the absence of extraneous carbohydrate, the ciliate produces large amounts of succinic acid, with smaller quantities of lactic and acetic acid, and assimilates some carbon dioxide.

7. Cell-free, centrifuged homogenates contain enzymes capable of hydrolysing starch, glycogen and maltose to glucose; they are also able to phosphorylate glycogen and starch to form glucose-1-phosphate and contain phosphoglucomutase and oxoisomerase.

8. Cell-free preparations convert fructose-1:6-diphosphate to triosephosphate, which is fermented to lactic acid. This process is stimulated by arsenate, and inhibited by iodoacetate.

9. Homogenates of the ciliate display both succinic and lactic dehydrogenase activity; in the presence of atmospheric oxygen, however, they oxidize lactic, but not succinic, acid.

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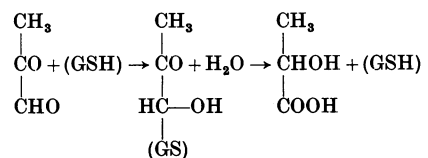# Glyoxalase: the Role of the Components

By E. M. CROOK and K. LAW

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Since the discovery by Lohmann (1932) that reduced glutathione (GSH) is the coenzyme of glyoxalase, several workers have reported that methylglyoxal and GSH form a compound and have suggested that this might be the true substrate for the enzyme or an intermediate in the reaction. Thus Jowett & Quastel (1932) observed that SH groups titratable by iodine decreased when methylglyoxal was added to GSH solution and suggested a reaction sequence:

$$\begin{array}{ccc} CH_3 & CH_3 & CH_3 \\ | & | & | \\ CO + (GSH) \rightarrow & CO + H_2O \rightarrow & CHOH + (GSH) \\ | & | & | \\ CHO & HC-OH & COOH \\ & | & \\ & (GS) & \end{array}$$

Platt & Schroeder (1934) studied this interaction of methylglyoxal and GSH, showing that under the usual conditions of temperature, pH and concentration of reagents, 80 % of the GSH was combined. However, they reported, as had most earlier observers, that the rate of transformation of methylglyoxal was constant until the reaction was almost complete. They commented upon the difficulty of reconciling the two observations, since, if the methylglyoxal-GSH compound were the substrate for the enzyme and its formation were only 80 % complete at the initial concentration of the reagents, the reaction rate would be expected to fall off from the beginning of the reaction. Giršavičius & Heyfetz (1935) created further difficulties when they showed that addition of enzyme to the equilibrium mixture of methylglyoxal and GSH caused a further decrease of SH concentration almost to zero, and that this low figure was maintained until the reaction was almost complete, when the whole of the GSH rapidly reappeared. They suggested that the enzyme drove the combination of methylglyoxal and GSH to completion, a concept entirely out of harmony with our present ideas of the role of enzymes as catalysts. The problem was clarified by Yamazoye's (1936) demonstration that the compound formed from methylglyoxal and GSH in the presence of rabbit-liver glyoxalase was different from that formed in its absence. He was able to isolate both. The compound formed in the absence of enzyme was very labile and behaved as a mixture of GSH and methylglyoxal. This, Yamazoye called the 'Chemical compound' and its properties are closely similar to those of the compounds described by Schubert (1935). The 'Biological compound', formed in the presence of the enzyme, did not react with iodine and gave no tests for SH, keto or aldehyde groups. Both compounds, however, contained GSH and methylglyoxal in equimolecular proportions. The biological compound, above pH 7·0, slowly broke down to form GSH and lactic acid. Yamazoye was able to show that this breakdown was catalysed by his crude rabbit-liver glyoxalase.

Later, Hopkins & Morgan (1948) demonstrated that ox-heart glyoxalase consisted of two components. One of these, called by them the 'enzyme', in the presence of GSH, converted methyl- and phenylglyoxals to the corresponding hydroxy acids at equal rates. The second component they called the 'factor' and this, when added to the system, accelerated the conversion of methylglyoxal but did not affect the rate of conversion of phenylglyoxal. In the absence of 'enzyme', the 'factor' had no effect on either glyoxal. Hopkins & Morgan (1948) also showed that phenylglyoxal inhibited the accelerating effect of the 'factor' upon the conversion of methylglyoxal. They suggested that this was due to combination of phenyl-

glyoxal with the 'factor' thereby preventing it from acting.

It seemed possible that the results of Hopkins & Morgan might be most easily interpreted by assuming that their 'enzyme' was responsible for catalysing the formation of Yamazoye's 'biological compound' of methylglyoxal and GSH. As he had shown this to break down slowly into lactic acid and GSH, the fairly slow activity of the 'enzyme' would be explained. Further, as Yamazoye had shown that crude glyoxalase accelerated this breakdown of his intermediate, it seemed likely that there was another enzyme present in crude preparations, and that Hopkins & Morgan had separated this and called it 'factor'. The difference in behaviour of methyl- and phenylglyoxals with the 'factor' could then be explained in terms of the specificity of this second enzyme. In this paper evidence for the correctness of this hypothesis is presented. A preliminary account of this work has been given (Crook & Law, 1950), and Racker (1950, 1951) has described similar experiments carried out independently.

EXPERIMENTAL

Enzymes. Acetone-dehydrated ox-heart powder, prepared and extracted by the method of Hopkins & Morgan (1948), was used as a source of both 'enzyme' and 'factor'. The 'enzyme' was purified by a method worked out by Dixon & Morgan (1948) and 'factor' was prepared according to Hopkins & Morgan (1948). 'Factor' was considered to be free from 'enzyme' when there was no reaction above the controls. The criteria for judging freedom of the 'enzyme' from 'factor' are much less certain. Arbitrarily it was decided to regard the 'enzyme' as free from 'factor' when the rates of conversion of equimolecular concentrations of methyl- and phenylglyoxal were equal. In this paper the nomenclature of Hopkins & Morgan (1948) will be used and inverted commas will not be used from now on.

Preparation of the glyoxal-GSH compounds. Chemical and biological compounds were prepared in the solid state by the methods of Yamazoye (1936). In certain experiments small quantities of biological compound were prepared in solution by allowing the enzyme to act upon a mixture of appropriate quantities of the glyoxal and GSH for approximately 20 min., precipitating the enzyme with sufficient 10 % (w/v) salicylsulphonic or trichloroacetic acid to bring the pH to 2·0, filtering off the precipitate and readjusting the pH to 7·3. Solutions were always used at once since the biological compound is unstable at the higher pH.

Reaction rate. This was measured manometrically in Warburg manometers at 25° following the technique of Hopkins & Morgan (1948). The flasks contained 0·4 ml. of 0·2M-NaHCO$_3$ solution, water and enzymes. In the side arm were placed 0·3 ml. containing 2·5 mg. of GSH and 0·3 ml. containing 2·1 mg. of methylglyoxal or 4·0 mg. of phenylglyoxal, adjusted to pH 7·3. The total volume was 2·0 ml. The flasks were gassed at room temperature with 95 % N$_2$– 5 % CO$_2$ (v/v) for 10 min. The reaction rate was measured between 5 and 25 min.

Rate measurements were also carried out by following the change in SH groups by iodine titration. Final concentra-

tions of reagents and the temperature were the same as for the manometric experiments. Initial samples were taken immediately after addition of enzyme. Samples (2 ml.) were removed at suitable intervals into 2 ml. of 10% (w/v) trichloroacetic or 20% (w/v) salicylsulphonic acid solution; 5 ml. of 0·0025 N-I_2 were then added, the solution allowed to stand for 3 min. for any chemical compound between the glyoxal and GSH to react with the iodine, and the excess iodine back-titrated with 0·0025 N-$Na_2S_2O_3$ using starch as indicator.

RESULTS

Role of enzyme and factor. Fig. 1 (upper part) shows the course of the liberation of CO_2 from bicarbonate when crude acetone heart-powder
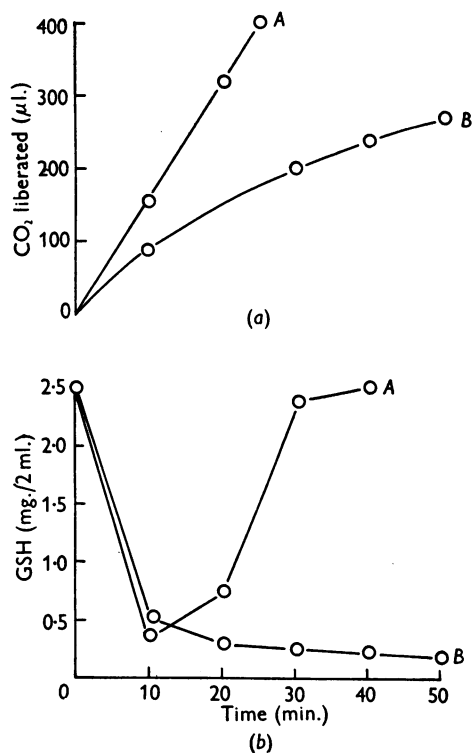
go to completion, may require several hours. In the lower portion of Fig. 1 is shown the simultaneous changes in the concentration of SH groups expressed as mg. GSH in 20 ml. of reaction mixture. With both the glyoxals the concentration of GSH falls rapidly and to approximately the same extent in the initial stages of the reaction. The subsequent behaviour is different. With methylglyoxal the reaction follows the course described by Yamazoye (1936), the GSH rapidly reappearing and reaching a very little lower than the initial value at approximately the same time as the evolution of CO_2 ceases, i.e. when all the methylglyoxal has been converted into lactic acid. With phenylglyoxal the
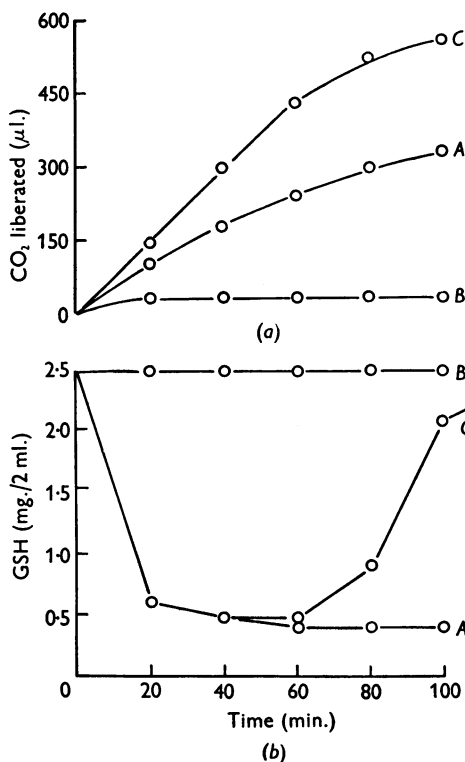

Fig. 1. (a) Liberation of CO_2 from 0·2 M-bicarbonate buffer by extract of heart acetone powder (9 mg. dry matter), using 2·5 mg. GSH with 2·1 mg. methylglyoxal (curve *A*), and with 4 mg. phenylglyoxal (curve *B*) in a total vol. of 2 ml. at 25°. (b) Uptake and liberation of GSH by extract of heart acetone powder (90 mg. dry matter) with methylglyoxal (curve *A*) and phenylglyoxal (curve *B*). The amounts used were 10 times those of the manometric experiment in a total vol. of 20 ml.

Fig. 2. (a) Liberation of CO_2 from 0·2 M-bicarbonate buffer by 0·75 mg. purified enzyme (*A*), 0·5 mg. purified factor (*B*) and enzyme + factor (*C*), using 2·1 mg. methylglyoxal as substrate with 2·5 mg. GSH in a total vol. of 2 ml. at 25°. (b) Uptake and liberation of GSH by 3 mg. purified enzyme (*A*), 7 mg. purified factor (*B*), and enzyme + factor (*C*) with methylglyoxal as substrate. The amounts of all components were 10 times those used in the manometric experiment.

extract acts upon methyl- or phenylglyoxal. With methylglyoxal the reaction is rapid (325 μl. CO_2 in 20 min.) and is practically complete in half an hour. With phenylglyoxal the reaction is much slower (150 μl. CO_2 in 20 min.) and, although it will finally

behaviour of the GSH is quite different. It remains at a low concentration and may even go on decreasing for periods up to 1 hr. It is at this stage that the long-continuing slow output of CO_2 is observed in the manometric experiments.

Fig. 2 shows the results of similar experiments with methylglyoxal and purified enzyme and factor. Factor alone is inert both in the production of CO_2 and in causing a disappearance of SH groups. Pure enzyme in the absence of factor behaves with methylglyoxal in a manner very similar to the behaviour of the crude extract with phenylglyoxal. The CO_2 output is slow and long continued and the GSH concentration decreases rapidly to a low value and remains thus for many hours. In one experiment it remained low for 5 hr. and had not regained its initial concentration for 10 hr. However, when

prepared in solution. It will be seen that in the absence of factor GSH is liberated quite slowly from each (curves A and B). The rate of liberation from the phenyl compound is little affected by the addition of factor (curve C), whereas the rate of liberation from the methylglyoxal compound is much increased (curve D). A similar result was obtained manometrically. The results are shown in Table 1.
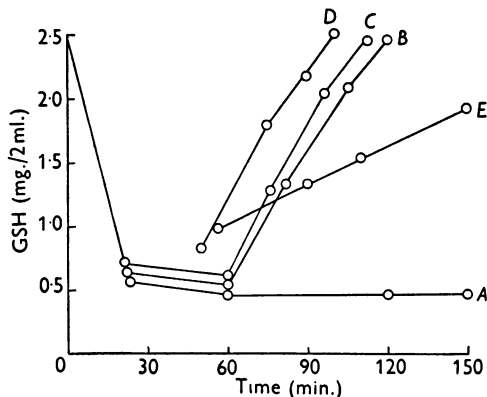

Fig. 3. The formation and breakdown of the methyl-glyoxal-GSH compound by enzyme and factor. GSH, 25 mg.; methylglyoxal, 21 mg.; buffer and enzyme in an initial vol. of 20 ml., incubated at 25°. (A) Action of 2 mg. enzyme alone; (B) action of 2 mg. enzyme + 3 mg. factor added initially; (C) addition of factor after 40 min.; (D) enzyme precipitated with salicylsulphonic acid after 20 min., pH readjusted to 7·3 and factor added; (E) enzyme precipitated after 20 min., pH readjusted to 7·3—no addition of factor.

enzyme and factor are mixed the properties of Fig. 1 are restored and the familiar U-shaped curve is observed.

For the experiment shown in Fig. 3, purified factor and purified enzyme were used. Curves A and B are given by enzyme alone and by enzyme + factor and follow the same course as before. For curve C, the experiment was started with enzyme alone but, after the GSH concentration had fallen to its lowest value, factor was added. Curve D is obtained if the enzyme is precipitated after 20 min. and factor added. This experiment shows that the factor always has the effect of liberating GSH from combination whether it be added with the enzyme, some time later, or after the enzyme has been removed. On the other hand, the spontaneous breakdown of the glyoxal-GSH compound is quite slow (curve E).

Specificity of factor. The behaviour of the phenyl- and methylglyoxal compounds with factor is shown in Fig. 4. For this experiment the compounds were
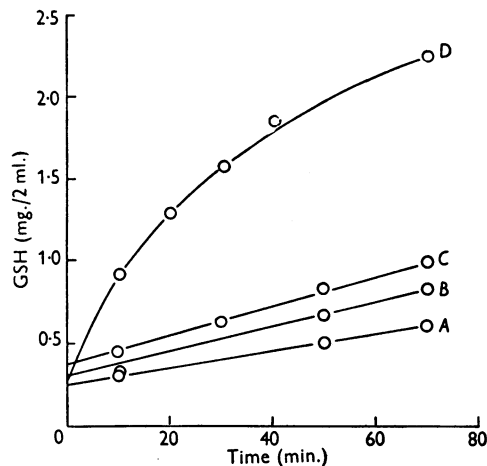

Fig. 4. Specificity of factor. 21 mg. methylglyoxal or 40 mg. phenylglyoxal + 25 mg. GSH incubated at 25° with 2 mg. enzyme in buffer, the total vol. being 5 ml. After 20 min., enzyme precipitated with salicylsulphonic acid, the pH readjusted to 7·3 and the solution diluted 3·3 times (equivalent to that in the manometric experiment, Table 1). (A) Methylgloxal as substrate—no addition after precipitation of enzyme; (B) phenylglyoxal as substrate—no addition after precipitation of enzyme; (C) phenylglyoxal as substrate—addition of 3 mg. factor after precipitation of enzyme; (D) methylglyoxal as substrate—addition of 3 mg. factor after precipitation of enzyme.

Table 1. *Manometric demonstration of the specificity of factor using the intermediate compounds formed between glutathione and phenyl- and methylglyoxal*

(21 mg. methylglyoxal or 40 mg. phenylglyoxal and 2 ml. 0·2 M-$NaHCO_3$ + 25 mg. GSH incubated with enzyme, total vol. 5 ml. After 20 min. the enzyme precipitated with salicylsulphonic acid, and the pH readjusted to 7·3. 0·6 ml. of the solution measured into the side arm of the Warburg flask, factor and bicarbonate being in the main compartment; total vol. 2 ml.)

	Evolution of CO_2 (μl.)			
Time (min.)	Phenyl-glyoxal compound alone	Phenyl-glyoxal compound + factor	Methyl-glyoxal compound alone	Methyl-glyoxal compound + factor
10	49	52	40	80
20	72	79	59	119
30	84	94	72	145

Composition of the intermediate compound. Yama-zoye's (1936) finding that this contains equi-molecular amounts of glyoxal and GSH indicates that it should give rise to equimolecular amounts of GSH and lactic acid on decomposition.

Table 2. *Equivalence of the reduced glutathione (GSH) and lactic acid (CO_2) liberated on decomposition of the intermediate compound of methylglyoxal and reduced glutathione*

(In each Warburg flask 4 mg. biological compound pre-pared from methylglyoxal by the method of Yamazoye (1936) together with 0·4 ml. 0·2M-$NaHCO_3$ and water to total vol. 2·0 ml. In side arms 0·6 mg. of ox-heart factor in 0·6 ml. water. Controls without factor. Gassed at room temperature; reaction at 25°. Theoretical CO_2 calculated on SH liberated on basis of 1 equiv. of CO_2 per equivalent of SH. Experimental CO_2 represents difference between flasks with and without factor. Control CO_2 output 6–7 μl.)

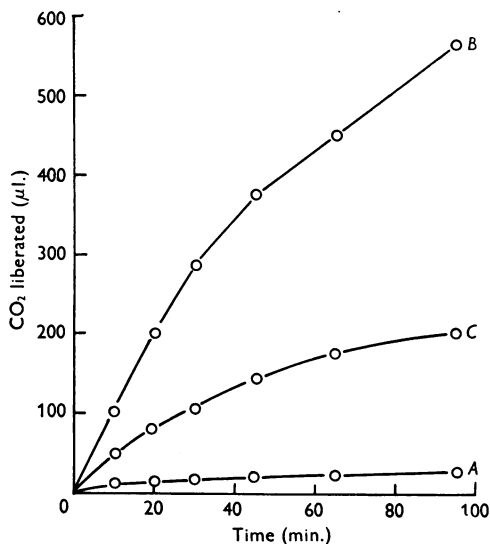|	Amount of GSH (mg.)	Amount of CO_2 (μl.)
Initial GSH content	1·06	—
GSH in control after reaction	0·94	—
GSH in flask with factor	2·41	—
GSH liberated	1·5	—
Theoretical CO_2 equiv.	—	110
Experimental CO_2 found	—	111

Fig. 5. Action of purified and crude enzyme on the chemical compound of methylglyoxal and GSH. CO_2 output at 25° from 0·2M-bicarbonate buffer and 10 mg. chemical compound in the presence of 0·3 mg. pure glyoxalase enzyme (curve A), 0·3 mg. pure enzyme + 5 mg. methyl-glyoxal (curve B) and crude extract of heart acetone powder containing 9 mg. dry matter (curve C). Total vol. in each vessel 1·5 ml.

To check this, 4 mg. quantities of biological compound of methylglyoxal and GSH were placed in pairs of Warburg vessels together with bicarbonate. One flask of each pair

contained 0·6 mg. factor purified to stage 6 of Hopkins & Morgan (1948); the other served as control. The liberation of CO_2 was followed until it had ceased (after 15 min.). The contents of each flask were then washed out, acidified with salicylsulphonic acid and the GSH titrated with iodine.

The compound contained approximately 20 % of free GSH, and this was usually found not to have increased in the controls, i.e. there was little spontaneous breakdown of the compound. The results of a typical experiment are shown in Table 2.
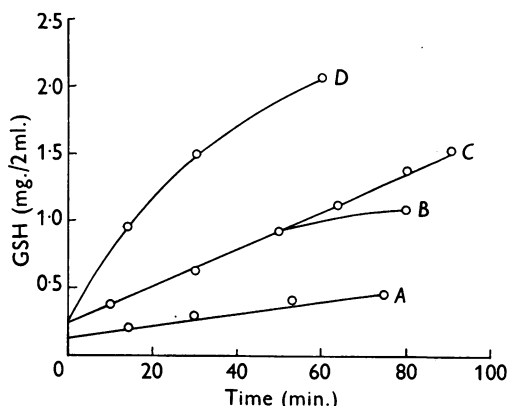
Fig. 6. Effect of extra methyl- or phenylglyoxal on the break-down of the methylglyoxal compound. Methylglyoxal, 21 mg.; GSH, 25 mg.; buffer and 3 mg. enzyme in a total vol. of 20 ml. were incubated for 20 min. at 25°. The enzyme was precipitated with salicylsulphonic acid, and the pH readjusted to 7·3. (A) The methylglyoxal-GSH compound without any addition; (B) 21 mg. methyl-glyoxal added to the compound; (C) 40 mg. phenyl-glyoxal added to the compound; (D) 3 mg. factor added to the compound.

Effect of methyl- and phenylglyoxal on the biological compound. Yamazoye (1936) has shown that the chemical compound when investigated by chemical tests behaves as though it were simply a mixture of methylglyoxal and GSH. It would therefore be expected to react with factor-free enzyme as would any other mixture of methylglyoxal and GSH, i.e. a slow but steady reaction. However, Fig. 5, curve A, shows that this is not so. The system is inert. However, when extra methylglyoxal is added, reactivity is normal (curve B). The presence of factor (curve C) also renders the system reactive even in the absence of extra methylglyoxal. The accelerating effect of extra methylglyoxal is exerted upon the breakdown of the biological com-pound as is shown in Fig. 6. Here the compound was prepared in solution from the usual initial concen-trations of reagents and enzyme, and the enzyme precipitated after 20 min. Curve A shows the rate of spontaneous liberation of GSH in the presence of

0·75 mg. methylglyoxal per ml., i.e. the concentration usually remaining when the intermediate compound is made in this way. Curves B and C show the effects of adding 1 mg. of methyl- and 2 mg. of phenylglyoxal per ml. The reaction rate has been increased approximately threefold in each case.
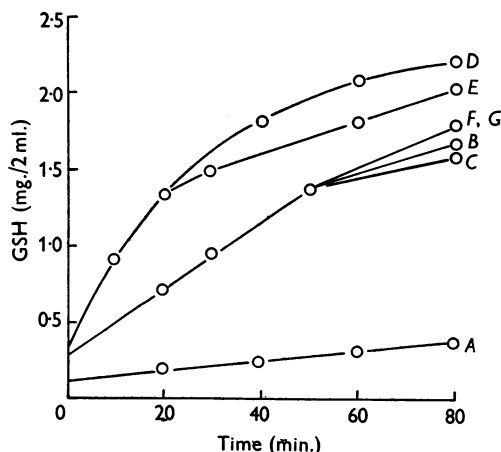

Fig. 7. Experiment to show the point in the reaction sequence where phenylglyoxal inhibits the acceleration by factor of the conversion of methylglyoxal to lactic acid. 25 mg. GSH, 21 mg. methylglyoxal, and 40 mg. phenylglyoxal added as shown above. Initial volumes, 20 ml., contained 4 ml. 0·2 M-NaHCO₃ + purified enzyme and were incubated for 20 min. at 25°. Enzyme pptd. with 10 % (w/v) salicylsulphonic acid, pH adjusted to 7·3, 2 ml. 0·2 M-NaHCO₃ added, and addition of factor and phenylglyoxal made, as indicated. All volumes adjusted to be equal, by addition of water where necessary.

Curve	Material incubated with enzyme	Material added after precipitation of enzyme
A	GSH + methylglyoxal	Nil
B	GSH + methylglyoxal	Methylglyoxal
C	GSH + methylglyoxal	Phenylglyoxal
D	GSH + methylglyoxal	Factor
E	GSH + methylglyoxal	Factor + phenylglyoxal
F	GSH + methylglyoxal + phenylglyoxal	Nil
G	GSH + methylglyoxal + phenylglyoxal	Factor

Curve D shows the effect of adding factor. It is thus clear that extra amounts of these glyoxals accelerate the overall reaction by increasing the rate of breakdown of the intermediate compounds. Thus, although the reaction rate may increase with GSH concentration in the lower ranges of concentration of these compounds as has been found by many authors, there will be a tendency for the reaction to slow down as a unimolecular ratio of GSH to glyoxal is approached. Such an effect presumably explains the well known fact that GSH becomes inhibitory beyond a certain critical concentration (Platt & Shroeder, 1934).

Inhibition by phenylglyoxal. Phenylglyoxal inhibits the accelerating effect of factor upon the conversion of methylglyoxal to lactic acid. Hopkins & Morgan (1948) ascribed this to combination of the phenylglyoxal with the factor thus preventing it from acting. The present experiments show that factor does not act directly upon either of the glyoxals, nor upon the intermediate compound formed by the enzyme between phenylglyoxal and GSH, but only upon the intermediate between methylglyoxal and GSH. Phenylglyoxal could therefore exert its inhibitory effect not only by combining with factor but also by competing for the available GSH, thus preventing the formation of a compound susceptible to the action of factor. These possibilities were tested experimentally.

The intermediate compound of methylglyoxal was prepared in the presence and in the absence of phenylglyoxal and the subsequent liberation of SH groups from it was followed after removal of the enzyme by trichloroacetic acid precipitation. The results are shown in Fig. 7. Curve A shows the usual slow spontaneous liberation of SH from the intermediate compound, while curves B and C show how this is accelerated by increase in the concentration of the glyoxals. Methyl- and phenylglyoxal have equal effects when added in equivalent concentrations. From curve D it can be seen that the effect of factor is much greater than that of the extra glyoxals and is not markedly inhibited by the presence of phenylglyoxal (curve E). However, when phenylglyoxal was present in the initial stages of the reaction, when the enzyme is acting, the intermediate compound has quite different properties. Although its rate of spontaneous breakdown is unchanged (curve F) and is fast due to the high total methyl or phenylglyoxal concentration, this rate is unaffected by the addition of factor (curve G), a property which in these experiments has been associated with the phenylglyoxal-GSH intermediate compound. Thus in the presence of both glyoxals it is the phenylglyoxal-GSH intermediate which is preferentially formed. As the breakdown of this compound is not accelerated by factor, the inhibitory effect of phenylglyoxal found by Hopkins & Morgan (1948) is understandable.

DISCUSSION

There seems little doubt that the conversion of glyoxals to the corresponding hydroxy acids by the addition of one equivalent of water under the influence of glyoxalase proceeds by way of an intermediate compound with glutathione. The relationships between the two catalysts involved also seems clear. That named 'glyoxalase' by Hopkins & Morgan (1948) is undoubtedly responsible for catalysing the formation of the intermediate compound and appears to be of low specificity so far as

32

the type of glyoxal is concerned. In order to explain the high specificity of glutathione as a 'co-enzyme', the specificity of 'glyoxalase' for the SH compound must, however, be high. Spontaneous breakdown of the intermediate accounts for the glyoxalase activity of the pure enzyme.

The second catalyst, which is here shown to be identical with the 'factor' of Hopkins & Morgan (1948), strongly catalyses the breakdown of the intermediate formed from methylglyoxal, thus accelerating the overall reaction. The specificity of this second enzyme appears to be higher than that of the first since its accelerating effect upon the intermediate formed from phenylglyoxal is shown to be low.

In heart tissue the activity of 'enzyme' appears to be greater than that of 'factor' when measured by the relatively greater rate at which SH groups disappear in the presence of methylglyoxal in comparison with their rate of reappearance. This suggests that in this tissue the rate-limiting step is the factor-accelerated breakdown of the intermediate. The spontaneous breakdown of the intermediate is certainly the rate-limiting step in the absence of factor. In the absence of factor the amount of 'enzyme' can be reduced much below that usually used without affecting the overall velocity. In heart extracts, too, the factor concentration is sufficiently low not to affect the velocity of breakdown of the phenylglyoxal intermediate. However, an investigation of the glyoxalase system in liver (unpublished experiments) indicates that in this tissue the enzymes are either more reactive or present in greater concentration than in heart. With factor preparations from liver it can be shown that the specificity of factor for the methylglyoxal intermediate is not absolute since high factor concentrations also catalyse the breakdown of the phenylglyoxal intermediate to some degree.

These experiments give little evidence for the type of intermediate compound involved or for the step at which the water molecule is incorporated. However, we would agree with Racker (1951) that an ester of thiolactic acid which could hydrolyse to lactic acid and GSH seems most likely to account for the observed properties, e.g.

$$
\begin{array}{ccc}
CH_3 & & CH_3 \\
| & & | \\
CHOH & \rightarrow & CHOH \\
| & & | \\
CO(SG) + HOH & & COOH + (GSH)
\end{array}
$$
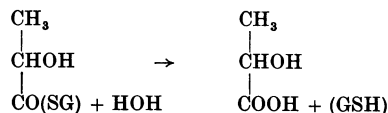
The route suggested by Racker for the formation of this ester is, however, capable of numerous variations according to the order in which one postulates the various keto-enol tautomerizations, whether one assumes that GSH adds across $C=C$ or $C=O$, and whether one invokes the hydrated or the anhydrous forms of the glyoxals. The reason adduced by Racker for preferring the anhydrous to the hydrated form of the glyoxal for the initial condensation with GSH is not valid. He suggests that the ensuing removal of water to form a $C=C$ double bond, necessary if the hydrated glyoxal is the reacting species, would imply easy reversibility of the glyoxalase reaction as a whole since it is then analogous to easily reversible systems such as enolase and fumarase. While agreeing that no sign of reversibility can be detected in the glyoxalase system, we do not agree that this necessarily eliminates an enolase type of water removal since the irreversibility may well lie in the earlier glyoxal-glutathione condensation. It thus seems too early to make any decisions in favour of one particular set of intermediates.

There are no data bearing on the relationship of the 'biological' and 'chemical' compounds of the glyoxals and glutathione. It is possible that the 'chemical' compound is an intermediate in the formation of the 'biological' compound, although its low reactivity towards factor-free enzyme in the absence of excess methyl or phenylglyoxal might argue against this hypothesis. From Racker's (1951) results it would appear that he has found the methylglyoxal-GSH compound to be rather more stable than it has proved in our hands. However, we have worked at a somewhat higher pH (7·3–7·4 instead of 6·6) and, in general, our time scale has been longer. In addition, determinations of the rate of breakdown were done in the presence of excess methylglyoxal, which has been shown to accelerate this reaction. Apart from such small points the two sets of results, which were obtained quite independently with enzymes from different sources and using different methods of estimation, show a most satisfactory measure of agreement.

SUMMARY

1. The enzyme glyoxalase has been shown to consist of two parts. The first of these, identical with the glyoxalase enzyme of Hopkins & Morgan (1948), catalyses the formation of an intermediate compound between methyl- or phenylglyoxal and reduced glutathione. This is the biological compound of Yamazoye (1936), and under physiological conditions of temperature and pH, slowly breaks down to form glutathione and the hydroxy acid corresponding to the glyoxal. The enzyme alone is therefore able slowly to convert glyoxals to the corresponding hydroxy acids.

2. The second part of glyoxalase, identical with the glyoxalase factor of Hopkins & Morgan (1948), catalyses the breakdown of the intermediate formed from glutathione and methylglyoxal but not the intermediate formed from phenylglyoxal.

3. The compound of phenylglyoxal is preferentially formed when both methyl- and phenyl-

glyoxals are present together, and therefore phenylglyoxal prevents the factor from accelerating the conversion of methylglyoxal to lactic acid as was shown by Hopkins & Morgan (1948).

4. The presence of uncombined methyl- or phenylglyoxal accelerates the breakdown of the intermediate compounds.

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Studies in Carotenogenesis

5. CAROTENE PRODUCTION BY VARIOUS MUTANTS OF *PHYCOMYCES BLAKESLEEANUS* AND BY *PHYCOMYCES NITENS*

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The general conditions governing carotene synthesis in *Phycomyces blakesleeanus* have been worked out in detail (Garton, Goodwin & Lijinsky, 1951) and the polyenes which accompany β-carotene (the major component) in traces have also been identified (Goodwin, 1952). As a number of morphological mutants of *P. blakesleeanus* have been isolated and were available, it was considered desirable to examine them quantitatively and qualitatively for carotene synthesis. It was thought possible that some metabolic block might have occurred which would help in the elucidation of the pathway of biosynthesis of carotene. The mutants examined were *P. blakesleeanus* var. *piloboloides* (+ and − strains), *P. blakesleeanus* mutant *gracilis* (+ strain only), *P. blakesleeanus* mutant *pallens* (− strain). *P. nitens* (+) was also examined.

A short account of this work has already appeared (Goodwin & Griffiths, 1952).

EXPERIMENTAL

Cultures. All the cultures used were obtained from the Centraalbureau voor Schimmelcultures, Baarn, Holland.

Cultural methods. The fungi were grown and analysed for dry weight, lipid and carotene production as described in detail by Garton *et al.* (1951). The standard medium containing 2·5 % (w/v) glucose was used throughout.

Identification of the polyenes present. The methods described by Goodwin (1952) were used.

RESULTS

The polyenes produced. A chromatographic examination of the polyenes present in the various mutants was carried out. All the mutants contained the same polyenes as are found in the parent strain, namely: lycopene, neurosporene, α-, β-, γ- and δ-carotene, phytofluene and a phytoene-like substance. These were also present in essentially the same relative amounts as in the parent form of *P. blakesleeanus*.

Production of carotene. Typical results obtained with the various strains are recorded in Table 1 and Figs. 1 and 2. In the first place it will be seen from Fig. 1 that there are no significant differences between the three (−) strains in either the rate of synthesis or the amount of carotene finally produced. There is also no major difference between the amount of dry weight and lipid synthesized by these strains (Table 1).

When the (+) strains are considered (Fig. 2), *piloboloides* and *nitens* fall into line with the (+) strain of the parent in synthesizing about half as much carotene as the (−) strain (Garton *et al.* 1951), but the same amount of dry weight and lipid (Table 1). The mutant *gracilis* (+), however, is different in two respects: (*a*) it synthesizes as much carotene as the (−) strains of the other variants, and (*b*) the rate of synthesis in young cultures is very