CCXIII. TRIOSE DEHYDROGENASE. I.

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THE existence of the trioses as intermediary compounds in mammalian carbohydrate metabolism has often been postulated. Since *dl*-glyceraldehyde was first synthesised by Wohl [1898] a number of investigations have been made on the changes which it may undergo in the animal body. References to these are given by Stöhr [1932]. It appears that the compound is capable of complete oxidation in the animal body, and that the liver has the power of converting it into lactic acid, glycogen or hexose. The production of *d*-sorbose from *dl*-glyceraldehyde [Embden, Schmitz and Wittenberg, 1913] is readily explicable on stereochemical grounds. This observation is of importance as it appears to indicate that glyceraldehyde may first be converted into dihydroxyacetone under the conditions prevailing in the liver cells.

An even more extensive series of investigations has been carried out on the behaviour of dihydroxyacetone in mammalian metabolism. The liver can convert dihydroxyacetone into glucose or glycogen. This triose, in contradistinction to glyceraldehyde, has the power of relieving insulin convulsions [Reeves and Hewitt, 1926]. It is not however antiketogenic.

Most of the work hitherto carried out on the trioses has demonstrated only their capacity to play a part in the synthesis of hexoses or glycogen, or to undergo internal oxidation-reduction to lactic acid. The possibility that the trioses might arise during carbohydrate catabolism and undergo direct oxidation has not been seriously entertained. The fact that dihydroxyacetone is autoxidisable in aqueous solution has long been known, and Wind [1925] has studied the influence of heavy metal ions on the autoxidation of both trioses in alkaline phosphate buffer. Euler, Eriksson and Brunius [1928] showed that dihydroxyacetone solutions were capable of reducing methylene blue in the presence of phosphate buffer. As far as we are aware the only observation hitherto made on the catalysis of triose dehydrogenations by an enzyme of mammalian origin is that of Lebedev [1926] who showed that the purified Schardinger enzyme preparation from milk catalysed this reaction.

We have studied the catalytic action of a liver extract prepared by the method of Harrison [1931] on the trioses, on acetaldehyde and on hypoxanthine in the presence of methylene blue.

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EXPERIMENTAL.

The reduction velocity as a function of substrate concentration.

In order to establish the identity or non-identity of the enzymes responsible for the dehydrogenations studied by the "competition" method, it was necessary to determine the optimum concentration of each substrate. In the following experiments an enzyme preparation which had been dialysed at 0° nearly free from ammonium sulphate was used. In general 4 to 6 2-litre changes of distilled water were used in the course of 48 to 72 hours. At the end of this time the last dialysate gave only an opalescence with barium chloride solution. 10 g. of the enzyme powder yielded about 40 cc. of solution. Since the enzyme solution was deeply pigmented, the time for apparently complete reduction of the methylene blue was observed. The velocities recorded in the following curves and tables are the reciprocals of the reduction times in minutes multiplied by 100. In every case the contents of the Thunberg tubes consisted of 1 cc. of the enzyme solution and 0.5 cc. of a solution of 1 part of methylene blue in 5000 parts of phosphate buffer. The phosphate buffer was Clark and Lubs p_H 7.2 buffer made up to be 10 times as concentrated as that given by Clark *viz.* 0.5 *M* with respect to phosphate. When diluted with 1 volume of water and 2 volumes of enzyme solution the resulting mixture had a p_H of 7.1 determined electrometrically. The total volume of the contents of the Thunberg tube was made up to 2.0 cc. with the substrate solution and water where necessary. The reduction times were in every case measured at 37°. The reduction time for the enzyme solution alone under these conditions is of the order of 5 hours.

dl-Glyceraldehyde. Crystalline glyceraldehyde prepared as indicated in a previous paper [Clift and Cook, 1932] was used in these experiments. Owing to the sparing solubility of the dimeric form in water, the strong solutions

Fig. 1. Initial reaction velocity as a function of glyceraldehyde concentration. Ordinates: relative velocity. Abscissae: glyceraldehyde concentration in millimols. per litre.

necessary were made by dissolving the glyceraldehyde in the minimum amount of water and allowing it to stand at room temperature for several days in the presence of toluene (to prevent infection) until the water could be removed *in vacuo* over sulphuric acid without the glyceraldehyde crystallising out. Although

glyceraldehyde reduces methylene blue in the presence of phosphate buffer, and in the absence of enzyme, control experiments showed that the velocity under these conditions was negligible compared with that of the enzymic reduction. The Michaelis curve for *dl*-glyceraldehyde is shown in Fig. 1. Within the limits of experimental error this curve is represented by the well-known equation

$$v = \frac{Vx}{K_m + x},$$

where v = initial velocity of the reaction,

x = concentration of substrate,

V = velocity of reaction when x is large,

K = Michaelis constant of the system.

Dihydroxyacetone. Freshly prepared solutions of monomeric dihydroxyacetone (prepared as described by Clift and Cook [1932]) were used in every case. Under the conditions described above the velocity of methylene blue reduction by dihydroxyacetone in the absence of the enzyme is not negligible compared to that of the enzymic reaction. Fig. 2 shows the Michaelis curve for methylene blue reduction in the presence of the enzyme. The lower curve shows the relationship between the non-enzymic reduction velocity and the concentration of dihydroxyacetone. Even when allowance is made for the non-enzymic reduction velocity, the curve does not fit the Michaelis equation, the velocity in high substrate concentrations being higher than this equation indicates.

Fig. 2.

Fig. 3.

Fig. 2. Initial reaction velocity as a function of dihydroxyacetone concentration. Ordinates: relative velocity. Abscissae: dihydroxyacetone concentration in millimols. per litre. Upper curve: in the presence of the enzyme. Lower curve: in the absence of the enzyme.

Fig. 3. Initial reaction velocity as a function of acetaldehyde concentration. Ordinates: relative velocity. Abscissae: acetaldehyde concentration in millimols. per litre.

Acetaldehyde. The relationship of the reduction velocity to the concentration of acetaldehyde is illustrated in Fig. 3. This curve shows that the aldehyde dehydrogenase is inhibited by high substrate concentrations. Acetaldehyde, added in high concentrations to the enzyme preparation causes the appearance

of a precipitate. Since a certain amount of acetaldehyde boils off when the Thunberg tubes are evacuated, no quantitative conclusions can be drawn from the shape of the curve in high concentrations of acetaldehyde. It will be observed that the aldehyde dehydrogenase present in the liver extract has a very different velocity-substrate concentration curve from the Schardinger enzyme studied by Dixon. The optimum aldehyde concentration is 0.1 *M* and the concentration giving half the maximum reduction velocity is 0.013 *M*.

Hypoxanthine. The reduction velocities with various concentrations of hypoxanthine are shown in Table I.

Concentration (millimols. per litre) of hypoxanthine	Reduction velocity
1.7	8.3
1.4	8.9
1.0	11.0
0.68	10.9
0.34	10.9
0.17	11.1

The identity of the dehydrogenases.

An attempt to ascertain whether the same or different enzymes are responsible for the dehydrogenations of glucose, the trioses, acetaldehyde and hypoxanthine has been made by allowing the preparation to act on these substrates taken in pairs each at the optimum or nearly optimum concentrations. According to the generally accepted theory of enzyme action, if the same enzyme is responsible for the oxidation of two substrates the reduction velocity for a mixture of the two substrates in optimum concentration should lie between the reduction velocities for the two substrates separately. If different enzymes are responsible, then the velocity for a mixture should be the sum of the velocities for the two components separately. In the results of such experiments shown in Table II, the above conditions were used. The concentrations of the various substrates were: glucose 0.25 *M* [Harrison, 1931], dihydroxyacetone 0.2 *M*, glyceraldehyde 0.15 *M*, acetaldehyde 0.1 *M*, hypoxanthine 0.0007 *M*. Since the velocity-substrate concentration curves for the trioses show that the reaction velocity continually increases with increasing concentration of the substrate, it is to be anticipated that if the same enzyme is responsible for both dehydrogenations the velocity for the dehydrogenation of the mixture of the ketose and the aldose will be somewhat greater than the mean of the velocities for each separately. Table II shows this to be the case. Hence it may be tentatively concluded that the same enzyme is responsible for catalysing both reactions.

The reduction velocity for a mixture of glucose with either triose is slightly less than the sum of the velocities for the components separately. This discrepancy is probably not due to experimental error since a number of experiments gave figures similar to those quoted. Since, however, the velocity for the mixture is considerably greater than the velocity for either glucose alone

Table II.

Substrate	Reduction time mins.	Observed velocity	Sum of observed velocities
Glucose	11.9	8.4	
Glyceraldehyde	8.1	12.4	
Glucose + glyceraldehyde	5.9	17.0	20.8
Glucose	14.3	7.0	
Dihydroxyacetone	7.6	13.2	
Glucose + dihydroxyacetone	5.5	18.2	20.2
Glucose	9.8	10.2	
Acetaldehyde	3.2	31.2	
Glucose + acetaldehyde	3.2	31.2	41.4
Glucose	11.1	9.0	
Hypoxanthine	5.6	17.9	
Glucose + hypoxanthine	3.9	25.6	26.9
Glyceraldehyde	7.4	13.5	
Dihydroxyacetone	6.3	15.9	
Glyceraldehyde + dihydroxyacetone	4.9	20.4	29.4
Glyceraldehyde	7.4	13.5	
Acetaldehyde	3.0	33.3	
Glyceraldehyde + acetaldehyde	3.1	32.2	46.8
Glyceraldehyde	7.4	13.5	
Hypoxanthine	3.8	26.4	
Glyceraldehyde + hypoxanthine	2.6	38.5	39.9
Dihydroxyacetone	7.7	13.0	
Acetaldehyde	4.0	25.0	
Dihydroxyacetone + acetaldehyde	4.5	22.2	38.0
Dihydroxyacetone	6.2	16.1	
Hypoxanthine	3.8	26.3	
Dihydroxyacetone + hypoxanthine	2.3	43.5	42.4
Acetaldehyde	3.6	27.8	
Hypoxanthine	5.6	17.9	
Acetaldehyde + hypoxanthine	4.4	22.7	45.7

or a triose alone, it seems probable that different enzymes are responsible for the two catalyses. This conclusion is supported by the fact that the activity of the extract on glucose decreases by about 50 % in the course of 8 hours at room temperature, while no difference can be detected in the activity of the extract on the trioses under these conditions.

Methylene blue reductions carried out with mixtures of glucose or a triose with acetaldehyde show that the velocity with the mixture is the same as the velocity with acetaldehyde alone. This seems to indicate that acetaldehyde inhibits the glucose and the triose dehydrogenase. If this interpretation is not put upon the phenomenon, the alternative assumption that the enzymes responsible are identical, leads to contradictory results. Thus with the latter assumption one would argue that the aldehyde dehydrogenase and the dihydroxyacetone dehydrogenase are identical. Since hypoxanthine and acetaldehyde appear to compete for the same enzyme, the hypoxanthine dehydrogenase would be identical with the dihydroxyacetone dehydrogenase. But the competition experiment shows that this is not the case.

Corroborative evidence as to the non-identity of the aldehyde and triose dehydrogenases was obtained by a study of the oxygen uptakes in the Barcroft

differential manometer. Hypoxanthine and acetaldehyde show an oxygen uptake in the presence of the enzyme. The action of the enzyme preparation on the trioses, however, is merely to effect a partial inhibition of the autoxidation which these substances show in phosphate buffer. Harrison showed that the glucose dehydrogenase is anaerobic. It seems possible but unlikely that the same enzyme should be capable of activating certain hydrogen donors with respect to oxygen as the acceptor, while it is unable to do so with others. Thus the competition experiments indicate that the extract contains three distinct dehydrogenases—one acting on glucose, one on the trioses, the third on hypoxanthine and acetaldehyde.

DISCUSSION.

The work of Neuberg and his school has shown that methylglyoxal is a probable intermediary between polysaccharides and lactic acid in mammalian metabolism. A probable main course of the oxidative degradation of carbohydrate in muscle is given by Toenniessen and Brinkmann [1930]. Very little is known at present of the mechanism of carbohydrate oxidation in other tissues. We regard the observation that an enzyme capable of activating the trioses as hydrogen donors exists in the liver as of importance in so far as it indicates that these substances may be involved in the oxidative breakdown of carbohydrate in that organ. This possibility is of course open to the objection that the appearance of these very reactive substances in liver metabolism has never been observed.

Our observation raises the interesting question as to what are the oxidation products of the two trioses when dehydrogenated by the enzyme. The similarity in the behaviour of the enzyme with respect to the two substrates indicates that the product may be the same in both cases. The enzyme possibly acts on one triose only or on a common enolic form. The fact that the relationship between the initial reaction velocity and the dihydroxyacetone concentration approximates to linearity in high substrate concentrations indicates that under these conditions the factor limiting the velocity is a non-enzymic reaction, thus lending support to this hypothesis. Lebedev [1926] showed that the oxidation product of glyceraldehyde by the Schardinger enzyme preparation was probably glyceric acid. Our observations agree with his in so far as the enzyme acts more rapidly on the aldose than on the ketose. Lebedev showed that his enzyme was incapable of bringing about a Cannizzaro transformation of glyceraldehyde into glycerol and glyceric acid. Work is now in progress with a view to deciding whether the liver enzyme is capable of bringing about this change and to identifying the products of the oxidation of the trioses by methylene blue. Preliminary experiments have shown that the oxidation is in both cases attended with the production of more acid than can be accounted for by the HCl liberated by the methylene blue which is reduced.

SUMMARY.

1. An extract made from ox-liver contains an enzyme capable of activating glyceraldehyde and dihydroxyacetone as hydrogen donators to methylene blue.

2. The question of the specificity of the enzymes present in the liver extract is discussed. It is concluded that in the liver extract studied there are three distinct dehydrogenases, one acting on glucose, another on the two trioses and the third on hypoxanthine and acetaldehyde.

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REFERENCES.

- Clift and Cook (1932). *Biochem. J.* **26**, 1788.
Embsden, Schmitz and Wittenberg (1913). *Z. physiol. Chem.* **88**, 210.
Euler, Eriksson and Brunius (1928). *Svensk. Kem. Tidskr.* **40**, 163.
Harrison (1931). *Biochem. J.* **25**, 1016.
Lebedev (1926). *Z. physiol. Chem.* **160**, 97.
Reeves and Hewitt (1926). *J. Physiol.* **61**; *Proc.* xxxv.
Stöhr (1932). *Z. physiol. Chem.* **206**, 211.
Toenniessen and Brinkmann (1930). *Z. physiol. Chem.* **187**, 137.
Wind (1925). *Biochem. Z.* **159**, 58.
Wohl (1898). *Ber. deutsch. chem. Ges.* **31**, 1796, 2394.