Biosynthesis of melatonin: enzymic conversion of serotonin to N-acetylserotonin

Recently LERNER and coworkers[1] isolated melatonin (5-methoxy-N-acetyltryptamine), a derivative of N-acetylserotonin, from beef pineal glands. Previous studies from our laboratory have described an enzyme in pineal extracts which transfers a methyl group from S-adenosylmethionine to N-acetylserotonin to form melatonin[2,3]. It was suggested that the biosynthesis of melatonin involved first the acetylation of serotonin, followed by methylation of N-acetylserotonin. MCISAAC AND PAGE[4] have reported the conversion of serotonin to N-acetylserotonin *in vivo*. We wish to report the presence of an enzyme in rat liver and beef pineal gland which can acetylate serotonin in the presence of an AcCoA-generating system.

N-Acetylserotonin, formed enzymically, was assayed as follows: aliquots of the incubation mixture were diluted with 10 vol. water and placed in a boiling-water bath for 1 min. The precipitated material was removed by centrifugation and the supernatant fluid passed over a small Permutit column to remove the substrate, serotonin. The N-acetylserotonin was then assayed fluorometrically with excitation wavelength, 295 mμ; fluorescence wavelength, 360 mμ.

As shown in Table I a soluble supernatant fraction of rat liver was able to acetylate serotonin in the presence of an AcCoA-generating system. The N-acetylserotonin formed in this manner was further identified by paper chromatography, as well as by enzymic conversion to melatonin with a purified hydroxyindole-O-methyl transferase from beef pineal glands[2,3].

TABLE I

ACETYLATION OF SEROTONIN BY RAT LIVER

	N-acetylserotonin formed (μg/h)
Complete system	5.8
Minus Liver	< 0.3
Minus AcCoA-generating system	0

Livers from rats, pretreated with iproniazid to inhibit monoamine oxidase, were homogenized in 3 vol. of ice-cold water and centrifuged at 78,000 × *g* to obtain a soluble supernatant fraction. The complete incubation system contained 0.3 ml of soluble supernatant, 0.34 μmole serotonin, 50 μmoles Tris buffer, pH 8.0, 15 μmoles EDTA, 0.5 μmole CoA, 12.5 μmoles acetyl phosphate, phosphotransacetylase* (0.04 mg protein), and water to a total volume of 1.5 ml. Incubations were carried out at 37°.

Soluble supernatant fractions obtained from rat brain and beef pineal glands were also shown to acetylate serotonin. However, the serotonin-acetylating system in the brain and pineal gland was less active and more labile than that in the liver. Since beef pineal extracts also contain hydroxyindole-O-methyl transferase it was possible to show the over-all conversion of serotonin to melatonin using a partially

Abbreviations: CoA, coenzyme A; AcCoA, acetyl-coenzyme A; AMe, S-adenosylmethionine; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

* A crude preparation of phosphotransacetylase was kindly supplied by Dr. ROY VAGELOS, National Heart Institute.

TABLE II

FORMATION OF MELATONIN FROM SEROTONIN BY BEEF PINEAL EXTRACTS

	Melatonin formed (μg/h)
Complete system	0.7
Minus AMe	0.0
Minus AcCoA-generating system	0.0

A soluble supernatant fraction obtained from beef pineal glands was fractionated with ammonium sulfate and the protein that precipitated between 33–60% satn. was dissolved in water and used as the source of the enzymes. The complete incubation mixture contained ammonium sulfate fraction (9.5 mg protein), 1 μmole AMe, 0.34 μmole serotonin, 50 μmoles Tris buffer, pH 8.0, 15 μmoles EDTA, 0.5 μmole CoA, 12.5 μmoles acetyl phosphate, phosphotransacetylase and water to a total volume of 1.5 ml. Melatonin was assayed by a procedure described elsewhere[5].

purified preparation from beef pineal glands, an AcCoA-generating system and AMe (Table II).

The experiments described here and elsewhere show that the biosynthesis of melatonin proceeds from serotonin as follows:

$$\text{Serotonin} \xrightarrow{\text{AcCoA}} \text{N-acetylserotonin} \xrightarrow{\text{AMe}} \text{Melatonin}$$

Further studies are now in progress on the purification and properties of this enzyme system.

Laboratory of Clinical Biochemistry, National Heart Institute, and Laboratory of Clinical Sciences, National Institute of Mental Health, National Institutes of Health, Bethesda, Md. (U.S.A.)

HERBERT WEISSBACH
BETTY G. REDFIELD
JULIUS AXELROD

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A comparison of the thermal dissociation of two polynucleotide helices in H_2O and D_2O

In studies of the infrared spectra of nucleic acid derivatives it is desirable to work in aqueous solution, since this eliminates the necessity of assumptions about possible changes in spectra or tautomeric form in going from the usual infrared solvents to the biologically important solvent, water. D_2O has the advantage over water of possessing a window in the region of double bond absorption, and spectra of alkylated model compounds[1], nucleotides[1,2], polynucleotides[1], and of nucleic acids[3] have been studied in this solvent. In the use of D_2O there is the implicit assumption (though a