PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 153, 251-253 (1976)

Carbohydrate Energy Sources for Chinese Hamster Cells in Culture[1] (39521)

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Most mammalian cells in culture are supplied glucose as a source of energy (1) and it is not clear how many other carbohydrates will permit growth. With the view of clarifying this situation and perhaps providing for the development of additional genetic markers by which to characterize cells in culture, we have tested a number of substances for their ability to replace glucose in the growth medium.

Materials and methods. 1. Cell lines and culture conditions. Three Chinese hamster cell lines were studied: strain CHO-K1 was isolated from hamster ovary (2), strain V79 from fetal lung (3), and strain CHS from spleen in this laboratory (ERICR).

Routine maintenance of these cultures in F12 medium supplemented with fetal calf serum was as described previously (4, 5).

2. Source of the carbohydrates. The carbohydrates tested were of the highest purity available and were obtained from either Pfanstiehl Laboratories (Waukegan, Ill.) or Sigma Chemical Co. (St. Louis, Mo.).

3. Enzyme assays. To test the ability of these cell lines to grow on various carbohydrates, the carbohydrates were substituted for the glucose in F12 at an equal concentration (10^{-2} *M*) or, in the case of glycogen, at an equal weight. The serum supplement was the macromolecular portion of fetal calf serum (FCM) prepared by passing serum over a Sephadex G-50 column (6). FCM was added to 7% of the total volume. The cells were not permitted to attach to the plates prior to the addition of the carbohydrates. The solution used for washing and other manipulations of the cells was saline G (6) with glucose omitted. This is essentially a phosphate-buffered saline containing calcium and magnesium. The inoculum in the growth experiments was 200 cells/30-mm plate.

The cultural conditions for measurement of enzyme activity were to inoculate 5×10^5 cells/60-mm-diameter plastic petri dish. After 24 h of growth the cultures were dense but not confluent with many cells in mitosis. Cells were harvested with trypsin, centrifuged, and washed twice with an equal volume of G-saline without glucose, and finally resuspended at 10^7 cells/ml in 0.01 *M* phosphate buffer at pH 7.2. The cells were lysed by three cycles of freeze-thawing.

The ability of cell extracts of CHO-K1 to hydrolyze *p*-nitrophenyl derivatives of various carbohydrates was measured by adding extract to give 0.1 mg of protein to a 1-ml reaction mixture containing 0.1 *M* acetate buffer, pH 4.5, and 0.01 *M* *p*-nitrophenyl derivative. Incubation was at 37° for 15 hr. The reaction was terminated by the addition of 2 ml of 0.23 *M* Na_2CO_3, the solution clarified by centrifugation, and the absorbance measured at 415 nm.

Results and discussion. The results, stated as a plating efficiency (EOP), which is the number of cells forming colonies (greater than 50 cells per colony) divided by the number of cells plated, are given in Table I. There are only a few carbohydrates which permit colony formation for each of the strains, and there are differences between hamster strains. It should be noted that the cells were not permitted to attach prior to the addition of the carbohydrates, and, if the substances did not permit or if they inhibited attachment, these experiments would not detect the effect. That attachment is important is indicated by the fact that cells of strain CHO-K1 allowed to at-

[1] Supported by a grant from The American Cancer Society (VC-81D) to T.T.P., by General Research Support Grant (NIH) 5 S01 05357 to M.L.M., and by Contract No. 72-213 from The National Center for Toxicological Research of The U.S. Food and Drug Administration to T.T.P.

tach in the presence of glucose and then exposed to trehalose give the appearance of growth (colony formation), whereas cells given continuous trehalose exposure show no signs of colony formation. The basis of this phenomenon remains obscure.

There are no disaccharides which serve as energy sources. This is surprising since CHO-K1 cell extracts possess enzymes for hydrolyzing a number of *p*-nitrophenyl derivatives of some of the carbohydrates at pH 4.5 (Table II). Presumably these enzymes are lysosomal in nature and used for the degradation of polysaccharides synthesized by the cells and cannot be used to provide energy for cell growth.

TABLE I. CARBOHYDRATES TESTED AS ENERGY SOURCES FOR CHINESE HAMSTER CELLS

A. *Carbohydrates not serving as energy sources (EOP < 0.01)*	
Strain CHO-Kl	L-Arabinose, cellobiose, dulcitol, erythritol, α-L-fucose, α-D-fucose, galactolactone, galactose 6-phosphate, galacturonic acid, glucose-6-phosphate, glucuronic acid, α-methylglucoside, DL-α-glycerophosphate, lactose, guanosine diphosphate mannose, α-methylmannoside, mannitol, mannitol 1-phosphate, mannosamine, acetylmannosamine, melibiose, pyruvate, α-L-rhamnose, raffinose, salicin, sucrose, trehalose, turanose, D-xylose, D-arabinose, D-lyxose
Strain V79	L-Arabinose, cellobiose, dulcitol, erythritol, esculin α-L-fucose, α-D-fucose, inulin, lactose, mannose, melibiose, melizitose, α-L-rhamnose, raffinose, trehalose, turanose, D-lyxose
Strain CHS	Arabinose, fructose, galactose, lactose, mannose, melibiose
B. Carbohydrates serving as energy sources (EOP > 0.5)	
Strain CHO-Kl	Glucose, mannose (14- to 16-hr generation time), glucose-1-phosphate (20- to 24-hr generation time), maltose,[a] glycogen,[a] galactose (14- to 30-hr generation time), fructose (30- to 36-hr generation time)
Strain V79	Glucose (12- to 14-hr generation time, galactose (20- to 24-hr generation time), fructose (20- to 24-hr generation time), maltose[a]
Strain CHS	Glucose (16-hr generation time)

[a] An artifact, since the serum in the medium hydrolyzes maltose and glycogen to glucose.

TABLE II. HYDROLYSIS OF *p*-NITROPHENYL DERIVATIVES OF A VARIETY OF CARBOHYDRATES BY EXTRACTS OF CHINESE HAMSTER OVARY CELLS.[a]

p-Nitrophenyl derivative of	*p*-Nitrophenol (m*M*/0.1 mg of cell protein × 18 hr)
α-Glucoside	10.27
β-Glucoside	10.04
α-Galactoside	48.00
β-Galactoside	47.04
α-Mannoside	19.18
β-Mannoside	0.00
α-L-Fucoside	44.16
β-D-Fucoside	1.71
β-D-Xylopyranoside	0.60
β-Glucuronide	48.00

[a] pH = 4.5.

An effect of sugars on fibroblast morphology has recently been reported (7) but we have not observed any changes in morphology induced by sugars on the hamster lines studied here.

It should be noted that at least some batches of serum contain enzymes that degrade maltose and glycogen to glucose and which give the appearance of growth support. A similar observation has been made for starch as well as maltose (8). Fetal calf serum also appears to hydrolyze *p*-nitrophenyl-α-mannoside, but it is not known whether this is the same or a different enzyme.

Summary. Three Chinese hamster cell lines (CHO-K1, V79, and CHS) were tested to see which carbohydrates would support their growth in culture. There were differences between lines (mannose supported growth of CHO-K1 only), and glucose was the only carbohydrate that supported growth of all three strains. CHO-K1 cells possess enzymes for hydrolyzing some disaccharides which do not support growth. Fetal calf serum contains enzymes that hydrolyze maltose, glycogen, and α-mannosides.

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Received May 17, 1976. P.S.E.B.M. 1976, Vol. 153.