2. Pathway of Pyrimidine Synthesis de Novo

Higher organisms and many micro-organisms do not require exogenous pyrimidines and can synthesize pyrimidine nucleotides from simple precursors. In 1944 it became apparent that orotic acid is involved in the synthesis of pyrimidines \textit{de novo} and a few years later it was evident that it is a precursor of nucleic acids [70,71].

The small-molecule precursors of orotic acid were identified by Reichard and Lagerkvist [72]. During \textit{in vitro} incubation, liver slices incorporated labelled ammonia, bicarbonate and aspartate into pyrimidine components of RNA. When the slices were incubated with the labelled precursors in the presence of extracellular orotate, isotopes were found in this extracellular fraction. In this way, orotic acid was recognized as an intermediate in pyrimidine synthesis \textit{de novo}. The labelled orotate was chemically degraded and small molecules identified as the precursors of particular atoms of the orotate ring, as shown in the following scheme:

![Chemical structure of orotic acid](image)

Nutrition studies in bacteria indicated that carbamoyl aspartate (also known as ureidosuccinate) is an intermediate in pyrimidine biosynthesis. Finally, uridine nucleotides were found as end products of pyrimidine synthesis \textit{de novo} [73]. The sequence of reactions leading to the synthesis of UMP is designated as the orotate pathway or pyrimidine synthesis \textit{de novo} in distinction to the salvage pathway.

The salvage pathway utilizes preformed pyrimidines and purines for the synthesis of nucleic acids and is highly active in various types of cells. Uridine kinase plays a key role in the pyrimidine salvage pathway and its concentration is considered to reflect the relative efficiency of the system in utilizing preformed pyrimidines [74]. Adenosine kinase plays a similar role in making use of preformed purines [75]. It should be noted, however, that uridine and adenosine kinases are not the only enzymes involved in the salvage pathway and other deoxynucleoside kinases, phosphorylases, and phosphoribosyltransferases [76] also have important roles.

As already mentioned, carbamoyl aspartate was recognized as an intermediate in the synthesis of orotic acid [77]. In ureotelic livers, it is
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synthesized from ammonia, carbon dioxide and aspartic acid in a two-step process, involving the formation of carbamoyl phosphate. When using a rat liver enzyme preparation, ATP and glutamate are necessary for its synthesis. Carbamoyl phosphate is required for the synthesis of urea and arginine, in addition to the synthesis of pyrimidines.

Carbamoyl phosphate synthesis from ammonia represents one of the prominent activities in ureotelic livers [78]. The enzyme requires N-acetylglutamate and is distinct from the enzyme responsible for carbamoyl phosphate synthesis in extrahepatic tissues and in the livers of uricotelic animals. This second enzyme utilizes glutamine [79], rather than ammonia as the primary nitrogen donor and is found in mushrooms, Escherichia coli, yeast, Ehrlich ascites tumour and several other animal tissues [80]. This enzyme is carbamoyl phosphate synthetase II (ATP: carbamate phosphotransferase, EC 2.7.2.2) and catalyses the following reaction:

\[
\text{Glutamine} + \text{HCO}_3^- + 2\text{ATP} \xrightarrow{K^+, \text{Mg}^{2+}} \text{glutamate} + (\text{or ammonia}) \text{carbamoyl phosphate} + 2\text{ADP} + \text{P}_i
\]

It differs from the Type I enzyme [81] in that both glutamine and ammonia are substrates, although in animal cells glutamine is probably the physiological substrate. A highly active mitochondrial carbamoyl phosphate synthetase I is essential for the detoxication of ammonia through the urea cycle in mammalian livers.

Glutamine-dependent carbamoyl phosphate synthetase II [82,83] is sensitive to allosteric inhibition by UTP and to allosteric activation by 5-phosphoribosyl-1-pyrophosphate (PRPP). Ammonia- and N-acetylglutamate-dependent carbamoyl phosphate synthetase I is neither activated by PRPP nor inhibited by UTP. The sensitivity of the Type II enzyme to feedback inhibition by UTP supports its role in the control of pyrimidine synthesis de novo. The enzyme is widely distributed in animal tissues as well as in lower organisms, providing carbamoyl phosphate for pyrimidine biosynthesis [84,85]. The activity of glutamine-dependent synthetase in the tissues is relatively low so it can limit pyrimidine synthesis. Pyrimidine synthesis in the liver in vivo is subject to feedback control as can be demonstrated by the immediate stimulation of UMP synthesis by depletion of the end product [86]. A valuable tool in studies of UMP synthesis de novo is the depletion of hepatic UTP by D-galactosamine. Using isolated perfused rat livers the concentration of UTP can be reduced in the intact organ to below the normal physiological level [86]. Under these conditions the incorporation of bicarbonate is highly stimulated, providing evidence that glutamine-dependent carbamoyl phosphatase is the site of feedback regulation of liver pyrimidine nucleotide synthesis in vivo [87]. Furthermore, the measurement of the rate of incorporation of labelled precursors into orotic acid in slices of various rat tissues demonstrated the operation of a feedback control mechanism