Enzymic interconversion of hydrouracil and β-ureidopropionic acid*

Partially purified enzymic preparations from beef liver catalyze reaction 1,

1. Hydrouracil** + $H_2O \rightleftharpoons$ β-Ureidopropionic acid.

The enzyme catalyzing this reaction, provisionally termed hydrouracilase, is present in the livers of the calf, rat and pigeon, and is not detectable in brain, muscle and heart of the rat, baker's and brewer's yeast, or strain D 10 of Group D streptococci[1]. It is present entirely in the supernatant fraction of isotonic KCl homogenates of rat liver, and appears to require no additional cofactors.

The dehydration of β-ureiodopropionic acid to hydrouracil has an optimum at pH 5.0. At this pH the equilibrium between β-ureidopropionic acid and hydrouracil is reached with 60 and 40% respectively, as indicated by the experiments of Table I. The optimum pH for the reaction Hydrouracil $\longrightarrow$ β-ureidopropionic acid is 10.0; at this pH the reverse reaction does not proceed to any measurable extent, and is in fact negligible at pH 6.5. At pH 10.0 the enzyme fraction used in these experiments will form about 1 micromole of β-ureidopropionic acid from hydrouracil per minute per milligram protein at 38°.

TABLE I

ENZYMIC INTERCONVERSION OF HU AND UP

Exp.	*Min.*	*0*	*15*	*30*	*60*	*90*	*120*
A	+ 1 μ*M*, UP	0	7.6	10.8	12.0	16.4	16.0
B	+ 1 μ*M*, UP	0	12.1	16.0	20.5	22.1	24.6

Final substrate concentrations in micromoles per 2 ml: Acetate buffer pH 5.0, 500; UP 50 (Experiment A); HU 50 (Experiment B). 8.8 mg protein of a 34–40% acetone fraction of a water extract of beef liver acetone powders (fractionated at 0°, 0.1 *M* acetate buffer pH 5.5). This fraction does not cleave β-ureidopropionic acid to β-alanine, CO_2 and NH_3 to any appreciable extent. UP was measured colorimetrically using the ARCHIBALD[2] method for citrulline since UP has about the same chromogenicity as citrulline, whereas HU is only slightly chromogenic. Identity of UP was ascertained by paper chromatography.

We have observed also that crude extracts of rat liver acetone powder convert β-ureidopropionic acid to β-alanine, CO_2 and ammonia according to reaction 2,

2. β-Ureidopropionic acid + $H_2O \longrightarrow$ β-alanine + CO_2 + NH_3.

Reaction 2 is not reversible as written; however, upon the addition of carbamyl phosphate and β-alanine to bacterial[1] and mitochondrial rat liver preparations, synthesis of β-ureidopropionic acid has been demonstrated (unpublished experiments of the authors).

Hydrouracil has been isolated from beef spleen[3] and has previously been shown to be metabolized in rat liver slices to β-alanine[4]. Whether the present findings indicate an important pathway of pyrimidine metabolism is yet to be determined. At any rate the combination of reactions 1 and 2 could provide a mechanism for the formation of β-alanine.

SANTIAGO GRISOLIA***
DONALD P. WALLACH §

McIlvain Biochemical Cardiovascular Laboratories, Department of Medicine, University of Kansas Medical Center, Kansas City, Kans. (U.S.A.)

1 S. GRISOLIA, D. P. WALLACH AND H. J. GRADY, *Biochim. Biophys. Acta*, 17 (1955) 150.
2 R. M. ARCHIBALD, *J. Biol. Chem.*, 156 (1944) 121.
3 C. FUNK, A. J. MERRIT AND A. EHRLICH, *Arch. Biochem.*, 35 (1952) 468.
4 R. M. FINK, K. FINK AND R. B. HENDERSON, *J. Biol. Chem.*, 201 (1953) 349.

Received September 2nd, 1955

* Supported by Grant #H-1925, National Heart Institute, National Institutes of Health.
** The following abbreviations are used in this paper, HU, Hydrouracil; UP, β-Ureidopropionic acid.
*** Established Investigator of the American Heart Association.
§ Post Doctorate Fellow of the National Heart Institute, National Institutes of Health.