

# XLV. STUDIES ON THE INHIBITION OF GLYCOLYSIS BY GLYCERALDEHYDE

By ZELMA BAKER

*From the Biochemical Research Foundation of the Franklin Institute, Philadelphia, Pa.*

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THE effect of glycerinaldehyde on tumour glycolysis was first reported by Mendel [1929] who observed that, at concentrations as low as 0.001 *M*, glycerinaldehyde inhibited the anaerobic glycolysis of the Jensen rat sarcoma, and that at this concentration respiration was unaffected. Since that time other investigators have confirmed Mendel's original report and have made similar experiments on other tissues. Thus Ashford [1934] reported that glycerinaldehyde inhibits rabbit brain glycolysis, and Ashford & Dixon [1935] showed further that the increased aerobic glycolysis of brain caused by a high  $K^+$  concentration was also inhibited by glycerinaldehyde. Similarly, Needham & Nowiński [1937] in a rather complete study showed that chick embryo anaerobic glycolysis was very sensitive to glycerinaldehyde. Rosenthal [1930, 1] showed that both glucolysis and fructolysis of tumour but not methylglyoxal breakdown are inhibited by glycerinaldehyde. Liver, however [Rosenthal, 1930, 2], can ferment glycerinaldehyde at a significant speed. According to unpublished experiments referred to by Holmes [1934], glycerinaldehyde has no effect on lactic acid production from starch by muscle extract.

In the course of a comparative study on the effects of glycerinaldehyde on various tissues, many of these experiments have been repeated and confirmed. Since this work was completed the papers of Needham *et al.* [1937] on the carbohydrate metabolism of embryo have appeared; the work here reported on the effect of glycerinaldehyde on various tissues, especially tumour, and the conclusions drawn from this work agree well with the experiments reported by Needham & Nowiński [1937] on chick embryo.

## EXPERIMENTAL

The differential manometric apparatus of Dixon and Keilin was used for both aerobic and anaerobic experiments and the technique was that described by Elliott & Schroeder [1934]. Unless otherwise specified, the medium [Krebs & Henseleit, 1932] contained 0.24 % glucose. The experimental period was usually 90 min. Rat tissues were used for all experiments. Where not enough tissue was available from one animal for several experiments (as in the case with brain), the carefully sampled tissue from several animals was used. Tumour experiments were done on the Philadelphia No. 1 sarcoma [Waldschmidt-Leitz, McDonald *et al.* 1933], a rapidly growing sarcoma, relatively free from necrosis; in all cases the least necrotic slices were chosen for experiments. Twin or triple tumours 20–25 days old, implanted in one animal, were used. A very close agreement in the metabolism of twin tumours has been demonstrated by Bancroft *et al.* [1935].

Whenever possible, reagents to be added to the manometer vessels were made up in 12 times the desired final concentration; 0.25 ml. solution was then added to 2.75 ml. medium. The glycerinaldehyde used for these experiments was

obtained from Fraenkel and Landau and was recrystallized from water. Glyceraldehyde solutions were made up at least 1 day before use to permit dissociation of the polymeride. Most other solutions were prepared just before use. Pyruvic acid (Eastman Kodak Company) was redistilled at 12 mm. and the fraction distilling at 58–59° was used. The 5% stock solution was diluted and neutralized with bicarbonate before use. The Ba salt of phosphoglyceric acid was prepared according to the method of Vercellone & Neuberg [1935] and was converted into the sodium salt by shaking in a mechanical shaking machine with the calculated amount of Na<sub>2</sub>SO<sub>4</sub> solution; the BaSO<sub>4</sub> formed was centrifuged off and the solution brought to the correct volume and pH. The silver-barium salt of phosphopyruvic acid was synthesized by Kiessling's method [1935]. [Analysis, very kindly performed by Dr H. K. Alber and Dr J. Harrand: Found: C, 8.47; H, 0.94; P, 7.22; Ag, 24.74; Ba, 32.02%. Calc. for C<sub>3</sub>H<sub>2</sub>O<sub>6</sub>PAgBa+H<sub>2</sub>O: C, 8.41; H, 0.94; P, 7.24; Ag, 25.19; Ba, 32.07%.] The sodium salt of phosphopyruvic acid was prepared by dissolving the calculated amount of silver-barium salt in dilute HCl and H<sub>2</sub>SO<sub>4</sub>, removing the AgCl and BaSO<sub>4</sub> by centrifuging, neutralizing to pH 7.4 with NaOH and diluting to the proper volume.

Table I. *Effect of glyceraldehyde on the anaerobic glycolysis of glycolysing tissues*

Tissue	Concentration of glyceraldehyde <i>M</i>	Q <sub>A</sub> <sup>N</sup> *	Experimental period min.
Tumour, Philadelphia No. 1 sarcoma	—	+ 0.7*	90
	2.2 × 10 <sup>-3</sup>	+ 0.9*	
	—	+ 24.6	60
	2.2 × 10 <sup>-3</sup>	+ 1.1	
	—	+ 35.4	60
	2.2 × 10 <sup>-3</sup>	+ 2.2	
	1.1 × 10 <sup>-3</sup>	+ 12.7	
	0.55 × 10 <sup>-3</sup>	+ 29.0	
	—	+ 28.8	60
	2.2 × 10 <sup>-3</sup>	+ 1.8	
	—	+ 32.2	60
Brain	1.0 × 10 <sup>-4</sup>	+ 34.5	
	1.0 × 10 <sup>-5</sup>	+ 34.8	
	1.0 × 10 <sup>-6</sup>	+ 34.1	
	—	+ 10.0	90
	2.2 × 10 <sup>-3</sup>	+ 0.5	
	1.1 × 10 <sup>-2</sup>	+ 0.4	
	—	+ 15.9	90
	2.5 × 10 <sup>-3</sup>	0.0	
	—	+ 9.8	90
	2.5 × 10 <sup>-3</sup>	+ 2.4	
	Testis	—	+ 13.6
2.2 × 10 <sup>-3</sup>		+ 11.3	
1.1 × 10 <sup>-2</sup>		+ 2.8	
—		+ 12.4	90
2.2 × 10 <sup>-3</sup>		+ 8.8	
1.1 × 10 <sup>-2</sup>		+ 1.9	
Whole chick embryo, 5 day	—	+ 22.5	90
	2.2 × 10 <sup>-3</sup>	+ 8.6	
	1.1 × 10 <sup>-2</sup>	+ 1.1	
	—	+ 20.6	90
	1.1 × 10 <sup>-2</sup>	+ 2.2	

\* No glucose.

Table II. *Effect of glyceraldehyde on the anaerobic glycolysis of liver and kidney*

Tissue	Concentration of glyceraldehyde <i>M</i>	$Q_A^{N_2}$
Liver	—	+1.6*
	$2.2 \times 10^{-3}$	+3.5*
	—	+2.5
	$2.2 \times 10^{-3}$	+3.0
	—	+2.1*
	$1.1 \times 10^{-2}$	+8.9*
	—	+1.5
	$1.1 \times 10^{-2}$	+6.7
	—	+5.3*
	$1.0 \times 10^{-2}$	+9.0*
	—	+1.2†
	$5.0 \times 10^{-2}$	+7.1
	—	+1.8
	$2.2 \times 10^{-3}$	+1.8
	$1.1 \times 10^{-2}$	+4.3
—	+3.9	
$5.0 \times 10^{-2}$	+9.8	
Kidney	—	+2.7
	$1.1 \times 10^{-2}$	+2.5
	—	+4.2†
	$5.0 \times 10^{-2}$	+3.6

\* No glucose.

† Experimental period 2 hr.

Glycolaldehyde was synthesized by the method of Fischer & Taube [1927].

In Tables I and II, the effect of glyceraldehyde on the anaerobic glycolysis of a number of rat tissues is indicated. A distinct differentiation in the action of glyceraldehyde may be observed. As can be seen from Table I, glyceraldehyde inhibits the glycolysis of all the tissues commonly considered as glycolysing tissues—tumour, brain, testis and whole chick embryo (5 day). Tumour and brain are more sensitive to the action of glyceraldehyde than are testis and embryo, both the former tissues showing maximum inhibition at a concentration of  $2.2 \times 10^{-3} M$ , while at this concentration the glycolysis of testis and embryo is only partially inhibited.

Liver and kidney (Table II) show a quite different response. The anaerobic glycolysis of liver is stimulated by glyceraldehyde, the stimulation being a function of the concentration. The values in the table represent the average values over the 90 min. experimental period. If one considered only the first 30 min., considerably higher  $Q_A^{N_2}$  values would be obtained. It is interesting to note that the maximum activation observed occurred at the highest glyceraldehyde concentration used ( $5 \times 10^{-2} M$ ). Much higher concentrations were not possible due to the insolubility of glyceraldehyde. Kidney cortex, on the other hand, shows a still different behaviour. The anaerobic glycolysis of kidney cortex, which is normally only slight, is neither stimulated nor inhibited by even the highest concentrations of glyceraldehyde.

Aerobically, much the same type of differentiation can be observed. Table III shows the effect of glyceraldehyde on the aerobic metabolism of tumour, brain, testis and embryo. The aerobic glycolysis of tumour is much less sensitive to the effect of glyceraldehyde than is the anaerobic glycolysis; a concentration of  $2.2 \times 10^{-3} M$  which anaerobically produces practically complete inhibition,

inhibits aerobic glycolysis only 20–40%. No significant inhibition of tumour respiration occurs at this concentration. At a concentration 5 times greater ( $1.1 \times 10^{-2}M$ ) both the respiration and the aerobic glycolysis are significantly inhibited.

Brain and embryonic tissue normally have a very small aerobic glycolysis, and this is unaffected by glyceraldehyde. The respiration of brain is inhibited to about the same extent as tumour, while that of embryo is not quite so sensitive and higher concentrations are required to produce the same degree of inhibition. The aerobic glycolysis of testis is, unlike that of tumour, quite insensitive to glyceraldehyde, but the respiration of testis is inhibited to about the same extent as that of tumour and brain.

Table III. *Effect of glyceraldehyde on the aerobic metabolism of glycolysing tissues*

Tissue	Concentration of glyceraldehyde <i>M</i>	$-Q_{O_2}$	R.Q.	$Q_A^{O_2}$
Tumour, Philadelphia No. 1 sarcoma	—	15.0	0.98	+ 0.8*
	$2.2 \times 10^{-3}$	11.1	0.85	+ 2.7*
	—	11.2	—	—
	$2.2 \times 10^{-3}$	10.8	—	—
	—	10.0	—	—
	$2.2 \times 10^{-3}$	9.2	—	—
	—	8.7	0.72	- 0.3*
	$2.2 \times 10^{-3}$	9.7	0.72	+ 1.3*
	—	10.3	0.83	+ 24.0
	$2.2 \times 10^{-3}$	9.0	0.87	+ 13.3
	—	9.3	0.75	+ 16.6
	$2.2 \times 10^{-3}$	9.7	0.77	+ 13.0
	—	9.6	0.61	+ 22.5
	$1.1 \times 10^{-2}$	8.9	0.82	+ 3.9
	—	11.7	0.74	+ 30.3
	$2.2 \times 10^{-3}$	10.6	0.73	+ 18.2
	$1.1 \times 10^{-2}$	5.8	0.42	+ 7.5
	$5.0 \times 10^{-2}$	4.3	—	+ 4.6
	—	11.0	0.69	+ 23.5
	$1.1 \times 10^{-2}$	7.3	0.58	+ 5.9
—	8.7	0.80	+ 21.6	
$1.1 \times 10^{-2}$	9.2	0.77	+ 5.1	
Brain	—	14.8	0.93	+ 1.0
	$2.2 \times 10^{-3}$	12.9	0.90	+ 1.9
	$1.1 \times 10^{-2}$	5.0	0.67	+ 1.2
	—	15.0	0.98	+ 0.8
	$2.2 \times 10^{-3}$	11.1	0.85	+ 1.7
	—	11.5	0.98	+ 1.0
	$2.2 \times 10^{-3}$	9.1	0.97	+ 0.8
	$1.1 \times 10^{-2}$	5.1	0.89	+ 0.2
Testis	—	10.6	0.99	+ 8.9
	$1.1 \times 10^{-2}$	7.3	0.73	+ 9.5
	—	13.6	0.91	+ 9.2
	$2.2 \times 10^{-3}$	12.3	0.83	+ 10.7
	$1.1 \times 10^{-2}$	7.2	0.81	+ 10.9
	$5.0 \times 10^{-2}$	3.9	—	+ 7.7
Whole chick embryo, 5 day	—	13.5	0.95	+ 3.5
	$1.1 \times 10^{-2}$	12.8	1.04	+ 2.2
	—	14.3	1.01	+ 3.3
	$5.0 \times 10^{-2}$	3.8	0.86	+ 4.0

\* No glucose.

Aerobically, too, liver and kidney react differently from the glycolysing tissues to the presence of glyceraldehyde. The aerobic glycolyses of both liver and kidney are greatly stimulated by sufficiently high concentrations of glyceraldehyde (Table IV). In this respect kidney reacts differently aerobically and anaerobically, since no effect was observed on the anaerobic glycolysis of kidney. The striking difference in the sensitivity of the respiration of glycolysing as compared with non-glycolysing tissues is also worthy of note; the respiration of kidney is scarcely affected even by glyceraldehyde at the highest concentration used,  $5 \times 10^{-2} M$ ; at this concentration liver respiration is inhibited 40–50%.

Table IV. *Effect of glyceraldehyde on the aerobic metabolism of liver and kidney*

Experimental period 90 min.				
Tissue	Concentration of glyceraldehyde <i>M</i>	$-Q_{O_2}$	R.Q.	$Q_A^{O_2}$
Liver	—	9.4	0.88	+ 1.9
	$2.2 \times 10^{-3}$	8.3	0.83	+ 1.8
	$1.1 \times 10^{-2}$	8.7	0.73	+ 4.7
	—	12.3	0.71	+ 1.1
	$1.1 \times 10^{-2}$	17.2	0.72	+ 2.9
	—	11.6	0.77	+ 0.7
	$1.1 \times 10^{-2}$	12.5	0.70	+ 2.7
	$5.0 \times 10^{-2}$	6.6	0.53	+ 9.8
	—	12.1	0.85	+ 2.5
	$5.0 \times 10^{-2}$	7.5	1.01	+11.8
Kidney	—	22.0	0.80	- 0.8
	$2.2 \times 10^{-3}$	24.8	0.80	- 1.1
	$1.1 \times 10^{-2}$	28.7	0.76	+ 5.4
	—	28.4	0.86	- 1.5
	$1.1 \times 10^{-2}$	30.5	0.81	+ 2.7
	—	27.5	0.77	- 1.6
	$5.0 \times 10^{-2}$	25.2	0.77	+ 8.5

It is interesting to note that the lactic acid production by tumour slices from methylglyoxal in the presence of glutathione is not inhibited by glyceraldehyde (Table V). Needham & Nowinski [1937] have shown that this is also true for embryo brei.

Table V. *Effect of glyceraldehyde on the glycolysis of methyl glyoxal by tumour*

Addition	$Q_A^{N_2}$
—	+37.6
$2.2 \times 10^{-3} M$ glyceraldehyde	+ 1.3
$3.3 \times 10^{-2} M$ (0.24%) methylglyoxal + $10^{-3} M$ glutathione	+41.6
Methylglyoxal + glutathione + glyceraldehyde	+41.8

One experiment with glyceraldehyde was done on a very active rabbit muscle extract (prepared by grinding the muscle with sand and extracting with water for half an hour). The buffer mixture of McCullagh [1928] was used. Starch was used as the substrate and lactic acid produced was estimated by the method of Friedemann & Graeser [1933] (Table VI).

As can be seen from Exp. 3, in the absence of starch glyceraldehyde cannot serve as a substrate for lactic acid production in muscle. Exps. 4 and 5 show that glyceraldehyde does not inhibit lactic acid production by muscle extract from starch (compare control, Exp. 2).

Table VI. *Effect of glyceraldehyde on muscle extract glycolysis*

Exp. no.	Muscle extract ml.	Buffer ml.	Starch 2% ml.	Glyceraldehyde	H <sub>2</sub> O ml.	Lactic acid formed in 4 hr. mg.
1	10.0	5.0	—	—	10.0	14.4
2	10.0	5.0	5.0	—	5.0	45.0
3	10.0	5.0	—	5.0 ml. of 10 <sup>-1</sup> M	5.0	10.3
4	10.0	5.0	5.0	5.0 ml. of 10 <sup>-2</sup> M	—	44.5
5	10.0	5.0	5.0	5.0 ml. of 10 <sup>-1</sup> M	—	47.9

Using tumour tissue a study of the time required for full inhibition and the reversibility of the glyceraldehyde inhibition has been made. A strong solution of glyceraldehyde (sufficient to make the concentration  $2.2 \times 10^{-3} M$ ) was tipped into the vessels of the manometer after the tumour slices had been permitted to glycolyse anaerobically normally for 45 min. Readings were then taken every few minutes. After 15–20 min., the slope of the glycolysis curve was the same as that obtained in experiments in which glyceraldehyde had been present from the start, indicating that the inhibition was complete within 15–20 min. This inhibition is completely reversible. Tumour slices were placed in Ringer-Krebs medium containing  $2.2 \times 10^{-3} M$  glyceraldehyde, gassed with N<sub>2</sub>-CO<sub>2</sub> and allowed to stand for 30 or 45 min. At the end of this time the slices were carefully washed and their anaerobic glycolysis determined in the usual manner, with controls which had been similarly treated in ordinary Ringer-Krebs solution. Table VII indicates that the inhibition is completely reversible.

Table VII. *Reversibility of glyceraldehyde inhibition*

Treatment	Q <sub>A</sub> <sup>N*</sup>
Control, soaked in Ringer-Krebs solution for 45 min., washed	+32.0
Experimental, soaked in Ringer-Krebs + $2.2 \times 10^{-3} M$ glyceraldehyde, for 30 min., washed	+33.8
Experimental, soaked in Ringer-Krebs, + $2.2 \times 10^{-3} M$ glyceraldehyde, for 45 min., washed	+34.8

Mendel *et al.* [1931] first pointed out that pyruvic acid could prevent the inhibition by glyceraldehyde of the anaerobic glycolysis of tumours. I have been able to confirm this qualitatively but not quantitatively. The extent to which pyruvic acid can prevent the inhibition is apparently a function of the degree of inhibition (Table VIII). Thus at maximum inhibition pyruvic acid, even at a fairly high concentration, can restore only a part of the normal glycolysis (Exps. 1, 2 and 4). When the degree of inhibition is less, the restoration of glycolysis is more complete (Exps. 3 and 5). Phosphoglyceric and phosphopyruvic acids act like pyruvic acid, partially reversing the inhibition.

The inhibition of brain glycolysis by glyceraldehyde cannot be prevented by pyruvic acid [Holmes, 1934]. One experiment demonstrating this is shown in Table IX.

It is obvious from the experiments listed above that even the great increase in brain glycolysis caused by pyruvic acid is completely inhibited by glyceraldehyde. In its failure to respond to reactivation by pyruvic acid, brain differs from both tumour and embryo [*vide* Needham & Nowiński, 1937].

The power of several other substances to prevent the inhibition of tumour glycolysis by glyceraldehyde was also tested. Calcium  $\alpha$ -glycerophosphate,

Table VIII. *Reversal of glyceraldehyde inhibition in tumour*

Exp. no.	Addition	$Q_A^{N_2}$	$Q_{Pyr}$
1	—	+28.8	—
	$2.2 \times 10^{-3} M$ glyceraldehyde	+ 1.8	—
	$1.0 \times 10^{-3} M$ pyruvic acid	+24.8	—
	Glyceraldehyde + pyruvic acid	+18.5	—
2†	—	+37.4	+0.4
	$2.2 \times 10^{-3} M$ glyceraldehyde	+ 2.0	-2.4
	$2.2 \times 10^{-3} M$ pyruvic acid	+34.7	-0.6
	Glyceraldehyde + pyruvic acid	+20.3	-5.2
3*	—	+30.7	—
	$1.0 \times 10^{-3} M$ glyceraldehyde	+ 9.1	—
	$1.0 \times 10^{-3} M$ pyruvic acid	+32.6	—
	Glyceraldehyde + pyruvic acid	+25.0	—
4†	—	+36.2	0.0
	$2.2 \times 10^{-3} M$ glyceraldehyde	+ 1.6	-1.1
	$2.2 \times 10^{-2} M$ pyruvic acid	+33.9	-3.1
	Glyceraldehyde + pyruvic acid	+16.7	-6.8
5	—	+34.4	—
	$1.1 \times 10^{-3} M$ glyceraldehyde	+ 7.3	—
	$1.0 \times 10^{-3} M$ pyruvic acid	+32.9	—
	Glyceraldehyde + pyruvic acid	+26.0	—
6	—	+39.6	—
	$1.1 \times 10^{-3} M$ glyceraldehyde	+ 5.4	—
	$5.0 \times 10^{-3} M$ phosphoglyceric acid	+33.5	—
	Glyceraldehyde + phosphoglyceric acid	+24.5	—
7	—	+41.6	—
	$1.1 \times 10^{-3} M$ glyceraldehyde	+ 8.9	—
	$5.0 \times 10^{-3} M$ phosphoglyceric acid	+38.7	—
	Glyceraldehyde + phosphoglyceric acid	+18.3	—
8‡	—	+47.1	—
	$2.2 \times 10^{-3} M$ glyceraldehyde	+ 2.6	—
	$1.1 \times 10^{-3} M$ phosphopyruvic acid	+41.5	—
	Glyceraldehyde + phosphopyruvic acid	+16.3	—

\* Jensen rat sarcoma.

† Estimations of pyruvic acid indicated that only a small part of the lactic acid formed in the presence of both glyceraldehyde and pyruvic acid was due to pyruvic acid being reduced to lactic acid. The remainder of the lactic acid formed was due to a true restoration of glycolysis.

‡ Free phosphorus estimations indicated that some hydrolysis of the phosphopyruvic acid had occurred, but only enough to account for about 7% of the acid formation so that the additional must be due to a reversal of the inhibition by phosphopyruvic acid.

Table IX. *Effect of pyruvic acid on glyceraldehyde inhibition of brain*

Addition	$Q_A^{N_2}$
—	+ 9.8
$2 \times 10^{-3} M$ glyceraldehyde	+ 1.2
$2 \times 10^{-3} M$ pyruvic acid	+25.5
Glyceraldehyde + pyruvic acid	+ 2.4

sodium  $\beta$ -glycerophosphate, glutathione and muscle adenylic acid had no effect. These results are indicated in Table X.

The effect of a few other aldehydes on tumour anaerobic glycolysis has been investigated; these were glycolaldehyde, propaldehyde, butaldehyde and benzaldehyde. They were used at a concentration of  $2.2 \times 10^{-3} M$ , which in the case of glyceraldehyde is sufficient to produce essentially complete inhibition. At this concentration no inhibition occurred with any of the compounds investigated, indicating that the effect with glyceraldehyde is fairly specific and is not merely a function of the aldehyde group. It is interesting to note that

Table X. *Effect of various compounds on removing glycerlaldehyde inhibition in tumour*

Addition	$Q_A^{N_2}$
0	+31.2
$2.2 \times 10^{-3} M$ glycerlaldehyde	+ 1.0
$2.2 \times 10^{-3} M$ calcium $\alpha$ -glycerophosphate	+31.2
Glycerlaldehyde + $\alpha$ -glycerophosphate	+ 1.3
0	+35.4
$2.2 \times 10^{-3} M$ glycerlaldehyde	+ 1.2
$2.2 \times 10^{-3} M$ sodium $\beta$ -glycerophosphate	+36.5
Glycerlaldehyde + $\beta$ -glycerophosphate	+ 0.8
0	+29.5
$2.2 \times 10^{-3} M$ glycerlaldehyde	+ 1.0
$2.2 \times 10^{-3} M$ glutathione	+29.9
Glycerlaldehyde + glutathione*	+ 2.0
0	+30.4
$1.1 \times 10^{-3} M$ glycerlaldehyde	+ 7.7
$2.2 \times 10^{-3} M$ adenylic acid†	+36.9
Glycerlaldehyde + adenylic acid	+ 7.1

\* Hoffman-La Roche.

† Fraenkel and Landau.

glycolaldehyde, which is structurally similar to glycerlaldehyde, inhibits tumour glycolysis only about 30% at a concentration which is 5 times as great as the concentration of glycerlaldehyde required for almost complete inhibition. The results obtained with these aldehydes are listed in Table XI.

Table XI. *Effect of other aldehydes on tumour anaerobic glycolysis*

Addition	$Q_A^{N_2}$
—	+38.4
Glycolaldehyde, $2 \times 10^{-3} M$	+38.5
Glycolaldehyde, $1 \times 10^{-2} M$	+26.2
—	+34.9
Propaldehyde, $2 \times 10^{-3} M$	+35.2
Butaldehyde, $2 \times 10^{-3} M$	+32.4
Benzaldehyde, $2 \times 10^{-3} M$	+37.0

A few experiments have been done on the effect of dihydroxyacetone on the glycolysis and respiration of a number of tissues. Since these are by no means complete they are not included in this paper. For the sake of comparison with the glycerlaldehyde effects, however, a few results with the Philadelphia No. 1 tumour are listed in Table XII.

Table XII. *Effect of dihydroxyacetone on tumour metabolism*

Concentration of dihydroxyacetone	$Q_{O_2}$	R.Q.	$Q_A^{O_2}$	$Q_A^{N_2}$
—	9.3	0.75	+16.6	—
$2.2 \times 10^{-3} M$	8.3	0.73	+16.7	—
$1.1 \times 10^{-2} M$	7.3	0.88	+15.2	—
—	9.7	0.76	+18.2	—
$3.0 \times 10^{-2} M$	7.1	0.75	+14.5	—
—	—	—	—	+32.0
$3.0 \times 10^{-2} M$	—	—	—	+25.9

These results corroborate the previous report of Mendel [1930] that dihydroxyacetone is almost without effect on the aerobic or the anaerobic glycolysis of the Jensen rat sarcoma. Apparently, however, the respiration of the Jensen



tumour is more sensitive to dihydroxyacetone than is the Philadelphia No. 1 sarcoma. Other differences in the metabolism of these two tumours have been pointed out by Baker [1937].

Also glyceric acid, 2:3-butylene glycol, and glycerol had no effect on either the aerobic or the anaerobic metabolism of tumour tissue, although used at a concentration of  $M/50$ . This is illustrated by Table XIII.

Table XIII. *Effect of other compounds on tumour metabolism*

Addition $2 \times 10^{-2} M$	$-Q_{O_2}$	$Q_A^{O_2}$	$Q_A^{N_2}$
—	8.2	+18.6	—
Glyceric acid	9.8	+21.4	—
2:3-Butylene glycol	9.7	+20.5	—
Glycerol	9.0	+17.7	—
—	—	—	+34.3
Glyceric acid	—	—	+37.5
2:3-Butylene glycol	—	—	+37.6
Glycerol	—	—	+34.5

No effect on the growth of tumour could be produced by injecting glyceraldehyde into animals inoculated with a transplantable tumour. This is not surprising in view of the fact that this compound is so readily metabolized by liver and kidney as is indicated by the experiments reported here and by the experiments of Stöhr [1932] who showed that after feeding rats with glyceraldehyde, the glycogen content of the liver was increased.

#### DISCUSSION

In recent years more and more evidence has been brought forward in favour of the view that there are at least two different paths by which carbohydrate is broken down by animal tissues, depending on whether the substrate is glucose or polysaccharide (glycogen or starch) [Ashford & Holmes, 1929; Bumm & Fehrenbach, 1930, 1931; Gaddie & Stewart, 1934; Geiger, 1935; Needham & Lehmann, 1937]. In the light of all available data, this question has been very thoroughly discussed by Needham & Lehmann [1937]. The experiments reported here on the effect of glyceraldehyde on the glycolysis of various tissues may serve to emphasize still further the differentiation between the glucose-splitting, non-phosphorylating tissues and the glycogen-splitting, phosphorylating tissues.

It is of interest to note that although liver has been included here among the "non-glycolysing" tissues, and although it differs strikingly from the "glycolysing" tissues in its response to glyceraldehyde, liver often does show a high temporary aerobic and anaerobic glycolysis [Elliott & Baker, 1935; Elliott *et al.* 1937]. However, this tissue differs from the ordinary "glycolysing" tissue in that its glycolysis appears to take place wholly at the expense of glycogen stores [Rosenthal & Lasnitzki, 1928; Rosenthal, 1929] and, even in the case of starved animals, liver metabolism is completely independent of the presence of glucose.

#### SUMMARY

1. The effects of *dl*-glyceraldehyde on the metabolism of tumour, brain, testis, embryo, liver and kidney have been studied. A distinct differentiation in the response of the glycolysing as compared with the non-glycolysing tissues has been observed. The respiration and the anaerobic glycolysis of tumour,

brain, testis and embryo are inhibited by glyceraldehyde. Glycolysis in liver and kidney is stimulated by glyceraldehyde.

2. The inhibition of tumour glycolysis by glyceraldehyde is completely reversible.

3. The inhibition of tumour anaerobic glycolysis by glyceraldehyde can be largely prevented by pyruvic acid, phosphopyruvic acid and phosphoglyceric acid. The extent to which the inhibition can be prevented depends on the degree of inhibition. Glutathione, adenylic acid,  $\alpha$ - and  $\beta$ -glycerophosphates have no effect on the inhibition.

4. Lactic acid production from methylglyoxal by tumour slices is not inhibited by glyceraldehyde.

5. Lactic acid production from starch by muscle extract is not inhibited by glyceraldehyde.

6. The effect of glyceraldehyde is fairly specific for this aldehyde, since it is not produced by glycolaldehyde, propaldehyde, butaldehyde, or benzaldehyde.

7. Dioxycetone is without effect on the glycolysis of tumour tissue.

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