TIME COURSE STUDY ON THE INCORPORATION OF OROTIC ACID INTO 5-RIBOSYLURACIL PHOSPHATE OF RAT LIVER SOLUBLE RIBONUCLEIC ACID[1,2]

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The occurrence of 5-ribosyluracil phosphate (5-RUP) in the ribonucleic acid (RNA) of a variety of sources (Cohn, 1960; Davis and Allen, 1957; Dunn, 1959, and Osawa, 1960) raises the problem of how this compound is synthesized. Hall and Allen (1960) have recently reported that orotic acid was incorporated equally into the 5-RUP and uridylic acid of a yeast RNA fraction soluble in 1 M sodium chloride. This result was interpreted to indicate that either the pathway leading to the synthesis of uridylic acid was as direct as that leading to the synthesis of 5-RUP or that 5-RUP was synthesized directly from newly synthesized uridylic acid.

The results to be presented in this paper indicate that neither of the above interpretations are applicable in rat liver.

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EXPERIMENTAL

Female albino rats of the Sprague-Dawley strain, weighing about 170 g were used. Each rat received an intraperitoneal injection of 7.7 μc of orotic acid-6-C^{14} (specific activity, 3 mc/mM). At each of the time periods indicated in Figure 2, three rats were killed by a blow on the head and the livers quickly removed, pooled, and homogenized in cold 0.25 M sucrose-0.003M $CaCl_2$. Whole cells and nuclei were removed by centrifugation at 600 x g for 10 minutes. The supernatant was then centrifuged at 105,000 x g for 60 minutes. This supernatant was made up to 5% $HClO_4$ and the precipitated RNA and protein washed two times with 2% $HClO_4$. The RNA was hydrolyzed with 0.3 N KOH to its constituent mononucleotides as described by Osawa et al. (1958) and applied to Dowex-1-formate columns (1 cm x 20 cm). The nucleotides were eluted with a gradient elution scheme as shown in Figure 1. Fractions containing cytidylic acid, 5-RUP, and uridylic acid were pooled, diluted approximately 10 fold, brought to a pH of 7.0 to 8.0 and reabsorbed on small (1 cm x 1 cm) Dowex-1-formate columns. The nucleotides were eluted with 4 N formic acid and lyophilized. The nucleotides were further purified by paper chromatography in the isobutyrate solvent of Magasanik et al. (1950). The ultraviolet-quenching spots were eluted in a small volume of water and spectral ratios in acid and base determined to establish the purity of the nucleotides. For the calculation of each nucleotide concentration the following millimolar extinction coefficients were used under acid conditions: 5-RUP, 8.6 at 260 mμ (Cohn, 1960); uridylic acid, 9.9 at 260 mμ; cytidylic acid, 13.0 at 280 mμ. Calculations of the moles of 5-RUP/100 moles of uridylic acid from the 260 mμ readings of the column chromatograms gave a value of approximately 28, in good agreement with those of other workers for a variety of soluble RNA's (Dunn, 1959; and Osawa, 1960).

RESULTS AND DISCUSSION

As shown in Figure 2 the incorporation of orotic acid into 5-RUP occurs at a significantly greater rate than into uridylic acid. The specific activities of uridylic acid are 66.7, 72.0 and 58.5% of the specific activities of 5-RUP at the 4, 8 and 12-hour periods respectively.

The greater labeling of 5-RUP of soluble RNA indicates that the pathway of its synthesis from orotic acid is more direct than is that of uridylic acid or that the uridylic acid of RNA is diluted to a greater extent by (a) precursor pool(s) than is the 5-RUP of RNA. The alternate possibility that 5-RUP is synthesized from newly synthesized uridylic acid while the uridylic acid incorporated into RNA goes through a metabolic pool is doubtful but still cannot be discounted.

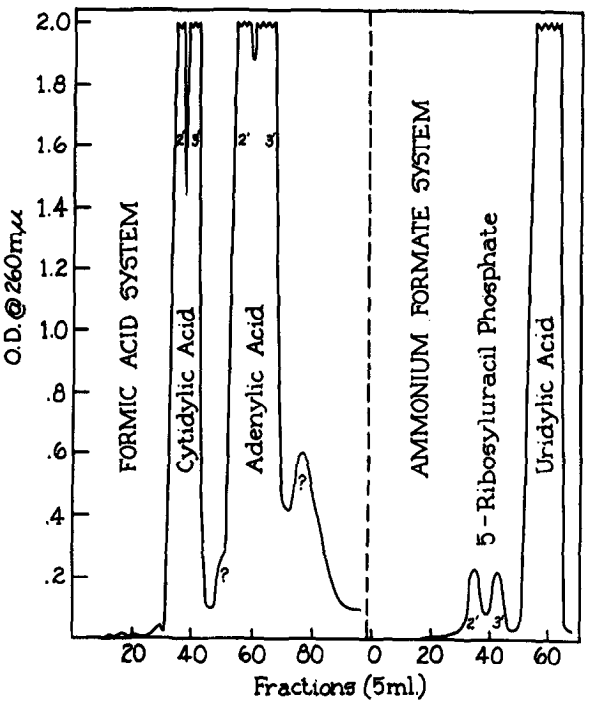

FIG. 1

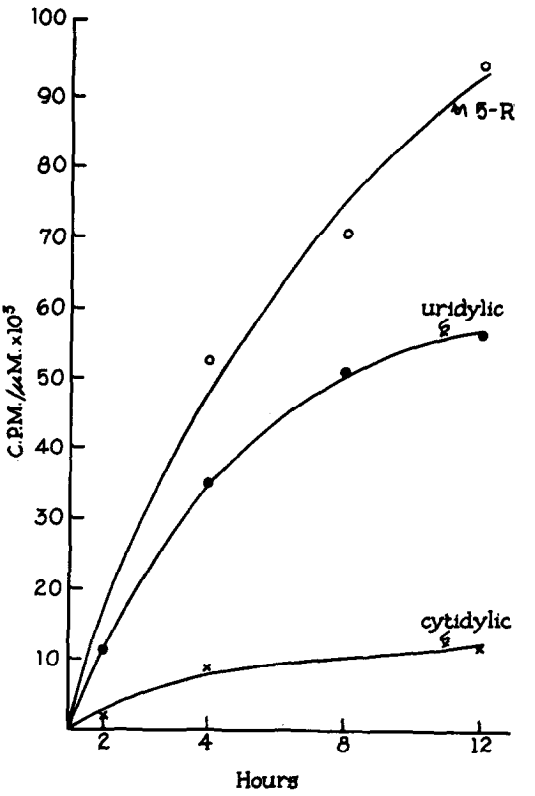

FIG. 2

Figure 1. Gradient elution chromatographic separation of individual mononucleotides of total RNA of rat liver. Dowex-1-formate column (1 cm x 20 cm). Conditions: Formic acid system, 1000 ml mixer containing water. Reservoir containing 0.5 M formic acid, fractions 0-33; 4 M formic acid, fractions 34-96. Column then washed with water until 260 mμ readings dropped to 0.00. Ammonium formate system, 1000 ml mixer containing water. Reservoir containing 1 M ammonium formate, pH 4.26.

Figure 2. Time course of the incorporation of orotic acid-6-C^{14} into 5-RUP, uridylic acid, and cytidylic acid of soluble RNA of rat liver.

In the interpretation of any results where the incorporation of a precursor into 5-RUP is compared to its incorporation into another constituent nucleotide of RNA it is important that one is dealing only with RNA which contains 5-RUP. In the experiment presented here the presence in the soluble RNA of RNA, which does not contain 5-RUP and whose turnover rate is slower, would have an effect on the interpretation of the data presented dependent on how much is present and to what degree its turnover is different. At present the best approach may be to isolate specific amino acid transfer RNA's in an experiment of this type.

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