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.

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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

much smaller one than is necessary in applying $CHCl_3$ or CCl_4 spectra to water) that results obtained in D_2O apply to H_2O. To investigate this point the dissociation of hydrogen-bonded polynucleotide* mixtures with increasing temperature (melting curve) was investigated in H_2O and D_2O, both as a matter of intrinsic interest and as background information for the infrared studies.

Melting curves of a number of polynucleotide mixtures have been reported[5,6], either u.v.-absorption or optical rotation being used to follow the thermal dissociation of the helices.

The melting temperatures, T_m, are defined in the present case as the maxima of the derivative curves for poly(A + U) and poly(I + C). This method has the advantage of permitting a characteristic temperature to be determined when very high or low temperatures make measurement of the full curve inconvenient or impossible. (This value of T_m is a few degrees higher then the temperature at which the absorption has had half its maximum increase).

The results, shown in Figs. 1 and 2, show that there is no difference in T_m in D_2O and H_2O within the limits of accuracy of the measurements (the values of T_m can be determined to within about 0.5°; any difference in T_m in D_2O and H_2O is less than 1°). It is concluded from these curves that the chemical properties measured in D_2O apply also to H_2O as a solvent.

Recent experiments have shown differences in melting-out behavior of a polypeptide[7], of ribonuclease[8], and of gelatin[9] in hydrogen and deuterium systems, but these results do not conflict with those cited above showing no difference in T_m in D_2O and H_2O. The relative stability of the helix in the two solvents will depend

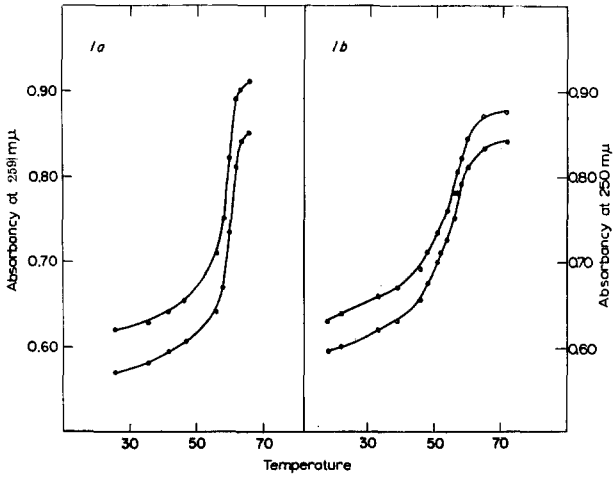

Fig. 1a. Poly A + Poly U, 0.1 *M* NaCl, 0.05 *M* sodium cacodylate, pH 7.2. Upper curve, H_2O; lower curve, D_2O. The temperature at half maximum increase of absorbancy is 59.3° in D_2O and 58.7° in H_2O.

Fig. 1b. Poly I + Poly C, 0.125 *M* NaCl, 0.05 *M* sodium cacodylate, pH 7.0. Upper curve, D_2O; lower curve, H_2O. The temperature at half maximum increase of absorbancy is 53.3° in D_2O and 53.0° in H_2O.

*The polynucleotides used are the homopolymers polyadenylic acid (Poly A), polyuridylic acid (Poly U), polycytidylic acid (Poly C) and polyinosinic acid (Poly I), prepared with polynucleotide phosphorylase[4].

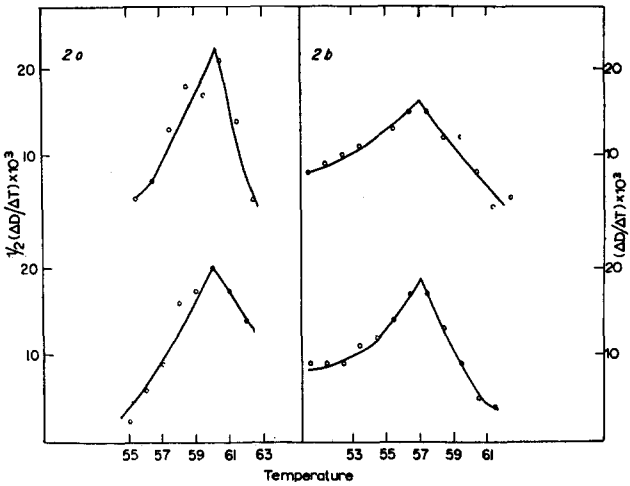

Fig. 2a. Poly A + Poly U; derivative plots of curves in 1a. Upper curve, $T_m = 60° \pm 0.5°$ in H_2O; lower curve $T_m = 60° \pm 0.5°$ in D_2O.

Fig. 2b. Poly I + Poly C; derivative plots of curves in Fig. 1b. Upper curve, $T_m = 57 \pm 0.5°$ inD_2O; lower curve, $T_m = 57° \pm 0.5°$ in H_2O.

primarily on whether the quantity (*Fp–p—Fp–s*)* is greater than, equal to, or less than the same quantity in D_2O. With the polynucleotides studied here the values must be very nearly the same in the two solvents**.

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** Dr. W. F. HARRINGTON has pointed out to the author the possibility that the ideas presented by GALLAGHER[10] on the effect of isotope substitution on hydrogen-bond length and strength may permit a prediction of the direction and possibly of magnitude of deuterium substitution on the melting temperature of polymers. At the present time, however, the necessary bond distances and the shape of GALLAGHER's curve (Fig. 1)[10] above 2.8 Å are probably not known with sufficient accuracy to make the prediction reliable in the case of the polynucleotides.

* Where *Fp-p* is the energy of the hydrogen bonds from one polymer chain to another in the helix and *Fp-s* is the energy of the hydrogen bonds from solvent to the polymer chains after they have dissociated.