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Conversion of Orotic Acid to Uridine Phosphates by Soluble Enzymes of Liver

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In rat liver *in vivo* radioactive orotic acid is converted to a group of uridine nucleotides before the label is incorporated into the pyrimidines of the ribonucleic acid [1]. These nucleotides, which are uridine-5-phosphate (UMP), uridine diphosphate (UDP), uridine triphosphate (UTP) and three compounds consisting of UDP linked to a carbohydrate, have recently been found in rat liver as well as in other sources (*cf.* Ref.[1,2]). Since it has furthermore been established [3,4] that orotic acid can be formed *in vitro* in rat liver from the essential metabolites aspartic acid, CO_2, and NH_3, it is apparent that the compound is an ideal precursor for the study of some of the enzymatic mechanisms in the normal biological synthesis of nucleic acids. The objective of the present project is to examine *in vitro* the nature of the ribosidation stage in the conversion of orotic acid to the uridine-5-phosphates.

The soluble enzymes of rat and pigeon liver are found to be capable of converting orotic acid-2-C^{14} to the uridine nucleotides

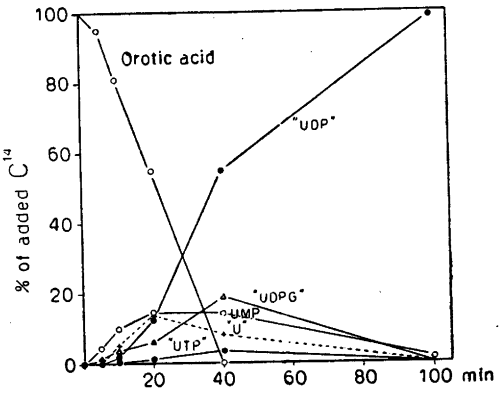

Fig. 1. The dialyzed supernatant fraction [5] from 400 mg of liver is incubated aerobically at 37° with 0.5 µmoles of orotic acid-2-C^{14} (500 000 c/min), 20 µmoles of hexose diphosphate, 3 µmoles of DPN and 120 µmoles of nicotinamide in a volume of 5.0 ml. The reaction is stopped by chilling the flask and adding perchloric acid to 0.4 N. The proteinfree extract is neutralized with KOH and chromatographed directly. ⊙ = *orotic acid;* + = *"U";* ○ = *UMP;* ● = *"UDP";* △ = *"UDPG";* ◐ = *"UTP".*

in vitro. The enzyme preparation is the dialyzed supernatant fraction of the liver after homogenization and high speed centrifugation (*cf.* Ref.[5]). The conversion of the orotic acid is obtained when fructose-1,6-diphosphate or ribose-5-phosphate with the cofactors DPN, ATP and Mg ion are added. A number of assisting and competing enzymes appear to be involved and the relative effectiveness of various combinations of these added components is different in preparations of pigeon liver as compared with rat liver.

The reaction products are separated by chromatography on 6 × 1 cm Dowex-2 (formate) columns by gradient elution with formic acid and formic acid-ammonium formate (*cf.* Ref.[6]). In addition to the unchanged orotic acid, five other radioactive peaks are found. One of these has been identified as UMP and three of them contain UMP which can be liberated by acid hydrolysis. They correspond in chromatographic behaviour to the components identified in rat liver *in vivo* [6] and have been designated here as "UDPG", "UDP" and "UTP" pending complete identification. The other radioactive fraction, "U", is found in the effluent from

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the sample placed onto the column. None of the fractions have as yet been found to contain a component resembling orotic acid.

In these experiments the UMP appears to be the first detectable product of the reaction. Fig. 1 shows the results of a time study of the reaction in which the isotope first appeared in the UMP and subsequently was distributed among the other uridine fractions and the "U" fraction.

In another time study similar to that of fig. 1, 4 μmoles of non radioactive UMP was added with the radioactive orotic acid. The incorporation of the label from the orotic acid in the "UDPG", "UDP" and "UTP" peaks was greatly diminished even though about 1 μmole of the added UMP was converted into the latter compounds. At the 40 minute time point of this experiment a large part of the radioactivity appeared in the "U" fraction in the form of uracil and uridine which were identified by chromatography on starch columns [7].

In a third time study it was found that 0.25 μmoles of added radioactive UMP, in the absence of orotic acid, was converted to the other uridine phosphate derivatives within 10 minutes.

The presence of 2 μmoles of non labeled uridine did not influence the incorporation of C^{14}-orotic acid into the UMP derivatives and thus uridine is excluded as an intermediate in the reaction.

The fixation into uridine phosphates of ribose formed from hexose metabolism may provide a useful system for evaluating the current concepts of ribose synthesis. The possibility is being examined that the ribotide formation from hexosediphosphate or ribosephosphate involves ribose-1,5-diphosphate as does the conversion of adenine to the adenine nucleotides [5]. The enzyme preparation is seen to be capable of degrading the UMP as well as of phosphorylating it, and the enzymes interconverting the "UDPG", "UDP" and „UTP" are also present.

The further investigation of these reactions by substrate limitation, inhibition and enzyme fractionation is continuing.

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The Structure of Selenium Diselenocyanate

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The crystal structure of selenium diselenocyanate [1,2], $Se(SeCN)_2$, has been determined from X-ray data, by two-dimensional Patterson and Fourier methods.

The unit cell dimensions, from oscillation and Weissenberg photographs (Cu$K\alpha$ radiation, $\lambda = 1.542$ Å) are: $a = 10.07 \pm 0.03$ Å, $b = 13.35 \pm 0.04$ Å, $c = 4.48 \pm 0.02$ Å. There are four molecules per unit cell. Absent reflections, $0kl$ when $k + l$ is odd, $hk0$ when h is odd. The space group is the centrosymmetric one, D_{2h}^{16}—$Pnma$.

Patterson projections along the a, b and c axes revealed the positions of the selenium atoms. In the subsequent Fourier syntheses, signs of the reflections were initially based on the selenium contributions alone. The final atomic coordinates, in fractions of corresponding cell edges, are:

	x	y	z
Se_1	0.540	0.250	0.492
Se_2	0.442	0.115	0.249
C	0.295	0.112	0.488
N	0.203	0.095	0.586

The reliability factor, $R = \Sigma\|F_{obs}| - |F_{calc}\|/\Sigma|F_{obs}|$, is 0.15, 0.12 and 0.13, respectively, for the $hk0$, $0kl$ and $h0l$ reflections, with an over-all value of 0.14 for the three zones.

The selenium diselenocyanate molecule possesses, by space group requirements, a

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