Chapter 13

CULTURE OF MAMMARY TISSUE: GLUCOSE TRANSPORT PROCESSES

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I. INTRODUCTION

The epithelial cells of the kidneys and the small intestine can transport glucose against a concentration gradient via a Na^+ - dependent transporter (Devaskar and Mueckler, 1991). In addition, in these same epithelial cells, Na^+-independent glucose transporters have been identified (Harris *et al.*, 1992). Mammary epithelial cells utilize large quantities of glucose

since the monosaccharide is the precursor of lactose, the major carbohydrate found in milk of most mammalian species. The types of glucose transporters present in mammary epithelial cells have never been determined. The objectives of the following experiments were to determine: (1) if glucose transport by mammary epithelial cells was Na^+-dependent or Na^+-independent, (2) changes in glucose uptake by mammary epithelial cells at different stages of growth, and (3) the effect of glucose concentration on its accumulation by mammary epithelial cells at different stages of growth.

II. MATERIALS AND METHODS

A. CELLS AND CULTURE CONDITIONS

An immortalized line of bovine mammary epithelial cells, the MAC-T cell line, was chosen as an experimental model. These cells form a tight monolayer in culture and have been demonstrated to differentiate when exposed to lactogenic hormones (Huynh *et al.,* 1991). Cells were grown in Dulbecco modified Eagle medium (Gibco) containing 5 μg/ml insulin, 50 mg/liter gentamicin sulfate (Gibco), and 10% fetal calf serum (Gibco). The glucose concentration in the medium was altered for certain experiments. The medium was changed every 2 days. Cells were seeded at a rate of 1.4×10^4 cells per 35-mm tissue culture dish.

B. EXPERIMENT 1: UPTAKE OF 2-[^{3}H]DEOXYGLUCOSE (2-DG) AND α-[^{14}C]METHYLGLUCOSE (MG) BY MAC-T CELLS

These two radiolabeled glucose analogues were chosen. The 2-DG is known to be accumulated in a Na^+-independent manner whereas MG is accumulated in a Na^+-dependent manner (Blais *et al.,* 1987; Malo, 1990). The uptake of 2-DG is specifically inhibited by phloretin (Pt) whereas the transport of MG is specifically inhibited by phlorizin (PZ). At Day 10 of culture the uptake of 1 μCi/dish of 2-DG and MG by MAC-T cells grown in DMEM with 25 m*M* glucose was measured in the absence and presence of PZ (200 μM), Pt (200 μM), or an excess of nonradioactive substrate (50 m*M*). The uptake was measured at Day 10 of culture according to the following procedure. Cells were rinsed three times with 2 ml of nonradioactive transport buffer composed of 200 mg/liter $CaCl_2$, 400 mg/liter KCl, 170 mg/liter $MgSO_4$, 125 mg/liter KH_2PO_4, 2.38 g/liter Hepes, 6.4 g/liter NaCl, 292.2 mg/liter glutamine, and mannitol to adjust the osmolarity

to 300 mOsmol. Cells were then incubated 15 min at 37°C in 2 ml of transport buffer after which the buffer was changed for radioactive and inhibitor-containing buffer (same composition as described above but with radioactive substrate and inhibitors desired) (1 ml/dish). Cells were incubated at 37°C for 5, 10, 15, 30, 45, 60, 90, 120, 180, and 240 min. At the end of the incubation, cells were washed three times with nonradioactive transport buffer containing 200 μM PZ and 200 μM Pt. Cells were then incubated at 37°C for 2 hr in 1 N NaOH (0.5 ml/dish). The extract obtained was analyzed for its radioactivity and protein content using the procedure of Lowry *et al.* (1951). All measurements were done in duplicate.

C. EXPERIMENT 2: UPTAKE OF 2-DG BY MAC-T CELLS AT DIFFERENT DAYS OF CULTURE

The uptake of 2-DG with and without Pt was measured on Days 3, 6, 9, 12, 15, 18, and 21 of culture according to the same procedure as in experiment 1 except that the uptake of 2-DG was only measured for 30 min to ensure that the uptake vs time curve was still in the linear portion. Cell number was determined at each time point. All measurements were done in triplicate.

D. EXPERIMENT 3: EFFECT OF GLUCOSE CONCENTRATION IN THE MEDIA ON THE UPTAKE OF 2-DG BY MAC-T CELLS AT DIFFERENT DAYS IN CULTURE

The uptake of 2-DG by MAC-T cells grown in the presence of 25, 5, or 0 mM glucose was measured on Days 3, 6, and 9 of culture following the same protocol as in experiment 2. The number of cells was determined for each condition.

III. RESULTS AND DISCUSSION

Bovine epithelial cells did not accumulate MG in a specific manner since the uptake was not altered by the presence of Pt, PZ, nor an excess of nonradioactive substrate (Fig. 1). The small accumulation of MG could be related to nonspecific uptake, diffusion, or binding to the cell surface. These results suggest that MAC-T cells grown under the present conditions do not express a Na^+- dependent glucose transporter. The significant inhibition of uptake of 2-DG by an excess of nonradioactive substrate and by Pt but not by PZ demonstrated that MAC-T cells express one or several Na^+-independent glucose transporters (Fig. 2). Specific uptake of 2-DG

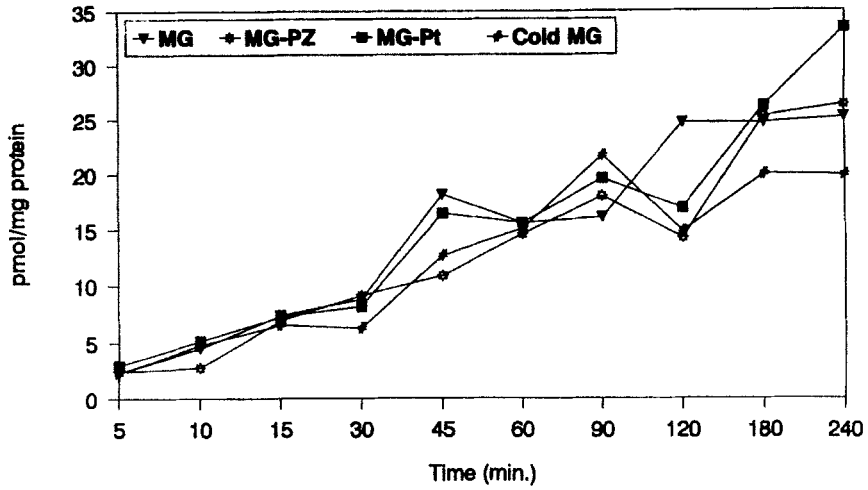

FIG. 1. Uptake of α-methylglucose (MG) by MAC-T cells at Day 10 of culture and in the presence of inhibitors phlorizin (PZ) or phloretin (Pt) or cold α-methylglucose.

was also observed in rat mammary acini (Threadgold *et al.*, 1982). These results are not surprising since mammary cells do not need to be able to transport glucose against a concentration gradient, blood concentration

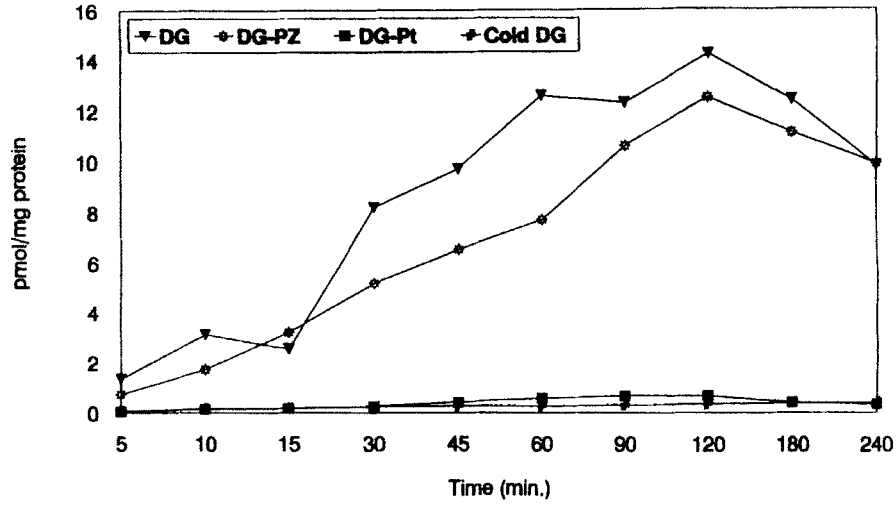

FIG. 2. Uptake of 2-deoxyglucose (DG) by MAC-T cells at Day 10 of culture and in the presence of inhibitors phlorizin (PZ) or phloretin (Pt) or cold 2-deoxyglucose.

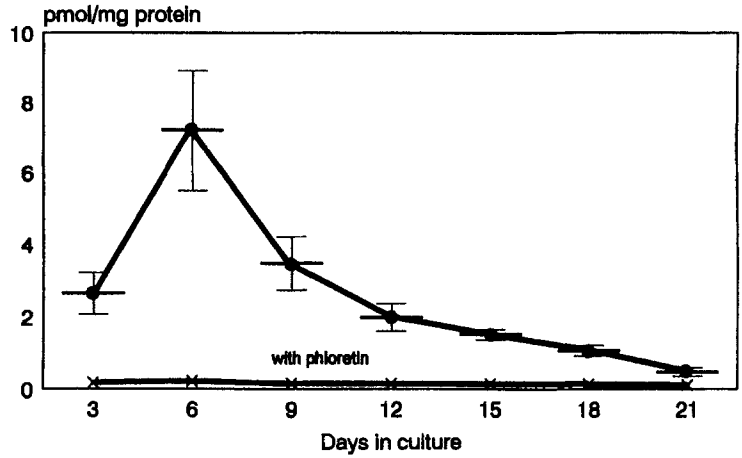

FIG. 3. Uptake of 2-deoxyglucose by MAC-T cells at different days of culture. The effect of phloretin treatment is indicated.

being higher than milk concentration: 0.05%vs traces (Larson, 1985). The uptake of 2-DG reaches a peak when expressed as pmol substrate/mg protein around Day 6 of culture well before confluency is attained between Days 12 and 15 (Figs. 3 and 4). The complete inhibition 2-DG uptake in the presence of Pt throughout the 21-day period confirmed that the uptake

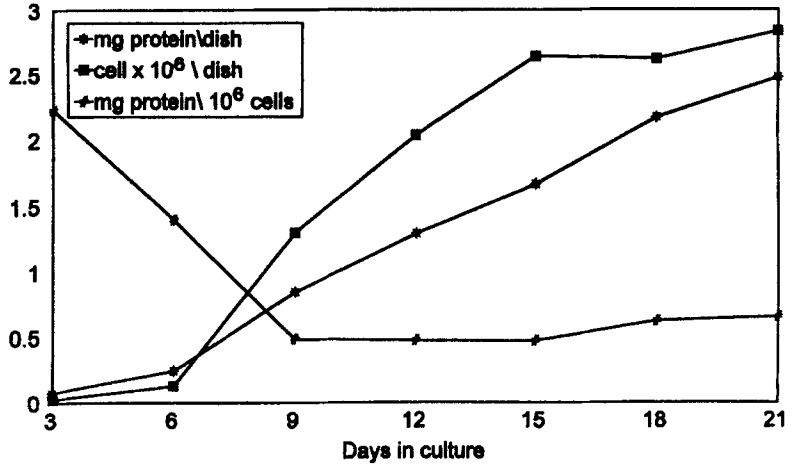

FIG. 4. Cell growth expressed as cell number and total cellular protein and protein/cell for MAC-T cells at different days of culture.

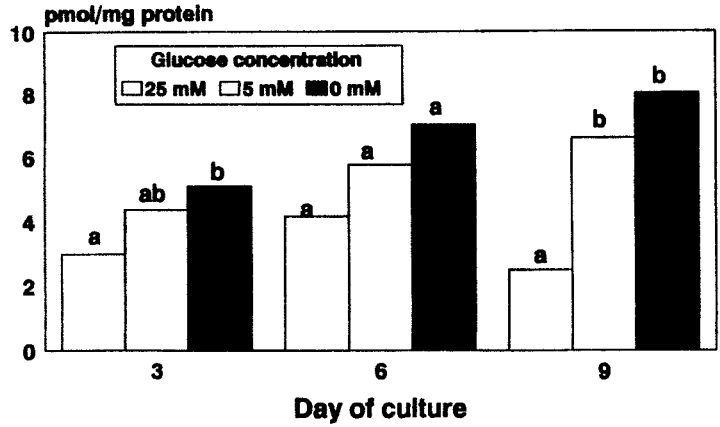

FIG. 5. Effect of media glucose concentration on the uptake of 2-deoxyglucose by MAC-T cells at different days of culture. The first column is 25 m*M*, the second column is 5 m*M*, and the shaded column represents media without glucose. Bars with different letters are significantly different ($P < 0.5$).

measured was specific and not due to nonspecific binding or transport or diffusion. The concommittent decrease in 2-DG uptake/mg protein and the increase in cell number or protein/dish after Day 9 can be explained by a reduced surface area per cell being in contact with the substrate as cells approach confluency or a reduced activity of the transporter as cells go from the growth state to the differentiation stage. The concentration of glucose in the medium had a significant effect on the uptake of 2-DG at Days 3 and 9 of culture (Fig. 5). The absence of glucose in the medium resulted in a significantly higher uptake of 2-DG/mg protein than in the presence of 25 m*M* glucose. These results demonstrated a negative feedback of glucose concentration on the expression and/or activity of the Na^+-independent glucose transporter(s).

IV. CONCLUSIONS

These experiments demonstrated that monolayers of bovine mammary epithelial cells do not accumulate glucose in a Na^+-dependent manner. The uptake of glucose reaches a maximum during the growth phase of the cells and diminishes as the cells reach confluency. The transport system(s) is regulated by the glucose concentration in the culture medium.

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