The Metabolism of Melatonin (N-Acetyl-5-methoxytryptamine) and 5-Methoxytryptamine*†

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(Received for publication, June 22, 1961)

The potent frog skin-lightening agent "melatonin" was isolated from pineal tissue and characterized as N-acetyl-5-methoxytryptamine by Lerner, Case, and Takahashi (1). Recently, Axelrod and Weissbach (2), studying the biogenesis of this hormone, have shown it to be the product of a specific methylating enzyme system in the pineal gland which acts on the substrate N-acetylsertotonin, a substance previously found to be a metabolite of serotonin by McIsaac and Page (3).

Another compound isolated from pineal tissue proved to be 5-methoxyindoleacetic acid (1), and there have been some differences of opinion regarding the biogenesis of this compound.

To study the metabolic fate of melatonin and the biogenesis of 5-methoxyindole acetic acid, N-acetyl-5-methoxytryptamine-β-C14 and 5-methoxytryptamine-β-C14 were synthesized.

EXPERIMENTAL PROCEDURE

Compounds—5-Methoxytryptamine was synthesized by the method outlined in Fig. 1. 5-Methoxyindole was converted to 5-methoxygramine (4). The quaternary methylsulfonate was prepared and condensed with KCN (5). The resulting nitrile was then reduced with LiAlH4 (6) to give crystalline 5-methoxytryptamine (m.p., 120-121°) (7). Melatonin (N-acetyl-5-methoxytryptamine) was obtained by acetylating 5-methoxytryptamine in a minimal amount of acetic anhydride. The reaction mixture was left overnight at 10°, diluted with water, made slightly alkaline, and extracted with CH2Cl2. The solvent was removed in a vacuum and the residue recrystallized from toluene (m.p., 115-116°) (found: C 66.88, H 6.87, N 11.57; C13H16N2O2 requires C 67.22, H 6.94, N 12.06).

5-Methoxyindole-3-acetic acid (m.p., 150-151°) was obtained by saponification of 5-methoxyindoleacetonitrile with NaOH. 10-Methoxyharmalan was prepared by boiling N-acetyl-5-methoxytryptamine with P2O5 in xylene (8).

N-Acetyl-5-Methoxytryptamine-β-C14 and 5-methoxytryptamine-β-C14, with specific activities of 989 PC per g and 775 PC per g, respectively, were synthesized by the preceding methods, the C14 being introduced by using KC14N. The products had the same melting points as the authentic compounds and the melting points of mixture with these were not depressed. These compounds were subjected to paper chromatography in Solvents A and B, and the chromatograms sprayed with Ehrlich’s reagent (p-dimethylaminobenzaldehyde) and scanned. Each compound gave only one indole spot and only one corresponding peak of radioactivity.

Animals—Male and female albino and hooded rats of 200 to 250 g, and female rabbits of Dutch or albino strains weighing 2.5 to 3 kg were used. Melatonin and 5-methoxytryptamine in aqueous solution were administered to rabbits and to rats by intraperitoneal injection or by stomach tube. Animals were fed on a standard diet, but were deprived of food, although not water, on the experimental day.

Chromatographic Method—Descending chromatography was employed for the detection of metabolites in urine and urine extracts. The solvents, Rf values, and color reactions of reference compounds are given in Table I. Radioactive chromatograms were used for radioautography and scanned with a scanogram RSC-5 (Atomic Accessories, Inc.). For the isolation of metabolites, a 34- x 2.5-cm cellulose (Whatman, standard grade) column and Solvent A were used.

Measurement of Radioactivity—Measurements were carried out on solid samples of "infinite thickness" on nickel planchets with an end window counter tube, the background of which was 18 c.p.m. The specific activities were determined by comparison with a stable polymer reference. A sample of 4 cm2, containing 0.1 μg of C14 per g of substance, gave approximately 250 c.p.m.

Urine and Tissues—Estimation of radioactivity in urine or tissues was made on the residue obtained by evaporating urine or tissue homogenates directly on the planchets.

Isotopic Dilution Methods—For the estimation of metabolites by isotopic dilution, urines were collected from each rat for 24 hours. The volume used depended upon the substance estimated.

5-Methoxyindoleacetic Acid—5-Methoxyindoleacetic acid (200 mg) was dissolved in an aliquot of urine and kept for 2 hours to equilibrate. The solution was then adjusted to pH 5 to 6 (HCl) and the 5-methoxyindoleacetic acid extracted with ether. The extract was evaporated to dryness and the residue recrystallized from benzene to constant specific activity (m.p. and mixed m.p., 120°).

10-Methoxyharmalan—10-Methoxyharmalan (50 mg) was dissolved in an aliquot of urine and kept for 2 hours to equilibrate. The solution was then adjusted to pH 9 to 10 and extracted with ether. The extract was evaporated to dryness and the residue was sublimed in a vacuum. The sublimate was dissolved in ethanol and 1 equivalent of picric acid added. The mixture was boiled and then left at 5° for 12 hours, after which time the precipitate was filtered and recrystallized from alcohol (m.p. and mixed m.p., 258°).

* A preliminary report of this work was presented at a meeting of the Biochemical Society (British) in May 1960.

† This investigation was supported financially by the Gertrude H. Britton Fund.
December 1961

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CH₃O

HCHO

NH(CH₃)₂

CH₂N(CH₃)₂

KCN

Li AlH₄

Saponification

NaOH

CH₃O

CH₂C₆H₄NHCO₂H

Acetylation

CH₃O

CH₂C₆H₄NH₂

CH₃O

CH₂C₆H₄CO₂H

Fig. 1. Radioactive melatonin, 5-methoxytryptamine, and 5-methoxyindoleacetic acid were synthesized according to this scheme.

TABLE I

<table>
<thead>
<tr>
<th>Compound</th>
<th>Rᵣ values in solvent</th>
<th>Fluorescence</th>
<th>Color of spots on paper with</th>
<th>φ</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin</td>
<td>0.48</td>
<td>0.54</td>
<td>Blue</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Hydroxyindoleacetic acid</td>
<td>0.15</td>
<td>0.80</td>
<td>Blue</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetylsertotonin</td>
<td>0.70</td>
<td>0.81</td>
<td>Blue</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Melatonin</td>
<td>0.84</td>
<td>0.85</td>
<td>Blue</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Methoxytryptamine</td>
<td>0.76</td>
<td>0.64</td>
<td>Blue</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-Methoxyindoleacetic acid</td>
<td>0.14</td>
<td>0.82</td>
<td>Blue</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-6-hydroxy-5-methoxytryptamine</td>
<td>0.37</td>
<td>0.26</td>
<td>Blue</td>
<td>Red</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-6-hydroxy-5-methoxytryptamine-O-sulfate</td>
<td>0.55</td>
<td>0.35</td>
<td>Blue</td>
<td>Pink (faint)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-6-hydroxy-5-methoxytryptamine-O-glucuronide</td>
<td>0.20</td>
<td>0.20</td>
<td>Blue</td>
<td>Pink (faint)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-Acetyl-5:6-dimethoxytryptamine</td>
<td>0.20</td>
<td>0.11</td>
<td>Yellow</td>
<td>Blue</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-Methoxyharmalan</td>
<td>0.76</td>
<td>0.87</td>
<td>Yellow</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* The sprays used for detecting compounds on paper were: 1, Ehrlich’s reagent (p-dimethylaminobenzaldehyde, 0.5% solution, in 1.5 n HCl); 2, 0.1% aqueous solution of Brentamine Fast Red B salt, followed by saturated NaHCO₃; 3, 0.5% aqueous solution of sodium nitrite and 0.1% solution of sulfanilic acid in 0.15 n HCl (1:30); 4, Naphthanil diazoblue B (tetrazotized di-O-anisidine), 3% solution, plus borate buffer, pH 9 (3:2); 5, 1,3-naphthalenediol and trichloroacetic acid in butanol; and 6, bromocresol green, 0.05% in ethanol.

Oxidizing System in Vitro—Use was made of the model oxidizing system described by Udenfriend et al. (9) which has been found to hydroxylate indoles in the 6-position (10).

RESULTS

Rate of Excretion and Distribution in Tissues of Administered 5-Methoxytryptamine-β-C¹⁴

The rate of excretion of metabolites in the urine and feces after administration of 3 mg of 5-methoxytryptamine-β-C¹⁴ to rats was measured by the activity present. More than 80% of the activity was excreted in the urine within 24 hours. The rate of elimination is shown graphically in Fig. 2.

The percentage distribution of 5-methoxytryptamine in, and the specific activity of, the tissues ½, 3, and 6 hours after administration are given in Table II.

Identification of Metabolites—Paper chromatography of the urine of rats given 5-methoxytryptamine-β-C¹⁴ revealed the presence of one major metabolite which had the same Rᵣ and color reactions in Solvents A and B as 5-methoxyindoleacetic acid. More than 90% of the radioactivity was associated with
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EXCRETION OF RADIOACTIVITY

Fig. 2. Total excretion of activity in the urine of rats dosed with 5-methoxytryptamine-β-C14.

FIG. 2. Total excretion of activity in the urine of rats dosed with 5-methoxytryptamine-β-C14.

TABLE II

Tissue distribution of 5-methoxytryptamine-β-C14 in rats

<table>
<thead>
<tr>
<th>Rat No. 1 (given 5 mg)</th>
<th>Rat No. 2 (given 5 mg)</th>
<th>Rat No. 3 (given 5 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue distribution after</strong></td>
<td><strong>Tissue distribution after</strong></td>
<td><strong>Tissue distribution after</strong></td>
</tr>
<tr>
<td></td>
<td>30 minutes</td>
<td>3 hours</td>
</tr>
<tr>
<td>Blood</td>
<td>µg/µl*</td>
<td>µg/µl%</td>
</tr>
<tr>
<td>Heart</td>
<td>0.16</td>
<td>10.3</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.11</td>
<td>0.47</td>
</tr>
<tr>
<td>Liver</td>
<td>0.1</td>
<td>0.71</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.16</td>
<td>8.1</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.07</td>
<td>0.25</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.23</td>
<td>4.1</td>
</tr>
<tr>
<td>Brain</td>
<td>0.033</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>37.63</td>
<td>7.83</td>
</tr>
</tbody>
</table>

* Specific activity.

Rate of Excretion and Distribution in Tissues of Administered N-Acetyl-5-Methoxytryptamine-β-C14

The rate of excretion of melatonin in the urine and feces after administration of 5 mg of N-acetyl-5-methoxytryptamine-β-C14 was measured by the activity present. Some 70% of the administered radioactivity was excreted in the urine and 20% in the feces in 24 hours. The rate of elimination is shown graphically in Fig. 3.

A rabbit was given 7 mg of N-acetyl-5-methoxytryptamine-β-C14 and killed after 1 hour. Urine, collected during this time and from the bladder post mortem, contained 12.4%. Bile collected from the gallbladder post mortem contained 0.5% of the administered radioactivity. Excretion of activity into the bile, therefore, represented 4% of the total.

The percentage distribution of N-acetyl-5-methoxytryptamine-β-C14 in the tissues of rats and specific activity of, 3, and 6 hours after administration are given in Table III.

Characterization and Attempted Isolation of Metabolites of Melatonin in Rabbit Urine

Melatonin, 0.8 g, was given to each of six rabbits and the 24-hour urine samples were collected and pooled.

The pooled urine gave a positive Ehrlich's reaction on paper and a positive color reaction with Brentamine reagent (Fast Red B salt), indicating the presence of phenolic compounds. A naphthoresorcinol reaction was strongly positive, indicating the presence of a glucuronide; however, the aqueous phase remained

this metabolite. The presence of a very minor amount, about 2%, of unchanged 5-methoxytryptamine was also shown by scanning.

5-Methoxyindoleacetic acid-β-C14, 10 mg, was given to each of two rats and the 24-hour urines were collected and chromatographed. Only one peak, with RF in Solvents A and B corresponding to 5-methoxyindoleacetic acid, was revealed by scanning.

5-Methoxytryptamine-β-C14, 3 mg, was given to each of three rats, and the 24-hour urines were collected and used for determination of 5-methoxyindoleacetic acid by the isotope dilution technique. 5-Methoxyindoleacetic acid, in an amount which represented 96.8% of the administered 5-methoxytryptamine, was found.

Rate of Excretion and Distribution in Tissues of Administered N-Acetyl-5-Methoxytryptamine-β-C14

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**TABLE III**

Tissue distribution of N-acetyl-5-methoxytryptamine-β-C14

<table>
<thead>
<tr>
<th>Rat No. 4 (given 5 mg)</th>
<th>Rat No. 5 (given 5 mg)</th>
<th>Rat No. 6 (given 5 mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue distribution after</strong></td>
<td><strong>Tissue distribution after</strong></td>
<td><strong>Tissue distribution after</strong></td>
</tr>
<tr>
<td></td>
<td>30 minutes</td>
<td>3 hours</td>
</tr>
<tr>
<td>Blood</td>
<td>µg/µl*</td>
<td>µg/µl%</td>
</tr>
<tr>
<td>Heart</td>
<td>0.084</td>
<td>10.8</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.06</td>
<td>0.23</td>
</tr>
<tr>
<td>Liver</td>
<td>0.23</td>
<td>16.2</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.22</td>
<td>3.1</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.02</td>
<td>13.7</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>0.02</td>
<td>13.7</td>
</tr>
<tr>
<td>Brain</td>
<td>0.02</td>
<td>13.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>44.46</td>
<td>9.8</td>
</tr>
</tbody>
</table>

* Specific activity.
intensely blue after extraction with ether or ethyl acetate, indicating that much of the color was due to the production of a chromophore other than that due to glucuronic acid. Preliminary chromatography disclosed the presence of a major metabolite ($R_f$ 0.55 in Solvent A) which gave positive Ehrlich and naphthoresorcinol reactions on paper, indicating it to be an indole glucuronide. A minor metabolite, $R_f$ 0.2, also gave a positive indole reaction. Since we assumed, erroneously, that the major metabolite was a glucuronide, an attempt was made to isolate it by the lead acetate precipitation method (11). The final fraction which should have contained the glucuronide gave a very weak naphthoresorcinol reaction, and chromatography revealed the presence of only a trace of the major metabolite. On chromatographing all the fractions obtained during the lead acetate precipitation, it was found that most of the major metabolite was present in the supernatant solution from the basic lead acetate precipitation. It was also found that, although the material gave a false positive naphthoresorcinol reaction, the color not being ether-extractable, the major metabolite was not a glucuronide since it had no optical activity and was not hydrolyzed by incubation with 3-glucuronidase. Hydrogen sulfide was then bubbled through this fraction, and the lead-free filtrate concentrated to a small volume. This was then subjected to cellulose column chromatography. Paper chromatography of the fractions containing the indole showed the presence of a hydroxyindole, $R_f$ 0.37 and 0.96, in Solvents A and B, respectively. Since this did not correspond to the major metabolite, $R_f$ 0.25 and 0.55, it indicated that, during the procedure, the major metabolite, presumably a conjugate, had been hydrolyzed to a free phenol. Confirmation of this and an indication of the position of the hydroxyl group were given by the immediately color reaction of the phenol and the delayed color reaction of the major metabolite when the paper was sprayed with acid-diazotized sulfanilic acid. This color reaction indicated that the free phenol was N-acetyl-5-methoxy-6-hydroxytryptamine and the major metabolite was its sulfate conjugate (12). Further confirmation was obtained by methylation of the phenol in tetrahydrofuran with an ethereal solution of diazomethane. The reaction mixture was subjected to paper chromatography. A product was observed ($R_f$ 0.2 and 0.1 in Solvents A and B, respectively) which gave a positive Ehrlich reaction but negative Brentamine and acid diazo reactions, indicating methylation of the hydroxy group. Electrophoretic mobility at pH 4.4 and 8 indicated that, like melatonin, there were no free hydroxyl or amine groups. The ultraviolet spectrum of this product was identical with that previously reported for a synthetic sample of N-acetyl-5-methoxy-6-hydroxytryptamine (13).

Minor Metabolite—Paper chromatography of the various fractions derived from the lead acetate procedure disclosed the presence of a minor metabolite concentrated in one of them. The minor metabolite was obtained from this fraction by cellulose column chromatography. On paper, the minor metabolite gave a positive Ehrlich reaction and a delayed positive acid diazo reaction, indicating that it was a conjugate of 6-hydroxy-melatonin (12). This was confirmed by incubation of a solution of the minor metabolite with glucuronidase. Paper chromatography of the incubation mixture demonstrated its disappearance ($R_f$ 0.2 in Solvent A) and the appearance of the free phenol, which was identified by color reactions and chromatography in two solvent systems, plus a trace of another indole compound ($R_f$ 0.8 in Solvent A). Thus, the minor metabolite was characterized as the glucuronide of N-acetyl-5-methoxy-6-hydroxytryptamine.

Characterization of Metabolites of Melatonin-$\beta$-$C^{14}$ in Rat Urine

Rats (200 g) were each given melatonin-$\beta$-$C^{14}$, 10 mg intraperitoneally, and the 24-hour urines were collected. The urines were treated with urease and concentrated (3) or treated with deactivated charcoal (14). The paper chromatographic pattern of these treated urines was qualitatively the same as the untreated urine. Paper chromatography of the treated urines in Solvent A and subsequent scanning showed three bands of radioactivity: one at $R_f$ 0.1 to 0.25, the second at $R_f$ 0.5 to 0.6, and the third at $R_f$ 0.7 to 0.9. These three bands were cut from the paper, the adsorbed material was eluted, and each was rechromatographed in Solvents A and B.

The band of low $R_f$ in Solvent A showed the presence of one radioactive spot, $R_f$ 0.22, which gave a positive Ehrlich reaction and a delayed acid diazo reaction. In Solvent B, the presence of two compounds was indicated, a minor spot, $R_f$ 0.19, and major one, $R_f$ 0.36. Both of these gave positive Ehrlich reactions, but only the latter gave a positive delayed acid diazo reaction. It was this latter compound which, on incubation with $\beta$-glucuronidase, gave the free phenol. The minor component, however, also disappeared on incubation with glucuronidase, but gave rise to an indolic compound with $R_f$ 0.83 and 0.80 in Solvents A and B, respectively.

The substance in the band of activity of intermediate $R_f$, on rechromatography, gave a single Ehrlich- and a delayed acid diazo-positive spot corresponding to the $R_f$ value of 0.55 in Solvent A. Chromatography in Solvent B again gave the appearance of a single Ehrlich-positive spot, $R_f$ 0.35. Continuous electrophoresis was employed to obtain a few milligrams of a chromatographically pure sample of this metabolite. It was an amber-colored amorphous powder, containing some microscopic crystals (m.p., 160–165°C). Hydrolysis, 1 mg in 1 ml of 1 N HCl for 30 minutes at 80°C, was used for determination of sulfate content (found: SO$_4$ 28.72%); C$_{12}$H$_{12}$O$_3$N$_2$SO$_4$ requires SO$_4$ 29.2%). These color reactions and sulfate estimations are consistent with the characterization of the major metabolite of melatonin as being N-acetyl-5-methoxy-6-hydroxytryptamine-0-sulfate.

The extract from the band of activity of high $R_f$ value, on chromatography in Solvent A, revealed the presence of a small amount of an indole and a larger amount of a compound which did not react with Ehrlich's reagent. The indole had the chromatographic characteristics of melatonin itself. The major spot ($R_f$ 0.86 in Solvent A), which gave negative reactions with Ehrlich's, acid diazo, and Brentamine reagents, gave a yellowish fluorescence under ultraviolet light. In Solvent B, this spot was resolved into two Ehrlich-negative spots, $R_f$ 0.78 and 0.90, respectively (cf. 10-methoxy-harmalan, $R_f$ 0.78 and 0.87 in Solvents A and B, respectively).

Quantitative Estimation of Metabolites of Melatonin-$\beta$-$C^{14}$ in Rat Urine

Quantitative estimation of the metabolites of melatonin was obtained by scanning radioactive chromatograms. The ethereal sulfate of N-acetyl-6-hydroxy-5-methoxytryptamine accounted for 55%, the glucuronide conjugate for 30%, and the other minor metabolites for 15% of the activity in the urine.
Melatonin $^3$H, 3 mg, was given to each of three rats and the 24-hour urines were collected for isotope dilution estimation of 5-methoxyindoleacetic acid and 10-methoxyharmalan.

5-Methoxyindoleacetic acid in the urine was found to account for 2% of the administered melatonin.

10-Methoxyharmalan in the urine was found to account for not more than 0.06% of the administered melatonin. As the range of the estimation was 0.0 to 0.06%, it is possible that the positive results could be due to adsorbed radioactivity which was not removed by two recrystallizations.

In Vitro Studies

With the use of tryptamine as a substrate for the model oxidizing system, the production of 6-hydroxytryptamine was identified by chromatography. Under identical conditions, however, no evidence was found for the production of N-acetyl-5-methoxy-6-hydroxytryptamine from melatonin.

**Discussion**

Metabolism of 5-Methoxytryptamine—Erspamer (15) reported the presence of three indolic metabolites in the urine of animals given 5-methoxytryptamine and identified one of these as 5-methoxyindoleacetic acid. With the use of radioactive 5-methoxytryptamine, we have confirmed that 5-methoxyindoleacetic acid is, indeed, the major metabolite accounting for 96.8% of the fate of the amine. A trace of unchanged 5-methoxytryptamine was also observed in the urine.

The distribution of 5-methoxytryptamine in the tissues was not unusual, the only point worthy of mention being the presence of a significant amount of activity in the brain one-half hour after administration. This may well be related to the fact that animals exhibited abnormal behavior, beginning about 20 minutes after administration and lasting for 20 minutes.

Metabolism of Melatonin—In contrast to the clear-cut metabolic fate of 5-methoxytryptamine, the degradation of melatonin presents a complex picture. Melatonin was found to be rapidly metabolized and excreted, 70% in the urine and 20% in the feces, within 24 hours. Biliary excretion accounted for 4% of radioactivity eliminated within 1 hour. Only 10% could be accounted for in the tissues as soon as 3 hours after administration. A trace of unchanged melatonin was excreted in the urine, and there was little difference between rats and rabbits as to the fate, or mode, of excretion. Further, neither the type of animal used, albino or colored, nor the route of administration affected the metabolism significantly.

The major metabolic product of melatonin was identified as the ethereal sulfate of N-acetyl-6-hydroxy-5-methoxytryptamine by color reactions, sulfate analysis, and by its hydrolysis to the free phenol, which was then converted into the previously known compound, N-acetyl-5,6-dihydroxytryptamine (13). This is in agreement with the preliminary report of Kopin et al. (16).

Strongly positive naphthoresorcinol reaction obtained with the major metabolite was shown to be due to a dye formation and not to the presence of glucuronic acid, emphasizing the danger of relying on color reactions alone to indicate structure.

With the use of an oxidizing system in vitro, we were unable to confirm the previous report that N-acetyl-6-hydroxy-5-methoxytryptamine could be produced from melatonin this way (16).

The metabolite present in the next greatest amount proved to be N-acetyl-6-hydroxy-5-methoxytryptamine-O-glucuronide. It has been reported previously that indole derivatives tend to conjugate to a much greater extent with sulfuric than with glucuronic acid (17).

5-Methoxyindoleacetic acid was found to be a metabolite of melatonin, but to account for only 2% of the dose, indicating that deacetylation plays no significant role. Similarly, demethylation of melatonin does not appear to occur since no N-acetylsertotonin was found.

The most intriguing finding was the presence of at least two metabolites which did not react with Ehrlich's reagent. There are three possibilities which could account for this, namely, ring opening, formation of a 2-oxindole, and cyclization to a harmalan. Cyclization has been shown to occur in the body with the drug Paludrine, which owes its antimalarial activity to a cyclized metabolite (18). The possible conversion of melatonin to 10-methoxyharmalan by cyclodehydration is of importance since such a compound is pharmacologically active. However, chromatography and isotope dilution studies failed to produce unequivocal evidence for its formation. The similarity of 10-methoxyharmalan to harmine, a known psychotomimetic compound (19), can be seen in Fig. 4.

The production of a 2-oxindole would also account for a negative Ehrlich-reacting compound. Lysergic acid diethylamide is metabolized to 2-oxylsergic acid, which is devoid of pharmacological action (20), so the oxidation of melatonin in the 2-position is a possibility.

Present Concept of Serotonin Metabolism—The isolation of melatonin and 5-methoxyindoleacetic acid from pineal glands (1) and the demonstration of the enzymatic O-methyl transferase in that tissue (2) have illuminated a new facet of the complex pattern of serotonin metabolism. The biogenesis of melatonin from

![Fig. 4.](http://www.jbc.org/) Cycloidehydration of melatonin yields 10-methoxyharmalan, which is similar to harmaline and harmicine.
serotonin has been clearly established (21). The biogenesis of 5-methoxyindoleacetic acid, however, is not so clear. Yet it is of importance since, if the substance arises by the direct O-methyla-
tion of 5-hydroxyindoleacetic acid, no physiologically active inter-
mediate is involved, whereas the alternative possibility entails postulation of the presence of the physiologically active amine, 5-methoxytryptamine (22). Since the amount of 5-
methoxyindoleacetic acid in the pineal gland is 10 times greater than the amount of melatonin (1), and since we have found that only 2% of the latter is converted into the former, if 5-methoxy-
tryptamine is present, it does not arise to any extent by the deacetylation of melatonin. Although the presence of 5-
methoxytryptamine in the pineal gland has not been demon-
strated, it seems probable that this substance should be formed since both the substrate and the enzyme necessary for its bio-
synthesis are present. 5-Hydroxyindoleacetic acid and 5-
methoxyindoleacetic acid are present in equal amounts (1), and the latter can arise from serotonin by either of the two pathways illustrated in Fig. 5. Since both of these pathways involve the same two enzymatic reactions, although in a different sequence, the limiting factor is the ratio of the rates of reaction of mono-
amine oxidase and hydroxyindole O-methyl transferase. If, for example, the monoamine oxidase pathway was blocked by an inhibitor, the conversion of serotonin to 5-methoxytryptamine would be a logical sequel.

Other factors, suggesting that 5-methoxytryptamine formation could occur physiologically and might be of some significance in the higher primates, are the high serotonin content, of human and simian pineal glands (23), and the greater activity of hydroxyindole-O-methyl transferase in simian pineal glands (24) in comparison with bovine pineal tissue.

The present concept of serotonin metabolism is, therefore, probably best represented as in Fig. 6.

Finally, it may be significant that the highest pineal gland serotonin content has been found in psychotic patients (23), and that methoxyindolealkylamines are more potent agents for causing abnormal behavior in trained animals than are the corre-
spanding hydroxy analogues (22).

**SUMMARY**

1. A study has been made of the metabolic fate of exogenous melatonin-β-C¹⁴ and 5-methoxytryptamine-β-C¹⁴ in rats and rabbits.
2. The activity of various tissues was estimated after ad-
ministration of compounds. Half an hour after administration of 5-methoxytryptamine-β-C¹⁴ to a rat, there was a significant amount of activity in the brain.
3. After administration of 5-methoxytryptamine-β-C¹⁴ to rats, 80% of the activity was excreted in the urine in 24 hours. After administration of melatonin-β-C¹⁴ to rats, 70% and 20% of the activity were excreted in the urine and feces, respectively.
4. 5-Methoxytryptamine was metabolized, 90% to 5-methoxy-
indoleacetic acid.
5. Melatonin was metabolized to N-acetyl-6-hydroxy-5-
methoxytryptamine, which was excreted as conjugates of sulfuric and glucuronic acids. A trace of unchanged melatonin was excreted and 2% was metabolized to 5-methoxyindoleacetic acid. The presence of two metabolites which did not react with Ehrlich’s reagent was established.

**Acknowledgments**—One of us (S. K.) is grateful to the Inter-
national Atomic Association for a research fellowship. We wish
to thank Dr. I. H. Page for his interest and helpful suggestions regarding the preparation of the manuscript, Mrs. I. Bahlor for her technical assistance, and Dr. A. Lerner for providing a sample of melatonin.

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