$\gamma\delta$ -Dioxovalerate as a Substrate for the Glyoxalase Enzyme System

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1. Crude $\gamma\delta$ -dioxovalerate was synthesized from laevulinate by two different methods and was purified by Sephadex chromatography. Some analytical reactions of the compound are described. 2. $\gamma\delta$ -Dioxovalerate is a substrate for glyoxalase I and the GSH derivative formed by this enzyme is hydrolysed by glyoxalase II to form D- α -hydroxyglutarate. The K_m of glyoxalase I for $\gamma\delta$ -dioxovalerate is 1.0×10^{-3} M at pH 5.8. 3. The u.v.-absorption spectrum of thiol ester, synthesized enzymically from $\gamma\delta$ -dioxovalerate and GSH by glyoxalase I, is almost identical with that for S-lactoylglutathione. Some optical properties of this thiol ester were measured. 4. Attempts to show reversibility of the glyoxalase system reactions with D- α -hydroxyglutarate as substrate were unsuccessful. 5. The possible metabolic role of the $\gamma\delta$ -dioxovalerate reaction is discussed. It is suggested that one of the metabolic functions of the glyoxalase system may be to provide a mechanism for the entry of this compound into the tricarboxylic acid cycle.

In the course of investigations of the metabolism of α -ketoaldehydes and their role in the regulation of cell division it was found that $\gamma\delta$ -dioxovaleric acid, one of the possible natural α -ketoaldehydes, can be a substrate for the glyoxalase enzyme system (glyoxalase I, S-lactoyl-glutathione methylglyoxal-lyase EC 4.4.1.5 and glyoxalase II, S-2-hydroxyacylglutathione hydrolase EC 3.1.2.6). This compound, presumably derived by transamination of δ -amino-laevulinate (Nemeth *et al.*, 1957; Neuberger & Turner, 1963; Kissel & Heilmeyer, 1969) has been postulated previously as an intermediate in the formation of succinate in the so-called succinate-glycine cycle. From earlier literature it is known that extracts from certain animal tissues, bacteria and yeasts contain 'ketoaldehyde mutase' (a mixture of glyoxalase I and II and GSH) and convert $\gamma\delta$ -dioxovalerate into D- α -hydroxyglutarate (Neuberger & Collatz, 1930; Mayer, 1931; Fujise, 1931). The precise nature of the biochemical reactions by which $\gamma\delta$ -dioxovalerate is transformed is not known, although one might suppose that this α -ketoaldehyde, like methylglyoxal, can react via glyoxalase I and glyoxalase II to form the corresponding D- α -hydroxy acid (see Scheme 1). Possible natural substrates for glyoxalase I and glyoxalase II previously tested include methylglyoxal (Racker, 1951), phosphohydroxypyruvic aldehyde (Weaver & Lardy, 1961) and hydroxypyruvic aldehyde (Reeves & Ajl, 1965).

In the present paper we report the enzymic formation of the GSH ester of $\gamma\delta$ -dioxovalerate by the action of glyoxalase I and the breakdown of this GSH derivative by glyoxalase II. Also described are some reactions and properties of $\gamma\delta$ -dioxovalerate

and S- α -hydroxyglutaryl-GSH. A preliminary report of these results has been published in abstract form (Jerzykowski *et al.*, 1971a).

Materials and Methods

Materials

$\gamma\delta$ -Dioxovalerate was synthesized in this laboratory and also by Dr. W. Szeja in the laboratory of the Institute of Organic Chemistry and Technology, Silesian Polytechnical University in Gliwice, Poland, by two different methods, that of Wolff (1890) and that of Kissel & Heilmeyer (1969). Crude preparations were further purified by chromatography on Sephadex G-10 columns as described in the text. Methylglyoxal was prepared from glyceraldehyde (Reanal-Finomvegyszergyár, Budapest, Hungary) by the method of Patterson & Lazarow (1955). The concentration of this compound in the distillate was determined by the method of Friedemann (1927). Glyoxalase I (for preparation of S-lactoyl-GSH and thiol ester from $\gamma\delta$ -dioxovalerate and GSH) was purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. This enzyme and glyoxalase II were also isolated from yeast and ox liver by Sephadex chromatography by the method of Jerzykowski *et al.* (1968). The fractions of highest specific activity were used as the source of the two enzymes in a soluble form. GSH was from E. Merck AG, Darmstadt, Germany, D- α -hydroxyglutaric acid (sodium salt from Sigma Chemical Co., St. Louis, Mo., U.S.A., and Sephadex G-10 from Pharmacia Fine Chemicals AB, Uppsala, Sweden. Other chemicals were of the highest purity available.

Enzyme assays

Unless otherwise stated glyoxalase I and glyoxalase II were assayed by a modification of the methods of Racker (1951), as described by Jerzykowski *et al.* (1968).

Enzyme units. All enzyme activities are expressed as 1 μmol of substrate transformed/min, at 25°C.

Measurements of kinetic constants of glyoxalase I and glyoxalase II

Determination of the K_m of glyoxalase I. The reaction mixture contained 300 μmol of acetate buffer, pH 5.8, 3 μmol of neutralized (with NaOH) GSH, glyoxalase I (Boehringer, 5 μg of protein) and $\gamma\delta$ -dioxovalerate or methylglyoxal over the concentration range 0.08–1.66 mM in a total volume of 3 ml. The reaction was started with glyoxalase I and the increase in absorbance at 240 nm during the initial 60 s was measured. Temperature was 25°C. Additional measurements were performed in the presence of $\text{Co}(\text{NO}_3)_2$ at 1 mM concn.

Determination of the K_m of glyoxalase II. The reaction mixture contained 150 μmol of Tris-HCl buffer, pH 7.4, glyoxalase II (from ox liver, 40 μg of protein) and *S*- α -hydroxyglutaryl-GSH or *S*-lactoyl-GSH over the concentration range 0.06–0.66 mM in a total volume of 3 ml. The reaction was started with glyoxalase II and the decrease in absorbance at 240 nm during the initial 60 s was measured. Temperature was 25°C.

Chromatography

Paper chromatography of α -hydroxyglutarate and other organic acids was performed by the method of Sheid & Hirschberg (1965). Organic acids on the paper were detected by spraying with 0.04% ethanolic Bromophenol Blue (pH 8).

Analytical methods

Both $\gamma\delta$ -dioxovalerate and methylglyoxal were standardized by the method of Friedemann (1927). These compounds were also determined with *o*-phenylenediamine by the method of Milligan & Baldwin (1967) or by the method of Dische & Robbins (1934) with α -methylindole. $\gamma\delta$ -Dioxovalerate and methylglyoxal were also determined by a modification of the procedure of Racker (1951). In this case the cuvette contained: 3 μmol of neutralized (with NaOH) GSH, 300 μmol of phosphate buffer, pH 5.8, in a total volume of 3 ml and 0.1–0.5 ml of solution of $\gamma\delta$ -dioxovalerate or methylglyoxal. Finally after mixing and stabilization of the absorbance 0.1 ml of glyoxalase I (about 1 unit) was pipetted into the cuvette. Readings were made at 30 s intervals at 240 nm and absorbance increases were extrapolated

to zero time. The molar extinction coefficient of *S*-lactoyl-GSH was taken as $\epsilon = 3370 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$. Spectrophotometric measurements were made with either a Unicam SP. 500 spectrophotometer or a Unicam SP. 800 recording spectrophotometer.

O.r.d. (optical rotatory dispersion) measurements were made with a Jasco model ORD/UV-5 optical-rotatory-dispersion recorder (Japan Spectroscopic Ltd., Tokyo, Japan).

Results

Preparation of $\gamma\delta$ -dioxovalerate

Laevulinic acid was brominated to form 3,5-dibromolaevulinic acid. After crystallization this compound (m.p. 114°C) was hydrolysed to give $\gamma\delta$ -dioxovaleric acid as described by Wolff (1890). However, the product always contained some impurities and chromatography showed at least three substances, probably isomers of $\gamma\delta$ -dioxovaleric acid. One of these substances inhibited the glyoxalase I reaction. A crystalline ketoaldehyde was not prepared, but a sufficiently pure substance was obtained after chromatography on a column of Sephadex G-10. The crude $\gamma\delta$ -dioxovaleric acid (300 mg) was placed on a column (1.1 cm $\times$ 65 cm) of Sephadex G-10 and the ketoaldehyde was eluted with water; fractions (2 ml) were collected. The eluate was analysed for α -ketoaldehyde by the method using *o*-phenylenediamine (at 336 nm), α -methylindole (at 543 nm) and the reaction with glyoxalase I and GSH (at 240 nm). A single peak was detected at about 37 ml by all three criteria. The fractions with highest concentration of $\gamma\delta$ -dioxovaleric acid were taken for further investigations.

$\gamma\delta$ -Dioxovaleric acid was also obtained by the method of Kissel & Heilmeyer (1969). This product, purified by Sephadex chromatography as described above, in the reaction with glyoxalase I did not differ from the product obtained by the method of Wolff (1890).

Preparation of dinitrophenylosazone and dithiosemicarbazone

The 2,4-dinitrophenylosazone and dithiosemicarbazone of $\gamma\delta$ -dioxovalerate were prepared as described by Neuberg & Collatz (1930) and Veibel (1931). They melted at 245–247°C and 224–225°C respectively, values which are in agreement with those previously reported (Kissel & Heilmeyer, 1969; Veibel, 1931; Neuberg & Collatz, 1930).

Analytical reactions of $\gamma\delta$ -dioxovalerate

Reactions typical for α -ketoaldehydes were given by $\gamma\delta$ -dioxovalerate. (1) $\gamma\delta$ -Dioxovalerate reacts

with H_2O_2 by the method of Friedemann (1927). This method was used to determine the concentration of $\gamma\delta$ -dioxovalerate in all preparations. Owing to the presence of a carboxylic grouping in the $\gamma\delta$ -dioxo-valeric acid, the soluble compound was neutralized before the Friedemann (1927) reaction was carried out. It should be noted that the $\gamma\delta$ -dioxovaleric acid concentration calculated from this neutralization reaction was in agreement with the results obtained by the method of Friedemann (1927).

(2) $\gamma\delta$ -Dioxovalerate gives a quantitative reaction with *o*-phenylenediamine by the method described by Milligan & Baldwin (1967) for methylglyoxal. The absorption spectrum was identical with that obtained with methylglyoxal and *o*-phenylenediamine. Some optical properties of the reaction product of $\gamma\delta$ -dioxovalerate in analytical conditions are: u.v.-absorption maxima, 336, 244 and 213 nm; the ϵ of the quinoxaline (the product of the condensation of *o*-phenylenediamine and α -ketoaldehyde) $\epsilon = 9.0 \times 10^3 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at 336 nm. In the same conditions the ϵ for methylglyoxal was $8.2 \times 10^3 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ at 336 nm.

(3) Both methylglyoxal and $\gamma\delta$ -dioxovalerate give identical colour reaction with α -methylindole. The absorption spectra were also identical ($\lambda_{\text{max.}} = 543 \text{ nm}$) but the molar extinction coefficient for methylglyoxal was approximately 3.5 times that for

$\gamma\delta$ -dioxovalerate (in analytical conditions described by Dische & Robbins, 1934).

Stability of $\gamma\delta$ -dioxovalerate to acid and alkali

The α -ketoaldehydes are very labile under certain conditions. On standing at pH 10 (adjusted with NaOH), $\gamma\delta$ -dioxovalerate rapidly loses its activity as a substrate for glyoxalase I (Fig. 1). Under comparable conditions methylglyoxal undergoes internal oxidation-reduction to form lactate. At pH 2 in HCl $\gamma\delta$ -dioxovalerate was stable for at least 2 h.

Reaction of $\gamma\delta$ -dioxovalerate with glyoxalases

Racker (1951) demonstrated that the glyoxalase reaction involves two different enzymes. Glyoxalase I catalyses the reaction between the GSH and a suitable α -ketoaldehyde to form a GSH ester with an absorption maximum at 235 nm, and glyoxalase II hydrolytically cleaves the ester to form a D- α -hydroxy acid and free GSH. By using $\gamma\delta$ -dioxovalerate as substrate we have been able to demonstrate the glyoxalase I and glyoxalase II reactions. Fig. 2 shows that this compound is a substrate for glyoxalase I and that the thiol ester formed is hydrolysed by glyoxalase II. The similarity of this enzymic reaction to the comparable reactions of methylglyoxal (Racker, 1951),

Fig. 1. Stability of $\gamma\delta$ -dioxovalerate in alkali

A portion (2 ml, 40 μmol) of $\gamma\delta$ -dioxovalerate and 2 ml of 0.02–0.04 M-NaOH [to obtain pH 7.5 (●) or pH 10 (▲)] were incubated at 30°C. At the indicated times, samples (0.2 ml) were withdrawn and after neutralization with HCl analysed for ketoaldehyde content (with glyoxalase I, as described under 'Analytical methods').

Fig. 2. Demonstration of the formation of the GSH ester of $\gamma\delta$ -dioxovalerate by glyoxalase I and its hydrolysis by glyoxalase II

The experimental cuvette (1 cm light-path) contained 1.5 μmol of neutralized GSH, 270 μmol of phosphate buffer, pH 6.8, and 0.1 ml (1 μmol) of $\gamma\delta$ -dioxovalerate in a total volume of 3 ml. The reaction was started by addition of 0.1 ml of glyoxalase I (about 0.15 unit). When the glyoxalase I reaction was completed 0.2 ml of glyoxalase II (about 0.25 unit) was pipetted and the decrease in absorbance at 240 nm was recorded. The reference cuvette contained phosphate buffer. The glyoxalase I and glyoxalase II were added as indicated in the figure at arrows (1) and (2) respectively.

Table 1. *O.r.d. measurements*

For details see the Materials and Methods and Results sections.

Sample	Cuvette contained 0.1 M-acetic buffer, pH 5.8 (100 μ mol/ml) plus:	Observed o.r.d. at 295 nm (1 cm light-path)	$\left(\frac{[\alpha]_{295}^{22}}{\text{degrees}} \right)$ (g/ml per dcm)
(I), D- α -Hydroxyglutaryl-GSH	<i>S</i> - α -Hydroxyglutaryl-GSH (2 μ mol/ml) + glyoxalase I (0.1 unit)*	+0.036°	+434 $\pm$ 20
(II), D- α -Hydroxyglutarate + GSH	GSH (2 μ mol/ml) + D- α -hydroxyglutarate (2 μ mol/ml) + glyoxalase I (0.1 unit)* + glyoxalase II (0.05 unit)†	−0.008°	
(III), Blank with GSH	GSH (2 μ mol/ml) + glyoxalase I (0.1 unit)* + glyoxalase II (0.05 unit)†	−0.016°	
(II)−(III), (D- α -Hydroxyglutarate)		+0.008°	+266 $\pm$ 20

* Less than 1 μ g and † less than 5 μ g of protein/ml of mixture which may cause an error less than 1%.

phosphohydroxypyruvic aldehyde (Weaver & Lardy, 1961) and hydroxypyruvic aldehyde (Reeves & Ajl, 1965) shows that the reaction takes place as in the scheme proved by these authors. Hence it may be assumed that the thiol ester formed by glyoxalase I is *S*-hydroxyglutaryl-GSH and the product obtained by cleavage of this ester is D- α -hydroxyglutarate. To prove the structure of *S*-hydroxyglutaryl-GSH it was shown that the u.v.-absorption spectrum produced after glyoxalase I reacted with either γ -dioxovalerate or methylglyoxal was almost the same from 220 to 300 nm and agreed with those obtained by Racker (1951) for *S*-lactoyl-GSH, by Weaver & Lardy (1961) for 3-phosphoglyceryl-GSH, by Reeves & Ajl (1965) for glycyl-GSH and also with those reported by Wieland & Köppe (1953) for synthetically obtained *S*-acyl derivatives of GSH.

Chromatography of D- α -hydroxyglutarate

D- α -Hydroxyglutarate was identified qualitatively as a product of the glyoxalase system reactions by ascending paper chromatography on Whatman no. 1 paper, with butanol–acetic acid–water (12:3:5, by vol.) as solvent, for 18 h. A sample from the reaction mixture, containing approx. 5 μ g of the acid, gave a spot with the same R_F value (0.09) as authentic D- α -hydroxyglutaric acid (not its lactone).

O.r.d. measurements

In the visible region the optical rotation values for D- α -hydroxyglutarate are insignificant, even for the molybdate complex (Martius, 1955). For this reason o.r.d. measurements of the products of glyoxalase system reactions were made over the range 350–250 nm. The following spectra were traced: (I), product of enzymic reaction of γ -dioxovalerate and GSH (*S*- α -hydroxyglutaryl-GSH); (II), product of

hydrolysis of this compound by glyoxalase II (α -hydroxyglutarate + GSH); (III), blank with identical concentrations of buffer, GSH, glyoxalase I and glyoxalase II. The difference spectrum of spectra (II) and (III), corresponding to the α -hydroxyglutarate spectrum, suggests the D(+) configuration for the hydroxy acid.

At 295 nm *S*- α -hydroxyglutaryl-GSH showed maximum o.r.d. For this wavelength the quantitative data are given in Table 1.

Reversibility of the glyoxalase I and glyoxalase II reactions

No thiol ester formation could be demonstrated in systems containing D- α -hydroxyglutarate, GSH and glyoxalase II, or after addition of ATP and GTP.

Stability and some other properties of *S*- α -hydroxyglutaryl-GSH

S- α -Hydroxyglutaryl-GSH was more labile than *S*-lactoyl-GSH. *S*- α -hydroxyglutaryl-GSH is stable in neutral solutions, but it was rapidly destroyed as a substrate for glyoxalase II at pH 1.5 and 9.0 and also in boiling water. Some optical properties of this thiol ester in neutral solution are: λ_{max} , 235 nm; ϵ = 2940 litre $\cdot$ mol^{−1} $\cdot$ cm^{−1} at 240 nm.

Effect of pH on glyoxalase I and glyoxalase II activities

To examine the pH-dependence of glyoxalase I with γ -dioxovalerate as substrate a series of measurements in 0.1 M-phosphate buffer covering the pH range 5.0–8.0 were performed. An optimum over the range pH 5.4–6.0 was obtained. For comparison the pH-dependence with methylglyoxal as substrate was also examined (Fig. 3). The pH-dependence of glyoxalase II with hydroxyglutaryl-GSH and lactoyl-GSH as substrates is given also in Fig. 3.

Fig. 3. Effect of pH on glyoxalase I and glyoxalase II activities

Activities of both enzymes were measured in 0.1M-phosphate buffers. Glyoxalase I. Reaction mixtures contained 1.5 μ mol of neutralized GSH, 1 unit of glyoxalase I (Boehringer) and 1.5 μ mol of $\gamma\delta$ -dioxovalerate (●) or methylglyoxal (○) in a total volume of 3 ml. Glyoxalase II. Reaction mixtures contained 0.2 unit of glyoxalase II (from ox liver) and 1.5 μ mol of *S*- α -hydroxyglutaryl-GSH (□) or *S*-lactoyl-GSH (■) in a total volume of 3 ml.

Kinetic studies on glyoxalase I and glyoxalase II

For glyoxalase I in the presence of excess of GSH double-reciprocal plots of initial velocities against $\gamma\delta$ -dioxovalerate and methylglyoxal concentration (see Materials and Methods section) yielded straight lines and the following K_m values: for $\gamma\delta$ -dioxovalerate at pH 5.8 $K_m = 1.0$ mM; in the presence of 1 mM- Co^{2+} [$\text{Co}(\text{NO}_3)_2$] (stimulating effect) the K_m value was 0.83 mM. For methylglyoxal as substrate K_m of 1 mM was found. The values of $V_{\max}$ under the same experimental conditions (protein content 5 μ g) are: for $\gamma\delta$ -dioxovalerate 0.79 μ mol/min, for $\gamma\delta$ -dioxovalerate in the presence of Co^{2+} , 1.03 μ mol/min; for methylglyoxal 1.48 μ mol/min. The K_m value for glyoxalase II and *S*-hydroxyglutaryl-GSH was 0.25 mM and for comparison the corresponding value for *S*-lactoyl-GSH as substrate was 0.5 mM. For glyoxalase II (protein content 40 μ g) the comparable calculated $V_{\max}$ values are: for *S*-hydroxyglutaryl-GSH, 0.35 μ mol/min, and for *S*-lactoyl-GSH, 0.77 μ mol/min.

Discussion

For many years methylglyoxal has been postulated to be a natural substrate for the glyoxalases. At one time this compound was thought to be an essential component of glycolysis, but Lohmann (1932) disproved this. Subsequently it was demonstrated that methylglyoxal was produced from glycolytic inter-

mediates but nonenzymically and in small quantities (Riddle & Lorenz, 1968). In considering possible natural substrates of the glyoxalases, Elliott (1960) has shown that methylglyoxal is a product of the deamination of aminoacetone and has postulated that the glyoxalase system may participate in a cycle for the degradation of glycine and threonine (see, e.g., Elliott, 1958; Urata & Granick, 1963). However, it should be pointed out that the reaction of the formation of methylglyoxal from aminoacetone was found only for an enzyme from ox plasma, and the so-called 'disappearance' of aminoacetone, in which the formation of methylglyoxal has been postulated, occurs only in some tissues and some animals (see, e.g., Green & Elliott, 1964; Urata & Granick, 1963). Hence it is possible that the other α -ketoaldehydes occur as substrates of the universally distributed glyoxalase system.

Among the possible natural substrates for the glyoxalase system that had been considered but not yet tested was $\gamma\delta$ -dioxovalerate. The putative succinate cycle, postulated by Shemin and co-workers (Nemeth *et al.*, 1957) and subsequently completed by Tubbs & Greville (1961), involves the conversion of δ -aminolaevulinate into $\gamma\delta$ -dioxovalerate. This α -ketoaldehyde can be metabolized into D- α -hydroxyglutarate and this substance dehydrogenated by D- α -hydroxy acid dehydrogenase, possibly yielding α -oxoglutarate. Oxidative decarboxylation of the α -oxoglutarate and the condensation of δ -aminolaevulinate from glycine and succinyl-CoA completed the cycle. As to whether this cycle takes place in animal tissues, it may be noted that the $\gamma\delta$ -dioxovalerate can be formed from δ -aminolaevulinate by a number of animal tissues (see, e.g., Kowalski *et al.*, 1959; Spryshkova & Poznanskaya, 1966; Kissel & Heilmeyer, 1969). From earlier literature it is known that extracts of certain animal tissues and cells converted $\gamma\delta$ -dioxovalerate into D- α -hydroxyglutarate (Neuberg & Collatz, 1930; Mayer, 1931; Fujise, 1931). Further evidence for these reactions is given in the present paper. $\gamma\delta$ -Dioxovalerate can be converted into *S*-hydroxyglutaryl-GSH, with kinetic values comparable with those of methylglyoxal, and further into D- α -hydroxyglutarate by glyoxalases, enzymes universally distributed in animal tissues. D- α -Hydroxy acid dehydrogenase has been shown to be also present in various mammals, and bearing in mind the wide substrate specificity of this enzyme (Tubbs & Greville, 1961; Cammack, 1969), there seems to be a strong possibility that D- α -hydroxyglutarate is also a natural substrate, yielding further α -oxoglutarate (Scheme 1).

Another possible way in which $\gamma\delta$ -dioxovalerate may be formed was considered by Tait (1968). Since there are some systems present in tissues where no δ -aminolaevulinate synthetase has been detected, and taking into account the equilibrium of the transaminase reaction towards δ -aminolaevulinate forma-

Scheme 1. Summary of reactions found and proposed for the formation and destruction of $\gamma\delta$ -dioxovalerate in animal tissues

(1) and (2), suggestive evidence for glyoxalase system (glyoxalase I and glyoxalase II) reactions are presented in the text and also by Jerzykowski *et al.* (1971a). (3) Indirect evidence is provided that an α -hydroxy acid dehydrogenase is active against a number of α -hydroxy acids (Tubbs & Greville, 1961; Cammack, 1969). (4) Transamination-reaction evidence is provided that $\gamma\delta$ -dioxovalerate may transaminate with alanine and glutamic acid giving δ -aminolaevulinate, and δ -aminolaevulinate may transaminate giving $\gamma\delta$ -dioxovalerate (Kowalski *et al.*, 1959; Spryskhova & Poznanskaya, 1966; Kissel & Heilmeyer, 1969; Jerzykowski *et al.*, 1971b). (5) Indirect evidence is provided that an α -ketoaldehyde dehydrogenase may be active against $\gamma\delta$ -dioxovalerate (Monder, 1967; Jerzykowski *et al.*, 1971c). (6) α -Oxoglutarate oxidase complex forms succinyl-CoA (citrate cycle reaction). (7) δ -Aminolaevulinate synthetase reaction. (8) Porphyrin synthesis reactions. (9) Unknown intermediate reaction, postulated by Tait (1968).

tion from $\gamma\delta$ -dioxovalerate, it was suggested by Tait (1968) that this latter substance is formed from another intermediate, not δ -aminolaevulinate, and could more probably be converted into δ -aminolaevulinate and further into porphyrins. If this is true, then the reaction demonstrated by us, and hence the glyoxalases and GSH, may possibly play some role in the regulation of porphyrin synthesis by the conversion of some part of $\gamma\delta$ -dioxovalerate into tri-carboxylate-cycle intermediates.

However, the metabolic significance of the reaction described in this report is still to be elucidated. It has been postulated (e.g. Szent-Györgyi *et al.*, 1967) that α -ketoaldehydes may have a special function as regulatory molecules in cell division. They also

possess anti-tumour activity (Jerzykowski *et al.*, 1970). Study of the precise function of $\gamma\delta$ -dioxovalerate in metabolism from this point of view could prove of value.

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