

Proceedings of the Swedish Biochemical Society

Papers read at the meeting in Lund, June 3-4, 1954.

The Biosynthesis of Ribonucleic Acid Pyrimidines in Ehrlich's Ascites Tumor

Ulf Lagerkvist and Peter Reichard

Department of Biochemistry, Karolinska Institutet, Stockholm, Sweden

In a previous communication we have proposed the following scheme for the synthesis of orotic acid in rat liver slices¹. L-aspartic acid + CO₂ + NH₃ → L-ureidosuccinic acid → dihydro-orotic acid → orotic acid.

Reichard² has presented strong evidence that orotic acid is a normal intermediate in the biogenesis of polynucleotide pyrimidines and therefore the scheme given above probably represents the actual pathway of pyrimidine synthesis in rat liver. The pyrimidine biogenesis of the Ehrlich ascites tumor³ has now been investigated for comparison.

Two different experimental approaches were used. In the first one tumor cells were incubated in Krebs-Henseleit medium with any of the following N¹⁵ labeled substances: Ammonia, aspartic acid, L-ureidosuccinic acid, orotic acid, uracil, dihydrouracil, ureidopropionic acid, β-alanine. The ribonucleic acid pyrimidines were isolated and their isotope content determined. In experiments with N¹⁵-ammonia and aspartic acid the distribution of the isotope in the pyrimidine ring was determined⁴.

In the second approach the tumor cells were incubated with an isotopically labeled precursor (known to give an incorporation of isotope into the polynucleotide pyrimidines) in the presence of a pool of the non labeled substance suspected to be an intermediate (ureidosuccinic acid, dihydroorotic acid or orotic acid). Both the substance under investigation and the polynucleotide pyrimidines were isolated at the end of the incubation and their isotope content was determined.

The results of these two experimental approaches show that the same precursors as in rat liver slices can be used by the ascites tumor for the synthesis of polynucleotide pyrimidines. The question of these substances being also

normal intermediates tends to be obscured by permeability effects and will be discussed.

1. Reichard, P. and Lagerkvist, U. *Acta Chem. Scand.* **7** (1953) 1207.
2. Reichard, P. *J. Biol. Chem.* **197** (1952) 391.
3. Klein, G. and Révész, L. *J. Natl. Cancer Inst.* **14** (1953) 229.
4. Lagerkvist, U. *Acta Chem. Scand.* **4** (1950) 543.

Formation *in vitro* of Uridine Phosphates from Orotic Acid

Robert B. Hurlbert and Peter Reichard

Department of Biochemistry, Karolinska Institutet, Stockholm, Sweden

The transformation of orotic acid into different uridine phosphates has been demonstrated in rat liver *in vivo*¹. Since it was shown that one or several of these nucleotides are precursors of polynucleotide pyrimidines and since orotic acid has been demonstrated to be a normal intermediate in pyrimidine biosynthesis in rat liver² an investigation of the enzymatic conversion of C¹⁴-orotic acid into uridine phosphates has been started.

The enzymes were obtained either from the dialyzed supernatant of homogenized liver (rat and pigeon) after high speed centrifugation or from extracts of pigeon liver acetone powder. In the presence of ribose-5-phosphate, Mg⁺⁺ and ATP (or fructose-di-phosphate DPN, Mg⁺⁺ and ATP) C¹⁴-orotic acid was converted into several radioactive compounds, one of which was identified as uridine-5-phosphate (UMP). The other compounds gave rise to UMP upon acid hydrolysis and were probably pyrophosphates of uridine (*e. g.* uridine-diphosphate and uridinetriphosphate). In some cases enzymatic breakdown of the uridinephosphates to uridine and uracil took place. In a time study it was found that UMP was the first uridine compound formed from orotic acid. This result was confirmed by the finding that C¹⁴-UMP was rapidly transformed into radioactive uridinepyro-