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6-Hydroxylation, the major metabolic pathway for melatonin

LERNER *et al.*[1–4] have demonstrated the presence of a melanocyte-contracting substance, melatonin (N-acetyl-5-methoxytryptamine), in bovine pineal glands and in the peripheral nerves of man, monkey, and cattle. AXELROD AND WEISSBACH[5] found a methyl transferase in pineal glands which can O-methylate N-acetylserotonin to melatonin. Little is known about the metabolic fate of this hormone in the body. Although LERNER *et al.*[6] have demonstrated 5-methoxyindole-acetic acid in bovine pineal glands, the origin of this compound is uncertain.

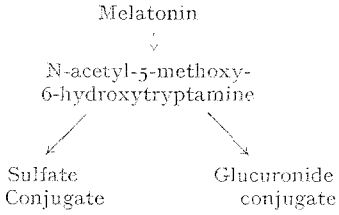
In order to study the metabolic fate of this hormone, [2-^{14}C]melatonin was synthesized from [2-^{14}C]serotonin, and administered intraperitoneally to albino male rats and the urine collected. Chromatography of the urine on Whatman No. 1 paper in isopropanol–5 % ammonia (8:2) and subsequent scanning for radioactive peaks indicated the presence of at least three radioactive compounds (R_F = 0.14, 0.54 and 0.75) the second peak (R_F = 0.54) containing about 80 % of the excreted radioactivity. Only two peaks (R_F = 0.25, 0.80) were found in butanol–acetic acid–water (4:1:1). None of the peaks had R_F's corresponding to melatonin, 5-methoxytryptamine, or 5-methoxyindoleacetic acid. A similar distribution of radioactivity was found on chromatography of the urine of rats which had received [^{14}C-*methoxy*]-melatonin or [^{3}H-*acetyl*]melatonin intraperitoneally indicating that the major metabolites retained both the 5-methoxy and the N-acetyl groups.

After the administration of a large dose of melatonin (20 mg), chromatography of the urine and spraying with Ehrlich's reagent indicated the presence of two indolic compounds, having the same R_F's as the first two radioactive metabolites in the isopropanol–ammonia system (R_F = 0.14, 0.54). These observations indicated that the α-position of the indole ring was unsubstituted. Hydrolysis of the eluted major component with a sulfatase preparation (Gluculase, Endo Products, N.Y.) in the presence of an antioxidant (ascorbic acid) or under N_2 resulted in the formation of a compound having a different R_F in butanol–acetic acid–water (4:1:1) [R_F = 0.67]. No change in R_F followed treatment with bacterial β-glucuronidase (Sigma Chemical Co., St. Louis). The hydrolysed compound reacted with diazotized *p*-nitroaniline to form a purple-colored derivative indicating the presence of a free phenolic group. Both hydrolysed and unhydrolysed metabolites gave red-colored compounds with acidic diazotized sulfanilic acid, the compound containing the free phenolic group reacting more rapidly. This reaction is characteristic of 6-hydroxyindoles and their sulfates[7,8]. Additional evidence for the identity of this metabolite as N-acetyl-5-

methoxy-6-hydroxytryptamine was obtained by treating melatonin with the model oxidizing system[9] which has been shown to hydroxylate indoles on the 6-position[10]. Incubation of melatonin with this system resulted in the formation of a compound having the same R_F's and color reactions as the major excretion product of melatonin after hydrolysis.

The indolic compound having an R_F of 0.14 in isopropanol–5 % ammonia (8:2) was also isolated and shown to have an R_F of 0.11 in the butanol–acetic acid–water system. Hydrolysis of this compound with β-glucuronidase resulted in the formation of two indolic compounds, one having the same R_F and color reactions as the compound tentatively identified as N-acetyl-5-methoxy-6-hydroxytryptamine.

These observations indicate that the principle pathway of metabolism of melatonin is as follows:



The methods of preparation of the radioactive compounds used will be reported later.

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