D-XYLOSE AND XYLITOL: PREVIOUSLY UNRECOGNIZED SOLE CARBON AND ENERGY SOURCES FOR CHICK AND MAMMALIAN CELLS

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SUMMARY: D-xylose and xylitol, previously considered inert sugars in human and mammalian metabolism, have been found to support excellent growth from small inocula of chick embryo fibroblasts, NIL syrian hamster, chinese hamster ovary cells and human skin fibroblasts. Both, as well as D-ribose, enter cells by simple diffusion and as such exhibit no competition with other pentoses or hexoses. Neither D-ribose nor L-xylose can be used as sole carbon source by the cells cited. Cells grown on glucose possess demonstrable constitutive levels of D-xylulokinase; the enzyme activity is derepressed several-fold by growth of the cells on D-xylose.

D-xylose and its reduction product xylitol have been recognized as metabolizable substrates in bacteria for some time (1). Their utilization depends in principle upon inducible aldopentose isomerases, ketopentokinases and pentitoldehydrogenases (2). Specifically, D-xylose isomerase, D-xylulokinase and ribitol dehyrdogenase are the ensymes whose derepression is required and for which D-xylose or xylitol can serve as inducers.

D-xylose has been considered a sugar of restricted metabolic utility in mammalian cells (3,4). Indeed, D-xylose has been considered poorly metabolized sugar in humans where, as such, it is routinely used to measure monosaccharide absorption from the intestine (5) in what is termed the "D-xylose absorption test."

We have discovered that many cell lines and strains of cells recently isolated from chick, mice and humans utilize D-xylose or xylitol as sole carbon and energy source and can reproduce indefinitely on these compounds alone.

Unlike the transport of D-xylose in the intestine where it utilizes the same transport mechanism as glucose (6), in cultivated cells D-xylose entry appears to depend upon simple diffusion. In any event, glucose and xylose do not compete with each other for uptake as they do in the intestine (6). Similarly, xylitol does not compete with glucose nor with xylose for entry.

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Cells grown on D-xylose or xylitol are markedly derepressed for glucose uptake, as if deprived of carbon source (12). In contrast, cells grown on xylose or xylitol are not derepressed for L-phenylalanine uptake.

MATERIALS: Hanks' balanced salt solution (BSS) (7) without glucose (BSS, glc^-) and phosphate buffered saline (PBS) pH7.0 were prepared from reagent grade salts. Eagle's minimal essential medium (MEM) and Eagle's Basal Medium (BME) (10) with or without glucose (glc^+ or glc^-) were prepared by GIBCO.

D-xylose, D-glucose, D-mannose, D-ribose, xylitol, etc. were obtained from Sigma Chemical Company as well as from J. T. Baker Chemical Company. D- ^3H - glucose 540 mCi/mM, D- ^{14}C - glucose 150 mCi/mM, D- ^{14}C - xylose 10 mCi/mM or 3.14 mCi/mM and D- ^{14}C - ribose 49.9 mCi/mM, were obtained from New England Nuclear. The radiolabeled sugars and amino acids were diluted in BSS glc^- (pH 7.2-7.5) to adjust the molarity and the specific activity of each solution as indicated in each experiment. Biofluor scintillation fluid was purchased from New England Nuclear. Multiwell tissue culture plates (3008) were purchased from Falcon Company, Oxnard, California.

D-xylulose was prepared by refluxing D-xylose in dry pyridine after the method of Glatthaar and Reichstein (1935) as reported (1966) by Mortlock and Wood (17).

METHODS: Secondary chick embryo fibroblasts (CEF) cultures were prepared by trypsinization as previously described (9) and inoculated at 2-5 x 10^5 cells per ml into MultiWell plates. The cells were allowed to grow in BME glc^-) supplemented with 4% fetal calf serum near or up to confluency (3-4 days) without change of medium.

At this point, the monolayers were washed twice with BSS glc^- and reincubated for an 18-24 hour period in BME glc^- with 4% dialyzed fetal calf serum, supplemented or unsupplemented with D-glucose, D-xylose or xylitol as indicated for "preconditioning" (12).

Sugar Uptake Assays: At the end of the "preconditioning" period, the monolayers were washed by rinsing them twice with BSS glc^- and chilled on ice, whereupon 0.3 ml of ice cold radiolabeled sugar solution was added per well. The plates were allowed to float on a 37°C water bath for the pulse period (usually 5 minutes) desired.

The uptake was stopped in one motion by immersing the plate in a beaker containing cold PBS. After draining, 0.5 ml of 5% trichloroacetic acid (TCA) was added to each well and the plates were placed at 4°C overnight. The TCA soluble phase was obtained, mixed with 10 ml scintillation fluid and counted on a Beckman LS-230 scintillation counter.

Protein was estimated from the TCA-insoluble phase as described by Lowry, et al (15).

Amino Acid Uptake Assays: The procedures for measuring amino acid uptake were essentially the same as those for sugar uptake assays.

RESULTS

Adaptability of Cells to Growth on D-Xylose or Xylitol: NIL (Syrian hamster) cells, Chinese hamster ovary (CHO) cells and chick embryo fibroblasts (CEF) grow with little or no lag when subcultured from D-glucose-containing medium to D-xylose or xylitol-containing medium (BME or MEM). Excellent growth was also observed with normal human fibroblasts (NHF) as well as fibroblasts from patients suffering with Cystic Fibrosis (CFHF) (Fig. 1).

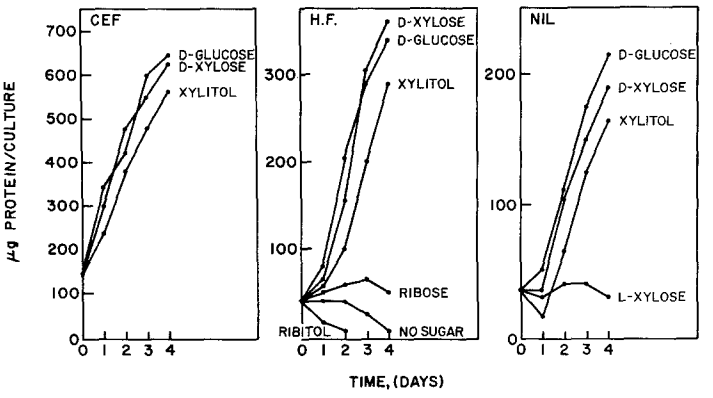

Figure 1.

Growth of Cells on Various Sugars and Sugar Alcohols

Subcultures of trypsinized monolayers growing in glucose of three different cell types, CEF (Chick Embryo Fibroblasts), HF (Human Fibroblasts) and NIL (Syrian Hamster Fibroblasts) were grown from small inoculum in bottles or MultiWell tissue culture plates. The subcultures were provided with medium (BME or MEM) supplemented or unsupplemented with sugars as indicated. (glucose 5.5mM, pentoses 6.6mM) The amount of protein per culture is used to assess growth at daily intervals without change of medium. (Similar results have been obtained by cell counts, data not shown).

Neither L-xylose, D-ribose or ribitol supported growth of any of the cells tested (Fig. 1.) Similarly, mannitol, dulcitol, inositol did not support cellular growth, while in sorbitol the cells grew poorly (data not shown). Indeed, ribitol at 6 mM concentration proved very toxic to cells, causing them to detach from the monolayer within 12 hours. To reduce the level of contaminating glucose or other monosaccharides in the medium of subculture to a mimimum, dialyzed calf or fetal calf serum was used as the course of the required serum supplement (4-10%). In the absence of added hexose or pentose, the cells showed little or no increase in numbers or mass and began to deteriorate within 72 hours of plating or shifting to sugar-free medium (Fig. 1). The implication is that the cells growing on glucose have the capacity to utilize D-xylose and/or xylitol and that the continued growth of the culture does not represent a selection of the kind observed by Chang (7) in his growth of variant clones from human conjunctival or Hela cells on D-xylose or D-ribose. NIL, CHO and HF cells have been subcultured for months on D-xylose on which the doubling time varies little from that maintained on glucose or combinations of glucose and xylose (data not shown).

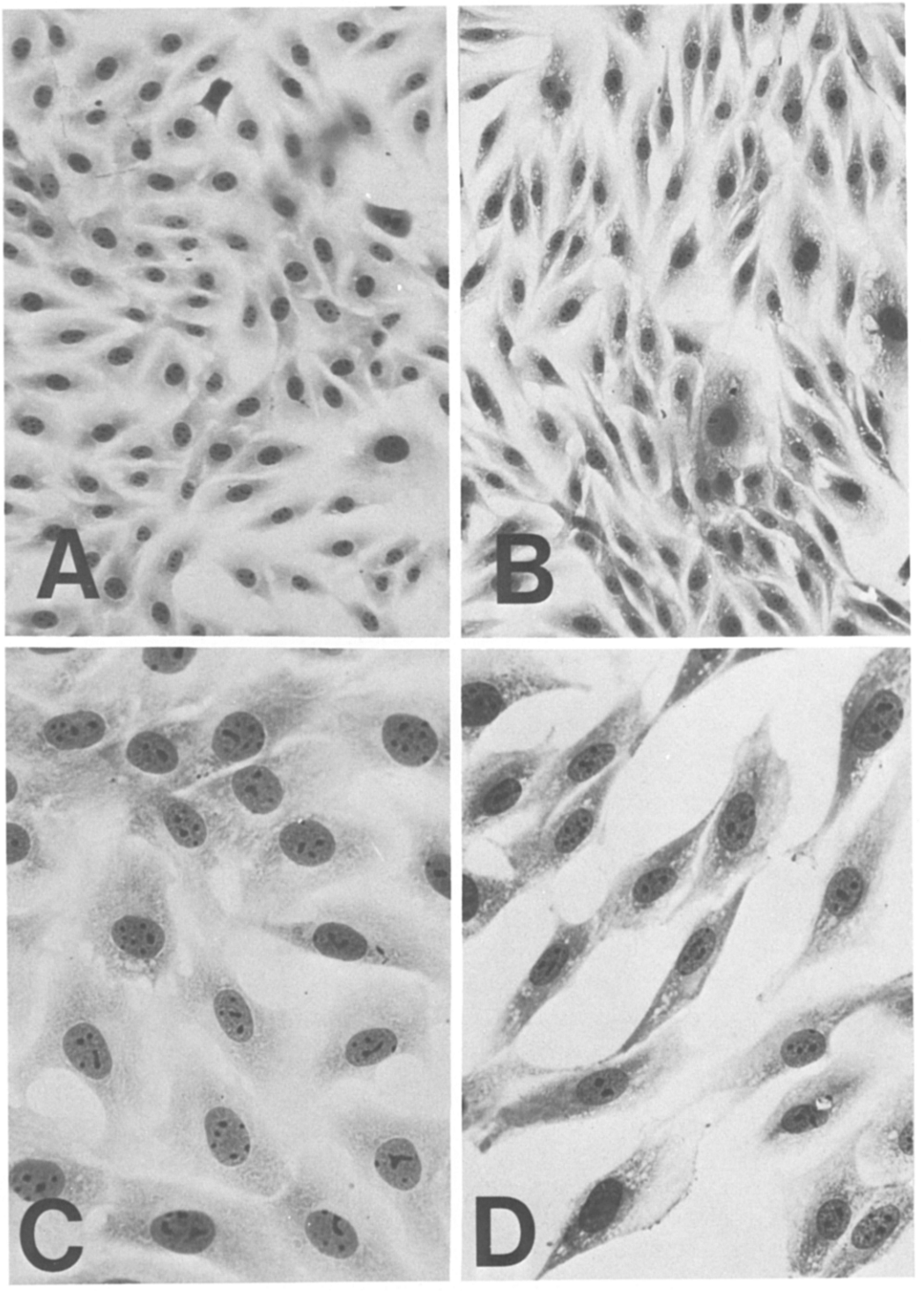

Figure 2.

Light microscope (100 x A and B, 400 x C and D) photographs of NIL cells grown for several generations in D-glucose (A,C) or D-xylose (B,D) stained with Hemtatoxylin and Eosin).

Growth on D-xylose: Effects Upon Cell Phenotype: Cells grown for several generations on D-xylose or xylitol as monolayer cultures had a characteristic cellular morphology compared to cells of the same age and density growing on glucose as sole carbon source. The cells are thin and long with pointed ends and so alligned that the cultures have the appearance of streaming when viewed under low power in the light microscope; even the cellular "vacuolization" pattern is different when compared to that of cells grown in glucose (Fig. 2). The same changes are observed in non-confluent cells growing on glucose and shifted to D-xylose or xylitol-containing medium. The modifications are observed within 12 hours and affect virtually the entire cell population within 24 to 30 hours. If cell growth is arrested by serum deprival (12) at the time of the medium shift, the same morphologic changes take place within a similar time period (data not shown). These properties are easily (within 12-24 hours) reversed to the more common stellate shapes when D-xylose is replaced by glucose in fresh medium.

Glucose, Xylose and Amino Acid Uptake After "Preconditioning": Chick cells "preconditioned" for 24 hours on D-xylose or xylitol-containing medium are derepressed for glucose uptake compared to cells growing on equivalent concentrations of glucose (Table I). Indeed, the derepression of uptake was first demonstrated (12) in cultures shifted for only 24 hours from glucose to D-xylose-containing medium in which the cells are unable to replicate because of the absence of serum.

TABLE I

Effect of Conditioning Sugar on Rate of Uptake of Glucose and Xylose

Conditioning Sugar			Picomoles/mg Uptake Protein/5min.	
glucose	xylose	xylitol	^{14}C-D-glucose	^{14}C-D-xylose
--	--	--	750 ± 40	289 ± 25
+	--	--	128 ± 30	230 ± 55
--	+	--	752 ± 50	245 ± 45
+	+	--	88 ± 32	232 ± 75
--	--	+	790 ± 18	250 ± 65

Secondary CEF cells were grown in glucose-containing medium to near confluency. The cells were washed and provided with fresh medium containing D-Xylose, D-Glucose, a combination of the two, Xylitol or No Sugar. After 24 hours, the cells were again washed and the uptake of ^{14}C-D-Glucose (UL) (0.5μCi/ml; 2×10^{-5}M) or ^{14}C-D-Xylose (UL) (1μCi/ml; 10^{-4}M) determined in 5 minutes pulses.

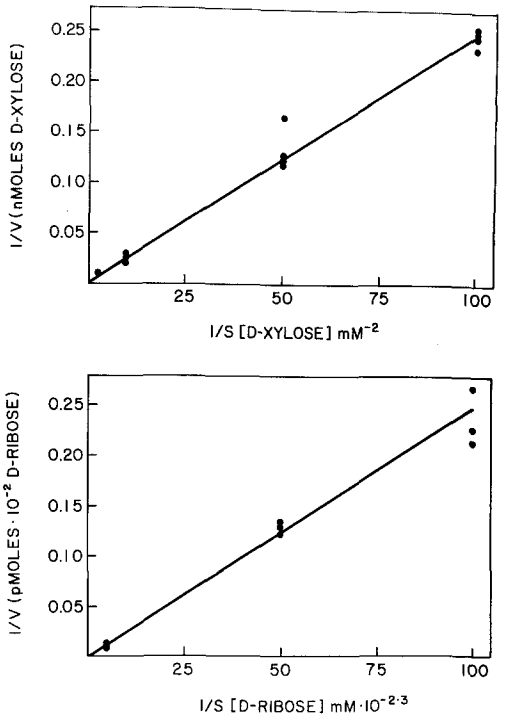

Figure 3.

Lineweaver-Burke Plot of D-Xylose and D-Ribose as a Function of Concentration

CEF cells were subjected to 5 minutes pulses of ^{14}C-D-Xylose (UL) (1 μCi/ml) or ^{14}C-D-Ribose (1C) 1 μCi/ml at concentrations as specified. All points represent the averages of quadruplicates. The same pattern was obtained whether cells were grown on glucose or xylose or no sugar.

Unlike glucose uptake, amino acid transport is little affected by starvation or the replacement of glucose by D-xylose or xylitol. The rate of L-phenylalanine transport increases by a factor of 2 (data not shown) when cells are starved for 24 hours, but is unaffected by growth on D-xylose or xylitol in lieu of glucose. Isoleucine transport was not significantly modified by either starvation or sugar substitution (data not shown).

D-xylose: Entry by Simple Diffusion - Lack of Competition with Other Sugars

D-xylose entry does not demonstrate staturation kinetics (13), even at concentrations as high as 50 mM (Fig. 3) which can be interpreted to mean that there is no carrier-mediated entry or that the carrier has a Km so high that it is not saturated at 50 mM D-xylose concentration. D-ribose entry is strikingly similar to that of D-xylose; it evidently enters also by simple diffusion (Fig. 3).

TABLE II

Lack of Competition for Entry Between D-Glucose and D-Xylose

Competitor	Concent. (M)	Labeled Sugar	Concent. (M)	D-Glucose Uptake
--	--	^{3}H-D-Glcuose	(5×10^{-6})	*56 ± 2
D-Xylose	(10^{-5})	"	(")	62 ± 14
"	(10^{-4})	"	(")	74 ± 20
"	(10^{-3})	"	(")	58 ± 6
--	--	^{14}C-D-Glucose	2.5×10^{-5}	**870 ± 80
D-Xylose	4×10^{-2}	"	(")	768 ± 75
--	--	^{14}C-D-Glucose	(")	681 ± 24
Xylitol	2.5×10^{-3}	"	(")	738 ± 75
				D-Xylose Uptake
--	--	^{14}C-D-Xylose	10^{-4}	***292 ± 10
D-Glucose	10^{-3}	"	"	225 ± 50
"	3×10^{-3}	"	"	182 ± 25
--	--	^{14}C-D-Xylose	10^{-4}	400 ± 13
Xylitol	10^{-2}	"	"	345 ± 35

CEF Secondary cultures were grown to near confluency in MultiWell plates. The cell monolayers were washed and exposed to labeled and unlabled sugars as indicated for 5 minutes at 37°C. The values presented are averages of triplicate wells for each point with the average deviation for each set.

Uptake expressed as picomoles of sugar per mg of cell protein/5 minutes;
* ^{3}H-D-Glucose (2C) 2μCi/ml, (cells grown on glucose-containing medium up to the time of pulsing)
** ^{14}C-D-Glucose (UL) 0.5μCi/ml, (cells deprived of sugar for 24 hours prior to pulsing).
*** ^{14}C-D-Xylose (UL) iμiCi/ml

Glucose, which in chick embryo fibroblasts is transported by carrier-mediated diffusion through a well-characterized transport system (8, 9, 12), apparently does not share its sytem with D-xylose or xylitol (Table II). Similarly, D-xylose uptake is not affected by 30 times as much glcuose or 100 times as much xylitol in the uptake medium (Table II). Among other molecules tested at concentrations 10-100 times the concentration of D-xylose, L-xylose, D-ribose, D-galactose, D-mannose and D-fructose do not compete with D-xylose for entry. These results are consistent with a mode of entry not dependent upon a membrane carrier for D-xylose (data not shown).

D-Xylulokinase Content of Glucose and Xylose-Grown Cells: Chick cells and NIL cells possess D-xylulokinase activity even when growing on glucose (Table III) Taken as an index of the constitutive level of enzymes concerned with D-

TABLE III

D-Xylulokinase Activity in Glucose and Xylose-Grown Cells

Cell Extract	Enzyme Activity n Units/mg/protein* Exp. 1	Exp. 2
NIL-Glc	58 ± 3**	143 ± 30
NIL-Xyl	341 ± 25	319 ± 22
CEF-Glc	70 ± 9	78 ± 4
CEF-Xyl	244 ± 19	699 ± 33

Xylulokinase was measured by the assay of Mortlock and Wood (16). A total reaction of volume of 1.0 ml contained 6.0 µmoles of $MgCl_2$; 9 µmoles of glutathione; 50 µmoles of tris buffer (pH 7.5); 3.0 µmoles of ATP; 1. 5 µmoles of PEP; 0.6 µmole of NADH; 2.0 units of lactic dehydrogenase containing pyruvate kinase (Sigma Rabbit muscle); 9 µmoles of xylulose. The reduction in absorbance of NADH was measured as a function of time in minutes at 340 nm. Cell extracts were prepared (16) by sonication of cells removed from the surface by scraping into BSS glc^-.

* 1 unit defined as the amount of enzyme reducing absorbance at 340 nm by 1.0 absorbance unit per minute.
NIL-Glc - NIL Cells cultivated on D-glucose-containing MEM
NIL-Xyl - NIL Cells cultivated on D-xylose-containing MEM
CEF-Glc - Chick Embryo Fibroblasts grown on D-glucose-containing BME
CEF-Xyl - Chick Embryo Fibroblasts grown on D-xylose containing BME

** Average of 3 determinations ± S.E.

xylose and xylitol metabolism, this could account for the ability of the cells to continue growth with a minimal lag when shifted from glucose to D-xylose or xylitol. A 3- to 5-fold enhancement in the activity of the kinase in chick as well as in NIL cell populations occurs when cells are subcultured on D-xylose (Table III). In this regard, the chick and mammalian cells resemble bacteria (1, 2) in their response to these potential substrates in the growth medium.

DISCUSSION

There are several major and surprising features of the response of mammalian cells to D-xylose and xylitol: (1) the ability of both to support indefinitely rapid growth of virtually all cells tested as sole carbon and energy source; (2) the concomitant derepression of glucose transport, not shared by amino acid transport in cells growing on D-xylose or xylitol; (3) morphologic differences that are observed in all lines of cells cultivated

on both at physiologic concentrations even when an equivalent concentration of glucose (5.5 mM) is added to the medium. These last findings were documented by EM scanning photographs in a recent report (11); (4) the enzyme, D-xylulokinase, is demonstrable in cells cultivated for generations on glucose before exposure to exogenous xylose and is derepressed by growth on D-xylose or xylitol.

That cells should grow well on D-xylose and xylitol without signs of toxicity is perplexing in the light of the well-documented failure of most cells, including those used in this study, to thrive on ribose or ribitol. It has been tacitly assumed that ribose toxicity is due to the accumulation of ribitol. Added ribitol has proved highly toxic to our cells although we have not measured the extent of its permeation of the cells. We, as others, have been able to overcome the ribose toxicity by adding pyruvate to the culture medium (data not shown). L-xylose does not support cell growth, probably because the cells do not possess the isomerase to convert L-xylose to L-xylulose, an intermediate in the normal pathway of glucuronic acid metabolism in mammalian cells.

The excellent growth of human fibroblasts on D-xylose or xylitol, opens the question of the metabolism of these naturally occurring sugars (14) by humans and deserves further exploration for physiologic as well as biochemical implications. Careful study of the xylose-metabolic pathway and the kinetics of induction by potential substrates has been initiated.

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