favour of energy production by the pentose phosphate pathway, the occurrence of this oxidative cycle in the early life of the seedling emphasizes its close association with the high metabolic activity at this stage.

The activities of the different enzymes in the extract, as judged from the number of units per ml. and from the activity in relation to the protein content, were low in comparison with reported activities of the enzymes in extracts from animal and bacterial systems. This is, however, to be expected because of the presence of considerable amounts of non-enzymic protein (storage protein) in the seeds and in the seedling at the early stages of germination.

Phosphoglucose dehydrogenase which has been partially purified has the pH optimum and other properties similar to those of the yeast enzyme. Unlike bacterial enzyme it is specific for TPN and inactive with DPN.

SUMMARY

1. The occurrence in extracts of germinating mung-bean seedling of the following enzymes of the oxidative pentose phosphate pathway has been shown: phosphoglucose dehydrogenase, phosphogluconate dehydrogenase, phosphoribose isomerase, phosphoribulose epimerase, transketolase, transaldolase and phosphoglucose isomerase.

2. During the initial period of germination (up to 48 hr.) the amounts and specific activities of the dehydrogenases increase with time. After 72 hr., however, both start decreasing and after 96 hr. the enzymes disappear from the extracts.

3. The enzyme phosphoglucose dehydrogenase has been partially purified and its properties have been found to be similar to those of the yeast enzyme. The enzyme is triphosphopyridine nucleotide-specific, and has been successfully used in the assay of glucose 6-phosphate.

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The Metabolism of Exogenous ¹⁴C-Labelled 5-Hydroxytryptamine in Rats

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(Received 6 February 1959)

In spite of intensive study of 5-hydroxytryptamine from both biochemical and biological aspects knowledge of its metabolism is still incomplete. The fate and distribution of 5-hydroxytryptamine in rats was studied by Erspamer (1955, 1956), but more specific information about an overall metabolism is still lacking. 5-Hydroxyindolylacetic acid was established as a metabolite by Udenfriend, Titus & Weissbach (1955) and Erspamer (1955). Weissbach, Redfield & Udenfriend (1957) found 5-hydroxyindolylacetaldehyde in homogenates of

guinea-pig liver. Curzon (1957) found 5-hydroxyindolylacetic acid *O*-sulphate in urine of argentaffinoma subjects and in urine of rats after administration of 5-hydroxytryptophan, 5-hydroxytryptamine and 5-hydroxyindolylacetic acid. Chadwick & Wilkinson (1958) established 5-hydroxytryptamine *O*-sulphate as a metabolite of 5-hydroxytryptamine in incubated rat-liver homogenates. Weissbach, Redfield & Udenfriend (1958) identified 5-hydroxytryptamine *O*-glucuronide as a normal metabolite of 5-hydroxytryptamine in the urine of

mice. The amount of this compound greatly increased when mice had been pre-treated with *iso*-propylisonicotinic acid hydrazide (iproniazid). McIsaac & Page (1958) investigated carcinoid human urine and identified, by paper chromatography, oxytocic activity and fluorescent spectra, besides 5-hydroxyindolylacetic acid and indicated two new metabolites of 5-hydroxytryptamine: 1'-*N*-acetyl-5-hydroxytryptamine and 5-hydroxyindolylacetic acid. They also claimed to have found in radioautographs obtained from urinary extracts of rats and rabbits given radioactive 5-hydroxytryptamine the same metabolites, with the addition of two other minor metabolites. One of these has been identified as 5-hydroxytryptamine *O*-glucuronide. However, most of the papers about new metabolites of 5-hydroxytryptamine are preliminary reports in which only few experimental details are given.

In the present work an attempt was made, by using 5-hydroxy[¹⁴C]tryptamine labelled at the end of the side chain (Keglević-Brovat, Kveder & Iskrić, 1957), to obtain some information about the overall metabolism of exogenous 5-hydroxytryptamine in rats, to establish the number of the main metabolites of 5-hydroxytryptamine excreted in urine and to determine chromatographic characteristics of these. A preliminary account of some of the results has been given by Keglević-Brovat, Supek, Kveder, Iskrić & Kečkeš (1958).

METHODS

5-Hydroxy[1'-¹⁴C]tryptamine, as the creatinine sulphate complex (Keglević-Brovat *et al.* 1957), with a specific activity of 0.69 mc/m-mole, was used in all experiments.

Animals and dosage. Adult male albino rats from our Institute stock (aged 3-4 months), maintained on a standard diet (Coward, 1947), were employed throughout. In all experiments the dose level was 2.9 mg./100 g. body wt. for 5-hydroxy[1'-¹⁴C]tryptamine creatinine sulphate (1.27 mg. of 5-hydroxytryptamine/100 g. body wt.). The compound was injected subcutaneously, dissolved in 1 ml. of 0.9% NaCl, and the syringe was rinsed twice with 1 ml. of the same solution, a metal three-way stopcock being used. During the experiment the animals were allowed water but not food.

Experiments 1-4. These are the experiments described by Keglević-Brovat *et al.* (1958).

Experiments 3-15. Each animal was kept in a metabolism cage designed for the separate collection of urine and faeces. Expts. 3-4, 10-12 and 13-15 were run simultaneously.

Collection of urine. In all experiments urine was collected separately for the periods 0-12 and 12-14 hr. in tubes cooled by ice. In Expts. 1-4 the whole urine was freeze-dried. In Expts. 5-6 urine as collected was investigated and only a small fraction was freeze-dried. In Expts. 7-15 all the urine samples were made up to 20 ml. and pipetted in 1 ml. portions into tared 2 ml. vials. About 15 vials of each urine sample were freeze-dried, sealed and reweighed. The weights of the dry residues agreed within

±2.5%. To keep identical drying conditions in Expts. 10-12 and 13-15 the urine vials were freeze-dried synchronously on the same pump.

Estimation of ¹⁴C in samples. Freeze-dried urine, faeces and tissues as well as untreated urine were subjected to wet combustion by the Van Slyke & Folch (1940) reagent in the apparatus by Weisburger, Weisburger & Morris (1952); the resulting CO₂ was absorbed in 1% (w/v) carbonate-free NaOH solution and converted into BaCO₃ by standard procedure (Calvin, Heidelberger, Reid, Tolberg & Yankwich, 1949). In Expts. 7-15 the vial containing dry residue corresponding to 1 ml. of the whole urine volume was cut in the middle and both parts were dropped into the combustion tube.

Determination of radioactivity. Samples of Ba¹⁴CO₃ (2.83 cm.²) were counted at infinite thickness on standard plates (Tracerlab Inc.; E-7 B). The counts were corrected when necessary for dead time, and the count rates of samples less than infinitely thick to the values of infinite thickness. Sufficient counts were recorded to give a standard error of ±5% for the lowest activities and ±3% for higher activities. Ba¹⁴CO₃ standards were prepared from the 5-hydroxytryptamine preparation used in these experiments.

Paper chromatography. Portions of freeze-dried urine were dissolved in water and subjected to descending chromatography on strips (54 cm. × 6 cm.) of Whatman no. 1 filter paper. The sample was applied to the paper as a streak about 5 cm. long. The four solvent systems used are listed in Fig. 2. Two-dimensional chromatograms were run in all combinations of the four solvent systems. In order to eliminate interfering substances some specimens of urine were treated with deactivated charcoal according to the procedure of Dalglish (1955). The phenolic eluates were evaporated *in vacuo* and the residue was subjected to one- and two-dimensional paper chromatography.

Detection of radioactive spots. Radioautographs were obtained from chromatograms kept in contact with Ferrania X-ray film for 5-10 days. One-dimensional chromatograms were scanned with an automatic scanner, consisting of a Geiger-Müller mica-window counter attached to an automatic recorder.

Elution of radioactive spots. Radioactive areas of the one-dimensional chromatograms were cut out and eluted with water. The transfer was performed as a continuous process, the eluates being dried simultaneously by a jet of air directed against the under side of the new paper strip (Moore & Boylen, 1953). The eluates were then rechromatographed separately in the three remaining solvent systems. Chromatograms obtained in this way were scanned, radioactive areas were re-eluted and individually subjected to chromatography in the original solvent system.

Tests for uronic acid. The eluates were checked by the carbazole method of Dische (1947) and the naphtharesorcinol test of Bray, Humphris, Thorpe, White & Wood (1952).

Bioassay. Oxytocic activity was tested on the isolated atropinized uterus of the rat in oestrus (Amin, Crawford & Gaddum, 1954).

SYNTHESES

All melting points are uncorrected.

5-Benzylxyindolylacetic acid ethyl ester (I). 5-Benzylxyindolylacetic acid (Stoll, Troxler, Peyer & Hofmann, 1955) (1.4 g., 5 m-moles) and *N*-carbonylglycine ethyl

ester, prepared according to Goldschmidt & Wick (1952) (0.7 g., 5.5 m-moles), were dissolved in toluene (10 ml.), warmed to 80° until the evolution of CO₂ ceased (about 1 hr.) and then refluxed at 110° for a further 1 hr. Toluene was removed *in vacuo*, the reddish gummy residue was dissolved in hot ethanol and filtered and kept at 0° overnight. Brown crystals (0.9 g.) separated. They were dissolved in ethyl acetate, treated with charcoal and precipitated by light petroleum (b.p. 60–80°). Two further recrystallizations from methanol-water gave white crystals (0.7 g., 38%) of m.p. 112–114° (Found: C, 69.1; H, 6.1; N, 7.8. C₂₁H₂₃O₄N₂ requires C, 68.8; H, 6.1; N, 7.7%).

5-Benzoyloxyindolylacetic acid (II). The ester (I) (366 mg., 1 m-mole) was moistened with ethanol, 0.5N-NaOH (2.5 ml.) was added and the mixture heated to 70° on a water bath with occasional shaking until it dissolved completely. It was then cooled and kept at room temperature for 3 hr. After acidification with acetic acid, small crystals separated. They were filtered off, washed with aq. 1% ethanol and dried (318 mg.). Crystallization from hot ethanol with addition of warm water to cause turbidity gave white crystals (268 mg., 79%) of m.p. 143–145° (Found: C, 67.6; H, 5.5. C₁₉H₁₈O₄N₂ requires C, 67.4; H, 5.4%).

5-Hydroxyindolylacetic acid (III). Compound (II) (280 mg., 1.16 m-moles), dissolved in methanol (10 ml.), was catalytically debenzylated at s.t.p., with 10% Pd-BaSO₄ (100 mg.). After 5 hr. the reduction was complete and the catalyst was removed by centrifuging. Methanol was completely removed by a stream of N₂, the residue dissolved in acetone (3 ml.) and the solution was filtered. To the clear solution CHCl₃ was added until a slight turbidity persisted, and kept at 0° for 3 days with slight further addition of CHCl₃. White, long prisms (166 mg., 80%), m.p. 183–185°, were obtained. After two recrystallizations the m.p. was 186–187° (134 mg., 66%) (Found: C, 58.0; H, 5.0; N, 11.4. C₁₂H₁₂O₄N₂ requires C, 58.1; H, 4.9; N, 11.3%). The infrared-absorption spectrum of this substance is shown in Fig. 1.

Hydrolysis of 5-hydroxyindolylacetic acid. Compound (III) (5 mg.), dissolved in 3N-HCl (2 ml.), was heated on a

boiling-water bath for 0.5 hr. The solution was evaporated to dryness *in vacuo*, the residue was dissolved in water and the solution again evaporated to remove the traces of HCl. The dark residue was dissolved in water and submitted to paper chromatography in four solvent systems.

RESULTS

Table 1 shows radioactivity recovered in urines from 15 rats. The volume of urine passed by each rat during the two periods of collection has no connexion with either the weight of total solids or the radioactivity recovered. The great differences in the recoveries of radioactivity in 0–12 hr. urines cannot be satisfactorily explained. Urine collected in the 12–24 hr. period showed a much lower radioactivity in all experiments and there is no evidence of a higher recovery to compensate for a low recovery in 0–12 hr. urines.

Paper chromatograms of urine from all experiments were scanned for radioactivity and always showed the same qualitative pattern. However, the quantitative relation of radioactivity between single metabolites differed slightly in individual experiments. Fig. 2 shows the typical diagrams of 0–12 hr. urine run in four solvent systems. Several radioactive compounds are excreted. The *R_F* values of individual radioactive compounds were determined by elution and rechromatography (Table 2). Only the compounds which retained after the whole procedure the same *R_F* in the original solvent system were accepted as metabolites. Other radioactive spots with no reproducible chromatographic behaviour were ascribed to degradation products.

The identification of 5-hydroxyindolylacetic acid and unchanged 5-hydroxytryptamine was established by comparing their chromatographic behaviour with pure synthetic substances. In addition, 5-hydroxytryptamine was checked by testing the eluates for oxytocic activity. In solvent system 4 the *R_F* of 5-hydroxytryptamine found in urine did not correspond with the *R_F* (0.33) of synthetic 5-hydroxytryptamine creatinine sulphate. Besides 5-hydroxyindolylacetic acid five other radioactive metabolites have been detected.

To judge from the radioactive areas of scanner diagrams, 5-hydroxyindolylacetic acid accounted for 50–60% of radioactivity applied to the chromatograms. Compounds A, B and C contained about 25–30% of the total radioactivity, C always being the most active among them. D, E and unchanged 5-hydroxytryptamine accounted for 10–15% of the radioactivity, of which the smallest part was due to 5-hydroxytryptamine. Compound F could not be taken decisively as a metabolite of 5-hydroxytryptamine, because in elution and rechromatography its presence was confirmed with

Fig. 1. Infrared-absorption spectrum of 5-hydroxyindolylacetic acid in Nujol (liquid paraffin). A Perkin-Elmer double-beam automatic recording spectrophotometer (NaCl prisms) was used.

Table 1. *Recovery of radioactivity in rat urine after injection of 5-hydroxy[1-¹⁴C]tryptamine*

The injected dose of 5-hydroxy[1-¹⁴C]tryptamine (in the form of creatinine sulphate complex) was 2.9 mg./100 g. body wt. (1.27 mg. of the free base/100 g. body wt.) with a specific activity of 0.69 mCi/m-mole. Standard BaCO₃ samples were prepared from the same preparation. In all experiments except Expts. 5-6 urine was freeze-dried. All measurements of radioactivity were on BaCO₃ mounted at infinite thickness on 2.83 cm.² plates. In Expt. 1 radioactive measurements were made with a Geiger-Müller mica-window tube and in Expts. 2-15 with a windowless flow counter (Tracerlab Inc., SC-16). Expts. 1-4 are from Keglević, Brovet, Suppek, Kveder, Iskrić & Kečkeš (1968). Mean values $\pm$ s.e.m. are given with ranges in parentheses; superior figures indicate the number of determinations.

Expt. no.	10 ⁻³ $\times$ Total radioactivity injected (counts/min.)	Urine excreted (ml.)		10 ⁻³ $\times$ Total radioactivity of urine (counts/min.)		Percentage of injected radioactivity recovered in urine	
		0-12 hr.		0-12 hr.		0-12 hr.	
		0-12 hr.	12-24 hr.	0-12 hr.	12-24 hr.	0-12 hr.	12-24 hr.
1	8.05	10.0	9.5	2.92 (2.79-3.05) ⁴	0.15 (0.14-0.16) ⁴	36.3 $\pm$ 0.70 (34.7-37.9) ⁴	1.9 $\pm$ 0.05 (1.8-2.0) ⁴
2	24.46	5.6	9.5	12.38 (11.31-13.54) ⁴	0.42 (0.36-0.51) ⁴	50.6 $\pm$ 1.92 (46.2-55.4) ⁴	1.7 $\pm$ 0.13 (1.5-2.1) ⁴
3	21.13	7.5	12.0	8.77 (8.31-9.31) ⁴	0.40 (0.34-0.45) ⁴	41.5 $\pm$ 1.07 (39.3-44.1) ⁴	1.9 $\pm$ 0.12 (1.6-2.1) ⁴
4	26.25	9.3	11.4	11.07 (10.92-11.26) ⁴	0.27 (0.24-0.29) ⁴	42.2 $\pm$ 0.29 (41.6-42.9) ⁴	1.0 $\pm$ 0.05 (0.9-1.1) ⁴
5	24.96	10.5	11.5	14.39 (12.00-18.56) ¹³	1.99 (1.91-2.04) ³	57.7 $\pm$ 2.44 (47.9-74.4) ¹³	7.9 $\pm$ 0.17 (7.6-8.2) ³
6	24.25	19.0	9.0	17.35 (16.50-18.84) ⁸	0.47 (0.46-0.49) ³	71.5 $\pm$ 1.07 (68.0-77.7) ³	1.9 $\pm$ 0.04 (1.9-2.0) ³
7	31.36	7.6	12.8	22.73 (21.52-24.35) ⁴	1.67 (1.60-1.72) ³	72.5 $\pm$ 1.94 (68.6-77.7) ⁴	5.3 $\pm$ 0.12 (5.1-5.5) ³
8	26.46	2.6	4.4	18.67 (17.69-19.53) ⁶	1.64 (1.52-1.72) ⁶	70.6 $\pm$ 1.02 (66.9-73.8) ⁶	6.2 $\pm$ 0.10 (5.8-6.5) ⁶
9	25.71	11.0	8.0	13.24 (13.13-13.34) ⁵	0.79 (0.73-0.84) ³	51.6 $\pm$ 0.19 (51.0-51.9) ⁵	3.1 $\pm$ 0.25 (2.8-3.3) ³
10	22.43	7.4	12.5	18.07 (17.27-18.69) ⁶	1.27 (1.24-1.29) ³	80.6 $\pm$ 0.87 (77.0-83.3) ⁶	5.7 $\pm$ 0.15 (5.5-5.8) ³
11	21.91	9.0	7.0	16.93 (16.31-17.39) ⁴	1.31 (1.29-1.35) ³	77.3 $\pm$ 1.17 (74.4-79.4) ⁴	6.0 $\pm$ 0.10 (5.9-6.2) ³
12	23.24	4.8	11.5	15.28 (14.23-16.12) ⁴	1.94 (1.88-1.99) ³	65.8 $\pm$ 2.09 (61.3-69.4) ⁴	8.3 $\pm$ 0.15 (8.1-8.6) ³
13	23.30	13.4	7.0	9.86 (9.12-10.62) ⁷	1.94 (1.88-2.00) ³	42.3 $\pm$ 0.93 (39.2-45.6) ⁷	8.2 $\pm$ 0.07 (8.1-8.3) ³
14	23.78	11.4	13.0	19.70 (18.54-20.60) ⁴	0.63 (0.62-0.65) ³	82.8 $\pm$ 1.95 (78.0-86.6) ⁴	2.7 $\pm$ 0.07 (2.6-2.8) ³
15	24.09	13.6	19.0	14.56 (13.92-15.10) ⁴	0.90 (0.83-0.94) ³	60.4 $\pm$ 1.00 (57.8-62.7) ⁴	3.8 $\pm$ 0.18 (3.5-3.9) ³

Fig. 2. Typical continuous scanning records of radioactive spots on chromatograms of 12 hr. urine of rats injected with 5-hydroxy[1-¹⁴C]tryptamine. The descending method on Whatman no. 1 paper was used. Solvent systems (by vol.) and times of run: 1, butanol-acetic acid-water (4:1:5; 19 hr.); 2, propan-2-ol-aq. NH₃ soln.-water (10:1:1; 22 hr.); 3, methanol-benzene-butanol-water (4:2:2:2; 14 hr.); 4, butanol-ethanol-water (4:1:1; 20 hr.).

certainly only in solvent systems 1 and 2. Its radioactivity amounted to not more than 2% of the total urinary activity.

All the compounds located gave a positive test with Ehrlich's reagent. Exposure of chromatograms to ultraviolet light revealed no fluorescence of radioactive spots. Compounds A and D gave a positive naphtharesorcinol and carbazole test for glucuronides.

Radioautographs obtained from two-dimensional paper chromatograms confirmed the location of metabolites found by one-dimensional technique. Combination of solvent system 2 followed by system 1 was the most efficient. Good results were also obtained with combination of system 4 followed by system 3. Other combinations of the four solvent systems gave a rather poor resolution.

A much better resolution in all systems was achieved by submitting urine to deactivated charcoal treatment (after Dalglish, 1955). The relative amounts of radioactive compounds changed after this treatment because of different absorption on the charcoal. 5-Hydroxytryptamine vanished completely and could not be detected either by scanning analysis or radioautography. The amount of 5-hydroxyindolylacetic acid was decidedly lower. Radioactive peaks of one-dimensional chromatograms in system 3 resolved after this procedure, giving compounds A, B, C and D as individual peaks. Two-dimensional radioautographs of charcoal-treated urine are shown in Fig. 3. All metabolites, including the compound F, could be detected. Moreover, two additional weak spots, D₁ and D₂, appeared. As they could not be detected by elution and rechromatography under milder conditions than charcoal treatment, they are believed to be degradation products. D₁ is the only radioactive spot not giving a positive test with Ehrlich's reagent.

Scanner diagrams and two-dimensional radioautographs of 12–24 hr. urine revealed the same

pattern as in 0–12 hr. urine, but of very low intensity.

R_f values of 5-hydroxyindolylacetic acid and of the products obtained after acid hydrolysis are summarized in Table 3. The R_f values of 5-hydroxyindolylacetic acid differ considerably from those of any radioactive metabolite found in rat urine. The substance was also added to the freeze-dried residue of 0–12 hr. urine and the mixture chromatographed in the usual manner. The R_f values of this compound were not changed by this procedure, and the spots were distinct from the radioactive spots in all four solvent systems used.

DISCUSSION

The dose of injected 5-hydroxytryptamine in the experiments described was rather high. The rats showed pronounced toxic effects in the first 6 hr. but then seemed to recover completely. Histological examinations revealed only mild renal injuries typical of 5-hydroxytryptamine. Therefore it seems that these experiments, in spite of the high dose, afford an insight into the normal metabolic pathways of exogenous 5-hydroxytryptamine in rats.

The results obtained in Expts. 1–4 (Keglević-Brovat *et al.* 1958) have shown that about 2% of administered radioactivity was expired, the most rapid elimination of ¹⁴CO₂ occurring 8–14 hr. after the injection. The low percentage of radioactivity found in faeces (about 2%) and retained in the body (about 6%), as well as in all 12–24 hr. urines, supports the presumption that the most important pathway of metabolism of 5-hydroxytryptamine is the excretion in urine during the first 12 hr.

However, in some experiments radioactivity amounting up to 40% of the total dose remained undetected. The great differences in radioactive recoveries obtained with freeze-dried 12 hr. urine

Table 2. R_f values and colour reaction of the main radioactive compounds in rat urine after injection of 5-hydroxy[1'-¹⁴C]tryptamine

Each radioactive spot of the original chromatograms of 12 hr. urine in solvent systems quoted in Fig. 2 was eluted and rechromatographed in the three remaining solvent systems; the separated radioactive spots were re-eluted and individually subjected to chromatography in the original solvent system. A–F are metabolites.

	R_f in solvent system				Intensity of colour with Ehrlich's reagent
	1	2	3	4	
5-Hydroxytryptamine	0.50	0.61	0.68	0.52	(+)
5-Hydroxyindolylacetic acid	0.78	0.32	0.68	0.52	+++
A	0.09	0.03	0.22	0.02	++
B	0.23	0.03	0.22	0.02	++
C	0.23	0.21	0.46	0.07	++
D	0.31	0.21	0.46	0.07	+
E	0.31	0.61	0.68	0.22	+
(F)	0.09	0.32	(0.46)	(0.07)	(+)

cannot, after all the precautions taken, be ascribed to experimental errors.

These findings and the following observations pointed to the possibility of existence of a volatile metabolite in 0–12 hr. urine. In some experiments, untreated urine and freeze-dried residues prepared from the same batch did not give identical radioactive recoveries. This difference was not observed in experiments where freeze-dried urine gave a high recovery of radioactivity (Table 1); in Expts. 14 and 15 some samples of urine as collected were also subjected to wet combustion, and the radioactive recoveries were within the range of recoveries obtained with the same freeze-dried urine; but in Expt. 13, where a low radioactive recovery was obtained with freeze-dried urine, untreated urine gave very inconsistent recoveries, ranging from

45 to 70%. In Expt. 5 the variations of radioactive recoveries were also very high (48–74%). A small amount of the same urine sample was freeze-dried and subjected to wet combustion, giving a recovery of only 42% of the total radioactivity. In Expt. 6, however, the recovery of radioactivity in untreated urine was high, with no great variations; a freeze-dried sample also gave the high recovery of 74%.

As the 5-hydroxytryptamine used had ¹⁴C at the end of the side chain, it is possible that a volatile compound is formed from the side chain of either 5-hydroxytryptamine or its metabolites. However, even if we accept the idea of the existence of a volatile radioactive compound, this cannot account for such great biological variations in the results obtained.

Fig. 3. Radioautographs of two-dimensional chromatograms of 12 hr. urine of rats injected with 5-hydroxy-[1-¹⁴C]tryptamine (5-HT). Before chromatography urine was treated with deactivated charcoal (Dalglish, 1955). 5-Hydroxyindolylacetic acid (5-HIAA) and A–F are metabolites; D₁ and D₂ are degradation products. Intensity of spots: black, high; cross-hatched, weak; broken line, very weak; +, position of 5-hydroxytryptamine in untreated urine.

Table 3. *Chromatographic behaviour of 5-hydroxyindolylacetic acid and its hydrolysis products*

For conditions of hydrolysis see text; for solvent systems see Fig. 2.

	<i>R_F</i> in solvent system				Colour with	
	1	2	3	4	Ehrlich's reagent	Ninhydrin
5-Hydroxyindolylacetic acid	0.63*	0.35	0.49	0.19	Deep blue	—
Products of hydrolysis	0.09	0.14	0.29	0.12	—	Blue-violet
Glycine†	0.77	0.33	0.61	0.57	Blue	—
5-Hydroxyindolylacetic acid†	0.06	0.12	0.25	0.04	—	Blue-violet
	0.80	0.33	0.61	0.53	Blue	—

* Value given by McIsaac & Page (1958) is 0.84.

† Run on the same sheet with hydrolysis products.

All radioactive metabolites of 5-hydroxytryptamine detected in urine were rather unstable compounds. The positive test with Ehrlich's reagent indicates the presence of the indole nucleus. The carbazole and naphtharesorcinol tests suggest that compounds A and D are glucuronides. Compounds A and B showed a very low oxytocic activity. In the urine of rats pre-treated with iproniazid, the amount of compound A increased considerably at the expense of 5-hydroxyindolylacetic acid (our unpublished data). From these findings compound A seems to be identical with 5-hydroxytryptamine O-glucuronide, identified by Weissbach *et al.* (1958) in the urine of mice pre-treated with iproniazid.

5-Hydroxyindolylacetic acid as a metabolite of 5-hydroxytryptamine was discussed by Erspamer (1955), and by Udenfriend, Titus, Weissbach & Peterson (1956), and was claimed to be found in carcinoid urine by McIsaac & Page (1958). We could not detect this conjugate in rats' urine. The R_f given by McIsaac & Page (1958) for this compound is not in accordance with the R_f of synthetically prepared 5-hydroxyindolylacetic acid (Table 3). The R_f of a second metabolite of 5-hydroxytryptamine, 1'-N-acetyl-5-hydroxytryptamine, in butanol-acetic acid-water (our solvent system 1), given by the same authors does not correspond to the R_f of any radioactive spots obtained in these experiments. Radioactive indican would not be expected to be formed from 5-hydroxytryptamine with ^{14}C in the side chain.

SUMMARY

1. 5-Hydroxy[1'- ^{14}C]tryptamine creatinine sulphate at a dose of 2.9 mg./100 g. body wt. (1.27 mg. of the free base/100 g. body wt.) was injected subcutaneously into 15 rats.

2. The outstanding feature of metabolism of 5-hydroxytryptamine was a rapid elimination of radioactivity within 12 hr.; the radioactivity excreted varied from 36 to 82% of the dose. In all experiments 12–24 hr. urine had a low radioactivity, corresponding to about 4% of the injected dose.

3. Besides 5-hydroxyindolylacetic acid, the major metabolite of 5-hydroxytryptamine, six radioactive compounds of unknown structure were found in 12 hr. urine. All of them gave a positive test with Ehrlich's reagent and two of

them are believed to be glucuronides. A small amount of unchanged 5-hydroxy[1'- ^{14}C]tryptamine was recovered in urine.

4. The synthesis and properties of 5-hydroxyindolylacetic acid are described. This compound was not detected as a metabolite of 5-hydroxytryptamine in rats.

The authors are indebted to Professor D. Hadži and Dr S. Detoni of the University of Ljubljana for the infrared spectra and to Dr L. Filipović and his staff (this Institute) for the microanalysis. We would also like to acknowledge the valuable technical assistance given to us by Mrs D. Orlić.

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