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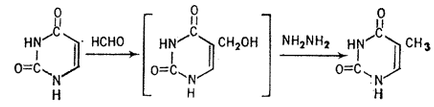
Thymine: A Possible Prebiotic Synthesis

Abstract. *A possible prebiotic synthesis of thymine has been achieved via the methylation of uracil with formaldehyde and hydrazine.*

Thymine is the only nucleic acid base that has not been synthesized under possible prebiotic conditions. We report here the successful synthesis of thymine under conditions presumably like those present on the primitive earth. The abiotic synthesis of the other nucleic acid bases, in particular the pyrimidines, uracil (*1, 2*), and cytosine (*3*), have been accomplished from simple precursor molecules. In addition, uracil could have arisen from cytosine by way of deamination (*3*). Attempts to synthesize thymine in a similar manner have not proved successful, and we therefore decided to investigate the methylation of uracil as a means of synthesizing thymine abiotically.

The catalytic addition above *p*H 7 of formaldehyde to uracil (*4, 5*), uridine (*4*), or its 5′-mononucleotides (*6*) has been reported. The 5-hydroxymethyl derivatives are obtained almost exclusively in each case. 5-Hydroxymethyluracil is found in the DNA of a group of *Bacillus subtilis* bacteriophages in the place of thymine (*7*). It is also a probable intermediate in the biological synthesis of thymine nucleotides (*8*). 5-Hydroxymethyluracil may be reduced to thymine, 5,6-dihydrothymine, or 5,6-dihydro-5-hydroxymethyluracil by catalytic hydrogenation (*4, 9*). Therefore, the reaction of uracil and formaldehyde in an ammoniacal solution in the presence of a reducing agent was investigated as a possible prebiotic method for producing thymine, as shown below.



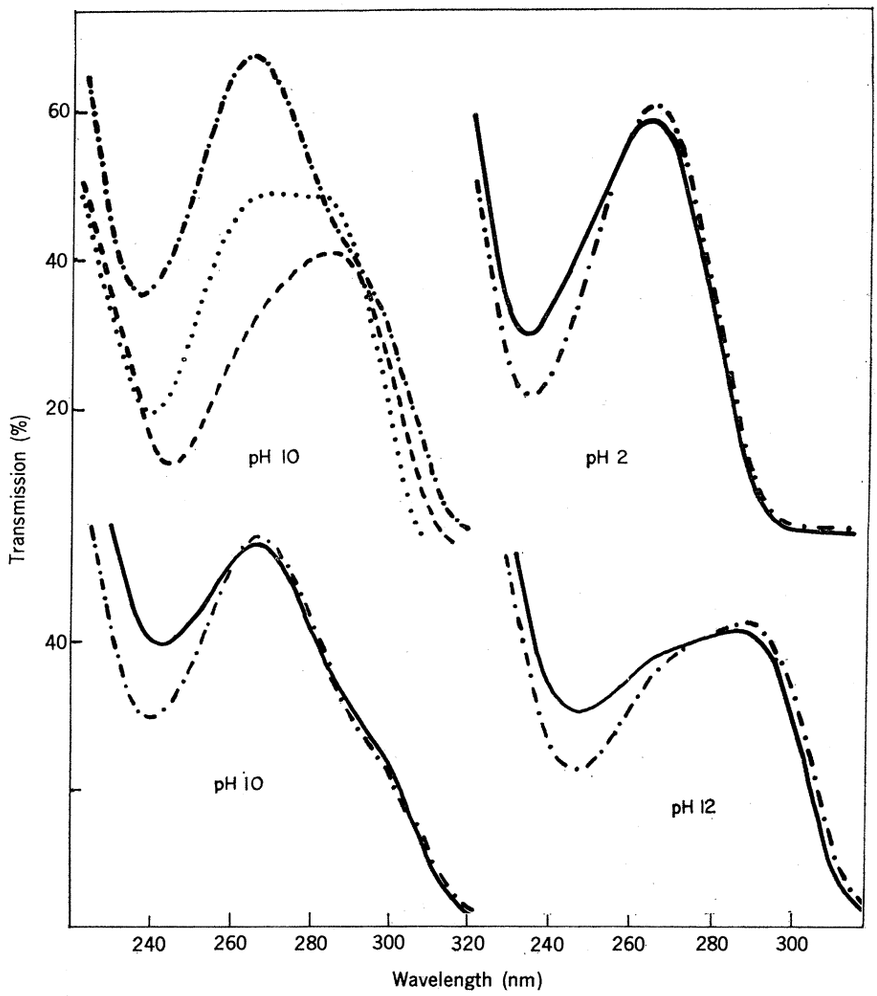


Fig. 1. Ultraviolet absorption spectra of the unknown compound (———), thymine (–·–·–), uracil (·····), and 5-hydroxymethyluracil (– – – –).

Hydrazine was chosen as the reducing agent, since this compound is formed by the action of electric discharges on ammonia (*10*). Also hydrazine will reduce the hydroxymethyl group of pyridoxine hydrochloride to a methyl group yielding 4-deoxypyridoxine (*11*).

In a typical experiment, thymine was obtained when uracil, paraformaldehyde, and hydrazine (0.005 mole, respectively) were heated in an ammoniacal solution (100 ml, *p*H 9) for 3 days at 70°C. The reaction mixture before heating had a *p*H of 8.5. On completion of the reaction, the solution was stirred with freshly washed Dowex 50 (H^+ form, 100 to 200 mesh, 3 g), filtered, and concentrated under reduced pressure at 40°C to a final volume of approximately 0.5 ml. The unreacted uracil, which precipitated out of solution during the concentration process, was removed by filtration. The thymine was isolated by means of two-dimensional preparative paper chromatography with two different solvent systems; the isolated product was finally purified by thin-layer chromatography on polyamide plates (*12*). Descending paper chromatography on Whatman No. 1 paper was used; the solvent system for the first dimension was 2-butanol saturated with water (thymine,

$R_F = 0.73$; unknown, $R_F = 0.73$). In the second dimension, 1-butanol, saturated with water and ammonia (100 : 1, by volume), gave R_F values of 0.60 and 0.58 for the thymine standard and unknown compound, respectively. A chloroform, acetic acid (95 : 5, by volume) solvent system was used in the polyamide thin-layer chromatography. Both the unknown compound and thymine had R_F values of 0.60 in this system. The unknown was eluted from chromatograms with distilled water. In control experiments, uracil and paraformaldehyde, or paraformaldehyde and hydrazine, or uracil and hydrazine, under identical conditions gave negative results. These experiments excluded the possibility of contamination giving rise to thymine. Apart from the evidence obtained from chromatographic procedures, the unknown compound was also identified as thymine by its ultraviolet adsorption spectra at three different *p*H's—2, 10, and 12 (Fig. 1). An inspection of the ultraviolet spectra of uracil, 5-hydroxymethyluracil, and thymine at *p*H 10 will indicate the value of recording the spectrum of the unknown compound at this *p*H. Conclusive evidence was obtained by mass spectral analysis of the isolated, pure compound. The analysis shows a molecular ion of mass number 126 with three major fragmentation ions of mass numbers 83, 55, and 28. Thymine under the same conditions yielded a molecular ion of 126 and an identical fragmentation pattern. Molecular ions are observed for pyrimidines, and the fragmentation pattern is dependent on the nature of the substituents and their positions on the pyrimidine ring (*13*). This excludes the possibility that the unknown compound is an isomer of thymine.

The above experiments were carried out in air. To exclude the possibility that oxygen was involved in the reaction, a reaction was carried out in a solution purged with nitrogen prior to the addition of hydrazine. The reaction vessel was tightly stoppered, and the solution was heated for 3 days as usual. However, during the isolation of the unknown compound, air was not excluded. Thymine was isolated as in previous experiments.

The yield of thymine in this reaction was found to be 0.1 percent, as determined by the isotope dilution method. This yield is low but is in accord with other prebiotic syntheses. Oró and Kimball (*14*) obtained a 0.5 percent yield for adenine from an 11.1*M* solution of ammonium cyanide. The yields obtained for uracil in the condensation of β-aminopropionamide with urea in ammoniacal solutions at 135°C were also less than 1 percent (*2*). Sanchez *et al.* (*3*) obtained 5 percent yields of cytosine by heating cyanoacetylene with potassium cyanate in an aqueous solution at 100°C for 1 day. Fox and Harada reported yields of more than 10 percent in their synthesis of uracil (*1*).

This abiotic formation of thymine, subject to confirmation, completes the list of nucleic acid bases synthesized under prebiotic conditions. Of interest is the similarity of this particular abiotic synthesis to the normal biological pathway (*8*).

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Calcium as a Mediator of Adrenocorticotrophic Hormone Action on Adrenal Protein Synthesis

Abstract. *Calcium stimulates leucine incorporation into protein during incubations of sections and cell-free preparations of the rat adrenal. Like adrenocorticotrophic hormone (ACTH) action, calcium enhances the transfer of amino acid from transfer RNA to protein. Stimulation of leucine incorporation by ACTH and cyclic adenosine monophosphate is best observed when sections are incubated in limiting calcium concentrations.*

While it has long been recognized that calcium is required during the steroidogenic action of adrenocorticotropin (ACTH) (*1*, *2*), the role of calcium is obscure. Adenosine 3′,5′-cyclophosphate (cyclic AMP) apparently mediates the ACTH effect on steroidogenesis (*3*), and Lefkowitz *et al.* (*4*) have found a requirement for calcium in the activation of adenyl cyclase by ACTH; but, in addition, calcium must act *after* generation of cyclic AMP, since the steroidogenic action of the latter also requires calcium (*5*). I have observed a marked effect of calcium on adrenal protein synthesis (*6*), and this effect may largely explain the calcium requirement during stimulation of steroidogenesis, since the latter is known to require continued protein synthesis (*7*).

In addition to steroidogenesis, ACTH controls various metabolic processes in the adrenal cortex, including, and perhaps most fundamentally, adrenal growth. The mechanism or mechanisms whereby ACTH controls adrenal growth is not well understood. Administration of ACTH leads to increased protein synthesis in cell-free preparations of the rat adrenal, and this is due to increased activity in the supernatant after centrifugation at 105,000*g* (105,000*g* supernatant) (*8*) and in the microsomes (*9*). The increase in supernatant activity precedes the increase in microsomal activity (*10*) and is due to a macromolecular factor which enhances the transfer of amino acid from the aminoacyl–transfer RNA (tRNA) complex to protein (*8*) and appears to be identified with the so-called "transfer enzymes" (*11*). The mechanism whereby ACTH increases transfer enzyme activity is unknown.

In accord with the hypothesis that cyclic AMP is an intracellular "second messenger" for ACTH (*3*), Ney (*12*)