The Catabolism of Uracil in Rat Liver Slices

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Fink *et al.*[1] have clearly established the conversion of dihydrouracil to β-alanine *in vitro*. β-Ureidopropionic acid could be detected [2]. 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 [3,4].

One μmole of uracil-4-^{14}C, dihydrouracil-4-^{14}C and β-alanine-1-^{14}C, 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 [5] indicate the following pathway of uracil catabolism in the rat: uracil → dihydrouracil → β-ureidopropionic acid → β-alanine → acetic acid + CO_2.

The difficulty in detecting dihydrouracil and β-ureidopropionic acid as intermediates in the uracil catabolism, is probably due to the rapid conversion of dihydrouracil to β-alanine.

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2. Fink, K. and McGaughey, C. *Federation Proc.* **13** (1954) 207.
3. Fritzson, P. *Acta Chem. Scand.* **9** (1955) 1239.
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Incorporation of ^{32}P into the Purine Ribonucleotides of *Tetrahymena pyriformis* in Heat-treated Cultures

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The incorporation of ^{32}P into synchronized cultures of *Tetrahymena pyriformis* has been investigated by a modification of the technique previously described [1]. In each experiment 3 one liter cultures were simultaneously submitted to intermittent heat-treatment as described by Scherbaum and Zeuthen [2]. 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-^{14}C, dihydrouracil-4-^{14}C and β-alanine-1-^{14}C, with rat liver slices. The results are expressed in per cent of the added activity.

Compound incubated	Time of incubation in hours	Radioactive compounds recovered after incubation (in per cent)				
		Uracil	Dihydro-uracil	β-Ureido-propionic acid	β-Alanine	$^{14}CO_2$
Uracil-4-^{14}C	0.5	87	4 ?	0	6	—
	2	75	3 ?	2 ?	14	6
Dihydro-uracil-4-^{14}C	0.5	0	0	3 ?	94	—
	1	0	0	0	97	3
	2					6
β-alanine-1-^{14}C	2					6

Table 1.

	1	2	3
Total cell number	76×10^6	55×10^6	54×10^6
Total dry weight, mg	147.6	278.2	350.4
^{32}P uptake (% of added)	82.5	48.7	44.7
Total acid soluble fraction, DU *	51	95.5	138
Total counts in acetone-ether extract	59×10^3	21×10^3	81×10^3
Specific activity of adenine nucleotides **	49 400	11 200	16 100
Specific activity of guanine nucleotides	103 500	27 300	53 200

period to be investigated 1 one liter culture was centrifuged in 750 ml tubes with funnel-shaped bottoms at 200 g for 5 minutes, the cells collected, resuspended in 50 ml of proteose-peptone medium and transferred back to the original shaking-aeration-temperature conditions. The isotope was added to this concentrated culture, and at the conclusion of the 20 minute incubation-period acetone powder was made of the ciliates. 1/10 of the powder dried *in vacuo* was submitted to a classical Schmidt-Tannhauser-Schneider procedure. The remaining 9/10 were extracted with 80 % saturated ammonium sulphate and the extract submitted to differential dialysis against Norite [1]. The nucleotides were isolated from the Norite eluate by subsequent chromatography in three different solvent systems.

The results (Table 1) are taken from one experiment, point 1 representing normal growth, point 2 the last 20 minutes of heat-treatment, point 3 the 20 minutes just prior to the first burst of division. The same amount of isotope was added to the cultures.

More detailed results will be presented on other points in support of the following working hypothesis, that the increase in cell size and nucleic acid content [3] in this induced abnormal growth is due to a failure of the cell to produce the normal precursors for nucleic acid synthesis, and that this is not due to a decrease in the permeability of the cell.

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* Density unit = Vol. (ml) × extinction at 260 mμ.

** Counts per minute per density unit.

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 H_2O^{18} 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.

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