Regulation of carbohydrate metabolism in cultured mammalian cells: energy provision in a glycolytic mutant

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The metabolism of radioactively labelled D-glucose, L-glutamine, and L-glutamate has been determined in a glycolytic mutant of Chinese-hamster ovary cells, R1.1.7, and in its parent, CHO-K1. The complete oxidation of glucose via the TCA-cycle is negligible in both cell types, but there is significant oxidation of carbon-1. CHO-K1 cells derive most of their energy from glycolysis and are independent of respiration in the short term. R1.1.7 cells are respiration-dependent and are rapidly killed by respiratory inhibitors. Both cell types oxidize L-glutamine and L-glutamate, but the oxidation of these substrates does not appear sufficient to satisfy completely the energy requirements of R1.1.7 cells.

We have described the isolation of a glycolytic mutant of Chinese-hamster ovary cells deficient in the activity of both glucosephosphate isomerase, EC 5.3.1.9 (GPI) and 3-phosphoglycerate kinase, EC 2.7.2.3 (PGK) (Faik & Morgan, 1977b; Morgan & Faik, 1980). The mutant grows readily on glucose, but unlike its wild-type parent does not excrete significant amounts of lactic acid into the culture medium and appears to be unable to derive energy from glycolysis. It was of interest, therefore, to investigate the metabolism of glucose in this mutant and to determine how it satisfies its energy requirements.

Here we describe the metabolism of radioactively labelled glucose, L-glutamine, and L-glutamate and conclude that Chinese-hamster ovary cells, like other mammalian cells, can satisfy their energy requirements by either glycolysis or respiration.

Materials and Methods

R1.1.7 is a ribose+ derivative of the Chinese-hamster ovary-cell line, CHO-K1 (Faik & Morgan, 1977b). Cells were grown in a modified version of Ham's F12 medium (Flow Laboratories) supplemented with 10 mM glucose and 4% foetal bovine serum (Gibco) as previously described (Faik & Morgan, 1977a). Cell numbers were determined with a Coulter Counter and protein by the Coomassie-blue method (Spector, 1978) with a standard of bovine serum albumin (Sigma).

To determine the metabolism of 14C-labelled substrates, cells were harvested in the mid-log phase by trypsinization, and 5 x 10^5 cells
were seeded into flasks (25 ml, Kontes Glassware) and incubated overnight at 37°C in 5% CO₂ in air in an humidified incubator. Cell sheets were rapidly but gently washed two times with saline G (37°C), and the appropriate labelling medium (2 ml) was added. The flasks were then tightly stoppered with serum caps bearing a plastic cup insert (Kontes Glassware) containing a filter-paper wick and incubated in a shaking water-bath (Gallenkamp) at 37°C. Duplicate flasks were removed at timed intervals. ¹⁴CO₂ was trapped in 1 M NaOH (0.2 ml) injected into the plastic cup and liberated from the medium by the injection of 10% TCA (1.0 ml) or 2 M PCA (0.15 ml). The flasks were left for at least 2 h, but usually overnight, before being further processed. The ¹⁴CO₂ liberated was determined by transferring the plastic cup to a scintillation vial (5 ml) and determining the radioactivity in a Packard Tri-Carb 3385 scintillation spectrometer. Acidified growth medium was neutralized and processed for lactic acid assay by the precipitation method and for glucose determination by the Glucostat method as previously described (Morgan & Faik, 1980). Cell sheets were washed with TCA or PCA, followed by ethanol, and then dissolved in 0.5 M NaOH and the incorporated radioactivity determined.

The sensitivity of the cells to inhibitors of respiration was determined by incubating duplicate sets of sub-confluent cell cultures in 35-mm dishes with various concentrations of respiratory inhibitors and observing the progress of toxicity by phase-contrast microscopy. Glucose-6-phosphate (G-6-P) accumulation in cells was measured by determining the G-6-P content of freeze-thawed cell extracts as previously described (Morgan & Faik, 1980).

Fig. 1. Relationship between cell number and rate of glucose utilization. Cells were plated at various densities overnight and then changed to Ham's F12 medium supplemented with 4% foetal calf macroserum, 20 mM Hepes, pH 7.5, and D-[U-¹⁴C]-glucose (0.5 μCi/μmol/ml). Incubation was continued for 2.5 h, samples (in duplicate) being removed at 0.5 and 2.5 h: (O), ¹⁴CO₂ evolution; (x), radioactivity incorporated.