Effect of Glycolaldehyde, Glyceraldehyde, and Related Compounds on Anaerobic Metabolism in the Rabbit Lens[1] (34855)

THOMAS G. SCHARFF AND OCIA L. MONTGOMERY
Department of Pharmacology, University of Louisville School of Medicine, Louisville, Kentucky 40202

The effects of a number of carbohydrate-like compounds have been studied in the mammalian lens. Kern (1), for example, studied the effects of pentoses, hexoses, and related compounds on metabolism and sugar transport in calf and rat lenses. Little work has been done in the lens, however, on the possible effects of similar compounds of 2- and 3-carbon size on metabolism and transport. These compounds are important because of their profound toxic effects (2–4) in tissue.

In this paper we report the effects of several 2- and 3-carbon substances on metabolism and/or transport of hexose in the rabbit lens under anaerobic conditions. Anaerobic conditions were chosen because this mode of metabolism in the lens is relatively unexplored and because complications arising from metabolic reactions proceeding to oxygen are avoided.

Methods. Young male white rabbits (4–6 lb) were sacrificed by air embolism, and the lenses were immediately removed. Experiments on intact lenses were carried out *in vitro* in the incubation tubes and oscillating device described by Merriam and Kinsey (5) or with the same oscillating device into which was placed tubes of the type shown in Fig. 1. The incubation medium was rabbit Ringer's solution (mEq/liter:$NaHCO_3$, 43.2; KH_2PO_4, 1.07; $MgSO_4$, 0.61; $CaCl_2$, 0.70; NaCl, 102.2; KCl, 5.12) containing 5mM glucose. Inhibitor concentrations were 5–20 mM. Lens homogenates were made in glass homogenizers and contained approximately three lenses per ml of a 0.15 M KCl–0.15 M $NaHCO_3$ solution. Lens homogenates show very little hexokinase activity (6). Hence glucose-6-phosphate was used in place of glucose in these preparations. All intact lenses or homogenates were incubated at 37° under a 5% CO_2–95% atmosphere.

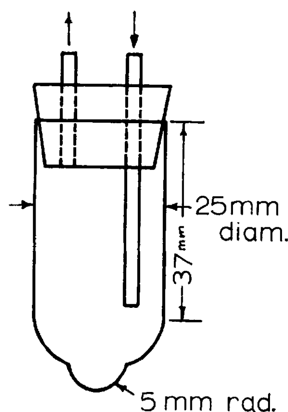

FIG. 1. Incubation tube for rabbit lenses or homogenates. A 5% CO_2–95% N_2 mixture was passed into the tube as depicted by arrows.

A commercial glucose oxidase method (Glucostat-Worthington Biochemical Corp.) was used in the estimation of glucose. Lactic acid was determined enzymatically (7) in the incubating medium. A chemical method (8) was used in the determination of lactic acid from $Ba(OH)_2$–$ZnSO_4$ extracts of lenses or of lens homogenates. The enzymatic and the chemical tests (respectively) for lactic acid were interfered with by glyoxylate and by DL-glyceraldehyde. Therefore, correction was made for glyceraldehyde in the enzymatic lactic acid determination, and lactic acid was measured chemically in the presence of glyoxylate. Glycoladehyde and glyoxylate were determined using the method of Dische and Borenfreund (9), glycollate by the method of Calkins (10), and glyceraldehyde by

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TABLE I. Effects of Rabbit Lenses of Several 2- or 3-Carbon Compounds on Anaerobic Glucose Utilization and Lactic Acid Leakage.[a]

Test compound	Incubation time (hr)	Glucose utilized		Lactic acid leakage[b]	
		μmoles per lens	% of control	μmoles per lens	% of control
Glycerol	6	11.2	110	9.3	100
Controls	6	10.2	100	9.3	100
DL-Glyceraldehyde	6	1.3	18	1.35	16
Controls	6	7.2	100	8.3	100
Propionaldehyde	6	3.0	57	3.2	45
Controls	6	5.3	100	7.1	100
Methylglyoxal	6	1.8	23	4.3	53
Controls	6	7.8	100	8.1	100
Ethylene glycol	3	5.0	96	5.2	96
Controls	3	5.2	100	5.4	100
Glycoaldehyde	6	0.6	7	1.2	14
Controls	6	8.8	100	8.5	100
Glyoxal	6	5.4	52	5.2	62
Controls	6	10.4	100	8.4	100
Glycollate	3	4.2	74	4.4	84
Controls	3	5.7	100	5.2	100
Glyoxylate	3	—	—	3.7	69
Controls	3	—	—	5.35	100

[a] All lenses were incubated at pH 7.5 in rabbit Ringers containing 5 m*M* glucose. Gas phase: 5% CO_2–95% N_2. All values given are averages of two lenses. Contralateral lenses used as controls in each case contained glucose without test compound. Compounds were tested at 5 m*M* concn (except DL-glyceraldehyde: 10 m*M*).

[b] Leakage of lactic acid, while a reflection of lactic acid production, is not a measure of the true production of lactic acid.

the method of Dische and Borenfreund (11). High-energy (acid-labile) phosphate compounds were determined by using a modified Fiske-SubbaRow method (12) before and after heating in 1N HCl for 10 min. Initial phosphate extraction for 5 min was made in ice-cold 10% trichloroacetic acid, followed by immediate centrifugation and neutralization. Manometric procedures were used in the determination of CO_2 from intact lenses in rabbit Ringer's buffered in 5% CO_2–95% N_2-bicarbonate.

Results and Discussion. The effects of a number of compounds on anaerobic metabolism of glucose in rabbit lenses *in vitro* were screened and are shown in Table I. The polyhydric alcohols, ethylene glycol and glycerol, had no effect. Propionaldehyde, glyoxal, methylglyoxal, and glyoxylate reduced moderately lactic leakage or glucose utilization under the given conditions. Greatest inhibition came from DL-glyceraldehyde and glycolaldehyde.

Since glycolaldehyde was so inhibitory to metabolism, further study was made of the effects of this substance in the lens. In Fig. 2 are seen the results of an experiment involving incubation of five pairs of lenses, one member of each pair serving as the zero-time control for intralenticular lactate and acid-labile phosphate content and for glucose present initially in the medium; the contralateral lens of each pair was incubated anaerobically for 3 hr in rabbit Ringer's solution containing a particular concentration of glycolaldehyde, after which time the system

(lens and medium) was analyzed for lactate. High-energy phosphate in the incubated lenses and glucose in the medium were also measured. The loss of high-energy phosphate, the actual production of lactic acid and the utilization of glucose were calculated from the initial values and from the values after incubation. A 76% inhibition of actual lactic acid production and a 68% inhibition of glucose utilization were seen with 5 m*M* glycolaldehyde. At this concentration about 88% of the high-energy phosphate content of the lens was lost during the incubation period. The effects of glycolaldehyde on either lactic acid production or on high-energy phosphate content tended to level off at the higher concentration of this compound. This finding is suggestive of an ever-increasing "resistance" of metabolism to the inhibitor, as long as glucose utilization was more than sufficient (as it was for all concentrations presented in Fig. 2) to account for lactic acid production. Failure of the glycolaldehyde (at 5 m*M*) to inhibit metabolism completely possibly could be explained on the basis that some of the metabolic sites (nucleus?) in the lenses were relatively inaccessible to the inhibitor, but available to glucose.

Under the anaerobic conditions described here, in both control lenses and in lenses treated with glycolaldehyde (Fig. 2) the ratio of glucose utilized to lactate produced was much lower than the value of 1:2 expected from glycolysis. This low ratio may have resulted from glucose utilization by other pathways. van Heyningen (13) and Pirie (14), for example, showed that significant fractions of glucose are metabolized via the sorbitol and α-glycerophosphate pathways in the lens. In a manometric experiment involving four pairs of lenses we found that intact lenses in a CO_2-bicarbonate buffer utilized 9.3 μmoles of glucose and produced 17.3 μmoles of CO_2. Only 8.3 μmoles of the CO_2 resulted from lactic acid leaking into the medium and liberating CO_2. Since glucose is not stored as glycogen in the mammalian lens (15), we conclude from our experiment that only about 45% of the glucose used (8.3 μmoles lactic acid assumed equivalent to 4.15 μmoles glucose) was utilized via glycolysis, the remainder being lost via other means. On the assumptions that CO_2 arose from metabolism via the hexosemonophosphate (shunt) pathway and that a 6:1 CO_2–glucose ratio holds for the shunt pathway, we calculated that about 16% of the glucose was utilized anaerobically in this pathway. This value is close to the 14% found (16) for the rabbit lens under aerobic conditions. Some of the remaining glucose perhaps may have been metabolized to glycerophosphate. Furthermore, a portion of the initial glucose undoubtedly remained in the lenticular extracellular fluid.

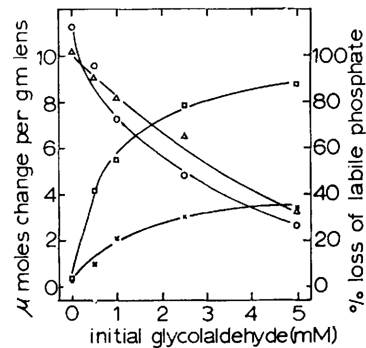

FIG. 2. Effects of varying concentrations of glycolaldehyde on glucose disappearance from the medium, lactic acid production, and high-energy (acid-labile) phosphate loss fom the lens. Rabbit Ringer's medium, pH 7.5; 5 m*M* glucose. Lenses were incubated for 3 hr in 5% CO_2–95% N_2 atmosphere. Values were obtained for each concentration of glycolaldehyde by calculation from paired lenses, one at zero time and one after 3 hr of incubation. ○ lactic acid formed per glens; Δ glucose disappeared per glens; × high-energy phosphate lost per glens; □ % high-energy phosphate lost.

Decreased glucose utilization and lactic acid production could be the result of an inhibitory effect of glycolaldehyde on transport of glucose or on metabolism of this sugar. That the effect was primarily on metabolism rather than on transport is seen in Table II. In six pairs of lenses it was noted that addition of glycolaldehyde to the incubating medium led to accumulation of glucose in the lens—a "backing up" of transported glucose which could not be metabolized. Use of the higher concentration of inhibitor led to higher accumulation of glucose, despite the fact

TABLE II. The Effects of Glycolaldehyde on Glucose Content and Lactic Acid Leakage from Intact Rabbit Lenses.[a]

Rabbit no.	Lens no.	Initial concn in medium (mM)	μmoles LA leaked to medium in 3 hr	% Decrease in LA leakage	μmoles glucose in lens	μmoles intracell. glucose per glens	μmoles excess intracell. glucose per g due to Gly
1	1	5 G	4.2	—	0.148	0.24	—
	2	5 G + 10 Gly	1.6	62	0.346	0.85	0.61
2	3	5 G	4.8	—	0.135	0.11	—
	4	5 G + 10 Gly	1.8	62	0.430	0.93	0.82
3	5	5 G	5.1	—	0.067	—0.075	—
	6	5 G + 10 Gly	2.0	61	0.359	0.76	0.84
Average							0.76
4	7	10 G	—	—	0.130	0.08	—
	8	10 G + 5 Gly	—	—	0.235	0.39	0.31
5	9	10 G	—	—	0.156	0.18	—
	10	10 G + 5 Gly	—	—	0.301	0.52	0.34
6	11	10 G	—	—	0.056	—0.13	—
	12	10 G + 5 Gly	—	—	0.229	0.35	0.48
Average							0.38

[a] In 3 hr of incubation weight changes in the lenses were minimal. Glucose was analyzed by means of a glucose oxidase method. Extracellular space of the lens was assumed to be 10%. It was assumed that glucose concentration in the medium and in the extracellular space of the lens were equal. G = glucose; Gly = glycolaldehyde.

that a lower glucose concentration was used at the higher inhibitor concentration (Table II). These findings are similar to those of Kern (1) who saw an accumulation of glucose in DL-glyceraldehyde-treated calf lenses.

Some of the compounds of Table I might have been poor inhibitors in intact lenses because the lenses might have been impermeable to these substances. Of particular interest in this regard were the less potent inhibitors, glycollate and glycoxylate. These oxidation products of glycolaldehyde carry a negative electric charge at pH 7.5. The relative values for uptake of these compounds and for glycolaldehyde are shown in Fig. 3. In 2 hr about 58% of the glycolaldehyde was taken up, whereas glyoxylate uptake was small and glycollate uptake was negligible. The toxic effects of glycolaldehyde apparently were not due entirely to conversion to glyoxylate, since the latter compound was not as inhibitory as glycolaldehyde in lens homogenates (Table III); nor was conversion of glycolaldehyde to glycollate responsible for the inhibitory effects of glycolaldehyde, since

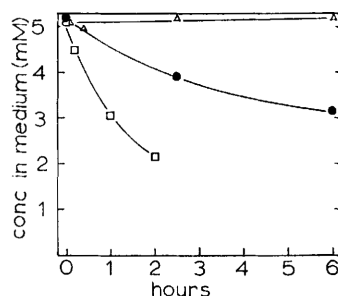

FIG. 3. Uptake of glycolaldehyde, glycollate, or glyoxylate by intact rabbit lenses. Medium as in Fig. 2, but volume equal to 0.8 ml. Initial concentration of test compounds 5 mM. □ glycolaldehyde (average of four experiments); ● glyoxylate (average of two experiments); Δ glycollate (average of two experiments).

the glycollate (Table III) had no effect on lactic acid production in homogenates.

Glyoxal and oxalate could possibly have been responsible for the inhibitory effects of glycolaldehyde on lenticular metabolism, but these compounds in other tissues are formed in negligible or minor amounts or cannot account (3, 17) for the marked inhibition by

TABLE III. Relative Inhibitory Effects of Glycolaldehyde, Glycollate, and Glyoxylate on Lactic Acid Production in Rabbit Lens Homogenates.[a]

Homogenate + additions	Lactic acid produced (% of control)
G-6-P (control)	100[b] (6)
G-6-P + glycolaldehyde	25.7 ± 4.4[c] (6)
G-6-P + glycollate	103.6 ± 11.3[c] (6)
G-6-P + glyoxylate	49.5 ± 4.8[c] (6)

[a] Numbers in parentheses refer to the number of homogenates used. Glucose-6-phosphate (G-6-P) concn: 5 m*M*. Concentration of test substance in each case: 20 m*M*. Incubation: 4 hr under 5% CO_2–95% N_2 at 37°. See Methods for additional details.

[b] Control value of lactic acid produced was 3.56 ± 0.16[c] (6).

[c] Mean ± standard error.

glycolaldehyde. A possibility is presently being investigated by us in which glycolaldehyde inhibits metabolism of glucose to lactic acid by inhibiting in the lens a Schiff base reaction similar to that involved in aldolase activity in other tissue (18).

Summary. Several short-chain aliphatic carbonyl compounds inhibited anaerobic metabolism in rabbit lenses. A very potent inhibitor, glycolaldehyde, inhibited lactic acid production and glucose utilization and lowered labile phosphate in the lens. Glycolaldehyde had no appreciable effect on the transport of glucose into the lenses. The effect of glycolaldehyde may have been a direct one or may have occurred through its metabolism to inhibitory oxidation products in the lenses. Glyoxylate and glycollate could not account completely for the inhibitory effects of glycolaldehyde in either intact lenses or in lens homogenates.

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