame technique), solvent (2) followed by solvent (1) was used. Radioautographs were prepared by placing papers in contact with X-ray film (Ferrania) for up to 2 weeks. One-dimensional chromatograms were scanned with an automatic Geiger–Müller scanner. For the detection of indoles, Ehrlich's reagent (2%, w/v, *p*-dimethylaminobenzaldehyde in 2N-HCl) was used and for glucuronic acid naphtharesorcinol reagent.

Separation of metabolite C. After administration of 5-hydroxy[1′-^{14}C]tryptamine, the charcoal-treated urine was chromatographed on Whatman 3MM paper in solvent (1). The radioactive area with R_F 0·25 was eluted and rechromatographed in solvent (3), where it resolved into individual peaks with R_F 0·22 and R_F 0·46. The latter, metabolite C, was eluted again and used throughout.

RESULTS

Enzymic hydrolysis of metabolite C

Metabolite C, eluted from chromatograms, was incubated with an excess of β-glucuronidase in phosphate buffer, pH 6·2, at 37° for 24 hr. After the addition of ethanol, the mixture was filtered and centrifuged and the supernatant concentrated in a stream of nitrogen and examined by paper chromatography. Metabolite C almost vanished, and a new spot, aglycone C, with R_F values given in Table 1, appeared. Glucuronic acid was identified by colour reaction and chromatography with an authentic sample.

Metabolism of 1′-N-acetyl-5-hydroxytryptamine and 5-hydroxytryptophol in rat-liver slices

R_F values from chromatograms prepared from incubation mixtures of 3 mg. of these compounds are given in Table 1. From the area and the intensity of the coloured spots it was obvious that the major part (80–90 %) of *N*-acetyl-5-hydroxytryptamine had remained unchanged but 60–70 % of 5-hydroxytryptophol was metabolized; of the three new spots formed the one identified as 5-hydroxyindolylacetic acid was the most intense.

Identification of 5-hydroxytryptophol as a metabolite of 5-hydroxytryptamine

It is evident from Table 1 that metabolite C could be a glucuronide of either *N*-acetyl-5-hydroxytryptamine or 5-hydroxytryptophol, both of which show almost identical chromatographic behaviour. To discover which of the two compounds is the aglycone of metabolite C, the isotope-trapping technique was applied.

Experiments in vitro. 5-Hydroxy[1′-^{14}C]tryptamine creatinine sulphate (5 mg., 0·605 μc/mg.) was incubated with rat-liver slices in the presence of 3 mg. of either *N*-acetyl-5-hydroxytryptamine or 5-hydroxytryptophol. Scanning records of one-dimensional chromatograms revealed radioactivity associated with the unmetabolized alcohol but none with the spot corresponding to the *N*-acetyl compound.

Experiments in vivo. Keglević *et al.* (1959) did not detect in rat urine any radioactive spot corresponding to *N*-acetyl-5-hydroxytryptamine or 5-hydroxytryptophol. It can be assumed that if these compounds are metabolites of 5-hydroxy-

Table 1. *R_F values of 5-hydroxyindoles and their metabolites*

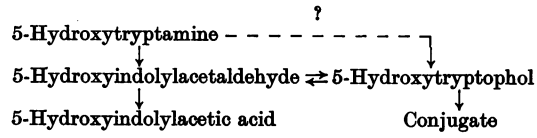
For composition of solvents and experimental conditions see text.

	R_F								
Solvent	(1)		(2)				(3)		
Metabolite C	—	0·25	—	—	—	0·21	—	—	0·46
Aglycone C	0·80	—	0·74	—	—	—	0·86	—	—
N-Acetyl-5-hydroxytryptamine	0·82	—	0·77	—	—	—	0·89	—	—
5-Hydroxytryptophol	0·79	—	0·76	—	—	—	0·87	—	—
5-Hydroxyindolylacetic acid	0·78	—	—	—	0·32	—	—	0·68	—
Metabolite E	—	0·31	—	0·61	—	—	—	0·68	—
Extract from rat-liver slices incubated with:									
N-Acetyl-5-hydroxytryptamine	0·78	0·32	0·77	0·63	—	0·24	0·84	—	0·42
5-Hydroxytryptophol	0·77	0·29	0·75	0·60	0·35	0·22	0·85	0·63	0·43

tryptamine, they are further metabolized. 5-Hydroxy[^{14}C]tryptamine was therefore administered together with a large dose of either *N*-acetyl-5-hydroxytryptamine (12 mg.) or 5-hydroxytryptophol (17 mg.). One-dimensional paper chromatography of a 24 hr. sample of urine showed again the radioactivity associated with the alcohol, but not with the acetyl compound. On two-dimensional chromatograms an intensely coloured spot appeared in the position of metabolite C after administration of either compound but an increase in intensity of the 5-hydroxyindolylacetic acid spot was observed only after 5-hydroxytryptophol was given. In addition, the spot corresponding to metabolite F of Keglević *et al.* (1959) was intensified in colour and radioactivity only when the alcohol had been given. From comparison of the chromatograms and radioautograms from the experiments both *in vitro* and *in vivo*, it was clear that the radioactivity coincided with the coloured spot of metabolite C only when 5-hydroxytryptophol was administered. Furthermore, the same coincidence was observed for metabolite E of Keglević *et al.* (1959).

DISCUSSION

It has been shown that 5-hydroxytryptophol is an intermediate metabolite of 5-hydroxytryptamine in rats. The alcohol is not excreted free in the urine, but forms conjugates or undergoes oxidation to 5-hydroxyindolylacetic acid. The fact that the alcohol is metabolized to this acid suggests that a significant amount of the latter may be formed in this way in the course of the metabolism of 5-hydroxytryptamine by a route such as that shown in Scheme 1.



Scheme 1

Weissbach *et al.* (1961) have indicated the possibility of a minor pathway of tryptamines to indolylacetic acids through an 'amine oxidation' insensitive to the known inhibitors of monoamine oxidase. Metabolite C, identified now as a 5-hydroxytryptophol *O*-glucuronide, is a major metabolite of 5-hydroxytryptamine. Since after the administration of 5-hydroxytryptamine together with $Na_2{}^{35}SO_4$ the radioactivity was found to coincide with the spot E (our unpublished result), this metabolite may be a sulphate conjugate of 5-hydroxytryptophol.

McIsaac & Page (1959) found that up to 25 % of 5-hydroxytryptamine given to rats was excreted in urine as *N*-acetyl-5-hydroxytryptamine. Weissbach *et al.* (1961) observed the excretion of less than 5 % as this compound. By the isotope-trapping technique we were unable to detect the formation of any *N*-acetyl derivative from 5-hydroxytryptamine. As the acetyl compound and 5-hydroxytryptophol have almost identical chromatographic properties it is likely that the cited authors dealt in fact with the alcohol.

SUMMARY

1. The metabolism of 1′-*N*-acetyl-5-hydroxytryptamine and 5-hydroxytryptophol in rat-liver slices was studied. The former compound remained mainly unchanged (80–90 %), whereas 60–70 % of 5-hydroxytryptophol was metabolized, being partly oxidized to 5-hydroxyindolylacetic acid and partly conjugated.

2. 1′-*N*-Acetyl-5-hydroxytryptamine and 5-hydroxytryptophol were chromatographically indistinguishable but only the latter was shown to be a metabolite of 5-hydroxytryptamine. A scheme for the metabolism of 5-hydroxytryptamine is suggested.

3. A major metabolite of 5-hydroxytryptamine excreted in rat urine is a 5-hydroxytryptophol-*O*-glucuronide.

4. The chemical synthesis of 5-hydroxytryptophol is described.

REFERENCES

Axelrod, J., Kopin, I. J. & Mann, J. D. (1959). *Biochim. biophys. Acta*, **36**, 576.
Dalgliesh, C. E. (1955). *J. clin. Path.* **8**, 73.
Desaty, D., Hadžija, O., Iskrić, S., Keglević, D. & Kveder, S. (1962). *Biochim. biophys. Acta*, **62**, 179.
Elderfield, R. C. & Fischer, B. A. (1958). *J. org. Chem.* **23**, 949.
Goldstein, M., Friedhoff, A. J., Pomerantz, S. & Simmons, C. (1960). *Biochim. biophys. Acta*, **39**, 189.
Keglević, D., Supek, Z., Kveder, S., Iskrić, S., Kečkeš, S. & Kisić, A. (1959). *Biochem. J.* **73**, 53.
Keglević-Brovet, D., Kveder, S. & Iskrić, S. (1957). *Croat. chem. Acta*, **29**, 351.
Koo, J., Avakian, S. & Martin, G. J. (1959). *J. org. Chem.* **24**, 179.
McIsaac, W. M. & Page, I. H. (1959). *J. biol. Chem.* **234**, 858.
Smith, A. A. & Wortis, S. B. (1960). *Biochim. biophys. Acta*, **40**, 569.
Stadie, W. C. & Riggs, B. C. (1944). *J. biol. Chem.* **154**, 687.
Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1949). *Manometric Techniques and Tissue Metabolism*, 2nd ed., p. 119. Minneapolis: Burgess Publishing Co.
Weissbach, H., Lovenberg, W., Redfield, B. G. & Udenfriend, S. (1961). *J. Pharmacol.* **131**, 26.