The Catabolism of Uracil in Rat Liver Slices

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Fink et al. have clearly established the conversion of dihydrouracil to β-alanine in vitro. β-Ureidopropionic acid could be detected. Attempts to demonstrate the formation of dihydrouracil and β-alanine from uracil were unsuccessful.

We have now studied the catabolism of uracil in rat liver slices by means of labeled compounds. One μmole of uracil-4-14C, dihydrouracil-4-14C, and β-alanine-1-14C, respectively, were incubated with rat liver slices. The incubation mixture was analysed by means of paper chromatography in different systems. The distribution of the activity on the paper strips was determined.

The data recorded in Table 1 together with data from β-alanine catabolism indicate the following pathway of uracil catabolism in the rat: uracil → dihydrouracil → β-ureidopropionic acid → β-alanine → acetic acid + CO2.

The difficulty in detecting dihydrouracil and β-ureidopropionic acid as intermediates in the

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uracil catabolism, is probably due to the rapid conversion of dihydrouracil to β-alanine.


Incorporation of 32P into the Purine Ribonucleotides of Tetrahymena pyriformis in Heat-treated Cultures

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The incorporation of 32P into synchronized cultures of Tetrahymena pyriformis has been investigated by a modification of the technique previously described. In each experiment 3 one liter cultures were simultaneously submitted to intermittent heat-treatment as described by Soherbaum and Zeuthen. 20 minute periods of incubation with isotope were used. 30—20 minutes before the beginning of the

Table 1. Amount of radioactive products after incubation of 1μmole of uracil — 4-14C, dihydrouracil-4-14C and β-alanine-1-14C, with rat liver slices. The results are expressed in per cent of the added activity.

<table>
<thead>
<tr>
<th>Compound incubated</th>
<th>Time of incubation in hours</th>
<th>Radioactive compounds recovered after incubation (in per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Uracil</td>
</tr>
<tr>
<td>Uracil-4-14C</td>
<td>0.5</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>75</td>
</tr>
<tr>
<td>Dihydrouracil-4-14C</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>β-alanine-1-14C</td>
<td>2</td>
<td>0</td>
</tr>
</tbody>
</table>

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Aspartate Carbamyl Transferase from *E. Coli*

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Extracts from lyophilized cells of *E. Coli* contain an enzyme which catalyzes the formation of ureidosuccinate (carbamyl aspartate) from aspartate and an "active" carbamyl compound 1. The recent discovery of carbamyl phosphate as the carbamyl donor in enzymic citrulline synthesis 2 has made possible a further investigation of the mechanism of carbamyl aspartate synthesis. With a 90 fold purified enzyme it was possible to demonstrate the stoichiometric reaction: carbamyl phosphate + aspartate → carbamyl aspartate + phosphate. The equilibrium of the reaction was shifted far towards synthesis of carbamyl aspartate. In the original bacterial extract ornithine and aspartate served about equally well as acceptors for the carbamyl group. With the purified enzyme, however, only aspartate out of ca. 30 different amino acids gave rise to a carbamyl compound.

In the absence of aspartate, there was no enzymic exchange of isotope between $^{32}$P-phosphate and carbamyl phosphate. Neither could any enzymic isotope exchange be observed between $^{14}$C-aspartate and carbamyl aspartate. These experiments tend to exclude the intermediate formation of a carbamylated enzyme. When the reaction was carried out in $\text{H}_2\text{O}$, no isotope was found in the reisolated carbamyl phosphate or in the carbamyl group of the carbamyl aspartate formed. A probable mechanism for the carbamyl transfer reaction will be discussed.