showed that enzymic oxidation to the oxides occurred for these two amines. Methylol transfer may occur through a tetrahydrofolic acid factor, and this point is being studied.

The amino acid oxide hydroxytransferase found in this work is so far known to be present only in a mouse liver homogenate; cellular fractionation procedures are in progress with liver and other tissues to determine its distribution in the cell and in other tissues.

Laboratory of Chemistry of Natural Products, National Heart Institute, National Institutes of Health, Bethesda, Md. (U.S.A.)

M. S. FISH
C. C. SWEELEY
N. M. JOHNSON
E. P. LAWRENCE
E. C. HORNING

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Enzymic synthesis of deoxyribonucleic acid

We have reported[1] the conversion of ^{14}C-thymidine via a sequence of discrete enzymic steps to a product with the properties of DNA*.

$$\text{Thymidine} \xrightarrow{\text{ATP}} \text{T5P} \xrightarrow{\text{ATP}} \text{TTP} \xrightarrow{\text{ATP}} \text{"DNA"} \qquad (1)$$

The thymidine product is acid-insoluble, destroyed by DNAase, alkali-stable and resistant to RNAase. We have now extended these studies to include adenine, guanine and cytosine deoxynucleotides, and with partially purified enzymes from *E. coli* we have studied further the nature of the polymerization reaction.

^{32}P-labeled deoxynucleotides were prepared by enzymic digestion of DNA obtained from *E. coli* grown in a ^{32}P-containing medium; the nucleotides were then phosphorylated by a partially purified enzyme. The principal product of T5P phosphorylation was separated as a single component in an ion-exchange chromatogram and identified as TTP. The ratios of thymidine:acid-labile P:total P were 1.00:2.03:3.08. Enzymic formation of the di- and triphosphates of deoxyadenosine and the pyrimidine deoxyribonucleosides has been observed[2] and the presence of pyrimidine deoxyribonucleoside polyphosphates in thymus extracts has been reported[3].

Polymerization of TTP requires ATP, a heat-stable DNA fragment(s), provisionally regarded as a primer, and two enzyme fractions (called S and P; previously[1] called A and B, respectively) each of which has thus far been purified more than 100-fold (Table I). Preliminary studies suggest that TDP can replace TTP and has the same requirements for incorporation into DNA; a decision as to the more immediate precursor requires further purification of the system.

"Primer" for the *crude* enzyme fraction was obtained (1) by the action of crystalline pancreatic DNAase on *E. coli* DNA or (2) on thymus DNA, or (3) by an *E. coli* enzyme fraction (SP) acting on DNA contained in it. However, "primer" for the *purified* enzyme fraction was obtained only with method (3); the action of pancreatic DNAase on either *E. coli* or thymus DNA did not yield "primer". These findings imply the existence of an activity in the crude enzyme fraction responsible for the formation of active "primer". The chemical properties of the unpurified 'primer" resemble those of a partial digest of DNA.

Utilization of the polyphosphates (presumably triphosphates) of adenine, guanine and cytosine deoxynucleosides for DNA synthesis occurs at rates approximately equal to those for TTP in crude enzyme fractions, but at appreciably slower rates with the enzyme purified for TTP polymerization (Table II). These changes in ratio suggest the presence of different enzymes for each of the deoxyribonucleoside triphosphates. Mixtures of these triphosphates, each tested at concentrations near enzyme saturation, gave additive or superadditive rates, further suggesting different enzymes for each of the substrates and a facilitation of polymerization by such mixtures.

Studies are in progress to define the mechanism of the polymerization reaction and the

* Abbreviations used are: DNA, deoxyribonucleic acid; ATP, adenosine triphosphate; T5P, thymidine-5'-phosphate; TDP, thymidine diphosphate; TTP, thymidine triphosphate; DNAase, deoxyribonuclease; RNAase, ribonuclease.

linkages and sequences in the DNA-like product formed. Further investigations with phage-infected *E. coli*[1] and studies with biologically active DNA may begin to clarify the question of how genetically specific DNA is assembled.

TABLE I

REQUIREMENTS OF THE PURIFIED SYSTEM

Extracts of *E. coli B* prepared by sonic disintegration were treated with streptomycin to yield a precipitate (fraction SP) and a supernatant fluid (fraction SS). Ammonium sulfate, gel and acid fractionation procedures applied to fractions SP and SS yielded fractions P and S, respectively. *E. coli* DNA was prepared by heating fraction SP (optical density at 260 mμ = 15) at 70° for 10 minutes. To produce "primer", 0.1 ml of *E. coli* DNA was combined with 40 γ of fraction SP; after 1 hour at 37° in the presence of $5 \cdot 10^{-3} M$ $MgCl_2$, the mixture was heated for 10 minutes at 80°. The complete system contained (in 0.3 ml) 0.014 μmole of TTP ($1.5 \cdot 10^6$ c.p.m./μmole), 0.1 μmole of ATP, 0.10 ml of "primer", 10 γ of fraction S, 1 γ of fraction P, 1 μmole of $MgCl_2$, and 20 μmoles of glycine buffer, pH 9.2. After incubation for 30 minutes at 37°, 0.05 ml of crude *E. coli* extract ("carrier") and 0.3 ml of 7% perchloric acid were added. The precipitate was washed, plated and its radioactivity measured.

	mμmoles DNA-P/hour
Complete system	1.48
No ATP	0.20
No "primer"	0.11
No enzyme fraction S	0.07
No enzyme fraction P	0.04

TABLE II

CONVERSION OF FOUR DEOXYNUCLEOSIDE TRIPHOSPHATES

The incubation mixtures and assays were as described in Table I except that (1) the concentrations of deoxynucleoside triphosphates were $1.5 \cdot 10^{-5} M$, and (2) the crude enzymes were 60 γ of fraction SP and 240 γ of fraction SS.

Triphosphates	*Tested with crude enzymes*	*Tested with purified enzymes (for TTP)*
	mμmoles DNA-P/hour	
Thymidine (T)	0.8	5.48
Deoxyguanosine (G)	0.6	0.98
Deoxycytidine (C)	0.8	1.44
Deoxyadenosine (A)	0.6	1.28
T + G	2.2	14.6
T + G + C	4.4	19.6
T + G + C + A	6.4	22.0*
T + G + C + A (no "primer")	2.0	0.28

* 65% conversion of substrate.

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Department of Microbiology, Washington University School of Medicine, St. Louis, Mo. (U.S.A.)

ARTHUR KORNBERG
I. R. LEHMAN
MAURICE J. BESSMAN
E. S. SIMMS

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