ledge his indebtedness to Professor A. Tiselius for his interest in this work. The technical assistance of Mrs Betty Malmström and Mr Bert Erstrand is also gratefully acknowledged.

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The Mechanism of the Glyoxalase I Reaction, and the Effect of Ophthalmic Acid as an Inhibitor

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The glyoxalase system catalyses the two reactions:

Methylglyoxal + glutathione → *S*-lactoylglutathione → lactic acid + glutathione

and each reaction is catalysed by a separate enzyme, glyoxalases I and II respectively (Racker, 1951; Crook & Law, 1952). The work described is concerned with glyoxalase I only, which, for brevity, will be referred to simply as glyoxalase. The thiol group of glutathione plays a part in the reaction, and ophthalmic acid, a tripeptide, isolated from calf lens, which is structurally similar to glutathione but lacks the thiol group (Waley, 1958), might be expected to inhibit the reaction. This has been demonstrated. Synthetic analogues of glutathione are known to act as inhibitors in the glyoxalase reaction (Kermack & Matheson, 1957).

In an enzymic reaction in which there are two substrates, it is possible that the two substrates might react with each other before reacting with the enzyme. In general, this does not happen. The evidence presented here, however, suggests that this occurs with the glyoxalase reaction. In that case, the intermediates in the complete glyoxalase system will be as set out in Scheme I.

A preliminary account of this work has already appeared (Cliffe & Waley, 1959).

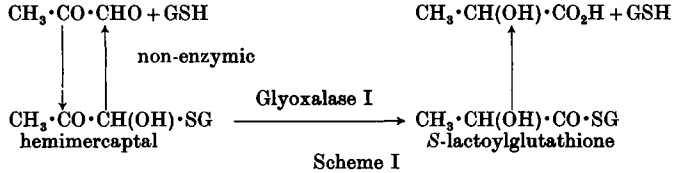
EXPERIMENTAL

Materials

Methylglyoxal was purified and standardized as described by Kermack & Matheson (1957), and the glutathione (GSH) was obtained from The Distillers Co. Ltd. (Biochemicals). Ophthalmic acid and norophthalmic acid were synthetic samples (Waley, 1957, 1958). We are indebted to Dr K. W. Daisley for the preparation of glyoxalase I, which had been purified to step 4 in the procedure of Racker (1951), and was free from glyoxalase II.

Methods

The formation of *S*-lactoylglutathione was followed by the increase in $E_{240\ m\mu}$ in a Beckman DU spectrophotometer (Racker, 1951); optical cells with 1 cm. light-path were used. The reactions were carried out in 33 mM-potassium



Scheme I

phosphate buffer pH 6·6, at room temperature which was held at 20±1°. Readings were taken against a blank cell containing water and buffer. Each day a portion of the stock glyoxalase I preparation was diluted with 0·1 M-potassium phosphate buffer, pH 7·4, that contained crystalline bovine serum albumin (100μg./ml.); 0·1 ml. of the diluted enzyme solution was used in each reaction mixture, whose total volume was 3 ml. As the extent of dilution of the stock solution of glyoxalase I (usually 200- to 500-fold) varied in different experiments, unit concentration of the enzyme is taken as 0·1 ml. of undiluted stock solution in 3 ml. Solutions of GSH were prepared immediately before use, and the pH was brought to 6·6 with aqueous potassium hydroxide.

Except where otherwise stated, the substrates were mixed and, 10 min. later, the solution of the enzyme was added; this ensured that the equilibrium concentration of the hemimercaptal had been attained before the enzymic reaction started. The molar extinction coefficients of the hemimercaptal and of *S*-lactoylglutathione are 220 and 3300 respectively.

RESULTS

Effect of substrate concentrations on the rate of the reaction

The effect of variation of the concentrations of methylglyoxal and of GSH on the rate of formation of *S*-lactoylglutathione is shown in Fig. 1. Each curve represents the results of two series of experiments. The lower curve shows one series of points for a fixed concentration (1 mM) of methylglyoxal and variable GSH concentration, and another series of points for a fixed concentration (1 mM) of GSH and variable methylglyoxal concentration. The upper curve is similar, except that the value of the fixed concentration was 2 mM. One feature is immediately obvious: within the experimental error, all the points for a given series lie on

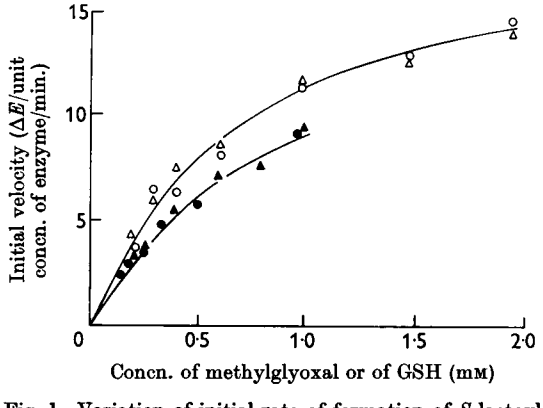


Fig. 1. Variation of initial rate of formation of *S*-lactoylglutathione with concentration of methylglyoxal when the concentration of GSH is 1 mM (●), or 2 mM (○), and with concentration of GSH when the concentration of methylglyoxal is 1 mM (▲) or 2 mM (△).

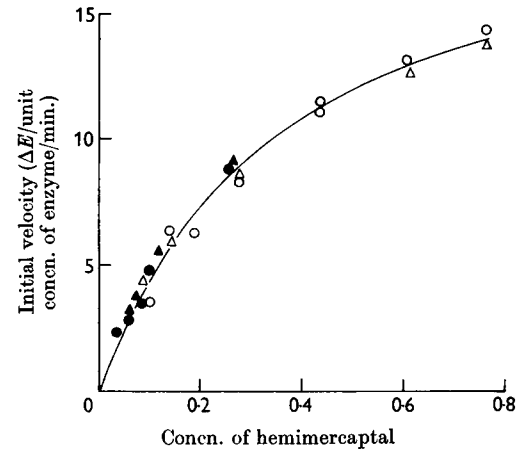


Fig. 2. Variation of initial rate of formation of *S*-lactoylglutathione with the calculated concentration of hemimercaptal. Each point corresponds to a point in Fig. 1, and the same symbols have been used. The concentrations of the hemimercaptal have been obtained from those of methylglyoxal and GSH; the dissociation constant of the hemimercaptal has been taken as 2 mM.

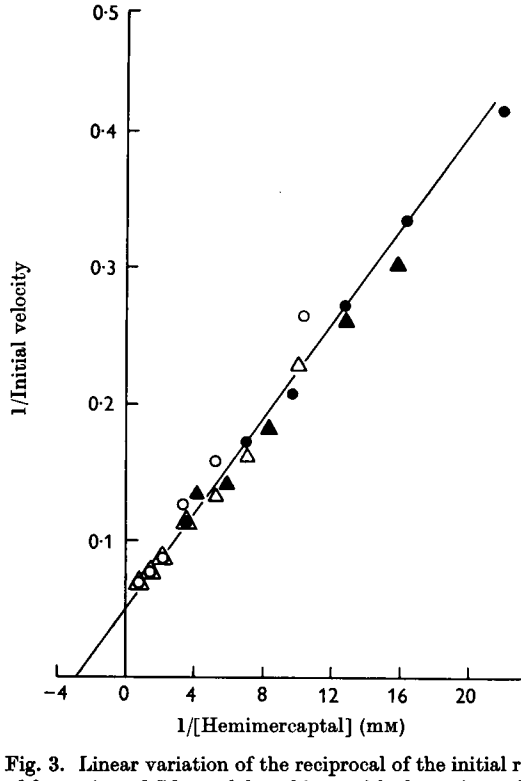


Fig. 3. Linear variation of the reciprocal of the initial rate of formation of *S*-lactoylglutathione with the reciprocal of the concentration of hemimercaptal. The symbols are as in Fig. 1.

the same curve. For example, the rate is the same when the concentration of methylglyoxal is 2 mM and that of GSH 1 mM as it is when the concentration of methylglyoxal is 1 mM and that of GSH 2 mM. It is clear that the velocity is a symmetrical function of the concentrations of methylglyoxal and of GSH. This is the test suggested by Segal, Kachmar & Boyer (1952) for a mechanism in which the two species, here methylglyoxal (M) and glutathione (G), unite to form an adduct (Q), which then combines with the enzyme (E):

$$M + G \rightleftarrows Q \qquad (1)$$
$$Q + E \rightleftarrows QE \rightarrow \text{product} + E$$

Now if the adduct (Q) is really the substrate (which is what this mechanism amounts to) then, when the velocity is plotted against the concentration of adduct, all the points should now lie on one curve. That this is so is shown in Fig. 2. The same feature is also seen in the linear plot obtained when the reciprocal of the velocity is plotted against the reciprocal of the concentration of the adduct (Fig. 3): all the points lie on one straight line. Statistically, there is no significant difference between the gradients and intercepts of the best straight lines for each four series of experiments.

To obtain the concentration of the adduct (Q) the equilibrium constant (K) of reaction (1) has to be known. This was determined by the method of Kermack & Matheson (1957) from measurements of the increase in E when solutions of methylglyoxal and of GSH are mixed. Our value (2 mM) for this equilibrium constant differs from theirs (5 mM), but the method is rather inaccurate, and the differing values are not important since the shape of the curve is not much affected by the exact value chosen for the calculated concentration of adduct.

This evidence, then, suggests that the formation of the adduct from methylglyoxal and GSH is a preliminary to the enzymic reaction proper. The mechanism of the formation of the adduct has therefore been investigated.

Mechanism of the formation of hemimercaptal

The rate of formation of the adduct was followed by the increase in $E_{240\ m\mu}$; the conditions of pH concentration, of buffer and temperature were the same as in the enzymic experiments. When the initial concentrations of methylglyoxal and of GSH were independently varied, the results (Table 1) showed that the initial rate was independent of the concentration of GSH, but linear with respect to the concentration of methylglyoxal. The reaction was rather rapid and so the initial rate could not be accurately measured, and the extent of reaction after a fixed interval of time (Table 2) is more suitable as a test of the kinetic scheme given below. A one-step bimolecular mechanism [reaction (1)], which requires that the extent of reaction should depend on the concentrations of both partners, is therefore excluded.

Since the rate-determining step involves only methylglyoxal, the simplest kinetic scheme is one in which the methylglyoxal (M) is converted into a more reactive species (M*) (the chemical nature of this change is discussed below), which then reacts with GSH (G) to give the hemimercaptal

$[CH_3 \cdot CO \cdot CH(OH) \cdot SG]$ (Q):

$$M \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} M^*$$
$$M^* + G \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} Q$$

where k_1, k_{-1}, k_2 and k_{-2} are the appropriate velocity constants. The rate of change of the concentration of M* is then

$$dm^*/dt = k_1 m - k_{-1} m^* - k_2 m^* g + k_{-2} q$$

where m^*, m, g and q denote respectively the concentrations of the species M*, M, G and Q in the mechanism written above. If M* is a reactive

Table 1. *Initial rate of formation of hemimercaptal*

The rates are measured by increase in $E_{240\ m\mu}$ from methylglyoxal and glutathione and are given as ΔE/sec.

Concn. of GSH (mM)	Concn. of methylglyoxal (mM)				
	1	2	3	4	5
1	0·0018	—	—	—	—
2	0·0019	0·0036	0·0056	—	—
3	0·0018	0·0039	—	0·0082	0·0100
4	0·0020	—	—	—	0·0090
5	0·0020	—	0·0057	—	0·0092

Table 2. *Extent of formation of hemimercaptal from methylglyoxal and glutathione after a fixed interval of time, and the first-order rate constant (k_1) obtained by the use of equation* (3) *(see text)*

Concn. of methylglyoxal (mM)	Concn. of GSH (mM)	$\Delta E_{240\ m\mu}$ after 37 sec.	Concn. of hemimercaptal formed (mM)	$10^3\ k_1$ (sec.$^{-1}$)
1	1	0·062	0·281	8·9
1	2	0·070	0·317	10·2
1	3	0·063	0·285	9·1
1	4	0·065	0·294	9·5
1	5	0·075	0·339	11·1
2	2	0·138	0·624	10·1
2	4	0·145	0·591	9·5
3	3	0·195	0·882	9·5
3	5	0·252	1·14	12·8
4	4	0·313	1·42	11·8
5	3	0·348	1·57	10·2
5	4	0·340	1·54	9·9
5	5	0·330	1·49	9·5

intermediate, so that the stationary state approximation may be applied,

$dm^*/dt = 0$, and $m^* = (k_1 m + k_{-2} q)/(k_{-1} + k_2 g)$.

Thus the rate of formation of the adduct (Q) is given by

$$dq/dt = k_2 m^* g - k_{-2} q,$$

i.e. $dq/dt = k_2 g(k_1 m + k_{-2} q)/(k_{-1} + k_2 g) - k_{-2} q$.

If the reactive intermediate (M*) reacts much more rapidly with GSH than it reverts to M, i.e. if $k_2 g \gg k_{-1}$, then for the early stages ofthe reaction, when $q \doteq 0$,

$$dq/dt = k_1 m.$$

As $dm^*/dt = 0$, $dq/dt = -dm/dt$, and $m = m_0 - q$, where m_0 is the initial concentration of M, we may write

$$-dm/dt = k_1 m, \quad \text{and} \quad q = m_0 (1 - e^{-k_1 t}). \qquad (2)$$

This equation shows that the amount of adduct (Q) formed after a fixed time (t) is proportional to the initial concentration of methylglyoxal, in agreement with the results in Table 2. Rearrangement of equation (2) gives equation (3), from which, together with the data in Table 2, the value of k_1 was found to be 0·01 sec.$^{-1}$

$$k_1 = (1/t) \ln [m_0/(m_0 - q)]. \qquad (3)$$

This reaction is discussed in more detail later, but we may mention here that the species symbolized by M* is probably free methylglyoxal and M is a hydrate of methylglyoxal. The rate-determining step in the formation of the hemimercaptal is then liberation of free methylglyoxal from its hydrate.

Interplay of the enzymic and non-enzymic reactions

Under suitable conditions, the rate of the non-enzymic reaction may affect the rate of formation of *S*-lactoylglutathione. Two sets of experiments were carried out, each at several enzymic concentrations. The sets differed in the concentrations of GSH and of methylglyoxal: in one set ('high methylglyoxal') the concentration of methylglyoxal was 2 mM and that of GSH 0·1 mM; in the other set ('low methylglyoxal') the concentrations were 0·1 and 2 mM respectively. In these experiments, the methylglyoxal was added last to the other constituents of the reaction mixture. Thus both the enzymic and non-enzymic reactions started at the same time. At the higher concentrations of enzyme the initial rate was too rapid to measure accurately, and the results in Fig. 4 are expressed as the change in E after 1 min. At the lower concentrations of enzyme the two curves are superimposable, and the velocity is a symmetrical function of the substrate concentrations, as already described. At the higher concentrations of enzyme, however, the two curves diverge. The 'high methylglyoxal' curve rises steadily with increase of enzyme concentration, but the 'low methylglyoxal' curve flattens off at a relatively low concentration of enzyme. In the latter, the rate of reaction is controlled by a non-enzymic step, which is (on our interpretation) the formation of the adduct from GSH and methylglyoxal. The rate of

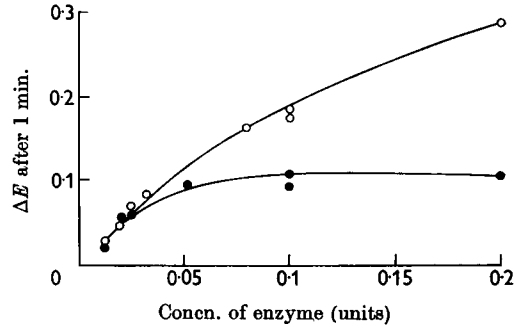


Fig. 4. Variation in rate of reaction (as measured by the ΔE after 1 min.) with the concentration of enzyme. The 'low methylglyoxal' cell (●) contained methylglyoxal (0·1 mM) and GSH (2 mM), and the 'high methylglyoxal' cell (○) contained methylglyoxal (2 mM) and GSH (0·1 mM).

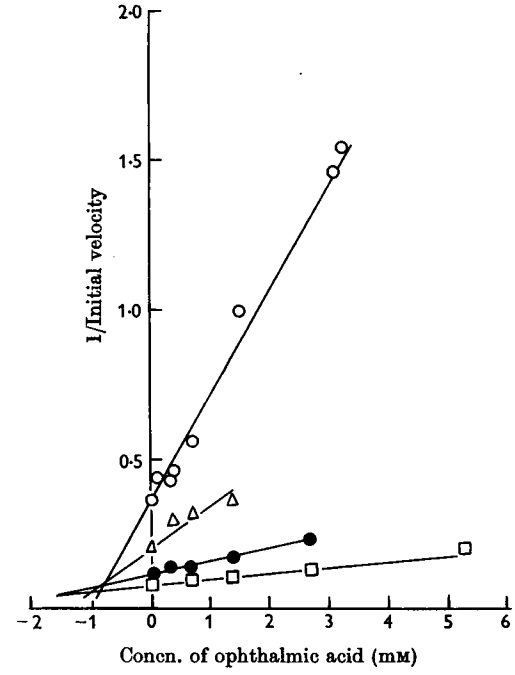


Fig. 5. Inhibition of the glyoxalase reaction by ophthalmic acid. The initial rates were measured for several concentrations of ophthalmic acid at GSH concentrations of 0·1 mM (○), 0·2 mM (△), 0·5 mM (●) and 1·0 mM (□). The concentration of methylglyoxal was 2·37 mM throughout.

formation of the adduct was shown above to depend on the concentration of methylglyoxal. These experiments, then, are consistent with the mechanism in which the adduct is the substrate.

Ophthalmic acid as inhibitor

The initial rates of formation of *S*-lactoylglutathione were measured for given concentrations of GSH and of methylglyoxal and various concentrations of ophthalmic acid; this procedure was repeated for several given concentrations of GSH. The results (Fig. 5) show that ophthalmic acid behaves as a competitive inhibitor; the equilibrium constant for the formation of the inactive enzyme–inhibitor compound, determined by the method of Dixon (1953), is 0·95 mM. Norophthalmic acid was also an inhibitor, but insufficient material was available for a determination of K_i for this compound.

DISCUSSION

Formation of the hemimercaptal from methylglyoxal and glutathione

The nature and composition of the mixture of hydrates and polymers in an aqueous solution of methylglyoxal is not known (Moulds & Riley, 1938). Aldehydes are hydrated (to a varying extent) in solution, and the stability of the hydrate is greater when electron-withdrawing groups are present (as in chloral hydrate). Thus methylglyoxal would be expected to be hydrated in solution. Moreover, α-dicarbonyl compounds are often coloured, and monomeric methylglyoxal is yellow, but the aqueous solution is colourless and has an absorption maximum at about 270 mμ. There is, then, little free methylglyoxal ($CH_3 \cdot CO \cdot CHO$) present in the aqueous solution. The adduct from methylglyoxal and GSH (Schubert, 1935; Yamazoe, 1936) is taken to be the hemimercaptal

$$[CH_3 \cdot CO \cdot CH(OH) \cdot SG]$$

and this seems the most likely structure on chemical grounds. The formation of this hemimercaptal requires free methylglyoxal, since the electrophilic carbon atom of the aldehyde group is absent in the hydrate or polymers derived from it. Hence the first step in the formation of the hemimercaptal is the liberation of free methylglyoxal. This accounts for the results given in Table 1, which show that the initial rate of formation of the hemimercaptal depends on the concentration of methylglyoxal and is independent of the concentration of GSH.

Kermack & Matheson (1957) used the increase in $E_{240\ m\mu}$ as a measure of the extent of hemimercaptal formation. Earlier workers (e.g. Platt & Schroeder, 1934) had reported that from 20 to 80 % of the GSH was not free in acid solutions of GSH and methylglyoxal. Knox (1960) suggested that there was a discrepancy between these figures and the value that Kermack & Matheson obtained for the equilibrium constant. Our value for the equilibrium constant indicates that approximately 30–40 % of the GSH is not free, at pH 6·6. The extent of hemimercaptal formation is known to be greater in more acid solution, and there does not seem to be any large discrepancy between our results and those of earlier workers.

Nature of the substrate in the glyoxalase reaction

The fact that the velocity of the formation of *S*-lactoylglutathione is a symmetrical function of the concentrations of methylglyoxal and GSH (Fig. 1) has been interpreted by the hypothesis that the hemimercaptal is the substrate for glyoxalase. We now have to consider whether other mechanisms could give the same result. Several mechanisms for enzymic reactions that involve two species yield a rate equation of the following type (Dalziel, 1957):

$$\frac{e}{v_0} = \Phi_0 + \frac{\Phi_1}{[S_1]} + \frac{\Phi_2}{[S_2]} + \frac{\Phi_{12}}{[S_1]\,[S_2]}, \qquad (4)$$

where e is the total concentration of the enzyme, v_0 is the initial rate, $[S_1]$ and $[S_2]$ are the initial concentrations of the two substrates, and the four parameters Φ_0, Φ_1, Φ_2, Φ_{12} are functions of the rate constants in the assumed mechanism. Practical considerations limit the extent to which the concentrations of the substrates can be varied, and so the fact that the results fit equation (4) is not, of itself, a satisfactory guide to the mechanism. Now, if the velocity is to be a symmetrical function of S_1 and S_2 it is necessary that

$$\Phi_1 = \Phi_2.$$

The mechanism put forward by Kermack & Matheson (1957) may be written:

$$E + S_1 \rightleftarrows ES_1 \text{ equilibrium constant } K_1$$
$$E + S_2 \rightleftarrows ES_2 \text{ equilibrium constant } K_2$$
$$ES_1 + S_2 \rightleftarrows ES_1S_2 \text{ equilibrium constant } K_3$$
$$ES_2 + S_1 \rightleftarrows ES_1S_2 \text{ equilibrium constant } K_4$$
$$ES_1S_2 \xrightarrow{k} \text{product} + E.$$

They assumed that the rate of formation of product from the ternary complex is small compared with the rates of the other steps; this is the 'equilibrium' assumption (Segal, 1959) which is necessary if the data are to fit equation (4), which now takes the form (Dalziel, 1957):

$$\frac{e}{v_0} = \frac{1}{k}\left[1 + \frac{K_4}{[S_1]} + \frac{K_3}{[S_2]} + \frac{K_1K_3}{[S_1]\,[S_2]}\right]. \qquad (5)$$

Kermack & Matheson also suggested that combination of the enzyme with one substrate did not affect its affinity for the other, i.e. that $K_1 = K_4$

and $K_2 = K_3$. For the velocity to be a symmetrical function of the concentrations of the two substrates it is thus necessary that $K_3 = K_4$, so that all the equilibrium constants have a common value (K'), i.e. the enzyme has the same affinity for both substrates, and equation (5) becomes

$$\frac{e}{v_0} = \frac{1}{k}\left[\left(1+\frac{K'}{[S_1]}\right)\left(1+\frac{K'}{[S_2]}\right)\right]. \qquad (6)$$

The fit of the data to equation (6) has been tested as follows. The results plotted in Fig. 4 of Kermack & Matheson's (1957) paper gave a value of 0·46 mM for K' [other mechanisms would also give the same value for this equilibrium constant (Frieden, 1957)]. This value of K' may be used to obtain a value of the rate constant, k, for the series of experiments (Kermack & Matheson, 1957) in which the concentration of methylglyoxal was 1·08 mM. The values of k and K' may then be used to calculate the rates in the experiments when the concentration of methylglyoxal was 2·16 mM. The calculated rates differ from the observed rates by an amount much greater than the experimental error. For example, the calculated rates (expressed as ΔE/min.) at concentrations of GSH of 0·162, 0·216 and 0·432 mM are 0·0182, 0·0229 and 0·0368 respectively, whereas the observed rates are 0·0230, 0·0285 and 0·0480. The conclusion is that the mechanism put forward by Kermack & Matheson does not accommodate the finding that the velocity is a symmetrical function of the concentrations of the substrates.

The most direct evidence that Kermack & Matheson (1957) put forward for their view that (free) glutathione was the substrate was based on an experiment in which the enzymic reaction appeared to proceed more slowly after an equilibrium concentration of the hemimercaptal had been attained. This experiment we have repeated, and our results (Fig. 6) show no such difference. The formation of hemimercaptal is quite rapid, and the attainment of equilibrium (as measured by the change in E) is characterized by a half-life of the order of 10 sec.

Petrovicki (1939) and Crook & Law (1952) found that the hemimercaptal from methylglyoxal and GSH was unreactive, as judged by the formation of lactic acid from *S*-lactoylglutathione. Excess of methylglyoxal (perhaps owing to the presence of impurities) apparently catalyses the hydrolysis of *S*-lactoylglutathione. The reported differences between the course of the overall reaction when the hemimercaptal is used (rather than a mixture of methylglyoxal and GSH) thus reside in the second (hydrolytic) step, and throw no light on the mechanism of the first step. We have, in fact, prepared the hemimercaptal by the method of Schubert (1935), and used it as the substrate: the rate of reaction was that expected on the assumption that the hemimercaptal rapidly forms the equilibrium mixture with methylglyoxal and GSH.

There are several mechanisms for an enzymic reaction between two substrates other than the one put forward by Kermack & Matheson (1957). These cannot be tested at all rigorously by the data at present available for the glyoxalase reaction; in all cases, the velocity would only be a symmetrical function of the concentrations of the substrates if several rate constants happened, fortuitously, to have values such that $\Phi_1 = \Phi_2$ in equation (4). Hence the simplest conclusion is that the hemimercaptal [$CH_3 \cdot CO \cdot CH(OH) \cdot SG$] is the substrate of glyoxalase.

This type of mechanism is not common in enzymic reactions. Another example may be provided by formaldehyde dehydrogenase; here Strittmatter & Ball (1955) have suggested that the substrate is *S*-hydroxymethylglutathione

[$CH_2(OH) \cdot SG$].

The reactions catalysed by glyoxalase I and by formaldehyde dehydrogenase are similar: both involve GSH and carbonyl groups, but that catalysed by glyoxalase is an intramolecular Cannizzaro reaction, whereas formaldehyde dehydrogenase requires diphosphopyridine nucleotide. Strittmatter & Ball also proposed a kinetic test for this type of mechanism. Consider two experiments, the first carried out at concentrations of methylglyoxal and GSH given by m_1 and g_1 respectively, and the second at concentrations

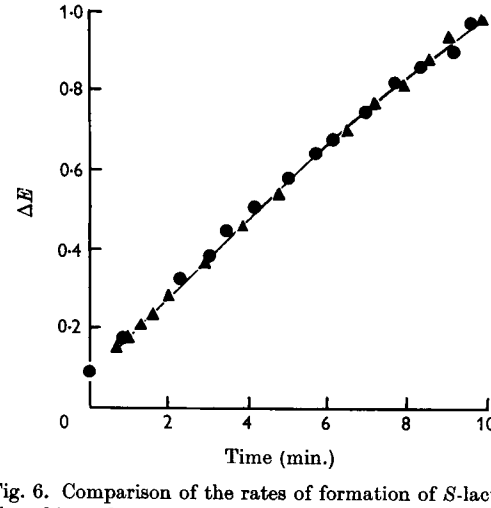


Fig. 6. Comparison of the rates of formation of *S*-lactoylglutathione from methylglyoxal and GSH, and from an equilibrium concentration of hemimercaptal. Both cells contained GSH (1 mM), and in one cell (●) methylglyoxal (2 mM) was present from the beginning, and in the other cell (▲) it was added after 70 min.; enzyme was then added immediately to both cells. The interval of time may be decreased to 5 min. without affecting the results.

given by m_2 and g_2. If the rate is the same in the two experiments, and if the rate depends on the concentration of hemimercaptal, then the concentration of hemimercaptal must be the same in the two experiments,

i.e. $$(m_1-q)\,(g_1-q) = (m_2-q)\,(g_2-q) \qquad (7)$$

or $$q = (m_2g_2-m_1g_1)/(m_2+g_2-m_1-g_1).$$

From smooth curves drawn through the experimental points (Fig. 1), pairs of values of the concentrations of the substrates at which the rates are equal were chosen. Values of q were then obtained from equation (7), and hence values of the equilibrium constant K were obtained. The values of K were in the range 1–2 mM, which is in reasonable agreement with the value (2 mM) obtained by the entirely independent method (the change in E on mixing the components) already described. The values for K, however, showed a trend towards higher values at lower concentrations of the substrates, which is not readily accounted for.

Rate of relatively high concentrations of glutathione

When the concentration of GSH is greater than that of methylglyoxal, further increase does not increase the rate of the enzymic reaction, but slightly decreases it (Kermack & Matheson, 1957). Thus, for a given concentration of methylglyoxal, there is an optimum concentration of GSH. This feature can be explained if the hemimercaptal (Q) is the substrate, and free glutathione (G) is an inhibitor. Then the mechanism (details of the formation of hemimercaptal are here omitted) would be:

$$M + G \rightleftarrows Q$$
$$E + Q \rightleftarrows EQ$$
$$E + G \rightleftarrows EG \text{ equilibrium constant } K_G$$
$$EQ \longrightarrow E + \text{product}.$$

This mechanism leads to the equation:

$$\frac{1}{v_0} = \frac{1}{V_{max.}} + \frac{K_m}{V_{max.}}\left(1+\frac{g}{K_G}\right)\frac{1}{q} \qquad (8)$$

where g is the concentration of free GSH, and q is the concentration of hemimercaptal. If g_0 is the total concentration of GSH, then after the equilibrium concentration of the hemimercaptal has been built up

$$g = g_0-q.$$

If m_0 is the total concentration of methylglyoxal, the equilibrium constant for the formation of hemimercaptal is given by

$$K = (m_0-q)\,(g_0-q)/q.$$

It may be shown that the rate, given by equation (8), is greatest when the total concentration of GSH has the value

$$g_0 = [m_0+K+(KK_G)^{\frac{1}{2}}]/[1+(K/K_G)^{\frac{1}{2}}].$$

The calculation is not given, since the data are not adequate to test the equation. If the value of K_G were about 1 mM, however, a curve similar to that obtained by Kermack & Matheson would be obtained, and this value of K_G seems quite reasonable, as it is similar to the value of K_i obtained with ophthalmic acid. It seems probable that the suggestion that free GSH is an inhibitor in the glyoxalase reaction can account for the variation of the rate with the concentration of GSH. GSH is also an inhibitor in the reaction catalysed by glyoxalase II (Wieland, Pfleiderer & Lau, 1956).

SUMMARY

1. Ophthalmic acid, an analogue of glutathione that occurs in calf lens, was a competitive inhibitor in the reaction catalysed by glyoxalase I.

2. The velocity of the enzymic reaction is a symmetrical function of the concentrations of methylglyoxal and glutathione (GSH). This result suggests that the hemimercaptal,

$$CH_3\cdot CO\cdot CH(OH)\cdot SG,$$

which is formed on mixing the components, is the substrate for glyoxalase I. Some supporting evidence for this suggestion has been provided, and a different mechanism, which had been proposed previously, has been excluded.

3. The initial rate of formation of the hemimercaptal is proportional to the concentration of methylglyoxal but independent of the concentration of glutathione. The rate-determining step in the formation of the hemimercaptal is probably the liberation of free methylglyoxal from a hydrate.

4. At low concentrations of methylglyoxal and high concentrations of enzyme the rate of the enzymic formation of *S*-lactoylglutathione is affected by the rate of the (non-enzymic) formation of hemimercaptal.

5. The retardation caused by a relatively high concentration of glutathione is ascribed to the action of free glutathione as an inhibitor (the hemimercaptal being the substrate).

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Studies in Detoxication

86. THE METABOLISM OF ^{14}C-LABELLED ETHYLENE GLYCOL*

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Ethylene glycol (ethane-1:2-diol) and its derivatives are regarded as relatively toxic substances to human beings, and the toxicology has been summarized by Browning (1953). This compound appears to be more toxic to man than to laboratory animals, for while oral LD_{50} doses vary from about 2 ml./kg. for cats to 13 ml./kg. for mice (Laug, Calvery, Morris & Woodard, 1939; Lehmann & Flury, 1943; Oettingen, 1943; Spector, 1956*a*), the minimum lethal dose for man is about 1·6 g./kg. Fatal poisoning from ethylene glycol in man is usually the result of the accidental drinking of anti-freeze fluid as a substitute for alcoholic beverages (e.g. Pons & Custer, 1946; Harger & Forney, 1959). The cause of death from ethylene glycol has been attributed to lesions of the central nervous system (Pons & Custer, 1946); renal damage due to deposition of calcium oxalate crystals in the renal tubules is usually not severe enough to cause death, although metabolic conversion into oxalate has often been postulated as the reason for the toxic effects. Most studies on the fate of the glycol in animals have been concerned with oxalate formation and excretion (see Oettingen 1943).

The only well established metabolite of ethylene glycol in man and animals is oxalic acid, which, however, accounts for less than 2% of the dose (see Oettingen, 1943). Glycolaldehyde, glyoxal, glycollic acid and glyoxylic acid are possible metabolites. Glycollic acid has been claimed to be a metabolite in rabbits (Mayer, 1903). Glucuronic acid conjugation of ethylene glycol has not been found (Fellows, Luduena & Hanzlik, 1947; Gessner, Parke & Williams, 1960). Unchanged ethylene glycol, however, is excreted by dogs (Nakazawa, 1950) and humans (Hjelt, Tamminen, Fortelius, Raekallio & Alha, 1958; Harger & Forney, 1959).

This paper shows that the major end product of ethylene glycol metabolism is respiratory carbon dioxide. The main metabolite excreted in urine is unchanged ethylene glycol; oxalic acid is a minor metabolite, the amounts of which depend upon the dose and the species of animal. Intermediates in the formation of carbon dioxide are glycolaldehyde and glyoxylic acid, and these were detected in liver-slice experiments.

* Part 85: Parke (1961).

MATERIALS AND METHODS

[$^{14}C_2$]*Ethane*-1:2-*diol*. [$^{14}C_2$]Ethylene (The Radiochemical Centre, Amersham, Bucks) was converted into ethanediol according to Milas & Sussman (1937). The [$^{14}C_2$]ethylene gas (26 mg., 0·5μc) was contained in an ampoule which was broken (with a piece of iron and a magnet) in a closed flask containing 1 ml. of a 5·8% (w/v) solution of hydrogen peroxide and 0·07 ml. of a 0·5% (w/v) solution of osmium tetroxide, both in anhydrous *tert.*-butyl alcohol. The flask was connected with an arrangement for adding, in the absence of air, non-radioactive ethylene. The reaction mixture was kept at room temperature for 72 hr. and then brought into contact with an excess of non-radioactive ethylene and kept until the appearance of black colloidal osmium oxide indicated total decomposition of the