Further studies are under way on the substrate affinities and specifities of glutamine and asparagine transaminases.

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[1] A. MEISTER AND S. V. TICE, *J. Biol. Chem.*, 187 (1950) 173.
[2] A. MEISTER, H. A. SOBER, S. V. TICE AND P. E. FRASER, *J. Biol. Chem.*, 197 (1952) 319.
[3] A. MEISTER, *Advances in Enzymol.*, 16 (1955) 185; *Biochemistry of the Amino Acids*, Academic Press, New York, 1957, p. 179.
[4] A. MEISTER AND P. E. FRASER, *J. Biol. Chem.*, 210 (1954) 37.
[5] F. SCHLENK AND A. FISHER, *Arch. Biochem.*, 12 (1947) 62.
[6] A. E. BRAUNSTEIN, in *The Enzymes*. ed. by P. BOYER, H. LARDY AND K. MYRBÄCK, Vol. 2, Academic Press, Inc., New York, 1960, p. 113.
[7] E. E. SNELL AND W. T. JENKINS, *J. Cellular Comp. Physiol.*, 54 Suppl. 1 (1959) 161.
[8] A. E. BRAUNSTEIN AND HSU TING SENG, *Biokhimiya*, 25 (1960), 758.
[9] HSU TING SENG AND M. LERMAN, (1960) in preparation.

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Terminal incorporation of [^{14}C]AMP into s-RNA by bacterial enzymes

AMP, derived from ATP, is incorporated into terminal positions of soluble ribonucleic acid (s-RNA) by enzymes derived from rat liver. This attachment of AMP has been shown to occur predominantly in positions adjacent to cytidylic acid of s-RNA, thus establishing the terminal ribonucleotide sequence and the specificity of the reaction[1]. The enzyme fractions responsible for the incorporation of terminal ribonucleotides into s-RNA have been obtained primarily from extracts of rat liver[1–4].

Since an analogous type of s-RNA has been found to exist in *Escherichia coli*[5], the presence of enzymes associated with its synthesis has been sought in extracts of this microorganism. The availability of enzyme systems from two markedly different sources (animal and bacterial) responsible for the same reaction would permit a detailed comparative study and lead to a better understanding of these reactions. In the present communication the existence is demonstrated in *E. coli* of an enzyme system responsible for the terminal incorporation of [^{14}C]AMP from [^{14}C]ATP into s-RNA, in positions adjacent to cytidylic acid; this enzyme system has been partially purified.

Extracts of *E. coli*[6] were centrifuged for 1 h at 100,000 × *g* and the pH of the supernatant fraction was adjusted to 3.8. The resulting isoelectric precipitate (Table I, Fraction 1) retained more than 90 % of the ability of the crude extract to incorporate [^{14}C]AMP into RNA when [^{14}C]ATP was the precursor (results not shown). As shown in Table I (Fraction 1), after alkaline hydrolysis of the isolated radioactive RNA, 60 % of the incorporated [^{14}C]AMP was liberated as adenosine and 40 % as AMP. Further purification resulted in the isolation of a fraction (Table I, Fraction 2)

Abbreviations: AMP, ADP, ATP, adenosine mono-, di- and triphosphate; s-RNA, soluble ribonucleic acid.

TABLE I

DISTRIBUTION OF RADIOACTIVITY IN ALKALINE HYDROLYSATES OF s-RNA

The incubation mixture consisted of 30 mμmoles of [^{14}C]ATP or [γ-^{32}P]ATP, of specific activity 0.88 μC/μmole and 5.0 μC/μmole, respectively, 100 μmoles tris(hydroxymethyl)aminomethane-HCl buffer, pH 8.0, 20 μmoles $MgCl_2$, 5 μmoles $MnCl_2$, 18 μmoles creatine phosphate, 0.2 mg creatine kinase[10], and 0.8 mg of *E. coli* enzyme; final volume, 1.3 ml. After incubation at 37° for 20 min, the samples were chilled in ice, and 8 ml of cold 0.4 *N* $HClO_4$ were added. The precipitates were washed and hydrolyzed with 0.3 *N* KOH overnight at 37°. The resultant acid-soluble fraction was fractionated by column chromatography[12].

Substrate	*Enzyme*	*Radioactivity in:*				
		Adenosine	*Cytidylic*	*Uridylic*	*Adenylic*	*Guanylic*
		as % of total				
[^{14}C]ATP	Fraction 1	60	—	—	40	—
[^{14}C]ATP	Fraction 2a	90–95	—	—	5–10	—
[γ-^{32}P]ATP	Fraction 2a	—	87.5	3.5	6.5	2.5
[γ-^{32}P]ATP	Fraction 2b	—	85.5	3.5	9.5	1.5

(a) 23.0 A_{260} units of *E. coli* s-RNA were added.
(b) 6.0 A_{260} units of s-RNA-β + -γ were added.

which incorporated [^{14}C]AMP into RNA in significant amounts only if s-RNA was added. In this case, the [^{14}C]AMP was incorporated mainly in terminal positions, as evidenced by the fact that, after alkaline hydrolysis of the radioactive s-RNA, 90–95 % of the incorporated radioactivity was recovered as adenosine. If a similar experiment was performed using [γ-^{32}P]ATP as the substrate, the ^{32}P in the alkaline hydrolysate of the isolated s-RNA was primarily associated with 2′(3′)-cytidylic acid. This occurred whether the s-RNA added was derived from *E. coli* or from rat liver. The s-RNA used in the latter case (s-RNA-β + -γ)[4,7] is known to contain only about 15 % terminal cytidylic acid groups; the remaining 85 % of the polynucleotide is composed primarily of adenylic acid and guanylic acid, with some uridylic acid being present. Thus, the bacterial enzyme system shows a distinct preference for attaching adenylic acid to cytidylic acid groups in terminal positions.

In order to ascertain the nature of the precursor for this reaction, the enzyme system was incubated with [^{14}C]ATP or [^{14}C]ADP, in the presence and absence of a phosphorylating system. The results presented in Table II indicate that optimal incorporation occurred when the phosphorylating system was present. The slight incorporation which took place in the presence of [^{14}C]ADP alone can be explained by myokinase activity, which is known to contaminate the enzyme preparation. Analysis of the acid-soluble fractions under these conditions showed appreciable degradation of the added [^{14}C]ATP, incubated in the absence of creatine phosphate and creatine kinase, as well as of the added [^{14}C]ADP; in the latter case, some [^{14}C]ATP was also formed. In the presence of the phosphorylating system, [^{14}C]ATP was the major constituent of the acid-soluble fractions. Accordingly, these results provide strong evidence that [^{14}C]ATP is the precursor in the reaction under study.

These experiments indicate that enzymes associated with the synthesis of s-RNA, which are essentially similar to those previously found in animal tissues[1–3] and probably similar to those recently reported[8,9], have been purified from bacterial

TABLE II

THE ABILITY OF [^{14}C]ADP AND OF [^{14}C]ATP TO SERVE AS PRECURSORS

The basic incubation mixture was the same as that described in Table I, except for the omission of creatine kinase and creatine phosphate from those samples marked (—). All samples contained 23.0 A_{260} units *E. coli* s-RNA. When [^{14}C]ADP was used, 30 μmoles were added (specific activity, 0.65 μC/μmole). After incubation at 37° for 20 min, 8 ml cold 0.4 *N* $HClO_4$ were added; the precipitates were washed repeatedly, plated and counted[11].

Substrate	*Creatine kinase + Creatine phosphate*	*[^{14}C]AMP incorporated mμmole*
[^{14}C]ATP	+	0.25
[^{14}C]ATP	—	0.18
[^{14}C]ADP	+	0.23
[^{14}C]ADP	—	0.09

extracts. Detailed comparative studies of the enzymes from these two sources will be presented at a later date.

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[1] E. S. CANELLAKIS, *Biochim. Biophys. Acta*, 25 (1957) 217.
[2] L. I. HECHT, P. C. ZAMECNIK, M. L. STEPHENSON AND J. F. SCOTT, *J. Biol. Chem.*, 233 (1958) 954.
[3] E. HERBERT, *J. Biol. Chem.*, 231 (1958) 975.
[4] E. S. CANELLAKIS AND E. HERBERT, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 170.
[5] S. LACKS AND F. GROS, *J. Mol. Biol.*, 1 (1959) 301.
[6] E. S. CANELLAKIS, M. E. GOTTESMAN AND H. O. KAMMEN, *Biochim. Biophys. Acta*, 39 (1960) 82.
[7] E. HERBERT AND E. S. CANELLAKIS, *Biochim. Biophys. Acta*, in the press.
[8] T. PREISS AND P. BERG, *Federation Proc.*, 19 (1960) 317.
[9] M. ALEXANDER, A. BRESLER, J. FURTH AND J. HURWITZ, *Federation Proc.*, 19 (1960) 318.
[10] S. A. KUBY, L. NODA AND H. LARDY, *J. Biol. Chem.*, 209 (1954) 191.
[11] E. S. CANELLAKIS AND E. HERBERT, *Biochim. Biophys. Acta*, in the press.
[12] E. S. CANELLAKIS AND R. MANTSAVINOS, *Biochim. Biophys. Acta*, 27 (1958) 643.

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Digestion of ribonuclease A with chymotrypsin and trypsin at high temperatures

We should like to describe some experiments which offer the possibility of locating the non-helical regions in native ribonuclease and also the portions of the polypeptide chain involved in the reversible, thermal transition[1]. These experiments were based on the hypothesis that the unfolded parts of a polypeptide chain are more susceptible than the helical portions to attack by proteolytic enzymes[2]. If the amino acid sequence is known, the positions of the enzymic cleavages may be determined. In contrast to