XXIV. THE GLYOXALASE ACTIVITY OF TISSUES.

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THE authors have already investigated [Jowett and Quastel, 1933] the glyoxalase activity of red blood corpuscles. A study has now been made of the glyoxalase activity of tissues with the use of tissue-slices.

An interesting finding, which former workers have not made clear, is that, owing to the high glyoxalase activity of tissues, diffusion of methylglyoxal sets a limit to the activity shown by tissue-slices of the usual thicknesses. The glyoxalase activity is proportional not to the weight of the tissue-slices, but to their surface areas. The observed activity depends on the diffusion coefficient of methylglyoxal in tissues as well as on the intrinsic activity of the cells.

This additional variable makes the designing and interpretation of experiments more difficult. Nevertheless we have been able to obtain a little further information on the part played by methylglyoxal in glycolysis.

Methods.

Rates of glyoxalase action have been measured, as in our former work [Jowett and Quastel, 1933], by the manometric method of Warburg. Methylglyoxal solution, contained in a side-tube, is added to the Ringer's solution after equilibration of the manometers at 37°. Manometric readings are begun 5–10 minutes later.

Because comparatively large weights of tissue are used (usually of the order 5–12 mg., no micro-balance being available), the rate of gas output is high, and measurements cannot be continued for long before changes in the methylglyoxal concentration become appreciable. The high rates however permit fairly accurate measurements of the velocity to be made over a short period.

Methylglyoxal was made by the method of Riley *et al.* [1932], a practically neutral solution being obtained¹. We have assumed that the gas-output in our anaerobic experiments corresponds to formation of lactic acid. The gas output is completely thermolabile.

The anaerobic glyoxalase activity of tissue-slices.

In Table I are given the results of experiments on the glyoxalase activity of tissue-slices under anaerobic conditions. The only substrate added is methylglyoxal, and the proportion of the gas output due to glycolysis is very small.

Slices of several thicknesses have been employed in each experiment. It is found that the value of $Q_M^{N_2}$ is greater the thinner the slice, and even with the thinnest slices used there is no evidence that $Q_M^{N_2}$ has reached a limiting value.

¹ For the methylglyoxal used in our former work we are indebted to Prof. C. Neuberg.

Table I. *Anaerobic glyoxalase activity of tissue-slices of varying thicknesses.*At 37°. N₂ + 5 % CO₂.Ringer's solution containing 0.025 M NaHCO₃.Initial methylglyoxal concentration M/100. $v_F = 3.2$ ml.

Measurements relate to the period about 7–25 minutes after addition of methylglyoxal.

 $Q_M^{N_2}$ = mm.³ CO₂ evolved per hr. per mg. dry weight. $S_M^{N_2}$ = mm.³ CO₂ evolved per hr. per cm.² of surface.

Tissue	Thickness of slice mm.*	$Q_M^{N_2}$	$S_M^{N_2}$
Guinea-pig kidney	0.22	105	240
	0.27	86	235
	0.41	74	(295)
	0.47	51	245
Guinea-pig liver	0.43	29.5	127
	0.43	31.0	133
	0.53	22.8	122
Jensen rat sarcoma	0.27	38.5	(103)
	0.37	34.5	128
	0.37	33.5	123
	0.42	30.5	128
Jensen rat sarcoma	0.32	33	116
	0.37	30	111
	0.45	25.5	116
	0.66	17.5	117
Rat kidney	0.17	110	191
	0.20	96	196
	0.27	70	191
	0.38	61	(235)

* The thicknesses of the slices are calculated by the approximate assumption that the volume of a slice in mm.³ is five times its dry weight in mg.

On the other hand S , the rate per unit surface¹, is constant within the experimental error. (The data have not been selected, and some irregularities are evident.)

These findings show that methylglyoxal in appreciable quantity is penetrating only a short distance into the slices. Only a thin outer layer of the slices contributes appreciably to the metabolism; hence the proportionality to the surface area. If sufficiently thin slices were studied, $Q_M^{N_2}$ should rise to a value almost independent of the thickness, and S should decrease as slices below the limiting thickness are reached. Our experiments have not been made with slices sufficiently thin to show these limiting phenomena, and consequently we have no direct method of deducing from our data either the maximum value of $Q_M^{N_2}$ or the limiting thickness.

It is clear that our values of $Q_M^{N_2}$, in common with those of former workers, do not measure the glyoxalase activity of tissue cells supplied with a definite concentration of methylglyoxal but give a mean activity averaged over all the cells of the tissue-slice, some of these cells obtaining practically no methylglyoxal.

The values of S , the activity per unit surface, are however characteristic for each tissue. S will be a function both of the rate at which cells can transform methylglyoxal into lactic acid and of the diffusion coefficient of methylglyoxal. Under certain conditions [Jowett, 1934] the value of S is given by the equation

$$S = \sqrt{2ADC_0} \quad \dots\dots(1),$$

¹ S is defined in Table I. In calculating surface both sides of the slice are counted, and the area due to the edges has been neglected.

where A is the amount of methylglyoxal consumed per unit volume of tissue adequately supplied with it¹, D is the diffusion coefficient of methylglyoxal in the tissue, and Co is the concentration of methylglyoxal in the outer medium.

The diffusion coefficient, D , may not change much from one tissue to another, and variations in S may be chiefly caused by differences in A , which measures the capacity of tissues to transform methylglyoxal when supplied with it. From the data in Table II it seems likely that A , and therefore the maximum $Q_M^{N_2}$, is higher for kidney than for liver and tumour tissue. The figures in Table II each relate to tissue from a different animal and show that S for any one tissue is fairly constant from one individual animal to another.

Table II. *Anaerobic glyoxalase activity of tissues.*

Conditions as in Table I.	
Tissue	$S_M^{N_2}$
Guinea-pig kidney	240, 240, 232, 271
Guinea-pig liver	127, 130
Jensen rat sarcoma	126, 115
Rat kidney	193, 175

In Table III are given the highest values of $Q_M^{N_2}$ which we have observed for various tissues. In each case, the figures provide a lower limit to the maximum value of $Q_M^{N_2}$. The values found by other workers are given for comparison. Conclusions as to the relative activity of glyoxalase in the tissues

Table III. *Anaerobic glyoxalase activity of tissue-slices.*

Conditions as in Table I.	
Tissue	Highest observed value of $Q_M^{N_2}$
Rat spleen	50
„ testis	60
„ brain	46
„ sarcoma (Jensen)	38
„ kidney	110
Guinea-pig brain	107
„ testis	54
„ kidney	105
„ liver	40
„ lung	40

Measurements by other authors.

Tissue	$Q_M^{N_2}$	Author
Rat liver	24	Warburg <i>et al.</i> [1924]
Flexner rat carcinoma	29	
Brain (rat?)	25-50	Meyerhof [1925]
Liver (rat?)	30	„
Diaphragm (rat?)	15	„
Rat liver	55	Rosenthal [1930]

cannot be drawn from these data. The thickness of the slices varies considerably, and furthermore the limiting thickness for maximum activity must also vary. We cannot yet estimate how far we are from the maximum values. For human red blood corpuscles we found [Jowett and Quastel, 1933] values of $Q_M^{N_2}$ about 55-75, which are presumably maximum. Other cells may be expected to show higher limiting values, and values up to 200 are quite conceivable.

¹ Regions partially but not adequately supplied are neglected.

The observed anaerobic glyoxalase activity of most tissues, using tissue-slices in 0.01 *M* methylglyoxal, falls off fairly rapidly as time passes. This is illustrated in Fig. 1, where the rates of gas output are plotted against time, taking the initial rate in each case arbitrarily to be 100. The first measurement of rate relates to a time about 10 minutes after methylglyoxal is added. While the velocity-time curves are similar for duplicate slices of any one tissue from the same individual animal, there is much variation from one animal of the same species to another. The variability from one tissue to another shows that the effect can only be due in a minor degree to the diminution in substrate concentration with time.

The behaviour of red blood corpuscles is shown in Fig. 2. The rate in 0.01 *M* methylglyoxal falls off slowly. In 0.02 *M* methylglyoxal the decrease in rate is greater. The decrease in 0.005 *M* methylglyoxal is chiefly due to the low substrate concentrations which are soon reached as the methylglyoxal is consumed. The experiment shows that, in a case where diffusion should be adequate to saturate the enzyme system with methylglyoxal, the maximum value of $Q_M^{N_2}$ is reached at a low methylglyoxal concentration. Raising the methylglyoxal concentration evidently has a toxic effect on the enzyme system, for the initial value of $Q_M^{N_2}$ is lower in the higher concentrations, and the rate of decrease in $Q_M^{N_2}$ is greater.

That tissue-slices are damaged by methylglyoxal is suggested by their change in appearance, on which Kisch [1932] has already remarked. Tissue-slices usually become tougher and firmer, and frequently swollen and opaque, after exposure to 0.01 *M* methylglyoxal in Ringer's solution. Slices of brain become fragile to handle. Loss of pigment, which normally takes place with slices of spleen and liver, is prevented. The changes in appearance are more rapid in higher methylglyoxal concentrations; in concentrations below 0.01 *M* they are less marked.

From these changes, it would not be surprising if the diffusion coefficient of methylglyoxal in tissues altered as time passed. The behaviour of rat testis, shown in Fig. 3, may be due to such changes. The observed glyoxalase activity goes through a maximum at the higher concentrations, which may be due to an initial lowering in the ability of methylglyoxal to diffuse, which then disappears as the membranes are further damaged. The diffusion of sodium lactate through muscle shows analogous changes [Eggleton *et al.*, 1928].

Fig. 4 illustrates the behaviour of guinea-pig liver. In this experiment 0.01 *M* methylglyoxal apparently had little toxic effect on the enzyme system, while higher concentrations exerted progressive inhibitory action. Both Figs. 3 and 4 illustrate the tendency for high methylglyoxal concentrations to produce higher apparent glyoxalase activity. We consider that this effect is due to a thicker layer of tissue being supplied with substrate.

It is only in the case of red blood corpuscles that we can safely attribute the effects of raising the methylglyoxal concentration entirely to toxic action. With tissue-slices we have as additional factors: (1) the better supply of methylglyoxal to the tissue, and (2) possible changes in the diffusion coefficient of methylglyoxal.

In carrying out experiments on the effect of varying conditions on the observed glyoxalase activity of tissue-slices, two methods have been used:

- (1) The measurement of activity per unit area of surface (*S*).
- (2) The use of tissue-slices of equal thickness, activity then being stated per unit weight (in terms of $Q_M^{N_2}$). By choosing slices carefully, and then cutting each slice into several smaller ones, which are distributed among the several manometric vessels, it is possible to obtain fairly reproducible behaviour, with

Fig. 1. Anaerobic glyoxalase activity of tissue-slices as a function of time.

Fig. 2. Glyoxalase activity of human erythrocytes, at several methylglyoxal concentrations.

Fig. 3. Glyoxalase activity of rat testis at various methylglyoxal concentrations.

Fig. 4. Glyoxalase activity of guinea-pig liver at various methylglyoxal concentrations.

occasional irregularities. This method, which was used in obtaining the data of Figs. 1, 3 and 4, is applicable in many cases where rectangular slices of reasonable size—for measurement of area—are hard to obtain.

From what has already been said, it should be clear that changes in the observed activity do not necessarily correspond with equal changes in the intrinsic glyoxalase activity of tissues. An additional point will be noted here. If the intrinsic activity falls, the methylglyoxal will penetrate further into the slice (no change in diffusion coefficient being supposed to take place), and as a result the observed change in activity will not be so great as the change in intrinsic activity. When equation (1) holds good, S will change proportionally to the square root of A , if D and Co are constant. The observed values of $Q_M^{N_2}$ will similarly change proportionally to the square root of the maximum $Q_M^{N_2}$, provided that the slices are incompletely saturated with substrate.

According to equation (1) S should vary as the square root of the concentration of methylglyoxal. At very low concentrations of methylglyoxal, according to Jowett [1934], S should vary instead proportionally to the concentration. That is, S varies as c^n , where n changes from 1 at low values of c to 0.5 at high values. Toxic effects are not considered. Measurements have been made of the values of S for guinea-pig kidney at a number of methylglyoxal concentrations; the data, shown in Fig. 5, refer to two different experiments. They appear to fit the linear equation best in dilute solutions and the square-root relation better in stronger solutions. The values of S are however not very suitable for testing the equations. In dilute solutions the substrate concentration is falling off rapidly with time, and in the stronger solutions toxic effects enter.

The influence of glucose on glyoxalase action.

If methylglyoxal is an intermediary in glycolysis, when methylglyoxal and glucose are both presented to a tissue competition between the two substances should occur. More strictly, the competition would be between added methylglyoxal and that produced from glucose. Addition of glucose should not increase the rate of production of lactic acid by a tissue saturated with added methylglyoxal. On the other hand, if glycolysis and glyoxalase action are quite independent, the rates of these processes may proceed independently and additively.

Table IV. *Anaerobic production of lactic acid from glucose and methylglyoxal by tissue-slices.*

Conditions as in Table I. The values relate to a period about 6–20 minutes after methylglyoxal is added. Where glucose is added, its concentration is 0.2 %, and it is present in the Ringer's solution before raising the temperature to 37°.

Tissue	No. of experiments	Substrate absent	Mean values of $Q_M^{N_2}$		Methylglyoxal + glucose
			Glucose	Methylglyoxal	
Rat spleen	2	2.0	9.5	43	46
Rat testis	4	0.5	8	41.5	40
Rat brain*	4	1.5	16	42.5	58
Guinea-pig testis	2	4.5	11	44	48
Guinea-pig kidney	2	—	8	99.5	98
Jensen rat sarcoma*	2	2.5	27	34	43.5

* The measurements of $Q_M^{N_2}$ in the absence of substrate and in presence of glucose were made in these cases before methylglyoxal was added to obtain the other values for $Q_M^{N_2}$. The effect is to exaggerate relatively the former figures.

Experimentally the difficulty is to saturate tissue-slices with methylglyoxal, and in the experiments given in Table IV we do not know how far this saturation is attained. Competition, if it is taking place at all, may only take place in a small portion of the tissue-slice, the central portions being supplied only with glucose.

The data suggest that some competition is taking place, except in the case of rat brain, and they therefore appear to support to some extent the view that methylglyoxal is an intermediary in glycolysis. Other interpretations are not excluded. We consider that further experiments on this subject should be restricted to tissues, such as leucocytes, which can be adequately supplied with methylglyoxal.

The effect of oxygen on glyoxalase activity.

The glyoxalase activity of human erythrocytes is less in oxygen than anaerobically [Jowett and Quastel, 1933]. With regard to tissue-slices, Meyerhof [1925] has stated that oxygen has no effect on the glyoxalase activity of brain and attributes the lower apparent activity of diaphragm and liver in oxygen to depression of glycolysis.

Respiration and glycolysis are complicating factors in experiments with tissue-slices. By omitting glucose from our Ringer's solution we consider we have avoided appreciable glycolysis in our experiments (except possibly with liver).

As regards respiration, we have adopted, in a first series of experiments, the assumption that respiration proceeds unchanged in presence of methylglyoxal and have corrected the observed manometric changes in oxygen on this basis, employing either measurements of our own for the manometric correction due to respiration, or data such as those of Dickens and Greville [1933].

We have found that the oxygen uptake of slices of guinea-pig liver and kidney is not affected immediately by 0.01 *M* methylglyoxal; appreciable inhibition develops in 30–60 minutes. We have therefore some evidence that Q_{O_2} is unaffected by methylglyoxal. That the R.Q. is unaffected has been assumed without direct experimental evidence.

The observed percentage effects of oxygen on the rate of glyoxalase action of tissue-slices are shown in Table V, the effects before and after correction for

Table V. *Effect of oxygen on observed glyoxalase activity of tissue-slices.*

Conditions as in Table I, except that activity is calculated per unit weight of tissue. Measurements relate to a period 6–20 minutes after addition of methylglyoxal. Rates in $O_2 + 5\% CO_2$ compared with those in $N_2 + 5\% CO_2$.

Tissue	No. of experiments	Mean percentage effect of oxygen	
		Uncorrected	Corrected
Guinea-pig liver	5	-18	-9
„ kidney	4	-16	-8
Rat testis	4	+4	+9
„ brain	3	+9	+11
„ spleen	3	-24	-14
„ kidney	1	-25	-14
Jensen rat sarcoma	2	-11	0

respiration being given. Evidently the initial effect of oxygen on glyoxalase activity is not large. As time passes, the apparent inhibitory effect of oxygen increases. This is shown in Figs. 6 and 7, in which are given the rates in oxygen (uncorrected for respiration) relative to those in nitrogen as a function of time.

The initial ratio is arbitrarily taken as 100 in each case. In every case the ratio falls off as time passes¹. If an inhibitory effect of oxygen is not present at first (Table V), it appears to develop as time passes. It is interesting to note that

Fig. 5. Glyoxalase activity of guinea-pig kidney per unit surface.

Fig. 6. Ratio of aerobic to anaerobic glyoxalase activity for various tissues.

Fig. 7. Rat testis. Ratio of aerobic glyoxalase activity to anaerobic glyoxalase activity.

rat testis shows anomalous behaviour (Fig. 7), the ratio passing through a minimum and then rising again.

In a second series of experiments we have measured aerobic glyoxalase action by the method of Negelein [1925], in which the decrease of bicarbonate concen-

¹ Data for Jensen rat sarcoma are not available.

tration during the experimental period is a measure of acid production. In this series the activity per unit surface of the tissue-slices has been measured. The data obtained are given in Table VI. In these experiments the p_H and bicarbonate concentration change considerably, and hence in each experiment the sizes of slices have been chosen so that the total glyoxalase action in the nitrogen-containing vessel is nearly equal to that in the oxygen-containing vessel.

Table VI. *Anaerobic and aerobic glyoxalase action of tissue-slices.*

$M/100$ methylglyoxal. N_2 or O_2 containing 1.5 % CO_2 .
3.2 ml. Ringer's solution containing 0.0073 M $NaHCO_3$.

Tissue	Experimental period mins.	$S_M^{N_2}$	$S_M^{O_2}$	Percentage effect of oxygen
Guinea-pig kidney	49	168	132	-21
„ liver	50	84	72	-14
„ „	34	80	66	-17
„ „	60	76	50	-34
„ „	50	94	69	-26
„ „	46	91	70	-23
„ „	66	80	62	-23
Cat brain*	37	—	—	-20
„ *	30	—	—	-17

* In the case of cat brain the activity per unit weight was measured.

The experiments show a quite definite inhibitory effect of oxygen on the acid production. Since lactate is being produced by glyoxalase action, in experiments of any length some lactate will be removed by oxidation. We consider, however, that the effect of lactate oxidation on our results will be small.

The real action of oxygen on glyoxalase activity of cells will, according to equation (1), be nearly twice the effect observed on the activity of tissue-slices¹. Accordingly, the effect of oxygen on the activity of cells may be greater than on that of erythrocytes, for which it amounts to about 20 %.

In considering the effect of oxygen on glyoxalase activity in relation to its possible bearing on glycolysis, it must be remembered that the effect of oxygen may be a function of the methylglyoxal concentration. As methylglyoxal combines with glutathione, the coenzyme of glyoxalase [cf. Jowett and Quastel, 1933], a high methylglyoxal concentration may partially protect glutathione from oxidation, and the effect of oxygen on glyoxalase activity may therefore be less at high methylglyoxal concentrations than at low ones. This expectation appears to be realised in the case of human erythrocytes (Table VII).

Table VII. *Glyoxalase activity of human erythrocytes.*

5 % CO_2 . $NaHCO_3$ 0.025 M . 0.2 % glucose present. Cells well washed with saline.

Exp. No.	Methylglyoxal 0.02 M			Methylglyoxal 0.005 M		
	$Q_M^{N_2}$	$Q_M^{O_2}$	$Q_M^{O_2}/Q_M^{N_2}$	$Q_M^{N_2}$	$Q_M^{O_2}$	$Q_M^{O_2}/Q_M^{N_2}$
1	62.5	55.5	0.88	67.5	56	0.83
2	63.5	55	0.86	75	58.5	0.78
3	46	44.5	0.97	59.5	46.5	0.78
		Mean	0.90			0.80

If the greater inhibitory effect of oxygen on glyoxalase activity is a general phenomenon at low methylglyoxal concentrations, the supposition that methyl-

¹ Because A , or the maximum $Q_M^{N_2}$, varies as S^2 .

glyoxal is an intermediary in glycolysis goes some way to explain the Pasteur phenomenon. Methylglyoxal as an intermediary will be present in tissues only in low concentrations, or glycolysis would proceed much faster than it does.

It may be remarked that the Pasteur phenomenon, *i.e.* the diminution of glycolysis in oxygen, is definitely in need of explanation. The view advanced by Meyerhof and Warburg, that in oxygen tissues can resynthesise lactic acid to carbohydrate, has so far been substantiated only for one mammalian tissue, liver [Cori and Cori, 1929]. Yet liver is the one tissue in which it is doubtful, from the data of Dickens and Greville [1933], whether glycolysis is affected by oxygen.

Lipmann [1933] has put forward the interesting suggestion that the glycolytic ferment may be reversibly oxidised, the oxidised form being inactive¹. The suggestion rests principally on experiments with dyestuffs which are considered to act solely as oxygen carriers. It is to be remembered, however, in considering Lipmann's experiments that a number of dyestuffs are toxic to ferments [*cf.* Yabusoe, 1925; Quastel, 1932], and it is possible that this toxicity is lost on reduction of the dyestuffs to their leuco-forms.

Glyoxalase activity in relation to glycolysis.

Experiments on muscle extracts have led Embden *et al.* [1933] to propose, and Meyerhof and Kiessling [1933] to support, a mechanism for glycolysis in which methylglyoxal has no place. Their work cannot however permit us to ignore the evidence that methylglyoxal is, in some tissues at least, a probable precursor of lactic acid. Many facts support or are in accordance with such a view.

(1) Tissues treated so as to inhibit glyoxalase activity produce methylglyoxal from hexosediphosphate [Neuberg and Simon, 1933] and from glyceraldehyde-phosphoric acid [Barrenscheen and Beneschovsky, 1933].

(2) All tissues investigated, with the exception of pancreas, can transform methylglyoxal into lactic acid.

(3) The kinetics of glyoxalase action satisfy the conditions required for participation in glycolysis.

(a) The rate of transformation of methylglyoxal into lactic acid by tissues is high and should proceed as rapidly as glycolysis at quite low methylglyoxal concentrations. The best evidence for this is provided by the present work. It had previously been in doubt whether the glyoxalase activity of tumour tissue was sufficiently high.

(b) The activity of glyoxalase extends over a range of p_H similar to or greater than that of the glycolytic enzyme-system [Kuhn and Heckscher, 1926; Lohmann, 1932].

(c) Glyoxalase action is only inhibited by substances which also affect glycolysis to an equal or greater extent. The actions of fluoride [Lohmann, 1933], iodoacetic acid [Dudley, 1931; Lohmann, 1933], and narcotics [Meyerhof, 1925], may be mentioned in this connection. Pancreatic extracts affect both enzyme systems [Harrison and Mellanby, 1930; Giršavičius, 1933] but quantitative comparisons have not been made.

(d) It also appears, from the present work, that the effect of oxygen on glycolysis is paralleled to some extent by its effect on glyoxalase action.

It must be admitted that the methylglyoxal theory meets with certain difficulties. Lohmann has shown [1932] that muscle extracts possessing no

¹ This suggestion is formally similar to the view already expressed by the authors [1933] that a partial explanation of the Pasteur phenomenon may be found in the reversible oxidation of glutathione—the co-enzyme of glyoxalase.

glyoxalase activity can break down glycogen to lactic acid. This proves that at least one tissue—or rather one tissue extract—has a mechanism of glycolysis not involving methylglyoxal. Many facts however favour the view that different tissues may have glycolytic mechanisms differing in detail, and even that one tissue may have several mechanisms for glycolysis (*cf.* Ashford, 1933). It can be suggested that several paths from carbohydrate to lactic acid may exist in tissues, and that methylglyoxal is not an intermediary in all of them.

The lactic acid produced by glyoxalase action is also apparently not stereochemically identical with that produced by glycolysis. It is not however certain that glycolysis produces only the *d*-form. It is, alternatively, conceivable that the glyoxalase action of undamaged cells at low methylglyoxal concentrations may produce only the *d*-form, for the variability in the rotations observed suggests that toxic effects may be playing a part.

SUMMARY.

1. An investigation of the glyoxalase activity of tissue-slices shows that diffusion of methylglyoxal into the slices limits the rate of reaction. Glyoxalase action is proportional to the surface areas of tissue-slices, not to their weights. The glyoxalase activity of tissues is greater than has been hitherto supposed.
2. It is possible that methylglyoxal and glucose compete as substrates.
3. Oxygen partially inhibits glyoxalase activity in most tissues.
4. Glyoxalase activity is discussed in relation to glycolysis.

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