INCORPORATION OF RIBONUCLEOTIDES INTO RNA

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Extracts of calf thymus nuclei catalyze the incorporation of isotope from CTP (CRP^{*}-P-P) into an acid insoluble polymer. This enzymic activity has now been purified more than 100-fold from acetone powder extracts of thymus gland by acetone and ammonium sulfate fractionations followed by chromatography on DEAE-cellulose columns. With the purified enzyme the reaction requires Mg^{++} and is completely dependent on the presence of RNA. The RNA requirement is highly specific-- RNA isolated from thymus gland by extraction with phenol (Kirby, 1956) is highly effective while RNA isolated by the same method from liver is completely inactive and RNA isolated from E. coli (kindly provided by Dr. P. Berg) and A. vinelandii shows only slight activity. In addition, the RNA isolated from thymus is inactive as an acceptor of the amino acid leucine when incubated with the corresponding amino acid activating enzyme purified from A. vinelandii (these experiments were kindly performed by Miss P. Ortiz). No activity is observed with CDP in place of CTP. However, the formation of pyrophosphate in the reaction has not been established because of the presence of pyrophosphatase in the enzyme preparations.

Evidence has been obtained, as shown in Table I, that CMP is added to the end of RNA chains. After incorporation of CTP-C^{14}, degradation of labeled RNA with alkali yields two-thirds of the total radioactivity as cytidine and the remainder as cytidylic acid. This pattern is similar to

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that observed in soluble RNA (Hecht, et. al. 1958). (See Table I) From an analysis of the product found on alkaline hydrolysis with CRP*-P-P as the substrate, it is evident (Table II) that CMP is added to all of the RNA nucleotides (Goldwasser, 1955) with some preference for addition to cytidine and adenosine end-groups. In this hydrolysis procedure, P^{32} initially introduced with CTP is transferred to the adjacent nucleotide.

The purified enzyme preparation has a high degree of specificity with respect to CTP. It does not catalyze the fixation of ATP or GTP to an appreciable extent. There is some incorporation of UTP into an acid-insoluble form but the ratio of UTP:CTP fixation varies between 1:2 and 1:50 during the course of purification, suggesting that these are distinct and separable enzymic activities. On the basis of these observations, it may be inferred that separate enzymes are required for ribonucleotide incorporation into RNA in thymus gland. This is in contrast to RNA synthesis with polynucleotide phosphorylase (Grunberg-Manago et. al, 1956) or DNA synthesis with the DNA "polymerase" (Lehman, et. al. 1958), where in each case a single enzyme appears to catalyze incorporation of all of the nucleotides concerned.

The most interesting properties of the enzyme reported here are the high degree of specificity with regard to both the nucleotide utilized and the RNA required. Work is in progress in an effort to further define these factors and the nature of the enzymes involved in RNA biosynthesis.

TABLE I

LOCALIZATION OF C^{14} IN ALKALINE HYDROLYSATE OF RNA

Product Isolated	CPM
C^{14} - cytidine	29,160
C^{14} - CMP (2' or 3')	17,100

The incubation conditions were as follows: C^{14} - (uniformly) - CTP 1 x 10^7 CPM/μmole, 30 mμmoles; thymus RNA, 21 μmoles of orcinol reacting material (Mejbaum, 1939); 160 μmoles of Tris buffer pH 8; $MgCl_2$ 20 μmoles;

100 µg. of an ammonium sulfate fraction of thymus acetone powder extract in a total volume of 2.5 ml were incubated for 60 minutes at 37°. The reaction was stopped by the addition of 0.5 ml of 7 per cent $HClO_4$ and the acid-insoluble product washed 4 times with 1 per cent $HClO_4$. The material was then dissolved with 1 ml of 1M NaOH and incubated overnight at 38°. Cytidine was isolated after passages of the diluted and neutralized fraction through a Dowex 1 Cl^- resin column. CMP was isolated after elution with 0.003 M HCl.

TABLE II

LOCALIZATION OF P^{32} IN ALKALINE HYDROLYSATES OF RNA

Product Isolated	cpm.
Uridylate (2' or 3')	6,000
Guanylate "	6,300
Adenylate "	14,700
Cytidylate "	31,000

The condition of the incubation was as in Table I with the exception that P^{32} labeled CTP (CRP^*-P-P) replaced C^{14}-labeled CTP. The nucleotides were isolated by paper electrophoresis in .05 M ammonium formate buffer, pH 3.5 after degradation of the RNA with 1M NaOH.

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