

enzyme activity. Experiments are now in progress to determine whether the increased serine aldolase activity results from activation of pre-existing enzyme or from the *de novo* synthesis of new enzyme, and to determine also the role of the hormone in this process.

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## Methylation of uracil deoxyriboside by soluble enzymes of thymus

Administration of serine-3-<sup>14</sup>C or <sup>14</sup>C-labelled formate or formaldehyde to animals leads to extensive incorporation of <sup>14</sup>C into thymine of tissue deoxynucleic acids (DNA)<sup>1-3</sup>, suggesting the synthesis of thymine from a uracil precursor. Of various (2-<sup>14</sup>C)uracil derivatives administered to rats the best precursor of DNA thymidine was found to be uracil deoxyriboside<sup>4</sup>, suggesting that in the intact animal the latter nucleoside is methylated to give thymidine. In confirmation of this view PRUSOFF, LAJTHA AND WELCH<sup>5</sup> observed that deoxyuridine stimulated the incorporation of <sup>14</sup>C-formate into DNA thymine by Ehrlich ascites tumour cells, and FRIEDKIN AND ROBERTS<sup>6</sup> reported the conversion of (2-<sup>14</sup>C)uracil deoxyriboside to DNA thymidine by bone marrow cell suspensions and minced chicken embryo. Both groups of workers reported an inhibition of the conversion of uracil deoxyriboside to DNA thymidine by aminopterin.

A synthesis of thymidine from uracil deoxyriboside and serine-3-<sup>14</sup>C has now been achieved in the presence of the clear supernatant from rabbit thymus homogenate. The reaction has been followed by isolating the synthesised thymidine with the aid of unlabelled carrier thymidine. After chromatography on paper using butanol-ammonia as solvent, the eluted thymidine was further purified by chromatography on Dowex-1-chloride. Untreated thymus supernatant catalysed the incorporation of <sup>14</sup>C from serine into thymidine in the presence of uracil deoxyriboside but, when boiled or treated with Dowex-1-chloride, incorporation did not occur. Thymus supernatant which had been treated with Dowex-1-chloride (100 mg/ml of supernatant) could be reactivated for optimum incorporation of <sup>14</sup>C from serine-3-<sup>14</sup>C into thymidine by the addition to the solution of tetrahydropteroyl glutamate ( $10^{-3}M$ ), adenosine triphosphate ( $10^{-3}M$ ) and reduced diphosphopyridine nucleotide as well as uracil deoxyriboside. Omission of tetrahydropteroyl glutamate caused a 20 to 30-fold decrease in the reaction rate. Pyridoxal phosphate, triphosphopyridine nucleotide and cobalamine do not increase the rate of incorporation.

In a preliminary experiment, <sup>14</sup>C from formate or formaldehyde was incorporated into thymidine at a much lower rate than from serine-3-<sup>14</sup>C. When uracil deoxyriboside was replaced by uridine, dihydrouridine or dihydrouracil deoxyriboside, the rate of incorporation of serine-3-<sup>14</sup>C into thymidine was lowered considerably, so that uridine, dihydrouridine and dihydrouracil deoxyriboside are not intermediates in the conversion of uracil deoxyriboside to thymidine.

The incorporation of serine-3-<sup>14</sup>C into thymidine with uracil deoxyriboside as precursor was much greater than incorporation into thymine using uracil as precursor, or into thymidine-5'-phosphate using uracil deoxyriboside-5'-phosphate as precursor. In a preliminary experiment, <sup>14</sup>C-thymidine formed by thymus extract was degraded to yield iodoform from the thymine methyl carbon<sup>1</sup>. The radioactivity of the isolated iodoform accounted for all the radioactivity of the thymine.

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