95. THE INHIBITION OF GLUCOLYSIS BY GLYCERALDEHYDE

BY LEONARD HUBERT STICKLAND

From the Department of Experimental Pathology and Cancer Research, University of Leeds

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THERE are three reasons why it seemed desirable to make a further study of the inhibition of glycolysis by glyceraldehyde. Firstly, from many tissues (e.g. brain, tumours, retina) whose glycolysis is completely inhibited by low concentrations of glyceraldehyde (0·001–0·005 *M*), extracts can be prepared in which glycolysis proceeds by the same series of reactions as in muscle extract. Now this series of reactions, studied in muscle extract, is only partially inhibited by even the highest concentrations of glyceraldehyde, up to 0·1 *M* [Holmes, 1934; Lehmann & Needham, 1938]. If this feebly inhibited system occurs in brain extract, why is the glycolysis of intact brain tissue readily inhibitable? Meyerhof *et al.* [1936] provided a possible explanation of this phenomenon when they showed that muscle extract contains an enzyme (aldolase) which brings about the condensation of various aldehydes with triosephosphate to form non-fermentable ketose-1-monophosphates; glyceraldehyde might in this way be rapidly removed from the system, so that no inhibition of glycolysis would be observed. Since then it seems to have been assumed that muscle extract would be inhibited by 0·001 *M* glyceraldehyde, if it were possible to maintain such a concentration in the system. However, Süllmann [1938, 1] raised strong doubts of the validity of this explanation. He confirmed that when muscle extract acts on hexosediphosphate in the presence of glyceraldehyde, the latter disappears and easily hydrolysable phosphoric esters (hexose-1-phosphates) appear; but when the substrate was glycogen no such changes could be detected. The difference presumably lay in the different concentrations of triosephosphate present in the two cases, the condensation taking place only when this concentration was high. Later in this paper two further reasons will be given for rejecting the aldolase condensation as an explanation of the absence of inhibition of glycolysis from muscle extract, and consequently a new answer to the above question must be found.

Secondly, Lehmann & Needham [1938] have shown that there is a difference between the action of freshly dissolved glyceraldehyde, which is in a dimeric form, and that of the monomeric form into which it is completely converted in the course of 3 days. The former inhibits glycolysis of glycogen in muscle extract in the same way as glucose does, the inhibition occurring at the stage

$$\text{glycogen} \rightleftharpoons \text{Cori ester},$$

while the latter has no such effect. This means that much of the work done with glyceraldehyde before 1938 must be reconsidered, as it is not known whether fresh or old solutions were used, though of course in some individual experiments it is possible to deduce this information from the results.

Thirdly, Orr & Stickland (unpublished results) have found that glycolysis in liver slices is unaffected by monomeric glyceraldehyde at concentrations up to

0·03 *M*. This glycolysis involves only the glycogen present in the liver cells, and not free glucose present in the medium [Rosenthal & Lasnitzki, 1928; Dickens & Greville, 1932; Orr & Stickland, 1941], while in those tissues in which glyceraldehyde does inhibit, the glycolysis is in every case a pure glucolysis.

These facts together suggested that it might be profitable to study the effect of glyceraldehyde on muscle extracts acting on glucose in the presence of hexokinase. This has been done before [Holmes, 1934; Needham & Lehmann, 1937, 1; Süllmann, 1938, 1, 2], but the results were contradictory; they agreed however in showing that glyceraldehyde has the same action on glycolysis in muscle extract whether the substrate is glycogen or glucose with hexokinase.

Experimental

Methods

Measurement of glycolysis. With tissue slices the usual manometric technique was used (see Berenblum *et al.* [1936] for details). The lactic acid was also determined chemically at the end of most experiments [Friedemann *et al.* 1927], and good agreement was usually obtained between chemical and manometric figures. With muscle extracts the chemical determination was relied on to give the total glycolysis, but the manometric readings were frequently used for following the time course of the lactic acid formation. The assumption that liberation of CO_2 is proportional to lactic acid formation throughout the course of an experiment ignores other minor causes of change in acidity, but cannot be a source of serious error.

Preparation of muscle extracts. Aqueous extracts of cooled rabbit muscle [Meyerhof, 1926], subjected to dialysis through collodion for 24 hr., were used in most experiments. Extracts made with 1 % Na_2HPO_4 from thoroughly washed rabbit muscle [Kendal & Stickland, 1937] were also used, and the same results were obtained with the two kinds of preparation.

Hexokinase. Hexokinase was prepared from baker's yeast by the method of Meyerhof [1927]. A single preparation was used for all the experiments, as it showed no noticeable change in activity when stored for 6 months at 0° with a little toluene. 1 ml. of this solution corresponded roughly to 1 g. of pressed yeast, and 1 ml. contained 18 mg. dry weight of organic matter.

Glucose (Analar), fructose (B.D.H. 'dextrose-free') and *dl*-glyceraldehyde (Schering-Kahlbaum) were used as obtained. For use with tissue slices an isotonic solution of glyceraldehyde was prepared by dissolving it in suitably diluted Ringer's solution, and the necessary dilutions were of course made with Ringer. For the muscle extract experiments a solution in distilled water was used. The solutions were in every case allowed to stand for at least 3 days before use, to ensure that the glyceraldehyde was entirely in the monomeric form. As it is not yet settled which is the active optical isomeride, or whether in some cases both may be active, concentrations are given in this paper in terms of the *dl*-compound. Needham & Lehmann [1937, 2] and Mendel *et al.* [1938], using respectively chick embryo brei and tumour slices, agree that only the *l* form is active, but Süllmann [1938, 1], using lens extracts, found the *d*-component to be equally active.

With tissue slices at low concentrations of glyceraldehyde the inhibition of glycolysis is usually small initially, and first becomes complete after some 15 min. With muscle extracts the glycolysis only in exceptional cases follows a linear course, and sometimes ceases after some 40 min. These two facts make the

expression of the degree of inhibition difficult, and in the present work the arbitrary course has been chosen of expressing the inhibition as the average value during the first hour of the experiment.

Results

The effect of glyceraldehyde on glycolysis in liver slices. The starting point of the experiments to be described here was the observation of Orr & Stickland that glyceraldehyde does not inhibit glycolysis in liver tissue. As these results have not hitherto been published they are given in Table 1.

Table 1. *Effect of glyceraldehyde on tissue glycolysis*

In the experiments with liver slices about 40 mg. tissue were used in each vessel, with brain slices about 20 mg. and with the Daels-Biltris tumour and the oesophageal mucosa about 15 mg.

The results are expressed as $Q^{N_2}_{CO_2}$ in the manometric experiments, as $Q^{N_2}_{LA}$ in the chemical.

The figures for the degree of inhibition refer in the case of the liver results to the chemically determined rate of glycolysis; with the other tissues only manometric results were obtained.

	Tissue		Concentration of glyceraldehyde (*M*)					
			0	0·0003	0·001	0·003	0·01	0·03
1	Liver (rat)	Manometric	12·7	—	10·8	15·3	—	19·9
		Chemical	10·8	—	10·1	12·0	—	13·1
		Inhibition %	—	—	6	−11	—	−21
2	Liver (rat)	Manometric	13·2	—	—	—	17·3	18·2
		Chemical	10·4	—	—	—	13·7	13·3
		Inhibition %	—	—	—	—	−32	−28
3	Liver (rat)	Manometric	8·8	—	—	9·1	—	16·2
		Chemical	7·3	—	—	8·5	—	9·5
		Inhibition %	—	—	—	−16	—	−30
4	Liver (rat)	Manometric	9·1	8·9	—	—	—	17·1
		Chemical	8·3	8·2	—	—	—	12·5
		Inhibition %	—	1	—	—	—	−50
5	Liver (rat)	Manometric	4·7	4·8	4·4	8·9	10·0	11·0
		Chemical	3·6	3·3	3·3	5·4	7·2	6·0
		Inhibition %	—	8	8	−56	−100	−67
6	Liver (rat)	Manometric	4·0	—	—	7·9	14·8	—
		Chemical	4·2	—	—	7·0	9·5	—
		Inhibition %	—	—	—	−67	−126	—
7	Brain (guinea-pig)	Manometric	15·8	5·0	2·5	2·5	2·0	—
		Inhibition %	—	68	84	84	87	—
8	Brain (guinea-pig)	Manometric	14·2	—	3·3	—	—	—
		Inhibition %	—	—	77	—	—	—
9	Daels-Biltris sarcoma (guinea-pig)	Manometric	25·4	25·3	13·4	4·7	2·0	3·0
		Inhibition %	—	0	47	81	92	88
10	Daels-Biltris sarcoma (guinea-pig)	Manometric	29·0	30·4	15·4	5·6	—	—
		Inhibition %	—	−5	47	81	—	—
11	Oesophagal mucosa (rabbit)	Manometric	17·9	—	12·5	8·9	3·3	—
		Inhibition %	—	—	30	50	82	—
12	Oesophagal mucosa (rabbit)	Manometric	15·3	—	—	7·5	3·3	—
		Inhibition %	—	—	—	51	79	—

The large increase in the manometric values of $Q^{N_2}_{CO_2}$ at high glyceraldehyde concentrations has been observed by Baker [1938]. The chemical results show, however, that this increase is due only to a small extent to greater lactic acid formation, and it must be attributed to the action of aldehyde mutase, which is present in liver, and has been shown to act readily on *d*-glyceraldehyde [Dixon & Lutwak-Mann, 1937].

The results with liver are open to two interpretations. (1) The glyceraldehyde has no inhibitory action. In this case the small increase in lactic acid formation might be due either to a stimulation of the breakdown of glycogen or to formation of lactic acid from glyceraldehyde itself. The greater proportional increase when the glycogen breakdown is slower (Exps. 5 and 6, Table 1) suggests that the latter may be true. The conversion of glyceraldehyde into lactic acid is known to take place in various tissues, the reaction probably proceeding through methylglyoxal and glyoxalase [Embden & Metz, 1932; Needham & Lehmann, 1937, 2]. (2) The normal glycolysis is completely suppressed, and the whole of the lactic acid found is derived from glyceraldehyde. This cannot be the case, for in one experiment with $M/100$ glyceraldehyde the quantity of lactic acid formed exceeded that of glyceraldehyde originally present, without any allowance for the part of the latter that suffered dismutation; and in several experiments with $M/300$, a concentration which completely inhibits the glycolysis of most tissues, the lactic acid was up to four times the amount of glyceraldehyde originally present, so the glycolysis cannot have been greatly inhibited. It may be concluded that the previous interpretation is much the more likely.

This leads us to the tentative hypothesis that tissues and extracts which attack glucose show a glycolysis which is inhibitable by low concentrations of glyceraldehyde, while those which attack only glycogen (liver and muscle extract) show a glycolysis which is only partially inhibitable even by high concentrations, if at all. It is then obviously worth while to study in greater detail than has previously been attempted the action of glyceraldehyde on muscle extracts acting on glucose in the presence of hexokinase.

Some observations on the kinetics of hexokinase action. Preliminary experiments with muscle extract and hexokinase gave contradictory results (Table 2).

Table 2. *Glyceraldehyde and muscle extracts*

2·5 ml. muscle extract, 4 mg. inorganic P as a mixture of Na phosphates at *p*H 7·5, 0·26% $NaHCO_3$, $M/2000$ adenyl pyrophosphate, 0·4% starch or glucose and 0·2 ml. hexokinase solution in a total volume of 5 ml. Incubated 1 hr. at 38° in $N_2 + 5\%$ CO_2. Results as mg. lactic acid per 5 ml.

		Glyceraldehyde concentration (M)							
		0	0·001	0·003	0·01	0	0·003	0·01	0·03
		Glucose + hexokinase				Starch			
1		13·3	0·4	0·5	0·4	4·8	5·7	5·5	4·8
	Inhibition %	—	97	96	97	—	−18	−15	0
2		13·0	12·5	0·9	0·6	3·0	2·8	2·9	—
	Inhibition %	—	4	93	95	—	7	3	—
3		9·2	9·2	9·7	0·5	4·6	4·7	4·1	—
	Inhibition %	—	0	−5	94	—	−2	12	—
4		14·0	14·0	13·9	—	7·1	7·3	7·3	—
	Inhibition %	—	0	1	—	—	−3	−3	—
5		6·8	6·7	6·9	8·1	4·7	—	4·7	5·1
	Inhibition %	—	1	−1	−19	—	—	0	−8

There is here no inhibition of the attack on starch at a concentration of $0{\cdot}01\,M$ glyceraldehyde, and in the two cases quoted none even at $0{\cdot}03\,M$. On the other hand glucolysis is completely inhibited by $0{\cdot}01\,M$ in three experiments out of four, by $0{\cdot}003\,M$ in two out of five and by $0{\cdot}001\,M$ in one out of five. A possible explanation of these variable results was found during a brief study of the kinetics of hexokinase action, and these experiments must now be described.

If the amount of lactic acid formed in 1 hr. from glucose is determined at varying hexokinase concentrations, then a curve of the type shown in Fig. 1 (curve *G*) is obtained. The relationship is unusual in showing an abrupt appearance of full activity with a small increase in hexokinase concentration; this critical concentration of hexokinase, below which practically no glucolysis takes place, is not a fixed quantity, but varies in different muscle enzyme preparations, the range being from about 0·1 to 0·5 ml. of the standard hexokinase solution per 5 ml. with the enzymes so far studied. With fructose as substrate the critical concentration of hexokinase is lower, lying between 0·02 and 0·08 ml./5 ml. Fig. 1 shows this effect in a case where the critical concentrations were 0·08 ml. for glucose and 0·02 ml. for fructose.

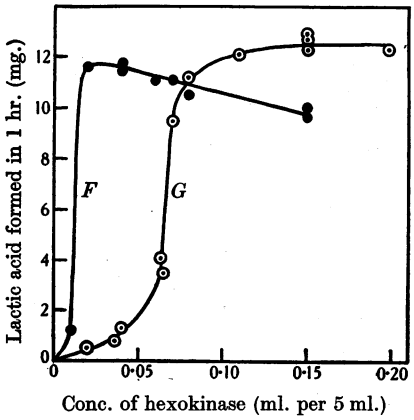

Fig. 1.

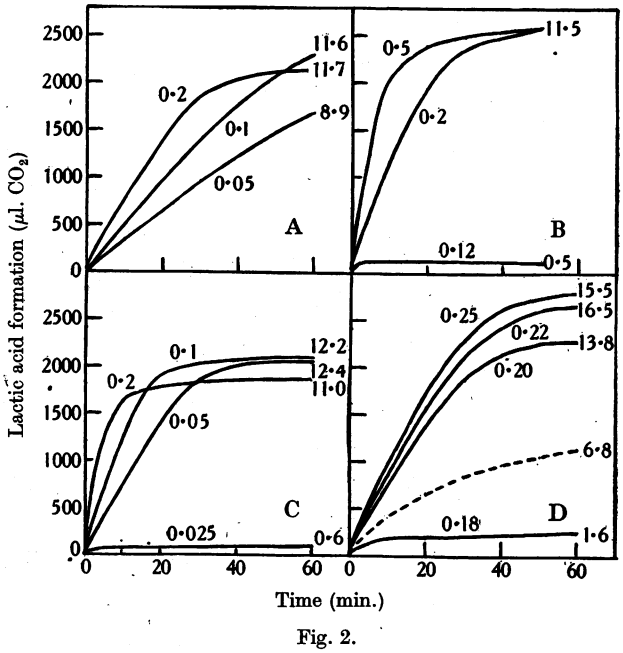

Fig. 2.

Fig. 1. The effect of increasing hexokinase concentration on glucolysis and fructolysis in muscle extract. Curve *G*, substrate glucose; curve *F*, substrate fructose.

Fig. 2. The course of glucolysis and fructolysis in muscle extract at different hexokinase concentrations. The curves were obtained manometrically, and the figure to the right of each curve gives the result of the chemical determination of lactic acid at the end of the experiment (mg./5 ml.). The hexokinase concentration (ml./5 ml.) is shown against each curve. The substrate was glucose in *A*, *B* and *D*, fructose in *C*. Curves *D* include for comparison the course of formation of lactic acid from starch (dotted line).

These results so far only deal with the total amount of lactic acid formed in 1 hr. If the time course of the reaction is followed, it is found that as the concentration of hexokinase is increased above the critical value the initial rate of glucolysis also increases, although the amount in 1 hr. remains roughly constant. Typical sets of curves showing the course of glucolysis and fructolysis at various hexokinase concentrations are given in Fig. 2. In general, it can be seen that at a concentration of hexokinase only just above the critical value the glucolysis starts relatively slowly and proceeds at a steadily diminishing rate; it may still be proceeding after 60 min. (Fig. 2 *A*) or may stop after some 40 min.

(Fig. 2, *B*, *C*). At higher concentrations, on the other hand, the glucolysis is initially much faster and very soon comes to a complete stop; the fastest case shown is in Fig. 2 *C*, where the glycolysis (in this case a fructolysis) has practically come to a standstill in 10 min. The effect of gradually increasing the concentration past the critical value is shown in Fig. 2 *D*. The cessation of the reaction at the same point at all hexokinase concentrations seems not to be due to the complete utilization of the substrate, for the amount of lactic acid formed in different experiment lies between 10 and 15 mg./5 ml., i.e. between 50 and 75 % of the glucose initially present. No explanation of these results can be offered, and they are not being further studied, as from the present point of view they are a side issue.

The importance of these phenomena in connexion with glyceraldehyde inhibition is this. In experiments with tissue slices it is commonly found that the inhibitory effect of glyceraldehyde is not immediately complete, but increases during the first 15–20 min. If the same were true in the case of muscle extract, then at high hexokinase concentrations, where the whole reaction may be finished in about 10 min., no inhibition can be expected. If any inhibition is to be found, the hexokinase concentration must be adjusted to produce a steady lactic acid formation, i.e. the concentration must be only just above the critical value.

Inhibition of glucolysis at controlled hexokinase concentration. Each muscle enzyme solution, as soon as it was prepared, was tested with a series of concentrations of hexokinase, to determine the critical value at which glucolysis first appeared. The effect of glyceraldehyde was then studied at the lowest hexo-

Table 3. *Inhibition of glucolysis in muscle extract by glyceraldehyde*

Details of experiments as in Table 1, except hexokinase concentration which is given below. Results as mg. lactic acid per 5 ml.

	Hexokinase concentration (ml./5 ml.)		Glyceraldehyde concentration (*M*)						
			0	0·001	0·003	0·01	0	0·01	0·03
			Glucose + hexokinase				Starch		
1	0·45		11·9	—	0·9	0·6	—	—	—
		Inhibition %	—	—	93	95	—	—	—
2	0·22		12·7	—	1·6	0·6	6·6	6·8	4·0
		Inhibition %	—	—	87	95	—	− 2	39
3	0·20		12·5	—	1·0	0·4	8·9	9·0	5·6
		Inhibition %	—	—	92	97	—	−1	37
4	0·50		14·1	—	0·9	0·6	—	—	—
		Inhibition %	—	—	94	96	—	—	—
5	0·20		8·7	7·6	2·1	0·7	8·0	8·7	6·8
		Inhibition %	—	13	78	94	—	−9	15
6	0·22		15·9	16·6	1·4	—	6·6	6·8	4·0
		Inhibition %	—	−5	91	—	—	−3	39
7	0·50		13·5	5·1	1·1	—	—	—	—
		Inhibition %	—	62	92	—	—	—	—
8	0·10		6·6	6·2	0·4	—	—	—	—
		Inhibition %	—	6	95	—	—	—	—
9	0·20		7·8	—	1·4	—	6·0	5·8	6·3
		Inhibition %	—	—	81	—	—	3	5
			Fructose + hexokinase				Starch		
10	0·05		13·2	—	0·7	0·5	9·3	8·5	5·8
		Inhibition %	—	—	95	96	—	8	38
11	0·015		8·0	—	1·3	0·7	6·2	6·0	4·6
		Inhibition %	—	—	84	91	—	3	27

kinase concentration which gave maximal, or in some cases slightly submaximal, lactic acid formation in 1 hr. The results of several such experiments are given in Table 3.

From these figures two facts are clear. First, 0·01 *M* glyceraldehyde has no effect on the rate of glycolysis of starch, and 0·03 *M* causes only a small inhibition (from 0 to 40 %). Secondly, 0·003 *M* glyceraldehyde causes almost complete inhibition of both glucolysis and fructolysis (on the average 90 %), while even 0·001 *M* in some cases gives a considerable degree of inhibition (Table 3, Exp. 7; cf. Table 2, Exp. 1). A curious point about these results is that a partial inhibition is rarely observed (in the whole of Tables 2 and 3 only one result lies between about 10 and 80 % inhibition). Usually when the concentration of an inhibitor is increased in threefold steps, the degree of inhibition increases slowly, as can be seen in the cases of the slices of Daels-Biltris sarcoma and of oesophageal mucosa in Table 1.

The removal of the inhibition of glucolysis by excess of hexokinase. The effect of increasing the hexokinase concentration on the degree of inhibition of glucolysis is shown in Table 4.

Table 4. *Reversal of glyceraldehyde inhibition by excess of hexokinase*

Conditions of experiment as in Table 2, except for hexokinase concentration, which is given below. Results as mg. lactic acid formed in 1 hr./5 ml.

	Substrate	Hexokinase conc. (ml./5 ml.)	Glyceraldehyde concentration (*M*) 0	0·003	0·01	Inhibition %
1	Glucose	0·10	10·7	—	1·8	84
		0·125	11·1	—	3·4	69
		0·16	11·0	—	11·5	−5
		0·20	10·8	—	10·9	−1
2	Glucose	0·22	13·1	1·3	—	90
		0·25	15·9	8·0	—	50
3	Fructose	0·02	9·6	—	1·2	87
		0·10	10·7	—	11·2	−5
		0·015	8·0	1·3	—	84
		0·02	9·6	6·3	—	34
4	Fructose	0·05	13·2	0·7	—	95
		0·20	12·3	11·9	—	3

These experiments show that, even within the range of hexokinase concentrations in which glucolysis is maximal (or nearly so), a very small change in that concentration may have a great effect on the degree of inhibition by glyceraldehyde. For instance, in Exp. 1 an increase of 60 % in the amount of hexokinase completely abolished an 84 % inhibition, and in Exp. 2 an increase of only 14 % reduced an inhibition of 90 % to one of 50 %. It has already been indicated that results of this nature are to be expected, and the explanation given of this effect is that at high hexokinase concentrations the glucolysis proceeds so fast that it is all over before the inhibiting action of the glyceraldehyde can come into play. This explanation is now seen to be insufficient. For example, in Exp. 2 (Table 4; see also Fig. 4) the small difference in hexokinase concentration caused very little difference in the course of the glucolysis; in each case the rate began to decrease after 15 min. and the reaction came to an end in 40 min., as measured by the manometric readings. The glyceraldehyde had the same opportunity to act in each, and yet the degree of inhibition was greatly different. It is clear that the hexokinase solution itself reverses the inhibiting effect of the glyceraldehyde. Whether this action is due to the hexokinase

protein or to some impurity in the solution remains undecided. Pyruvic acid at very low concentration is known to behave in this way [Mendel *et al.* 1931; see below], but determination of the total bisulphite-binding power of the preparation of hexokinase used in these experiments showed that pyruvic acid could be present only in amount quite insufficient to account for the above results.

The removal by pyruvic acid of the inhibition by glyceraldehyde of glucolysis in muscle extract. Mendel *et al.* [1931] found, using slices of Jensen rat sarcoma, that 0·001 *M* glyceraldehyde produced an inhibition of 82%. Addition of 0·00005 *M* pyruvate reduced the inhibition to 40%, of 0·0001 *M* to 20%, and of 0·001 *M* to nothing. To determine whether a similar phenomenon can be observed in muscle extract, experiments were carried out in the following way. A muscle extract was tested to find the concentration of hexokinase required to give good inhibition of glucolysis by 0·003 *M* glyceraldehyde, and then at these concentrations of hexokinase and glyceraldehyde the effect of various amounts of pyruvic acid was studied. In Table 5 the results obtained in two such experiments are given.

Table 5. *The reversal of glyceraldehyde inhibition by pyruvic acid*

Conditions of experiment, other than those given in the table, as in Table 2. Results as mg. lactic acid formed in 1 hr./5 ml.

1	Glyceraldehyde conc. (*M*)	0	0·003	0·003	0·003	0·003	0·003	—
	Pyruvate conc. (*M*)	0	0	0·003	0·0015	0·0008	0·0004	—
	Lactic acid formed	13·5	1·1	13·6	13·0	11·0	5·5	—
	Inhibition %	—	92	0	4	18	59	—
	Glyceraldehyde conc. (*M*)	0	0·001	—	—	—	0·001	—
	Pyruvate conc. (*M*)	0	0	—	—	—	0·0004	—
	Lactic acid formed	13·5	5·1	—	—	—	12·0	—
	Inhibition %	—	62	—	—	—	11	—
2	Glyceraldehyde conc. (*M*)	0	0	0·003	0·003	0·003	0·003	0·003
	Pyruvate conc. (*M*)	0	0·003	0	0·003	0·0015	0·0008	0·0004
	Lactic acid formed	15·9	16·4	1·4	16·5	17·1	15·5	15·9
	Inhibition %	—	—	91	0	0	2	0

The figures show that in Exp. 2 0·0004 *M* pyruvate (one-eighth of the inhibiting concentration of *dl*-glyceraldehyde) abolished the inhibition completely, though the same concentration had no effect on the rate of glycolysis in the absence of glyceraldehyde. In Exp. 1 the reversal was less complete at the lowest dilutions of pyruvate, but was still marked.

Now it is known that glyceraldehyde may be converted into lactic acid in some tissues [Embden & Metz, 1932; Needham & Lehmann, 1937, 2], and in particular that in muscle extract it reacts with pyruvic acid, in the same manner as phosphoglyceraldehyde, at the oxido-reduction stage in the Embden-Meyerhof scheme of glycolysis [D. M. Needham & Pillai, 1937], though only when present at a high concentration [Warburg & Christian, 1939]. Süllmann [1938, 2] found that in muscle extract the glycolysis of glycogen, inhibited by 0·1 *M* glyceraldehyde, could apparently be restored by 0·022 *M* pyruvate, but he found also that this lactic acid formation took place equally in the absence of glycogen, and must in fact be due to a direct reaction between glyceraldehyde and pyruvate. In the present case, where lower concentrations of the reactants have been employed, it appears that this explanation of the reversal of glyceraldehyde inhibition does not apply. Under the same conditions as in the above experiments (Table 5) only a trace of lactic acid was found when muscle extract was allowed to act on a mixture of 0·003 *M* glyceraldehyde and 0·003 *M* pyruvate in the absence of glucose.

The partial inhibition of starch glycolysis by high concentrations of glyceraldehyde. The view that the absence of inhibition of glycogen or starch glycolysis by low concentrations of glyceraldehyde in muscle extract is attributable to the removal of glyceraldehyde by the aldolase reaction has had great doubt cast upon it by Süllmann [1938, 1]. Two further small pieces of evidence against that view may now be mentioned.

First, it is reasonable to suppose that the faster the glycolysis is proceeding the faster will be the removal of the glyceraldehyde; i.e. if the measurements are made over some arbitrary period of time, such as 1 hr., the faster the glycolysis the less the inhibition. Exactly the contrary is found experimentally, for glucolysis, even at minimal hexokinase concentration, proceeds faster than the breakdown of starch, and yet is inhibited to a much greater extent. The assumption is made throughout, of course, that the stages in glucolysis after the primary attack on the sugar are similar to those involved in the glycolysis of glycogen.

Secondly, the condensation of triosephosphate and glyceraldehyde cannot be instantaneous, so if the above view were correct there should be an initial period during which inhibition would be observed, followed, after the removal of the glyceraldehyde, by a period of active glycolysis. Experimentally exactly the opposite is found, the inhibition increasing steadily for the first hour of the experiment. This result can obviously be observed best when the inhibition is partial (as measured over a period of 1 hr.), and results showing the course of the reaction in a few such cases are given in Fig. 3.

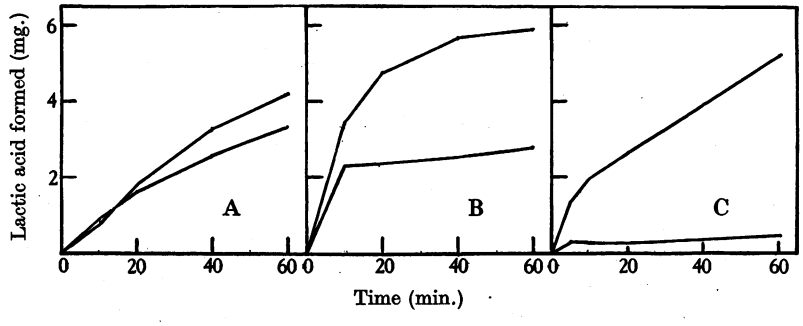

Fig. 3. The course of the inhibition of starch glycolysis by glyceraldehyde. Results by chemical determination of lactic acid. Glyceraldehyde concentrations: *A*, 0·01 *M*; *B*, 0·05 *M*; *C*, 0·03 *M*.

On the other hand, there are two observed facts which one might attempt to explain by this aldolase theory. First, the lifting of the inhibition by excess of hexokinase could be attributed to the increased rate of glucolysis, which would result in the more rapid removal of the glyceraldehyde. However, it has been shown that a very small increase in hexokinase concentration, causing only a slight increase in rate of glycolysis, is sufficient to remove the inhibition by glyceraldehyde (Table 4). Moreover, in the case in which an increase of 14 % in the hexokinase concentration reduced the inhibition from 90 to 50 % (Table 4, Exp. 2, and Fig. 4), the partially inhibited experiment showed the usual effect of the inhibition increasing with time. Secondly, when, for a variety of reasons (including age and dilution of the enzyme preparation and reduction of the phosphate concentration), the glycolysis of starch was proceeding very slowly, then this starch glycolysis was also largely inhibited by fairly low concentrations

of glyceraldehyde (0·01 *M* and sometimes 0·003 *M*). If the aldolase theory is to be rejected, then a new explanation must be found for this inhibition of starch glycolysis, and work is proceeding to try to discover its nature.

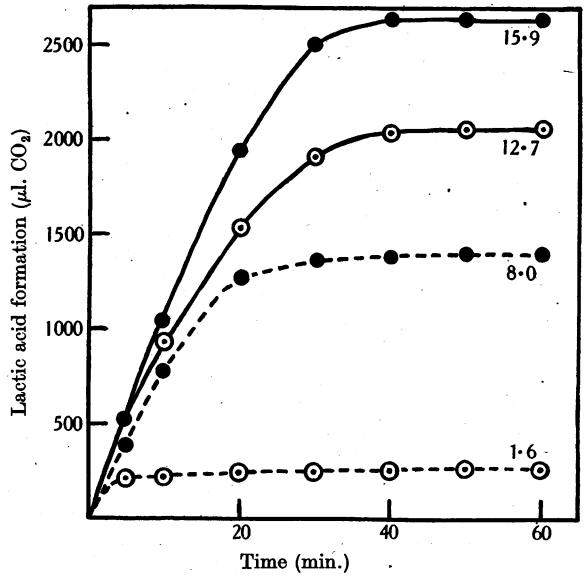

Fig. 4. The course of the inhibition of glucolysis by glyceraldehyde at different hexokinase concentrations. The curves were obtained manometrically, and the figure to the right of each curve gives the result of the chemical determination of lactic acid at the end of the experiment (mg./5 ml.). •—• indicates 0·25 ml. hexokinase solution per 5 ml., o—o 0·22 ml.per 5 ml. Dotted lines are the same inhibited by 0·003 *M* glyceraldehyde.

Discussion

The chief remaining difficulty in connexion with the inhibition of glycolysis by glyceraldehyde was that a large number of tissues, whose glycolysis is known to be inhibited by low concentrations of glyceraldehyde, have now been shown to contain the phosphorylating mechanism which is not inhibitable except by extremely high concentrations of glyceraldehyde (in red blood cells, Meyerhof [1932]; in brain, Euler *et al.* [1936], Geiger [1938]; in tumours, Boyland *et al.* [1937]; in embryo, Meyerhof & Perdigon [1939]; in retina, Süllmann & Vos [1939], Kerly & Bourne [1940]). The evidence for the presence of the whole phosphorylating system is in some cases not yet complete; for example, the disappearance of free phosphate, the need for adenosine triphosphate and coenzyme I, or the presence of the reaction between triosephosphate and pyruvic acid, or combinations of two or more of these and other such pieces of evidence, have been taken as proof of the existence of the whole system. On the other hand, there can be little doubt that further investigation will show that in every case these extracts do bring about glycolysis by the phosphorylating mechanism. Whether other mechanisms may exist as well is for the moment beside the point.

The results described here appear to go a long way towards explaining this discrepancy. They lead to the view, expressed in its simplest form, that the breakdown of glycogen to lactic acid by the enzyme system of muscle extract

(which is also present in all other tissues so far studied) is practically unaffected by glyceraldehyde, but that the breakdown of glucose by the same enzyme system is completely inhibitable. The glyceraldehyde therefore inhibits the initial stage of the process, which is catalysed by hexokinase.

The probable mode of action of hexokinase has been described by Euler & Adler [1935] and Meyerhof [1935]. The former, using a yeast preparation which they called heterophosphatese, showed that the addition of this enzyme enabled the hexosemonophosphate dehydrogenase system of Warburg & Christian to act also on hexoses. Meyerhof [1935] proved that the reaction which occurs is a stoichiometric one between hexose and adenosine triphosphate, leading to the formation of a hexosephosphate which can be converted into lactic acid by muscle extract, and that hexokinase and heterophosphatese behave in identical fashion. The effect of glyceraldehyde on this reaction remains to be studied. Iri [1939], arguing from the results of Willstätter & Rohdewald [1937] with leucocytes and yeast, thought that hexokinase must act by causing a synthesis of glycogen from glucose, and produced evidence that this synthesis does actually take place in the early stages of glucolysis in muscle extract. It seems unlikely, however, that glycogen is an intermediary in muscle glucolysis, for with optimal hexokinase concentration glucose is turned into lactic acid five times faster than is glycogen (Meyerhof [1927] and see Fig. 2), so that it would be necessary to assume the existence of a specially reactive form of glycogen as the first product of the synthesis.

Several points remain to be cleared up before this explanation of glyceraldehyde inhibition can be accepted as generally applicable. In some tissue slices, e.g. Jensen rat sarcoma [Mendel, 1929] and brain (Ashford [1934] and see Table 1), almost complete inhibition is observed with 0·001 *M* glyceraldehyde, whereas in the case of muscle glucolysis complete inhibition can be regularly obtained only with 0·003 *M*. Inhibition of muscle glucolysis by 0·001 *M* glyceraldehyde is variable and frequently absent, and the results indicate that this is possibly due to the presence in the latter cases of a slight excess of hexokinase. In some other tissue slices a higher concentration of glyceraldehyde is required to produce inhibition, e.g. chick embryo 0·005 *M* [Needham & Lehmann, 1937, 1], Daels-Biltris sarcoma 0·003 *M* and oesophageal mucosa 0·01 *M* (Table 1), and the speculation could be offered that in these tissues the effective concentration of hexokinase is higher. This reversal of glyceraldehyde inhibition by excess of hexokinase was not found by Needham & Lehmann [1937, 1] in chick embryo, but it must be remembered that they used a brei which, though free from intact cells, retained a considerable degree of cellular structure, and the hexokinase, which is a protein, may not have been able to reach the appropriate site. Their statement that 'In all cases brei made with distilled water to abolish cell integrity was used; hence it cannot be argued that hexokinase had no entrance to the glucolytic system' does not appear to me to be conclusive on this point.

Holmes [1934] and Needham & Lehmann [1937, 1] found no inhibition of glycolysis of either starch or glucose in muscle extract. They do not indicate the quantity of hexokinase used, and in the light of the present results it must be assumed that excess of this agent was present. Süllmann [1938, 2], on the contrary, found that at high concentrations of glyceraldehyde (0·05–0·07 *M*) glycolysis of glycogen or of glucose was equally inhibited (70–100 %). The explanation of this probably lies in the two facts (1) that his extracts were of very low activity (in only one experiment did the rate of glycolysis exceed 2·5 mg. lactic acid/5 ml. in 1 hr.), and (2) that the hexokinase concentration was of the

order of ten times that used in the present experiments. Glucolysis in certain other extracts has also been found not to be inhibited by glyceraldehyde, except at very high concentrations. Adler *et al.* [1937] found glucolysis by yeast preparations to be inhibited about 70% by 0·1*M* and about 15% by 0·03*M*, a result confirmed by Boyland & Boyland [1938].[1] The latter authors also found that in tumour extract 90% inhibition could be achieved only with 0·022*M* glyceraldehyde, the result being the same whether the substrate was glucose, fructose or glycogen. Kerly & Bourne [1940] showed that in their extract of retina about 80% inhibition could be attained only with 0·2*M* glyceraldehyde, but the same result was obtained even with hexosediphosphate as substrate, which indicates that at this extremely high concentration a different kind of inhibition may be involved. No attempt at a final explanation of such results can be made now, but the ready reversal of glyceraldehyde inhibition of glucolysis by excess of hexokinase may prove to be of importance in this connexion too.

Another way in which the inhibition of glucolysis in muscle extract differs from that in tissue slices is in the shape of the curve relating concentration of glyceraldehyde with degree of inhibition, this being much steeper in the case of muscle extract. Further study of the kinetics of these reactions and of the relation between hexokinase and glyceraldehyde will, it is hoped, help to show whether the present results have any bearing on the nature of glucolysis in tissue slices and its inhibition by glyceraldehyde.

Summary

Glycolysis in liver, a glycogen-fermenting tissue, is not inhibited by high concentrations of glyceraldehyde.

It is confirmed that in muscle extract glycolysis of starch is not greatly inhibited by high concentrations of glyceraldehyde, but on the other hand it is found that glucolysis by this system in the presence of hexokinase is completely inhibited by 0·003*M* glyceraldehyde.

This inhibition is reversed by a small excess of hexokinase. It is also reversed, as is the case in slices of glucolysing tissues, by very small amounts of pyruvic acid.

[1] But in this paper it is reported that glycolysis in muscle extract is inhibited at the stage of phosphorylation of glycogen, so that at least in these particular experiments the dimeric form of glyceraldehyde was probably concerned.

REFERENCES

Adler, Calvet & Günther (1937). *Hoppe-Seyl. Z.* **249**, 40.
Ashford (1934). *Biochem. J.* **28**, 2229.
Baker (1938). *Biochem. J.* **32**, 332.
Berenblum, Kendal & Orr (1936). *Biochem. J.* **30**, 709.
Boyland & Boyland (1938). *Biochem. J.* **32**, 321.
—— —— & Greville (1937). *Biochem. J.* **31**, 461.
Dickens & Greville (1932). *Biochem. J.* **26**, 1546.
Dixon & Lutwak-Mann (1937). *Biochem. J.* **31**, 1347.
Embden & Metz (1932). *Pflüg. Arch. ges. Physiol.* **230**, 526.
Euler & Adler (1935). *Hoppe-Seyl. Z.* **235**, 122.
—— Günther & Vestin (1936). *Hoppe-Seyl. Z.* **240**, 265.
Friedemann, Cotonio & Schaffer (1927). *J. biol. Chem.* **73**, 335.

Geiger (1938). *Nature, Lond.*, **141**, 373.
Holmes (1934). *Ann. Rev. Biochem.* **3**, 381.
Iri (1939). *J. Biochem.* **30**, 217.
Kendal & Stickland (1937). *Biochem. J.* **31**, 1758.
Kerly & Bourne (1940). *Biochem. J.* **34**, 563.
Lehmann & Needham (1938). *Enzymologia*, **5**, 95.
Mendel (1929). *Klin. Wschr.* **8**, 169.
—— Bauch & Strelitz (1931). *Klin. Wschr.* **10**, 116.
—— Strelitz & Mundell (1938). *Nature, Lond.*, **141**, 288.
Meyerhof (1926). *Biochem. Z.* **178**, 395.
—— (1927). *Biochem. Z.* **183**, 176.
—— (1932). *Biochem. Z.* **246**, 249.
—— (1935). *Naturwissenschaften*, **23**, 850.
—— Lohmann & Schuster (1936). *Biochem. Z.* **286**, 301, 319.
—— & Perdigon (1939). *C.R. Soc. Biol., Paris*, **132**, 186.
Needham (D. M.) & Pillai (1937). *Biochem. J.* **31**, 1837.
Needham (J.) & Lehmann (1937, 1). *Biochem. J.* **31**, 1210.
—— —— (1937, 2). *Biochem. J.* **31**, 1913.
Orr & Stickland (1941). *Biochem. J.* **35**, 479.
Rosenthal & Lasnitzki (1928). *Biochem. Z.* **196**, 340.
Süllmann (1938, 1). *Biochem. Z.* **296**, 325.
—— (1938, 2). *Enzymologia*, **5**, 372.
—— & Vos (1939). *Enzymologia*, **6**, 246.
Warburg & Christian (1939). *Biochem. Z.* **303**, 40.
Willstätter & Rohdewald (1937). *Hoppe-Seyl. Z.* **247**, 115, 269.