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THE EFFECT OF GLYCERALDEHYDE ON GLUCOSE METABOLISM IN EHRLICH ASCITES TUMOR CELLS*

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Abstract—The addition of D, L-glyceraldehyde to Ehrlich ascites tumor cells resulted in a stimulation of glucose-1-^{14}C conversion to $^{14}CO_2$, a decrease in glucose-6-^{14}C conversion to $^{14}CO_2$, a decrease in total glucose consumption, and a depression of lactate production. These changes were associated with decreased steady-state levels of ATP and increased levels of glucose 6-phosphate. Aldose reductase activity was demonstrated in Ehrlich ascites tumor cells and a mechanism proposed for the stimulation of $^{14}CO_2$ production from glucose-1-^{14}C by glyceraldehyde, based on glyceraldehyde acting as a substrate for aldose reductase.

ALTHOUGH it has been known since 1929 that DL-glyceraldehyde inhibits the production of lactate from glucose in some experimental tumors and normal tissues,[1] the mechanism of this action is still not definitively known. In recent years many investigators have clarified certain aspects of the problem, and the following information has been generally accepted. (1) L-Glyceraldehyde results in an inhibition of glucose phosphorylation by hexokinase.[2–4] The inhibition is not a direct one, but one in which L-glyceraldehyde condenses with dihydroxyacetonephosphate in the presence of aldolase to yield L-sorbose 1-phosphate which directly inhibits hexokinase.[4] (2) D-Glyceraldehyde inhibits glycolysis via the inhibition of triosephosphate dehydrogenase.[3–5] Moreover, Warburg has demonstrated the oncolytic activity *in vivo* of both L- and D-glyceraldehyde in experimental tumors of mice.[6]

It has been shown that the net inhibitory influence of DL-glyceraldehyde on glycolysis in tissues and extracts depends upon more than one mechanism and may vary with the conditions of the experimental design.[5] Various investigators have used tissue slices, homogenates, subcellular fractions, and enzyme extracts in their studies, and these differences in design make any comparison of results difficult. The differences include the concentration of DL-glyceraldehyde,[4, 5] the concentration of glucose in the case of hexokinase inhibition,[2, 5] and the concentration of triosephosphates in the case of triosephosphate dehydrogenase inhibition.[5]

In this communication data are presented which show the effects of D- and L-glyceraldehyde on some parameters of glucose metabolism in Ehrlich ascites tumor cells. It was ascertained that D- and L-glyceraldehyde stimulated the conversion of glucose-1-^{14}C to $^{14}CO_2$, whereas it inhibited the aerobic conversion of glucose-6-^{14}C to $^{14}CO_2$, and the conversion of glucose to lactate. Both D- and L-glyceraldehyde were found to serve as substrates for aldose reductase, and this observation has been used

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as the basis of an explanation for the mechanism of action of these optical antipods on glucose metabolism in Ehrlich ascites tumor cells.

EXPERIMENTAL PROCEDURES

Preparation of cells and enzymes. The Ehrlich ascites tumor cells were maintained in Swiss Webster mice by weekly peritoneal transfers. Mice with ascites 6–8 days old were sacrificed by cervical dislocation and the ascites fluid removed from the peritoneum. The cells were washed three times and then resuspended in calcium-free Krebs–Ringer phosphate buffer, pH 7·4,[7] to the desired protein concentrations.

Homogenates of Ehrlich ascites tumor cells were prepared in a ground-glass homogenizer at 0° for 1 min in 2 volumes of calcium-free Krebs–Ringer phosphate buffer, pH 7·4, and filtered through a double layer of cheesecloth. The whole homogenate was used immediately after preparation.

Partial purification of aldose reductase activity from Ehrlich ascites tumor cells was carried out by a slight modification of the procedure of Hayman and Kinoshita.[8] The cells were homogenized in 2 volumes of cold 0·0001 M sodium phosphate buffer, pH 7·0, and centrifuged at 10,000 *g* for 15 min. The precipitate was discarded, and the supernatant was brought to 50% saturation with solid ammonium sulfate which was added with stirring over a 15-min period. The mixture was centrifuged for 15 min at 15,000 *g* and the precipitate again discarded. Aldose reductase activity was precipitated from the supernatant at 75% ammonium sulfate saturation. The precipitate was taken up in a few milliliters of 0·05 M NaCl. The initial homogenate of Ehrlich ascites tumor cells used in the enzyme preparation had 5·13 g of biuret protein. The homogenate contained a total of 900 units of aldose reductase activity with a specific activity of 0·18 unit/mg protein/min. The purified enzyme preparation had a specific activity of 0·78, and 728 units of total activity were recovered. A unit of enzyme activity is based on the decrease in absorption of NADPH at 340 mμ. One unit is that amount of enzyme activity producing an optical density change of 0·001/min.

Assays. The conversion of D-glucose-1-^{14}C and D-glucose-6-^{14}C to $^{14}CO_2$ was assayed by the collection of $^{14}CO_2$ according to the procedure of Snyder and Godfrey.[9] Reactions were carried out at 30° with shaking in 25-ml Warburg flasks, which were closed with tight-fitting rubber serum stoppers. Labeled substrate and medium were added and a removable glass vessel placed on the center well. The serum stopper was then used to seal the flask and the reaction started by the injection of the homogenate or cells through the serum stopper. Glucose oxidation was assayed by the collection of the $^{14}CO_2$ derived from the oxidation of the radiolabeled substrate. At the end of the incubation period, 0·2 ml of 4 N H_2SO_4 was injected into the reaction mixture. The collection of $^{14}CO_2$ for subsequent liquid scintillation counting in a Packard spectrometer was accomplished by the injection of 0·2 ml of Hyamine (*p*-diisobutyl-eresoxyethoxyethyldimethyl-benzyl ammonium hydroxide in methanol) into the removable glass vial after the reaction has been terminated. The $^{14}CO_2$ was trapped in Hyamine by shaking the flask in a water bath at 30° for 1 hr. Control flasks containing no homogenate were treated exactly as the experimental flasks. Radioactive counting was done in 10 ml of a phosphor solution of *p*-dioxane:anisole:1,2-dimethoxyethane (6:1:1) containing 2,5-diphenyloxazole (PPO), 6 g/l. Recoveries of $^{14}CO_2$ by this procedure ranged from 95 to 100 per cent.

Aldose reductase activity was assayed by following the oxidation of NADPH by DL-glyceraldehyde according to the procedure of Hayman and Kinoshita.[8] Lactic acid assays were carried out enzymatically by the procedure of Hohorst which is based on the oxidation of lactate by NAD^+ in the presence of lactic dehydrogenase.[10] ATP was assayed enzymatically by the method of Adam, which is based on the conversion of 3-phosphoglycerate to 1,3-diphosphoglycerate in the presence of ATP and phosphoglycerol kinase.[11] Glucose 6-phosphate was assayed enzymatically by the glucose 6-phosphate dehydrogenase assay of Horecker and Wood.[12] Glucose was determined by the glucose oxidase procedure of Teller.[13] Protein determinations were done by the biuret method.[14]

Substrates and chemicals. D- and L-Glyceraldehyde were purchased from Calbiochem and purified by chromatography on Whatman 3 MM paper in a system of *n*-butanol: acetic acid:water (4:1:5).[15] The D- and L-glyceraldehyde aqueous solutions were freshly made every week. In some experiments the frozen aqueous solutions were concentrated under vacuum and heated to 80° for 30 min to convert any dimer that might have formed during the period of storage, back to the monomeric state.[16]

D-Glucose-1-^{14}C and D-glucose-6-^{14}C were purchased from New England Nuclear Corp. and purified according to the method of Merlevede *et al.*[17]

Glucose oxidase, lactic acid dehydrogenase, and glucose 6-phosphate dehydrogenase were obtained from Sigma Chemical Co. Phosphoglycerol kinase was obtained from Boehringer. All other chemicals were purchased from commercial sources.

RESULTS

The effect of D, L *and* DL-*glyceraldehyde on the conversion of* D-*glucose*-1-^{14}C *to* $^{14}CO_2$

The addition of D, L or DL-glyceraldehyde stimulated the conversion of glucose-1-^{14}C to $^{14}CO_2$. This is shown in Table 1 and Fig. 1. D-Glyceraldehyde resulted in a more

TABLE 1. THE EFFECT OF D- AND L-GLYCERALDEHYDE ON THE PRODUCTION OF $^{14}CO_2$ FROM D-GLUCOSE-1-^{14}C

Experiment	$^{14}CO_2$ (mμmoles/mg protein)
1. Control	7·6
2. D-Glyceraldehyde	14·1
3. L-Glyceraldehyde	10·8

The reaction mixtures contained 4 μmoles D-glucose-1-^{14}C (92,000 counts/min), Ehrlich ascites tumor cells in 0·7 ml of calcium-free Krebs–Ringer phosphate buffer, pH 7·4 (10·5 mg of Ehrlich ascites tumor cells protein), and 10 μmoles of D- or L-glyceraldehyde where indicated. Final reaction volumes were 1 ml, and incubations were carried out at 30° for 30 min.

marked stimulation than L-glyceraldehyde, whereas the DL-mixture effected an intermediate stimulation (cf. Fig. 1). The stimulatory effect of glyceraldehyde on the conversion of glucose-1-^{14}C to $^{14}CO_2$ was noted over a wide range of glucose (3–100 mM), glyceraldehyde (5–400 mM), and Ehrlich ascites tumor cells protein concentrations (3–50 mg protein). The course of the reaction was linear with time for 30 min (cf. Fig. 1). The conversion of glucose-1-^{14}C to $^{14}CO_2$ could have resulted from the metabolism of glucose via aerobic glycolysis or by the hexosemonophosphate shunt or both.

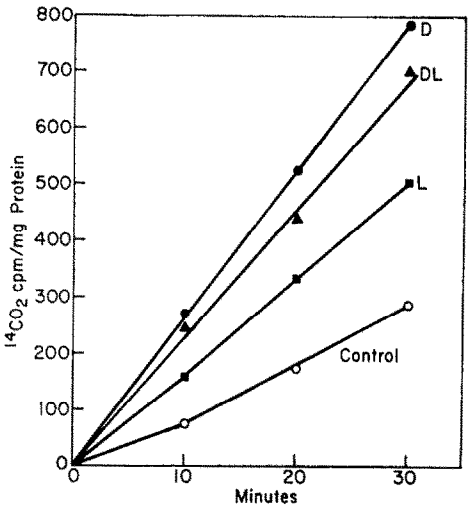

FIG. 1. The effect of D,L,DL-glyceraldehyde on the conversion of D-glucose-1-^{14}C to $^{14}CO_2$ by Ehrlich ascites tumor cells. The reaction mixtures contained 10 μmoles D-glucose-1-^{14}C (200,000 counts/min), Ehrlich ascites tumor cells in 1 ml of calcium-free Krebs–Ringer phosphate buffer, pH 7·4 (9·4 mg Ehrlich ascites tumor cells protein), and 3 μmoles of D,L or DL-glyceraldehyde were indicated. Final reaction volumes were 1·3 ml, and incubations were carried out at 30° for the indicated times.

The effect of D, L-*glyceraldehyde on the conversion of* D-*glucose*-6-^{14}C *to* $^{14}CO_2$

The addition of either D- or L-glyceraldehyde resulted in a decreased conversion of glucose-6-^{14}C to $^{14}CO_2$, as shown in Table 2. The inhibitory effect was noted over a

TABLE 2. THE EFFECT OF D- AND L-GLYCERALDEHYDE ON THE CONVERSION OF D-GLUCOSE-6-^{14}C TO $^{14}CO_2$

Experiment	$^{14}CO_2$ (mμmoles/mg protein)
1. Control	8·3
2. D-Glyceraldehyde	5·2
3. L-Glyceraldehyde	4·6

The reaction mixtures contained 5 μmoles D-glucose-6-^{14}C (158,000 counts/min) Ehrlich ascites tumor cells in 0·7 ml of calcium-free Krebs–Ringer phosphate buffer, pH 7·4 (12·8 mg of Ehrlich ascites tumor cells protein), and 10 μmoles of D- or L-glyceraldehyde where indicated. Final reaction volumes were 1 ml, and incubations were carried out at 30° for 30 min.

range of glucose (5–20 mM), glyceraldehyde (10–25 mM), and Ehrlich ascites tumor cells protein concentrations (8–20 mg protein).

The effect of D, L-*glyceraldehyde on metabolism of glucose by Ehrlich ascites tumor cells*

The addition of either D- or L-glyceraldehyde resulted in a marked depression to total glucose consumption and in conversion of glucose to lactate. These decreases of glucose uptake and glycolysis were accompanied by a depression of the steady-state levels of ATP as shown in Table 3. The effect of glyceraldehyde on the inhibition of the conversion of glucose to lactate has been known since the work of Mendel in 1929.[1]

These data and the data shown in Table 2 make it likely that the stimulatory effect of glyceraldehyde on the conversion of glucose-1-^{14}C to $^{14}CO_2$ is via some influence on the phosphogluconate oxidative pathway (hexosemonophosphate shunt; cf. Table 1, rather than on glycolysis which it decreases.

TABLE 3. THE EFFECT OF D- AND L-GLYCERALDEHYDE ON GLUCOSE CONSUMPTION, LACTATE PRODUCTION AND ATP LEVELS

Experiment	Glucose consumed	Lactate produced (mμmoles/mg protein)	ATP
1. Control	208	135	17·2
2. D-Glyceraldehyde	43	63	9·4
3. L-Glyceraldehyde	44	45	11.2

The reaction mixtures contained 33 μmoles D-glucose, Ehrlich ascites tumor cells in 2·3 ml of calcium-free Krebs–Ringer phosphate buffer, pH 7·4 (50 mg Ehrlich ascites tumor cells protein), and 100 μmoles of D- or L-glyceraldehyde where indicated. Final reaction volumes were 2·5 ml, and incubations were carried out at 30° for 30 min.

The effect of glyceraldehyde on the steady-state levels of glucose 6-phosphate in Ehrlich ascites tumor cells is shown in Table 4. The addition of D- or L-glyceraldehyde

TABLE 4. THE EFFECT OF D- AND L-GLYCERALDEHYDE ON GLUCOSE 6-PHOSPHATE LEVELS

Experiment	Glucose 6-phosphate (mμmoles/mg protein)
1. Control	5·6
2. D-Glyceraldehyde	8·7
3. L-Glyceraldehyde	9·9

The reaction mixtures contained 300 μmoles of D-glucose, Ehrlich ascites tumor cells in 2·3 ml of calcium-free Krebs–Ringer phosphate buffer, pH 7·4 (43 mg Ehrlich ascites tumor cells protein), and 1 m-mole of D- or L-glyceraldehyde where indicated. Final reaction volumes were 2·7 ml, and incubations were carried out at 30° for 30 min.

resulted in elevated glucose 6-phosphate levels. Glyceraldehyde increased the glucose 6-phosphate levels despite the decrease in glucose uptake and in conversion of glucose to lactate (cf. Table 3). This suggests that glyceraldehyde may have inhibitory effects both on the initial phosphorylation of glucose and at some other site prior to the formation of lactate. These inhibitory sites have been previously demonstrated and could account for the elevated glucose 6-phosphate levels.[3]

The experiments shown in Tables 1–4 are representative. They were repeated in duplicate at least three times and showed similar magnitudes and the same direction of change.

The effect of $NADPH^+$ *on the conversion of* D-*glucose*-1-^{14}C *to* $^{14}CO_2$

$NADP^+$ is a cofactor for glucose 6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase, two important initial enzymatic steps in the phosphogluconate oxidative pathway of glucose metabolism. This pathway affords a route of total combustion of glucose to CO_2, independent of the citric acid cycle.[18] $NADP^+$ was added

to homogenates of Ehrlich ascites tumor cells to see if it would stimulate the conversion of glucose-1-^{14}C to $^{14}CO_2$ as it has been shown to do in other tissues.[18] Figure 2 shows the stimulatory effect of the added $NADP^+$ on the production of $^{14}CO_2$ from glucose-1-^{14}C. This stimulatory effect was found over a range of $NADP^+$ concentrations (0·5–5·0 mM), and suggested the presence of an operative phosphogluconate oxidative pathway in the Ehrlich ascites tumor cells.

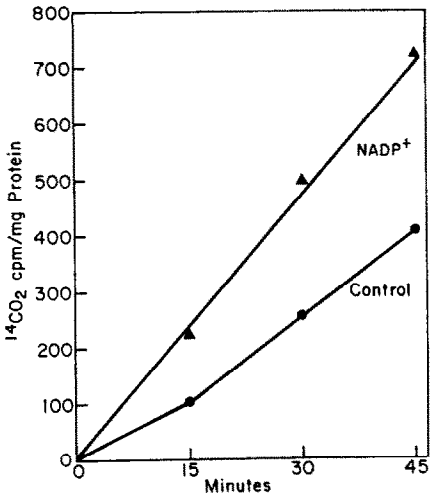

FIG. 2. The effect of $NADP^+$ on the conversion of D-glucose-1-^{14}C to $^{14}CO_2$. The reaction mixtures contained 10 μmoles D-glucose-1-^{14}C (400,000 counts/min), 6·5 mg of Ehrlich ascites tumor cells protein homogenate in 1 ml of calcium-free Krebs–Ringer phosphate buffer, pH 7·4, and 3 μmoles of $NADP^+$ where indicated. Final reaction volumes were 1·5 ml, and incubations were carried out at 30° for the indicated periods of time.

Demonstration of aldose reductase activity in Ehrlich ascites tumor cells

Landau and Merlevede have shown that an initial reaction of glyceraldehyde metabolism in the liver involves reduction of the glyceraldehyde to glycerol.[15] Metabolism

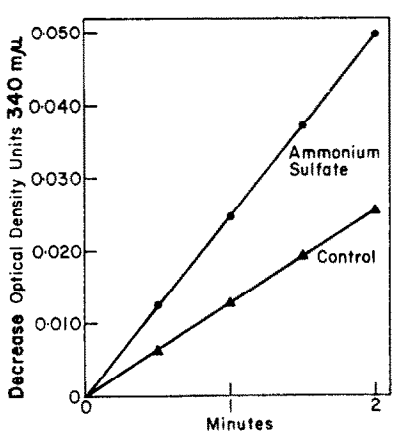

FIG. 3. The stimulation of aldose reductase activity from Ehrlich ascites tumor cells by sulfate. The reactions were carried out as in Table 5 except for the use of 0·5 μmole D-glyceraldehyde as the substrate in all reactions and the addition of 30 μmoles ammonium sulfate where indicated.

of D- and L-glyceraldehyde via aldose reductase was suggested as a possible pathway for this initial reductive step. Aldose reductase utilizes both D- and L-glyceraldehyde as substrates and requires NADPH as a cofactor.[8] Aldose reductase was purified from Ehrlich ascites tumor cells, and the active preparations obtained were further characterized by the stimulatory effect of added sulfate, as shown in Fig. 3.[8, 19]

The aldose reductase preparations were assayed with a series of aldoses previously reported as substrates for the enzyme.[8, 19] Methyl gloyoxal was included in the testing because of its structural similarity to glyceraldehyde and its reported oncolytic activity.[20] The capacity of the compounds tested to serve as substrates for aldose reductase is shown in Table 5. It was of interest that D- and L-glyceraldehyde and methyl glyoxal,

TABLE 5. ALDOSE REDUCTASE ACTIVITY WITH VARIOUS ALDOSE SUBSTRATES

Experiment	Aldose reductase activity (units/min)
1. D-Glyceraldehyde	94
2. L-Glyceraldehyde	58
3. Methyl glyoxal	67
4. L-Arabinose	13
5. D-Xylose	11
6. D-Ribose	8
7. D-Glucose	2

The reaction mixtures contained 67 μmoles potassium phosphate buffer, pH 6·2, 0·05 μmole NADPH, 0·5 μmole of the indicated aldose substrate, and 25 units of aldose reductase activity in 1 ml of 0·0001 potassium phosphate buffer, pH 6·5. Final reaction volumes were 1·2 ml, and reactions were followed at 350 mμ in a Zeiss PMQ 11 spectrophotometer at 30°. Initial velocities were linear for the first 3 min.

which served best as substrates for aldose reductase, have all been reported to be antitumor agents.[1, 6, 20]

The effect of various aldoses on the conversion of glucose-1-^{14}*C to* $^{14}CO_2$

In order to ascertain whether the capacity of glyceraldehyde to serve as substrate for aldose reductase and thereby help to catalyze the production of $NADP^+$ from NADPH, was related to its stimulatory effect on the conversion of glucose-1-^{14}C to $^{14}CO_2$ (cf. Table 1 and Fig. 2), other aldoses were tested. The data of Table 6 show that all the aldoses that served as substrates for aldose reductase resulted in a stimulation of the conversion of glucose-1-^{14}C to $^{14}CO_2$ by Ehrlich ascites tumor cells. Here

TABLE 6. THE EFFECT OF ALDOSES ON THE PRODUCTION OF $^{14}CO_2$ FROM D-GLUCOSE-1-^{14}C

Experiment	$^{14}CO_2$ (mμmoles/mg protein)
1. Control	6·4
2. D-Xylose	9·5
3. L-Arabinose	9·2
4. D-Ribose	10·1
5. Methyl glyoxal	34·7

The reaction mixtures contained 5 μmoles D-glucose-1-^{14}C (100,000 counts/min), Ehrlich ascites tumor cells in 0·7 ml of calcium-free Krebs–Ringer phosphate buffer, pH 7·4 (15 mg Ehrlich ascites tumor cells protein), and 10 μmoles of aldose where indicated. Final reaction volumes were 1 ml, and incubations were carried out at 30° for 30 min.

again, methyl glyoxal, which served as one of the most rapidly utilized substrates by aldose reductase (cf. Table 5), caused the greatest stimulation of $^{14}CO_2$ production from glucose-1-^{14}C.

DISCUSSION

Cancer cells produce large amounts of lactic acid from glucose even in the presence of oxygen. Cancer chemotherapy based on inhibition of the phosphorylation of glucose (hexokinase reaction) at substrate, coenzyme, and enzyme levels, has been under investigation by a number of groups.[21] Glyceraldehyde has been found to be a potent inhibitor of glycolysis in some normal tissues and tumors. Warburg has recently found that sublethal doses of D- or L-glyceraldehyde resulted in complete regression of Ehrlich ascites tumors in mice.[6] Because of the possibility that the effect of glyceraldehyde on glucose metabolism in tumor cells might afford a basis for antitumor therapy, investigators have studied the mechanism of action of glyceraldehyde on glycolysis. Lardy *et al.*[4] found that the inhibition of glycolysis in beef brain preparations by glyceraldehyde depended upon the condensation of L-glyceraldehyde with dihydroxyacetone phosphate to form L-sorbose 1-phosphate, which in turn inhibited hexokinase. These workers showed that only the L-form of glyceraldehyde was effective.

D-Glyceraldehyde also undergoes a condensation with dihydroxyacetone phosphate to form D-fructose 1-phosphate.[4] This compound is capable of inhibiting hexokinase only if it is converted to glucose 6-phosphate, which serves to inhibit glucose phosphorylation through a noncompetitive inhibition of hexokinase.[22] The control of hexokinase activity in cells is complex, and steady-state levels of glucose 6-phosphate may have very different effects depending on the modulating activities of inorganic phosphate,[23] ADP,[24] and metabolically related compounds such as 6-phosphogluconate and mannose 6-phosphate.[25] The decrease in glucose uptake found in our studies, and the decreased hexokinase activity found by others with DL and L-glyceraldehyde, made it unlikely that the levels of glucose 6-phosphate would be elevated in Ehrlich ascites tumor cells. However, Needham and co-workers have found that D-glyceraldehyde inhibits triosephosphate dehydrogenase, and this would explain the increased glucose 6-phosphate levels found, because the enzymatic block would impair the conversion of glucose 6-phosphate to lactate.[3]

Our data show that D- and L-glyceraldehyde both inhibit glucose consumption by Ehrlich ascites tumor cells and impair the conversion of glucose to lactate and of glucose-6-^{14}C to $^{14}CO_2$. This inhibition of glucose metabolism is associated with decreased steady-state levels of ATP and increased levels of glucose 6-phosphate.

D- and L-Glyceraldehyde have also been shown to result in a stimulation of the conversion of glucose-1-^{14}C to $^{14}CO_2$ in Ehrlich ascites tumor cells. The augmentation of glucose oxidation probably occurs via increased activity of the oxidative phosphogluconate pathway. This pathway is stimulated by $NADP^+$, and the conversion of glucose-1-^{14}C to $^{14}CO_2$ in homogenates of Ehrlich ascites tumor cells was shown to be increased by the addition of $NADP^+$.

Both D- and L-glyceraldehyde served as substrates for aldose reductase, which was demonstrated to be present in Ehrlich ascites tumor cell extracts. The enzyme catalyzes the reduction of aldoses by NADPH and generates $NADP^+$, which could stimulate the oxidative phosphogluconate pathway of glucose metabolism. This occurred when a number of aldoses which were found to serve as substrates for aldose reductase also

effected a stimulation of the conversion of glucose-1-^{14}C to $^{14}CO_2$. This scheme is shown in Fig. 4.

The possibility that the capacity to serve as a substrate for aldose reductase and the oncolytic activity of a compound are related, was suggested by the behavior of methyl glycoxal, a known oncolytic agent, as a substrate for aldose reductase and as a stimulator of the phosphogluconate pathway of glucose metabolism.

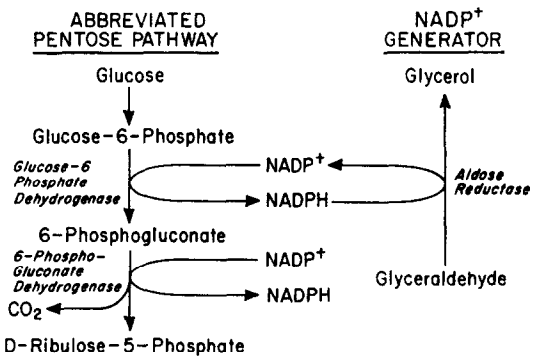

FIG. 4. Glyceraldehyde metabolism and the hexose monophosphate shunt.

The effects of D- and L-glyceraldehyde on glucose metabolism in Ehrlich ascites tumor cells are probably manifest at a number of sites and depend on more than one mechanism. The effects of glyceraldehyde on the depression of amino acid uptake into normal and neoplastic tissues are independent of its characteristic action on glycolysis[26] and are associated with the uncoupling of oxidative phosphorylation.[27] The literature on glyceraldehyde permits one to conclude that, whereas in whole-cell preparations or homogenates both D- and L-glyceraldehyde may impair glucose metabolism, they must do so by different pathways. The inhibitory effect of the L-isomer is manifest at the hexokinase site, whereas the D-isomer exerts its effects both at the hexokinase site and, probably more importantly, at the triosephosphate dehydrogenase site.

The inhibitory effects of glyceraldehyde decrease the utilization of glucose for energy production by Ehrlich ascites tumor cells. The increased activity of the oxidative phosphogluconate pathway could further impair the yield of energy from glucose, because glycolysis yields more energy to the cell than the oxidative phosphogluconate pathway.[28] The combination of decreased glucose uptake and depressed glycolysis in association with a shunting of glucose to a less energy-producing pathway of metabolism may in part be the basis for the oncolytic activity of both D- and L-glyceraldehyde. In our studies the combination of the decreased glucose consumption and increased activity of the phosphogluconate pathway were sufficient to account for the decrease in glycolysis.

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