Incorporation of Amino Acids into Protein in a System Containing Rat Liver Ribonucleoprotein Particles and Chick Liver Cell Sap

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It has been shown [1, 2] that isolated ribonucleoprotein particles from rat liver incorporate amino acids into protein when incubated with labelled amino acids, cell sap, adenosine triphosphate (ATP), and a system to regenerate ATP. The possible synthesis of a specific protein, rat serum albumin, by these particles has been studied in some detail [3]. It has been found, that a protein similar to rat serum albumin becomes radioactive during incubation. This albuminlike protein has the immunological properties of rat serum albumin but differs slightly from rat serum albumin in its electrophoretic mobility on cellulose acetate [4].

When the ribonucleoprotein particles are incubated under the above conditions but with cell sap from chick liver, the rat albumin-like protein becomes radioactive as well as a chick albumin-like protein. The proteins can be precipitated by antiserum to rat serum albumin or antiserum to chick serum albumin, respectively. The labelled chick protein can be separated from the incubation mixture by column chromatography on DEAE-cellulose followed by electrophoresis on cellulose acetate. If the labelled chick protein is then allowed to react with antichick serum albumin in Ouchterlony plates, a radioactive precipitate forms.

The possibility remains, however, that the radioactivity obtained in the chick albumin-like protein is due to non-specific but energy dependent addition of amino acids to a protein moiety which behaves immunologically and on DEAE-cellulose columns as chick serum albumin.

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Early Effects of Glucocorticoids on Nucleic Acid Metabolism of Rat Liver

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One important aspect of the action of adrenal glucocorticoids in mammalian tissue is their effects on several hepatic enzyme activities. It has become increasingly evident that the response of these enzymes to either cortisone or hydrocortisone *in vivo* actually represents rapid synthesis of new enzyme protein. In spite of this, investigations concerning the possible role of the nucleic acids in early steroid mediated enzyme induction in the mammalian liver are rather scarce [1-3].

By a procedure based on the recovery of RNA-uridine and DNA-thymine, it can be seen that cortisone or hydrocortisone administered to fasted, adrenalectomized rats induce a rapid accumulation of liver RNA, which occasionally can be observed as early as 6 h after the hormone injection. The DNA content remained constant. The increase in RNA can be located in the cytoplasm as well as in the nuclear fraction.

The underlying changes in RNA metabolism are presumably revealed by the increased relative specific activities attained in mixed nucleic acid purines of liver tissue exposed to radioactive bicarbonate *in vitro*, and in RNA-uridine after incorporation of orotic acid-6-^{14}C *in vitro* and *in vivo*.

The stimulatory effect of cortisone on RNA metabolism, reported by Feigelson *et al.* [2, 3] was demonstrated as increased incorporation rates of inorganic ^{32}P and glycine-^{14}C, preceding an early rise in the levels of RNA.

In this laboratory, by short time labeling *in vivo* with orotic acid, a response has been observed within an hour after cortisone injection.

The changes described take place concomitant with the synthesis of tryptophan pyrrolase, which is among the earliest enzymes so far recognized to be induced by the glucocorticoids. Puromycin prevents the induction of tryptophan pyrrolase, and inhibits in part the responses observed within the RNA metabolism. Pretreatment with Actinomycin D also eliminates the induction of tryptophan pyrrolase.

The results obtained support the idea that adrenal glucocorticoids affect enzyme activities

in the liver *via* RNA, and will be discussed within the frame of current concepts of regulatory mechanisms in the synthesis of proteins. The possibility that these hormones regulate the formation of "clusters" of secondary gene products the expression of which may, or may not, be modified by substrates or dietary manipulations, will be considered.

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Species Specificity of Amino Acyl RNA Synthetases from *E. coli* and Yeast

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Berg *et al.*[1] have reported that a methionyl RNA synthetase purified from yeast did only recognize 40 % of the methionine sites on *E. coli* amino acid acceptor RNA (acc RNA) that are available to the homologous enzyme. Benzer and Weisblum[2] using crude extracts of *E. coli* and yeast found striking differences in their ability to esterify non-homologous RNA depending on which amino acid was used. Rendi and Ochoa[3] noted that crude *E. coli* extracts could not attach leucine to yeast RNA nor could yeast extracts esterify *E. coli* RNA with this amino acid. In view of these findings it seemed of considerable interest to make a systematic study of the species specificity of amino acyl RNA synthetases with purified enzyme preparations from *E. coli* and yeast.

As part of an attempt to extend previous investigations of the nucleotide sequences in acc RNA from *E. coli*[4,5] to other microorganisms we have undertaken the purification of valyl-, leucyl-, and phenylalanyl RNA synthetases from yeast. The details of these procedures and of the purification of phenylalanyl RNA synthetase from *E. coli* will be reported elsewhere. Valyl- and leucyl-RNA synthetase from *E. coli* were prepared according to Bergmann *et. al.*[6] A comparison of the properties of the yeast enzymes and their counterparts from *E. coli* gave the following results.

All of the yeast enzymes with the exception of the leucyl RNA synthetase were able to esterify *E. coli* RNA to approximately the same extent as the *E. coli* enzymes as measured by the ratio of amino acid to RNA nucleotide. The maximal esterification of *E. coli* RNA obtained with yeast leucyl RNA synthetase was 60–70 % of the available sites. On the other hand, of the *E. coli* enzymes only the valyl RNA synthetase could esterify yeast RNA to the same extent as the homologous enzyme. The leucyl- and phenylalanyl RNA synthetases did not attach amino acids to the non-homologous RNA to a measurable extent.

Comparisons of the reaction velocities catalyzed by the enzymes with homologous RNA as compared to non-homologous RNA revealed some interesting differences. In all the cases tested the velocities with the non-homologous RNA were considerably lower. The value obtained with yeast valyl RNA synthetase and *E. coli* RNA was 10–15 % of the velocity with the "natural" substrate while the combinations yeast phenylalanyl RNA synthetase *E. coli* RNA and *E. coli* valyl RNA synthetase/yeast RNA gave only 2–6 % of the velocities with homologous RNA.

The results of analysis on the Hershey column[7,8] of amino acyl RNA's synthesized with homologous and non-homologous enzymes will be discussed.

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