Pseudouridine Biosynthesis in Rat Liver

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The biosynthesis of pseudouridine, 5-ribosyl-uracil¹, has been studied in rat liver by determining the specific activities of pyrimidine nucleotides in several RNA fractions after intraperitoneal injection of radioactive precursors. The methods were based on those of Potter *et al.* (for references see Ref. ²) as used in studies on nucleic acid metabolism in normal and regenerating rat liver. The animals were sacrificed at various time intervals after the injection of orotic acid-6-¹⁴C, the liver tissue homogenized and fractionated by differential centrifugation³. The RNA was extracted⁴, purified by repeated precipitations, then hydrolysed with alkali and the mononucleotides separated on Dowex 1, formate columns¹. The nucleotide fractions were desalted and further purified by paper chromatography before determination of specific activities by UV-spectroscopy and liquid scintillation counting. Data for microsomal RNA are given in Table 1. Similar data were obtained with soluble and nuclear RNA fractions also⁵.

The RNA uridylic acid is seen to be labeled preferentially and to reach highest specific activity. The relative specific activities cytidylic acid/uridylic acid are seen to be increasing with time. This is to be expected from the known precursor-product relationship between these two⁶. The findings are also in good agreement with similar time course studies in regenerating rat liver².

RNA pseudouridylic acid is seen to behave similar to cytidylic acid, however being labeled somewhat faster and reaching the specific activity of uridylic acid after 20 h.

Ureido-¹⁴C-carbamylaspartic acid, uridine-2-¹⁴C, and cytidine-2-¹⁴C were used for single time point experiments⁶ and the results found to support the orotic acid data.

These results are interpreted to indicate biosynthesis of RNA pseudouridine *via* uridine

Table 1. The specific activities in microsomal RNA nucleotides from liver following intraperitoneal injection of orotic acid-6-¹⁴C (specific activity 1.14×10^3 cpm/mμmole) to groups of 6 male Sprague-Dawley rats.

Time interval before sacrifice	50 min	4 h	12 h	20 h
Dose injected × 10 ⁶ cpm	56	50	50	78
Pooled liver weight in grams 6 rats	65	72	60	41
Counts per min per mμmole nucleotide				
Cytidylic acid	0.07	1.3	5.5	4.1
Pseudouridylic acid	0.30	2.9	9.4	8.6
Uridylic acid	0.70	3.7	11.1	8.3

nucleotides in agreement with studies in *Neurospora crassa* 36601⁷, and excluding pathways direct from orotic acid or *via* cytidine nucleotides.

Experiments⁵ with uracil-2-¹⁴C gave a somewhat different distribution of label, pointing either to the existence of uracil intermediates in the conversion of uridine nucleotides to pseudouridine derivatives, or to the possibility of alternate pathways for pseudouridine biosynthesis in rat liver, similar to the one reported in *Tetrahymena pyriformis*⁸.

1. Cohn, W. E. *J. Biol. Chem.* **235** (1960) 1488.
2. Hecht, L. I. and Potter, V. R. *Cancer Res.* **16** (1956) 999.
3. Keller, E. B. and Zamecnik, P. C. *J. Biol. Chem.* **221** (1956) 45.
4. Sibatani, A. *Biochim. Biophys. Acta* **25** (1957) 592.
5. Frøholm, L. O. *In preparation*.
6. Hurlbert, R. B. and Kammen, H. O. *J. Biol. Chem.* **235** (1960) 443.
7. Hall, J. B. and Allen, F. W. *Biochim. Biophys. Acta* **45** (1960) 163.
8. Heinrickson, R. L. and Goldwasser, E. *Federation Proc.* **21** (1962) 383 d.