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Identification of 5-Hydroxytryptophol as a Serotonin Metabolite In Man.* (31281)

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Although the metabolism of serotonin (5-HT) has been investigated intensively, the metabolic fate of this amine has not been defined completely. In man, the major metabolic route for 5-HT is oxidative deamination to the intermediate aldehyde and then oxidation to 5-hydroxyindoleacetic acid (5-HIAA)(1).

Evidence for a reductive pathway of 5-HT metabolism to the corresponding alcohol has recently been demonstrated in animals. Thus, Kveder *et al* found that 5-hydroxytryptophol (5-HTOH) was formed from 5-HT by rat liver slices and that 5-HTOH-O-glucuronide was a major 5-HT metabolite in rat urine(2). Bartholini, Pletscher, and Bruderer showed the formation of 5-HTOH from 5-HT during the incubation of isolated rabbit platelets with reserpine(3). Subsequently, Feldstein and Wong detected both the aldehyde and the alcohol derivatives of 5-HT in rat liver homogenates incubated with 5-HT-C^{14}(4).

This communication reports the isolation and identification of free 5-HTOH and 5-HTOH liberated from its glucuronide and sulfate conjugates in human urine and documents the *in vivo* conversion of 5-HT-C^{14} to 5-HTOH-C^{14} in man.

Extraction of free and enzymatically-released 5-hydroxytryptophol. Aliquots (75 ml) of 24-hour urine collections from patients with carcinoid tumors were adjusted to pH 11 with sodium hydroxide. Solid barium chloride was added to precipitate inorganic phosphates and sulfates which interfere with subsequent sulfatase hydrolysis. The samples were centrifuged to sediment the barium precipitates. The supernatant urine was then filtered and adjusted to pH 7. Separate 20 ml aliquots of the urine were saturated with sodium chloride and the free 5-HTOH was extracted with two volumes of ether. The ether extracts were then washed with 3 ml of 0.5 M phosphate buffer, pH 7, to remove traces of 5-HIAA.

The ether extracted urine was pooled and incubated with 15,000 units of bacterial

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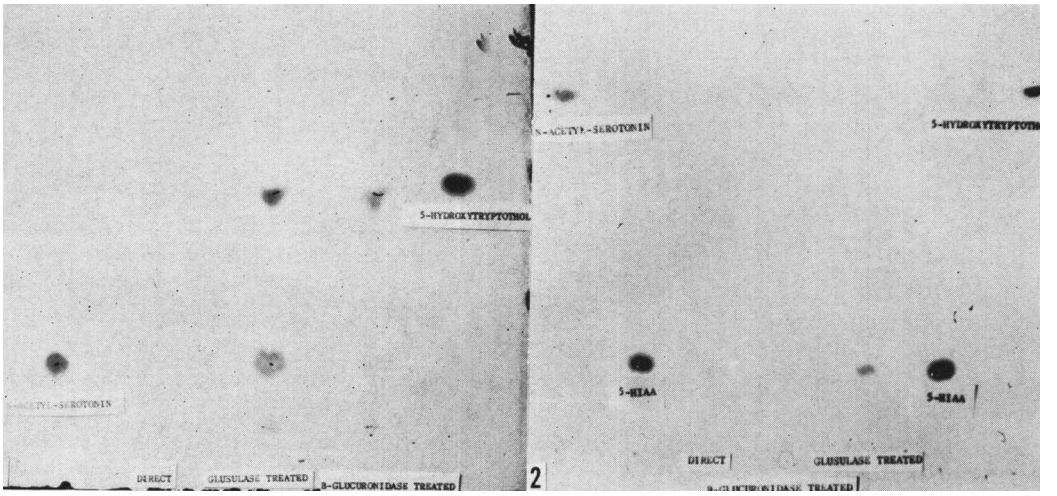

FIG. 1. Thin layer chromatography on silica gel G with ethyl acetate. Urine extracts from untreated, B-glucuronidase-, and Glusulase-treated urine were subjected to paper chromatography and the spots corresponding to 5-HTOH and N-acetyl-5-HT (R_f 0.72) subjected to thin layer chromatography.

FIG. 2. Thin layer chromatography of 5-hydroxyindoleacetic acid derived from enzymatic oxidation of isolated 5-hydroxytryptophol. Silica gel G; solvent system:isopropanol:ethyl acetate; ammonia:water (35:45:18:3). R_f isolated and standard 5-hydroxyindoleacetic acid: 0.28; standard 5-hydroxytryptophol: 0.90; standard N-acetyl serotonin: 0.90.

B-glucuronidase at 37° overnight and the 5-HTOH liberated from its glucuronide conjugate was extracted into 2 volumes of ether and washed as before.

Finally, the extracted urine was adjusted to pH 5.5 and incubated overnight with 0.2 ml of Glusulase, a preparation containing both B-glucuronidase and sulfatase activity. The 5-HTOT liberated from its sulfate conjugate was then extracted into ether as before. The three ether extracts containing the free 5-HTOH, the 5-HTOH liberated from its glucuronide conjugates and the 5-HTOH liberated from its sulfate conjugates were then evaporated with nitrogen over a small volume of ethanol.

Identification of isolated material as 5-hydroxytryptophol. The extracts were then subjected to ascending paper chromatography with isopropanol:ammonia:water (10:1:1) solvent system. A marginal strip was cut from each chromatogram after development and sprayed with an ethanol solution of 0.5% d-dimethylbenzaldehyde and one per cent HCl. The marginal strips contained standards of 5-HTOH, N-acetyl serotonin, and a portion of each extract. In all instances, a spot corresponding to R_f (0.72) to 5-HTOH and N-acetyl-5-HT was demonstrable in extracts from urine treated with B-glucuronidase or Glusulase.

The area of the paper chromatograms corresponding to authentic 5-HTOH and N-acetyl serotonin (R_f 0.72) was removed and eluted with ether. The ether was evaporated to a small volume and chromatographed on silica gel G with ethyl acetate as solvent. In this system, the 5-HTOH migrated with an R_f of 0.75 and the N-acetyl-5-HT with an R_f of 0.21. Both compounds were detected in all samples hydrolyzed with B-glucuronidase or Glusulase (Fig. 1).

The zones identical to 5-HTOH (R_f 0.75) and to N-acetyl-5-HT (R_f 0.21) were eluted from the silica gel with water. The activation and fluorescence characteristics of the compounds identified chromatographically as 5-HTOH and N-acetyl-5-HT were consistent with those of 5-hydroxyindoles. Thus, in water the activation wavelength (uncorrected) for both these compounds was 300 mu and the fluorescence wavelength was 350 mu. In 6 N HCl the fluorescence spectrum showed the typical shift to the visible range of 550 mu for N-acetyl-5-HT and to 560 mu for 5-HTOH. The fluorescence characteristics of

TABLE I. Excretion of Free and Conjugated 5-Hydroxytryptophol.

Subject	Date	μg 5-HTOH/24 hr Free	Sulfate	Glucuronide	Total
O.S.	3-14-65	34	378	414	826
O.S.	3-23-65	44	311	249	604
D.G.	10- 1-64	180	477	1,314	1,971

the isolated compounds were identical with those of authentic standards.

Enzymatic conversion of isolated 5-HTOH to 5-HIAA. A method has recently been developed in this laboratory for quantitative chemical determination of 5-HTOH in biological materials. The method is based on the quantitative conversion of 5-HTOH to 5-HIAA using a coupled enzyme reaction with purified liver alcohol dehydrogenase and rabbit liver aldehyde dehydrogenase preparations. The 5-HIAA formed enzymatically was extracted by the method of Udenfriend *et al* and determined fluorometrically(5).

The compounds identical in R_f to 5-HTOH and to N-acetyl-5-HT were eluted from the silica gel chromatograms with water and incubated in glycine buffer pH 9.6 with NAD, liver alcohol dehydrogenase and purified rabbit liver aldehyde dehydrogenase. The material from the area corresponding to authentic 5-HTOH was converted enzymatically to 5-HIAA. The 5-HIAA was measured fluorometrically and identified chromatographically (Figure 2). No 5-HIAA was formed from the material eluted from the area corresponding to N-acetyl-5-HT.

Excretion of free and conjugated 5-HTOH by patients with carcinoid tumors. The levels of the free and conjugated 5-HTOH in the urine of 2 subjects with carcinoid tumors were measured by conversion of the extracted 5-HTOH to 5-HIAA with the coupled enzyme reaction. The levels of 5-HTOH excreted as the free compound and as the glucuronide and sulfate conjugates are given in Table I.

TABLE II. Serotonin Metabolites in Human Urine After Administration of 5-Hydroxytryptamine-C^{14}-Creatinine Sulfate.

Metabolite	% of C^{14} in urine, mean ± S.D.
5-Hydroxyindoleacetic acid	82.3 ±4.8
Free 5-hydroxytryptophol	.4 ± .1
5-Hydroxytryptophol-O-sulfate	1.0 ± .5
5-Hydroxytryptophol-O-glucuronide	.9 ± .7
Total 5-hydroxytryptophol	2.3 ± .7

In vivo *conversion of 5-HT-C^{14}, to 5-HTOH-C^{14}.* Tracer amounts of 5-HT-C^{14}, usually 5 uc, were given orally to 5 normal subjects and 2 patients with carcinoid tumors and the urine was collected for 8 hours.

The 5-HIAA-C^{14} excreted during this 8-hour period was quantitatively extracted using the method of Udenfriend *et al*(5) and an aliquot of the phosphate buffer counted in a liquid scintillation spectrometer. The 5-HTOH-C^{14} was extracted with ether, pH 7, from unhydrolyzed urine and urine hydrolyzed as described previously(6). Aliquots of the phosphate buffer-washed ether were dried in counting vials for liquid scintillation counting. The activity in this ether extract was shown to consist only of 5-HTOH-C^{14} by thin layer chromatography in ethyl acetate. A 0.2 ml aliquot of the untreated urine was also counted and the per cent of the total C^{14} activity excreted as 5-HIAA and 5-HTOH was calculated. As shown in Table II, only 2.3% of the excreted C^{14} activity was attributable to 5-HTOH and its conjugates and 82.3 per cent to 5-HIAA.

These observations show that the conversion of 5-HT to 5-HTOH represents a regular but relatively minor metabolic pathway in man. The previous demonstration of 3-methoxy-4-hydroxyphenyl glycol sulfate as a metabolite of epinephrine and norepinephrine in man(7) and the present demonstration of 5-HTOH and its conjugates as 5-HT metabolites in man suggests that the aldehydes derived from oxidative deamination of other biogenic amines may also be metabolized to the corresponding alcohols.

Summary. 5-Hydroxytryptophol has been isolated, identified, and measured in urine of subjects with carcinoid tumors. In the urine of normal subjects and of patients with carcinoid tumors who ingested 5-HT-C^{14}, 2.3% of the excreted C^{14} was attributable to 5-HTOH and its conjugates and 82.3% to 5-HIAA. These data show that 5-HTOH is a 5-HT metabolite in man as well as in animals.

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Sensitivity of *Mycobacterium leprae* to Low Levels of 4,4'-Diaminodiphenyl Sulfone.* (31282)

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The most widely used drug for treatment of leprosy is 4,4'-diaminodiphenyl sulfone (DDS). Although it must be administered for years to achieve cure in lepromatous disease, it is eventually effective in nearly all cases(1). The usual dosage is 100 mg/day, and it produces 1-5 μg DDS/ml blood(2). DDS is also effective against *M. leprae* in mice(3,4). The lowest intake previously studied was 0.01% in the diet; it also was completely effective against the bacilli, and it produced about 3 μg DDS/ml serum(4).

In the present study in mice the range of DDS dosages tested has been extended to very low levels, without encountering the endpoint.

Materials and methods. In the experimental system employed(5,3,4), mice were injected in a rear foot pad with 5×10^3 *M. leprae*. The bacillary growth curve was then monitored by monthly harvests of foot pads from untreated mice and counts of the contained acid-fast bacteria (AFB). In the method for counting AFB the technique for applying the sample to the slide has been modified somewhat (unpublished). When the counts rose to a level near 1×10^6 AFB/mouse, harvests were made from each treated and control group. The strain of *M. leprae* used was in first mouse passage. Due to difficulties in the breeding colony, the line of mouse used previously, CFW, was not available in adequate numbers, and BALB/C mice were used instead.

DDS was administered in the diet starting on the day of infection. Since it was essential to ensure homogeneous distribution in the food, the drug was dissolved in 95% ethanol and mixed into the unpelleted diet with a liquid-solid twin-shell blender (Patterson-Kelly Co.). In this apparatus the liquid is sprayed into cavities formed in the rapidly mixing diet by a spinning feed bar so that aggregation of food particles and localized soaking does not occur. The same amount of ethanol was mixed into the control diet. Diets were always mixed in the order of increasing DDS concentration, and the apparatus was carefully cleaned after each day of use.

Sulfone (unconjugated) in blood was determined by the procedure of Simpson(6), modified for smaller volumes for measurement in the 3 ml cell of a Beckman DU spectrophotometer. To 1 ml of heparinized blood, 0.25 ml 0.2 M Na_2HPO_4 was added, and extraction was carried out with 6 ml ethyl acetate by mixing on a vortex mixer for 15 seconds. After centrifugation at 2000 RPM for 5 minutes, 5 ml of the organic phase was transferred by pipette (fitted with a safety bulb) to another tube, and extraction was carried out in the same way with 5 ml 1 N HCl. After the same centrifugation 4 ml of the aqueous phase was removed similarly to another tube. The efficiency of the extrac-

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