A new enzymic reaction concerned in the metabolism of γ-hydroxyglutamic acid

γ-Hydroxyglutamic acid is known as a metabolic product of L-hydroxyproline in rat liver[1]. Recently DEKKER[2] has reported the enzymic formation of glyoxylic acid from γ-hydroxyglutamic acid. In the present study we report the isolation from rat liver of a condensing enzyme which forms γ-hydroxy-α-ketoglutaric acid from pyruvic acid and glyoxylic acid. The enzyme was purified about 12-fold from an extract of an acetone powder of rat liver by fractionation with ammonium sulfate and acetone The acid was separated from other keto acids as 2,4-dinitrophenylhydrazone by paper chromatography. The stoichiometry (Table I) shows that one mole of the keto acid is produced from one mole of pyruvic acid and one mole of glyoxylic acid. The keto acid was isolated from the reaction mixture as the calcium

TABLE I

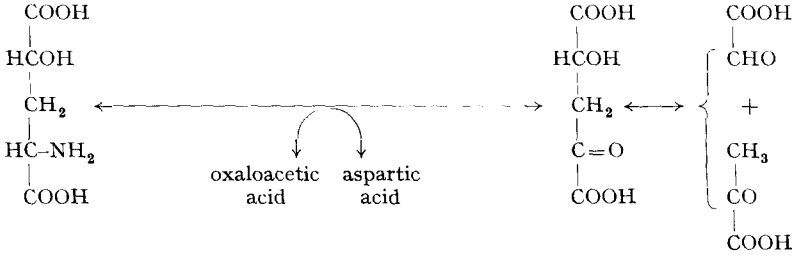
THE BALANCE SHEET OF THE ENZYMIC CONDENSING REACTION AND ITS REVERSIBILITY

The reaction mixture (3.0 ml) contained 30 μmoles potassium phosphate buffer (pH 7.4), 5 μmoles sodium glyoxylate, 5 μmoles lithium pyruvate, or 5 μmoles γ-hydroxy-α-ketoglutaric acid (obtained enzymically as calcium salt and standardized from its crystalline 2,4-dinitrophenylhydrazone), enzyme protein, 1 mg. Gas phase, N_2. Incubated for 60 min at 37°. There were little changes in the amounts of enzymic products, when air was used for gas phase during incubation. The amount of the product was measured as its 2,4-dinitrophenylhydrazone, after being extracted from the reaction mixture by ethyl acetate and separated by paper chromatography.

	The condensing reaction of pyruvic acid and glyoxylic acid			*The splitting reaction of γ-hydroxy-α-ketoglutaric acid*		
	Before incubaiton (μmoles)	*After incubation (μmoles)*	*Difference (μmoles)*	*Before incubation (μmoles)*	*After incubation (μmoles)*	*Difference (μmoles)*
Glyoxylic acid	5.0	2.70	2.30	—	2.47	2.47
Pyruvic acid	5.0	2.81	2.19	—	2.55	2.55
γ-Hydroxy-αKeto-glutaric acid	—	2.22	2.22	5.0	2.39	2.61

salt and the product of reductive amination of the acid with Raney-Ni + H_2 + NH_3 was shown to be γ-hydroxyglutamic acid by paper chromatography with four different solvent systems. γ-Hydroxyglutamic acid was also formed from the 2,4-dinitrophenylhydrazone of the acid by catalytic reduction with Pt + H_2 (ref. 3). Moreover, the keto acid was decarboxylated with ceric sulfate, which is generally used for α-keto acid decarboxylation, and the existence of the hydroxyl group of the acid was proved by the xanthate–molybdate test and the sulfur test[4] for secondary alcohols. From these results it may be concluded that the product of the enzymic reaction is γ-hydroxy-α-ketoglutarate. When the calcium-salt of the keto acid was used as substrate of the condensing enzyme, it was split into pyruvic acid and glyoxylic acid in equimolar amounts, which was the evidence that the reaction is reversible. Formaldehyde or acetaldehyde did not react with pyruvic acid or glyoxylic acid in the presence of the enzyme, which distinguishes the latter from the formaldehyde and pyruvic acid condensing enzyme reported by HIFT AND MAHLER[5]. γ-Hydroxy-α-ketoglutaric acid was formed also from authentic γ-hydroxyglutamic acid by the action of a transaminase obtained from the same source by ammonium sulfate fractionation (62–75 %). The transaminase preparation was completely free of the condensing

enzyme. In the transaminase reaction, the acceptors of the amino group of γ-hydroxyglutamic acid were oxaloacetic acid or α-ketoglutaric acid, from which aspartic acid or glutamic acid was formed respectively. Formation of amino acids was confirmed by paper chromatography with four different solvent systems. γ-Hydroxy-α-ketoglutaric acid formed by the transaminase had the same properties as the keto acid formed by condensation of pyruvic acid and glyoxylic acid enzymically, and was split into pyruvic and glyoxylic acid in equimolar amounts by adding the condensing enzyme (free of the transaminase). Accordingly, one of the metabolic pathways of γ-hydroxyglutamic acid in rat liver may be shown as follows:



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Calcification *in vivo* of implanted collagen

On the basis of nucleation experiments[1-3] *in vitro*, it has been suggested that formation of the mineral phase of bone is initiated by an interaction between calcium, phosphate and some as yet unidentified template on the collagen polymer. It has further been postulated that inhibitors[4] or promoters[5] of calcification, or differences in the reactivity of hard- and soft-tissue collagens[6], may be factors determining which of the collagenous tissues do or do not undergo mineralization. None of these ideas have been substantiated by evidence gained from experimentation *in vivo*.

In recent studies carried on as part of an investigation of collagen catabolism, we observed that rat-tail tendon and reconstituted collagen implanted in the peritoneal cavities of rats became calcified. The possibility of using a peritoneal implant