The inverse relation between liver carnitine and serum ketone bodies, presented here, is not a unique precedent. In a human disease termed 'systemic carnitine deficiency' the liver carnitine level is also depressed[13]. In such patients ketoacidosis has occasionally been observed[13-15].

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High glucose-induced enzyme activity changes in cultured cell lines established from the kidneys of Chinese hamsters with aglycosuria or spontaneous glycosuria

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Summary. Chinese hamster kidney epithelial-like cells derived from highly inbred nondiabetic *(AV)* and diabetic *(XA)* genetic sublines were passaged in medium containing 100 or 400 mg/dl glucose. The effect of high medium glucose on the activities of 5 enzymes involved in glucose metabolism was followed and significant glucose-dependent difference was observed. The effects, however, were opposite in cells derived from *AV* and *XA* sublines.

Diabetes-related changes in the activities of many enzymes involved in carbohydrate metabolism have been documented in the highly inbred Upjohn Chinese hamster colony[1,2]. These changes could arise from hormonal imbalances or, alternatively, a direct effect of hyperglycemia per se. In view of the fact that excessive nonenzymatic glycosylations of proteins have been increasingly found in diabetic human patients and animal models[3-10], an attempt to ascertain the effect of high glucose in the medium on the activities of enzymes involved in carbohydrate metabolism in kidney cell cultures was thus made. Pyruvate kinase (PK, EC 2.7.1.40), malate dehydrogenase (MDH, EC 1.1.1.37), isocitrate dehydrogenase (IDH, EC 1.1.1.42) and phosphoenolpyruvate carboxykinase (PEPCK, EC 4.1.1.32) were studied because of their pivotal roles in the glycolytic-gluconeogenic and citric acid cycles which are the major pathways in carbohydrate metabolism. UDP-Glucose: galactosylhydroxylysine-collagen glucosyltransferase (CGT, EC 2.4.1.66) was also included due to its implicated role in the development of diabetic microangiopathy[11].

Materials and methods. 2 lines of epithelial-like cells, designated CHK-XA_E and CHK-AV_E, were established from the kidneys of 2 8-week-old male Chinese hamsters derived from the diabetic *XA* and the nondiabetic *AV* genetic sublines respectively. The method used and the lineage of the animals are described elsewhere[12,13]. The CHK-XA and CHK-AV cells were further divided, at the 5th and the 3rd passages, into E-100 and E-400 sublines by culturing in medium containing 100 or 400 mg/dl glucose respectively in successive passages. The cells were grown until confluent in 25 cm^2 tissue culture flasks. The medium was removed and the attached cells were rinsed 5 times with ice-cold saline. 2 ml saline was added to each flask and the cells were removed with a rubber policeman. The cells were transferred to 12×75 glass tubes and then combined with 2 ml saline used to wash the flask. The cells were centrifuged at $180 \times g$ for 10 min, the supernatant was discarded and the cells were suspended in 0.25 ml saline. The cells were frozen-thawed rapidly 5 times and sonicated for 15 sec at 30 W using a Sonifier Cell Disruptor Model W185 (Heat Systems-Ultrasonics, Inc., Plainview, Long Island, NY). The samples were then centrifuged at $260 \times g$ for 10 min, the supernatant collected and stored on ice until use. All enzyme assays were done on the day the samples were prepared. Aliquots of 0.01-ml samples were assayed for PK, MDH and IDH; aliquots of 0.05 ml were assayed for PEPCK and CGT. The enzymes were assayed by methods previously described[1,14] with the exception that the precipitates formed in the CGT assay were washed 4 times in 1% (w/v) phosphotungstic acid, 0.5 N HCl instead of 16% (w/v) trichloroacetic acid[14]. 1 unit (U) of enzyme was defined as substrate turnover at a rate of 1 μmole/min. Proteins were determined by a modified Lowry's procedure[15]. The experiments were carried out in cells harvested on 4 successive passages with 4 sister flasks per passage resulting in a total of 16 observations for each enzyme in each subline. PK and PEPCK were studied in one series of experiments and MDH, IDH and CGT in another. All E-400 cells had been grown in 400 mg/dl glucose for at least 7 passages and they were matched with E-100 cells cultured pairwise in 100 mg/dl glucose under identical conditions. Studies on CHK-AC_E and CHK-XA_E were performed separately on different days whereas E-100 and E-400 cells in each cell line were always studied in pairs.

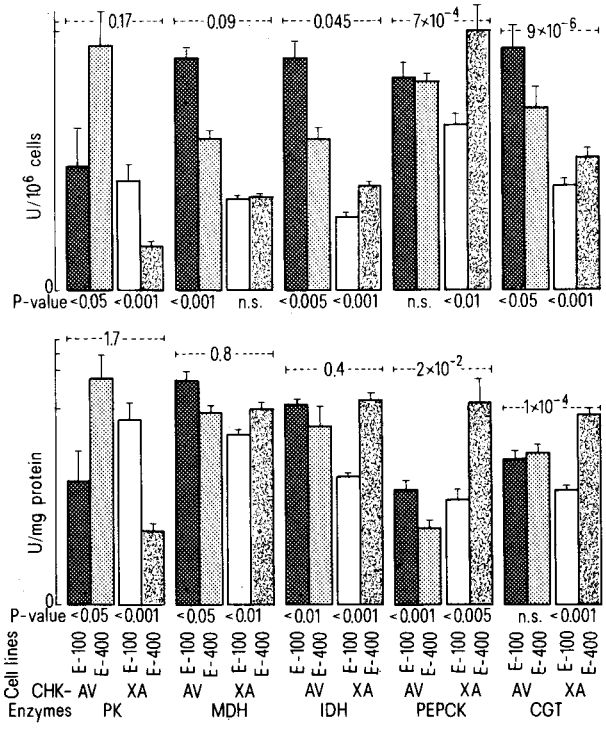
Results and discussion. Our data are expressed in terms of U per 1×10^6 cells or U per mg proteins in cell extracts and summarized in the figure. In CHK-AV_E cells, successive passages in 400 mg/dl glucose led to significant increase in PK activity (1.05 ± 0.27 U/mg and 0.081 ± 0.024 U/10^6 cells in E-100 vs 1.94 ± 0.21 U/mg and 0.157 ± 0.023 U/10^6 cells in E-400) and decrease in MDH (0.721 ± 0.035 U/mg protein and 0.080 ± 0.005 U/10^6 cells in E-100 vs

0.622 ± 0.026 U/mg protein and 0.052 ± 0.004 U/10^6 cells in E-400) and IDH (0.339 ± 0.008 U/mg protein and 0.039 ± 0.003 U/10^6 cells in E-100 vs 0.305 ± 0.009 U/mg protein and 0.026 ± 0.002 U/10^6 cells in E-400). The decrease in PEPCK was significant only when the activity was expressed in mU/mg protein (9.74 ± 0.66 in E-100 vs 6.47 ± 0.46 in E-400) but not in mU/10^6 cells (0.546 ± 0.042 in E-100 vs 0.539 ± 0.022 in E-400); whereas the difference in CGT activity was significant only when it was calculated in terms of μU/10^6 cells (8.43 ± 0.75 in E-100 vs 6.44 ± 0.52 in E-400) and not in terms of μU/mg protein (74.4 ± 4.4 in E-100 vs 77.6 ± 4.4 in E-400).

In CHK-XA_E cells, successive passages in 400 mg/dl glucose caused drastic decrease in PK activity (1.58 ± 0.16 U/mg protein and 0.079 ± 0.012 U/10^6 cells in E-100 vs 0.62 ± 0.08 U/mg protein and 0.029 ± 0.006 U/10^6 cells in E-400) and substantial increase in IDH (0.220 ± 0.006 U/mg protein and 0.013 ± 0.001 U/10^6 cells in E-100 vs 0.350 ± 0.012 U/mg protein and 0.018 ± 0.001 U/10^6 cells in E-400), PEPCK (8.92 ± 0.80 mU/mg protein and 0.428 ± 0.036 mU/10^6 cells in E-100 vs 17.3 ± 2.1 mU/mg protein and 0.666 ± 0.076 mU/10^6 cells) and CGT (58.4 ± 2.6 μU/mg protein and 3.60 ± 0.22 μU/10^6 cells in E-100 vs 96.4 ± 2.8 μU/mg protein and 4.62 ± 0.14 μU/10^6 cells in E-400). The high glucose-elicited increase in MDH was significant only when the activity was expressed in U/mg protein (0.546 ± 0.018 in E-100 vs 0.629 ± 0.024 in E-400) and not in U/10^6 cells (0.0321 ± 0.0013 in E-100 vs 0.0323 ± 0.0013 in E-400). Therefore, the effects of high glucose concentration in the culture medium on the activities of PK, PEPCK, MDH, IDH and CGT were diametrically different between kidney epithelial-like cells established from animals with aglycosuria and spontaneous glycosuria.

The high glucose-induced increase in PK and decrease in PEPCK activity observed in CHK-AV_E were not entirely unexpected and appeared to arise for the purposes of increasing the rate of glucose removal via glycolysis and suppressing glucose production by gluconeogenesis. The regulation of glucose homeostasis in CHK-AV_E cells relied more on the glycolytic pathway since the difference between E-100 and E-400 was much more pronounced in PK activity than in PEPCK activity. That the high glucose-elicited differences in PK and PEPCK between E-100 and E-400 in CHK-XA_E sublines were directly opposite to those in CHK-AV_E sublines suggests disorderliness in the regulation of glucose metabolism in cells derived from an animal with spontaneous glycosuria. The higher CGT activity in CHK-$XA_{E\text{-}400}$ cells than in CHK-$XA_{E\text{-}100}$ cells further attests to the failure to prevent overglucosylation in the presence of excessive glucose by cells of diabetic origin as opposed to the down-regulation of CGT activity observed in the CHK-$AV_{E\text{-}400}$ cells.

The physiological significance of the observed differences in MDH and IDH, both of which are citric acid cycle enzymes, between E-100 and E-400 sublines is more difficult to depict. One speculation remains that pentose phosphate shunt and lipogenesis were favored in CHK-AV_E cells when 400 mg/dl glucose was present in the culture medium. The fact that CHK-XA_E behaved in contrast to CHK-AV_E in MDH and IDH again suggests a lack of normal enzyme regulation in the cells established from a spontaneously diabetic animal. The high glucose-dependent changes in enzyme activities observed here may arise via several possible routes. 1. Glucose per se or its metabolite directly or indirectly modulates the expression of genes responsible for the enzyme activities. 2. Glucose per se or its metabolite serves as post-translational modifier on the enzymes via non-enzymatic or enzymatic reactions. 3. Different cell types are being selected in 100 and 400 mg/dl glucose. The present communication makes no attempt to exclude any of these possibilities and further studies are called for in order to elucidate the mechanism leading to the observations reported here.



The activities of pyruvate kinase (PK), malate dehydrogenase (MDH), isocitrate dehydrogenase (IDH), phosphoenolpyruvate carboxykinase (PEPCK) and collagen glucosyltransferase (CGT) in CHK-$AV_{E\text{-}100}$, CHK-$AV_{E\text{-}400}$, CHK-$XA_{E\text{-}100}$, and CHK-$XA_{E\text{-}400}$ cell lines established from the kidneys of male Chinese hamsters of the highly inbred *AV* and *XA* genetic sublines in the Upjohn colony by subculturing in medium containing 100 or 400 mg/dl glucose. The cells were epithelial-like morphologically. P-values were obtained by Student's t-test. Numbers across dotted lines indicate arbitrary scales of each enzyme activity, the actual values of which are given in the text.

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