Enzymic Formation of Uridine-5′-phosphate from Uracil

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Most mammalian tissues studied show a relatively poor utilization of uracil for pyrimidine nucleotide synthesis. In the Ehrlich ascites tumor, however, uracil was incorporated into polynucleotide pyrimidines as rapidly as orotic acid. In a study of the enzymic mechanism of this process it was found that uridine-5′-phosphate(UMP) formation from uracil in acetone powder extracts of the tumor proceeded according to:

$$\text{Uracil} + \text{ribose-1-phosphate} \xrightarrow[\text{phosphorylase}]{\text{nucleoside}} \text{uridine} + \text{phosphate}$$

$$\text{Uridine} + \text{ATP} \xrightarrow[\text{kinase}]{\text{uridine}} \text{UMP} + \text{ADP}$$

Both enzymes involved are known to occur in mammalian organs, *e. g.* liver, which form very little UMP from uracil.

We have now investigated the amount of these two enzymes in the ascites tumor and in rat liver. Furthermore, the breakdown of uracil to CO_2 was also studied, since it had been proposed that this process might determine uracil utilization in different tissues [2].

A significant difference was found in the amount of nucleoside phosphorylase as measured by a spectrophotometric assay. Uridine kinase was consistently *ca.* 10 times more active in extracts of the tumor than in liver extracts. A high concentration of uridine was necessary for optimum UMP synthesis. In accordance with earlier findings of other investigators we observed a quite rapid breakdown of uracil to CO_2 in rat liver preparations. No such degradation was observed in tumor extracts, however. In both cases a high TPNH level was maintained during the reaction.

It seems likely that uracil utilization by different tissues will depend on a competition between catabolic reactions which degrade uracil to CO_2 and the anabolic formation of UMP.

1. Reichard, P. and Sköld, O. *Acta Chem. Scand.* **11** (1957) 17.
2. Canellakis, E. S. *J. Biol. Chem.* **221** (1956) 315.

Dephosphorylation of Nucleoside Triphosphates by Rat Diaphragm *in vitro* *

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When the isolated rat diaphragm is incubated in saline phosphate medium at pH 7.4 at 37 °C a marked ATPase and adenylic acid deaminase activity is present. These properties have been studied by determination of the degradation products of ATP in the diaphragm during incubation. The products formed have been separated by an ion exchange resin method on Dowex-1. By the same technique the conversion products of nucleoside triphosphates added to the medium during incubation of rat diaphragm have been studied

The breakdown of ATP in the diaphragm has been studied in experiments where diaphragms from 20 rats, weighing 6—7 g, were incubated in 15 ml saline phosphate medium. During incubation for 30 min in oxygen, 25 % of ATP initially present in the diaphragm (2.2 μmole per g initial level) was broken down and almost quantitatively recovered as IMP and inosine. In anaerobiosis the breakdown of ATP was greatly accelerated, amounting to 90 % in 30 min. The same high rate of breakdown was observed in oxygen if 5×10^{-5} M 2,4-dinitrophenol or 0.01 M NaCN was present in the medium. In these experiments nucleotides were not detected in the medium which, however, contained a considerable amount of inosine.

The breakdown of nucleotides added to the medium was studied by incubating 2 hemidiaphragms weighing 250 mg in 2 ml saline phosphate medium. 10 μmole ATP added at the start of the experiment was rapidly broken down in oxygen atmosphere. Thus after 30 min 95 % of ATP was broken down and accumulation of ADP, AMP, IMP and inosine was demonstrated. The dephosphorylation and deamination probably did occur on the surface of the diaphragm muscle as no enzyme activity was liberated into the medium when 2 hemidiaphragms were incubated for 30 min. The experiments indicate that the following sequence of reactions takes place:

ATP → ADP → AMP → IMP → Inosine

* Supported by grants from *Eli Lilly and Co.* and from *Norwegian Research Council for Science and the Humanities.*