The Effects of 2-Deoxyglucose on the Growth and Metabolism of Cultured Human Cells*

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The glucose analogue, 2-deoxy-d-glucose, has been found to inhibit glycolysis and the growth of yeast (1, 2) and various animal tissues (3-7). It is phosphorylated by hexokinase (8), but is not believed to be further metabolized. Wick et al. (9) have reported that deoxyglucose 6-phosphate competitively inhibits the conversion of glucose 6-phosphate to fructose 6-phosphate by a purified phosphoglucomutase from kidney. Recently Kipnis and Cori (10) have presented evidence that deoxyglucose 6-phosphate noncompetitively blocks the transport of glucose. It has also been suggested that the phosphate inhibits glycolysis at the hexokinase level (9).

The experiments described in this paper show that deoxyglucose inhibits the growth of human cell cultures by inhibiting the utilization of glucose. They also indicate the sites of inhibition of glucose utilization in the glycolytic and oxidative pathways. The implications of these findings on the possible mode of action of deoxyglucose are discussed.

EXPERIMENTAL PROCEDURE

Two human cell lines were used in the present studies, the HeLa, derived from a human carcinoma, and a culture of intestine from normal embryonic human tissue. The cells were grown both in monolayers, adherent to glass, and in suspension. The composition of the medium for the stationary cultures has been described previously (11) as have methods for cultivation and assay of growth (12). The growth in suspension was carried out as described by McLimans et al. (13), with the use of Eagle's basal medium (18) supplemented with 5% dialyzed human serum.

Preparation of Cell-free Extracts—Cells grown in suspension were collected by centrifugation, washed twice with Krebs-Ringer salt solution, resuspended in 4 volumes of cold distilled water, and disrupted by sonic vibration in a 10-ke oscillator at 2 to 5°C for 10 to 15 minutes. Cellular debris was removed by centrifugation at 20,000 × g for 15 minutes. The resulting cell-free extracts contained from 5 to 10 mg of protein per ml as measured by a modification of the colorimetric method of Lowry (12). For assays of hexokinase activity the uncentrifuged crude extract was employed.

Analytical Methods—The procedures for measurement of growth, residual carbohydrate, and the amount of lactic acid produced have been previously described (14). Deoxyglucose was measured by the colorimetric method of Ball and Sanders with phenylhydrazine reagent. The other sugars employed did not interfere in this test. Glucose was analyzed by the method of Park and Johnson (15). In mixtures of a natural sugar, e.g. glucose and deoxyglucose, the sugar concentration was taken as the difference between the total reducing sugar and the independently assayed deoxyglucose, it having been established that the two sugars were additive in the colorimetric method employed. Orthophosphate was estimated by the method of Fiske and SubbaRow (16).

Hexokinase activity in the crude extracts was measured by (a) the disappearance of sugar in aliquots of the reaction mixture after purification of protein and phosphorylated sugars with Ba(OH)2 and ZnSO4 (17), and (b) the decrease in acid labile phosphate in aliquots of the reaction mixture (8). Glucose 6-phosphate dehydrogenase, phosphogluconate dehydrogenase, lactate dehydrogenase, and isocitrate dehydrogenase were measured by direct spectrophotometric assays.

Phosphorylated carbohydrate intermediates were isolated from the acid-soluble pool, after removal of nucleotides with charcoal (19), by precipitation with barium acetate (1 ml) and 4 volumes of ethanol overnight at 4°C. The dried barium precipitates were dissolved in 0.1 N HCl and converted to the potassium salts by addition of K2SO4 (1 ml). Chromatographic separation of the compounds was carried out with n-amyl acetate-glacial acetic acid-water (3:3:1) as solvent for 48 to 72 hours on Whatman No. 1 paper. The hexose phosphates were identified by internal standards.

Materials—Deoxyglucose was obtained from the Aldrich Chemical Company; deoxyglucose-6-P, TPN, DPN, and glucose-6-P from the Sigma Chemical Company; fructose-6-P and purified glucose 6-phosphate dehydrogenase from C. F. Boehringer and Sons, Germany. Glucose, fructose, and mannose were obtained from the Nutritional Biochemicals Corporation. The purity of the compounds was checked chromatographically.

RESULTS

Effects on Growth—At equimolar concentrations of deoxyglucose and glucose (5 mM), growth of both cell lines was markedly inhibited (Fig. 1) with microscopically visible effects after the second and third day. As shown in Fig. 2, glucose utilization and lactic acid production were inhibited concomitantly. Deoxyglucose did not, however, irreversibly destroy the ability of the cells to multiply. Even after 2 and 3 days of contact with the inhibitor, growth was restored simply by washing the

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The inhibitory effect of 2-deoxyglucose on the growth of HeLa cell cultures. - - - -, growth in the presence of equimolar (5 mM) concentrations of glucose and deoxyglucose. At the indicated times (arrows) the deoxyglucose containing medium was removed, the cells washed with balanced salt solution, and replaced with deoxyglucose-free medium.

The effect of 2-deoxyglucose on growth, glucose utilization, and lactic acid formation of suspension cultures of HeLa cells. ○, Δ, □, cultures growing in the presence of equimolar concentrations of glucose and deoxyglucose (5 mM); ●, △, ■ cultures growing on glucose (5 mM), no deoxyglucose.

The finding that deoxyglucose is phosphorylated to deoxyglucose-6-P by yeast hexokinase (1) was confirmed for the HeLa and intestine cell cultures. The ester also inhibited HeLa cell growth, although it was not as active as deoxyglucose itself (Fig. 3). This difference may reflect a decreased rate of transport of the phosphorylated compound; alternatively, the rate of dephosphorylation before transport may be limiting. Glucose-6-P was not as effective as glucose in reversing the inhibitory effects of deoxyglucose-6-P. When the inhibited cells were placed in a medium containing glucose-6-P, there was a 3-day lag before growth was resumed, as contrasted with the immediate resumption of growth when cells were placed in a glucose-containing medium.

Since deoxyglucose-6-P has been implicated as a metabolic inhibitor of hexose transport and phosphohexoseisomerase (9), its effect was also examined in HeLa cell-free extracts.

The inhibitory effect of 2-deoxyglucose could be competitively reversed by additional substrate, either glucose or mannose. However, fructose-grown cells required 5 to 10 times more fructose than either glucose or mannose to reverse the inhibitory effect of deoxyglucose.

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Effect of Deoxyglucose and Deoxyglucose-6-P on HeLa Hexokinase—Although it has been suggested that deoxyglucose or deoxyglucose-6-P inhibits hexokinase (9), this was not observed in HeLa cells even at a molar ratio of inhibitor to glucose of 10:1. In contrast, deoxyglucose actively inhibited the phosphorylation of fructose (Table I). These results suggest that deoxyglucose competes successfully with fructose for the active sites on HeLa cell hexokinase, a nonspecific hexokinase, but not with glucose. It is important to note that although deoxyglucose did not inhibit glucose phosphorylation, the latter inhibited the phosphorylation of deoxyglucose.

When HeLa extracts were incubated with fructose-6-P, it was found that deoxyglucose-6-P inhibited the reduction of TPN in the spectrophotometric assay. Since the extracts contained phosphohexoseisomerase, glucose-6-P dehydrogenase, and 6-phosphogluconic dehydrogenase, it was not possible to delineate the step or steps which deoxyglucose-6-P was inhibiting. The various reactions were therefore examined independently.

Effect of Deoxyglucose-6-P on Phosphohexoseisomerase—In an assay system based on the disappearance of fructose-6-P (25) it was found that deoxyglucose-6-P inhibited the formation of glucose-6-P by HeLa enzymatic preparations. At a ratio of deoxyglucose-6-P to fructose-6-P of 1:1 and 2:1, the degree of inhibition was, respectively, 38 and 64%.

Effect of Deoxyglucose-6-P on Glucose-6-P Dehydrogenase—As shown in Fig. 4, deoxyglucose-6-P inhibited the conversion of glucose-6-P to 6-phosphogluconate, as measured by its effect on TPN reduction. That the inhibitory effect was not on the subsequent TPN-dependent reaction, 6-phosphogluconic dehydrogenase, was shown by the lack of effect of deoxyglucose-6-P when 6-phosphogluconate was the substrate. Deoxyglucose had no effect unless the extracts were preincubated with it before the assay, i.e., presumably until it was phosphorylated. For reasons which are not yet clear, preincubation of enzyme preparations with deoxyglucose-6-P for periods up to 1/2 hour on hanced the inhibitory effects. The inhibition of glucose-6-P dehydrogenase was not competitive. At a given deoxyglucose-6-P concentration, the inhibition of TPN reduction was only partially reversed with increased concentration of substrate or TPN.

It is of interest that deoxyglucose-6-P also inhibited a purified preparation of glucose-6-phosphate dehydrogenase from yeast. However, the inhibition in this instance was only 60% of that obtained with the HeLa enzyme preparation under the same conditions.

Miscellaneous Effects—Contrary to previous reports (8-10), deoxyglucose-6-P was itself oxidized by the HeLa extracts, although at a much slower rate than glucose-6-P (cf. Fig. 4). This oxidation was specifically dependent on TPN, and DPN had no effect. Studies are in progress to determine the metabolic products of this oxidation. That deoxyglucose-6-P did not generally inhibit TPN-dependent enzymatic reactions was evidenced by the fact that it had no effect on the isocitric dehydrogenase and 6-phosphogluconic dehydrogenase reactions. In addition, deoxyglucose or deoxyglucose-6-P had no effect on the lactic dehydrogenase reaction.

**DISCUSSION**

In mammalian cell cultures, deoxyglucose and deoxyglucose 6-P act at various loci in the inhibition of carbohydrate metabolism and of growth. The present results are at variance with the hypothesis that deoxyglucose competes with glucose in the hexokinase reactions (9). At molar ratios of deoxyglucose and deoxyglucose-6-P to glucose of 10:1, neither compound inhibited phosphorylation of glucose. Kipnis and Cori (10) were also unable to inhibit glucose phosphorylation by rat muscle hexokinase with deoxyglucose-6-P. In contrast, the phosphorylation of fructose was inhibited by deoxyglucose. These results would be expected, since glucose has a greater affinity for hexokinase than does deoxyglucose, and fructose a lesser affinity (8).

In confirmation of Wieland et al., deoxyglucose-6-P acts by blocking the phosphohexoseisomerase reaction in the reverse direction (9). The present data further indicate that the forward reaction, fructose-6-P to glucose-6-P, is also inhibited. The latter effect, since the equilibrium of the reaction is in the forward direction, would clearly contribute to the inhibitory effect of deoxyglucose on cell metabolism.

The finding that deoxyglucose-6-P can directly affect the glucose-6-P dehydrogenase reaction in mammalian cells, in contrast to the enzyme of Leuconostoc mesenteroides (21), provides another possible mechanism for its inhibitory effect on carbohydrate metabolism. There is now ample evidence that in HeLa cells, a significant although not major portion of glucose is ox-
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dized via the hexose monophosphate shunt.2 Hiatt (20) has recently shown that nucleic acid ribose produced by the HeLa cell is synthesized in part by the oxidative pathway. The finding that glucose-6-P accumulates in cultures growing in the presence of deoxyglucose is in accord with the hypothesis of a block in the oxidation of glucose-6-P.

Kipnis and Cori (10) and Helmreich and Eisen (22) found that deoxyglucose, once it had entered the cell and had been phosphorylated to deoxyglucose-6-P, noncompetitively inhibited the transport of glucose, mannose, fructose, and deoxyglucose, whereas external deoxyglucose-6-P had no effect, presumably because it did not enter the cell. In our experiments, however, external deoxyglucose-6-P was able to inhibit the growth of cell cultures, and both deoxyglucose and deoxyglucose-6-P were competitive with glucose. In the converse situation it had been found that glucose and mannose inhibited the penetration of deoxyglucose, whereas fructose was ineffective in this respect (10). Although the present experiments offer no direct evidence on this point, it is to be noted that fructose was much less active than glucose or mannose in reversing the effect of deoxyglucose.

Although it has been previously reported that deoxyglucose-6-P is not metabolized (8, 9), the present results suggest that it is metabolized, although slowly (cf. Fig. 4), and that the products may themselves be inhibiting at other loci. It is pertinent that recently Brooks et al. (23) reported the finding of an unidentified ester, in addition to deoxyglucose-6-P, on chromatograms derived from skin grown on deoxyglucose; and Williams and Eagon (24) have found that extracts of Pseudomonas aeruginosa could oxidize deoxyglucose to 2-deoxy-δ-gluconic acid. No phosphorylated intermediates could be demonstrated, and pyridine nucleotides were not involved in the latter oxidation.

SUMMARY

2-Deoxyglucose, at concentrations equimolar to the glucose of the medium, inhibits glycolysis and growth of cultured human cells. Growth inhibition may be reversed by added glucose or mannose. Cells grown on fructose are more sensitive to the inhibitory effects of 2-deoxyglucose than are those grown on glucose and mannose, and higher concentrations of fructose than of glucose and mannose are required to reverse the inhibition. 2-Deoxyglucose and 2-deoxyglucose 6-phosphate have been shown to inhibit fructokinase, phosphohexoseisomerase, and glucose 6-phosphate dehydrogenase. The implications of these findings with respect to the primary site of action of 2-deoxyglucose are discussed.

REFERENCES


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