Mechanism of the decrease in hexose transport by mouse mammary epithelial cells caused by fasting

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The basal carrier-mediated uptake of 0.5 mM-3-*O*-methylglucose by mammary epithelial cells from lactating mice was calculated to be 227 ± 9 pmol/min per μ g of DNA (mean $\pm$ S.E.M., $n = 11$). Fasting the mice for 16 h overnight resulted in a decrease in this rate to 65 ± 4 pmol/min per μ g of DNA ($n = 10$). Refeeding the fasted mouse for 3 h before isolation of the cells restored the transport activity to 230 ± 12 pmol/min per μ g of DNA ($n = 12$). The $V_{\max}$ for equilibrium exchange entry of 3-*O*-methylglucose by intact cells was decreased from 6.6 ± 0.4 to 0.9 ± 0.2 nmol/min per μ g of DNA (mean $\pm$ S.E.M., $n = 3$) by fasting. The number of D-glucose-inhibitable cytochalasin-B-binding sites in a plasma-membrane-enriched fraction of the cells was also decreased from 5.7 ± 1.5 to 1.7 ± 0.1 pmol/mg of membrane protein (mean $\pm$ S.E.M., $n = 3$). Again, refeeding the fasted mouse for 3 h reversed both these effects. These results are consistent with a decrease in the number of functional glucose carriers in the plasma membrane of the mammary epithelial cells. Since the restoration of transporter activity after refeeding does not appear to require the synthesis of new protein, the effect of fasting probably involves not a loss of transporters, but a change in their orientation within the plasma membrane or a redistribution within the cell.

INTRODUCTION

Fasting in lactating animals causes a profound decrease in their lactational performance. Consistent with the overall decrease in milk production is a decrease at the cellular level in many of the processes essential for milk synthesis. These include lactose synthesis (Wilde & Kuhn, 1979; Bussman *et al.*, 1984), lipid synthesis (Robinson *et al.*, 1978; Jones *et al.*, 1984), glucose oxidation (Robinson & Williamson, 1977) and amino acid transport (Vina *et al.*, 1983, 1985). Studies both *in vivo* and *in vitro* of the lactating rat mammary gland have shown that glucose uptake by the gland decreases after long-term fasting (Hawkins & Williamson, 1972; Robinson & Williamson, 1977; Page & Kuhn, 1986). Unfortunately, these types of experiments cannot determine whether the effects of fasting are at the level of metabolism of the glucose or its transport into the cell.

Threadgold *et al.* (1982) have shown that the transport activity of the mammary acini of the lactating rat could be measured by using 2-deoxyglucose. They showed that the transport of hexose into the mammary cell is by a carrier-facilitated process. They reported, however, that the transport activity by the isolated acini was little affected by previous starvation of the donor animals or the addition of hormones to the cells. Subsequently their group performed some experiments *in vivo*, again utilizing 2-deoxyglucose (Threadgold & Kuhn, 1984). These studies showed that the transport of this hexose into the lactating mammary gland was depressed after an overnight fast. It would appear that the inhibition of transporter activity was reversed during the isolation and subsequent incubation of the cells.

We have previously shown that a preparation of mouse mammary epithelial cells can be utilized to study the glucose-transport system of the mammary epithelium (Prosser & Topper, 1986; Prosser *et al.*, 1987). Using this

cell preparation, we have shown that the transport of hexose by the mouse mammary gland is also mediated by a specific carrier and that the activity of the carrier is increased almost 40-fold from the virgin to the 10-day-lactating state. This development could be simulated *in vitro* by 2–3 days of explant culture in the presence of insulin and insulin-like growth factor-I with cortisol and prolactin. In contrast, insulin exerted only a small, acute, stimulatory effect on glucose transport by the cells from pregnant mice, and no detectable acute effect on cells from fed lactating mice. It seemed that the more important role of insulin was in the attainment of the very high basal rate of transport, which required prolonged exposure of the cells to this hormone.

In this paper it is shown that this cell preparation can also be used to examine the effects of an overnight fast on the carrier-facilitated transport of hexose by the mammary epithelium. The mechanism by which these effects are manifested was examined.

EXPERIMENTAL

Materials

Collagenase (Type III) was from Cooper Biomedical (Freehold, NJ, U.S.A.). Bovine serum albumin, 3-*O*-methylglucose, Hepes, cycloheximide, ouabain and cytochalasin B and E were from Sigma (St. Louis, MO, U.S.A.). Medium 199, containing Hanks salts and 5.5 mM-glucose, was from Gibco (Grand Island, NY, U.S.A.). Medium 199, containing Hanks salts, but without glucose was from the NIH Media Unit. Each were buffered to pH 7.6 with 20 mM-Hepes. 3-*O*-[³H]-Methyl-D-glucose, L-[1-¹⁴C]glucose, L-[4,5-³H]leucine, [4,5-³H]cytochalasin B and [¹⁴C]urea were from New England Nuclear (Boston, MA, U.S.A.).

The mice used in this study were of the C₃H/HeN

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strain obtained from the NIH animal care centre. The mice were used at 8–12 days lactation. Each dam was nursing five to seven pups. The animals were kept under the standard regimen of light and dark and fed *ad libitum* on a standard chow-based diet. The mice were fasted by removal of the food for 16 h from 18:00 until 10:00 h the following day. If the mice were to be refed, the food was replaced at the end of the period of fasting and the mice allowed to feed for the specified length of time. In all cases the pups were kept with the dams, and were frequently observed to suckle during the time of fasting.

Mammary epithelial-cell preparation

An epithelial-cell-enriched population was prepared from the abdominal mammary glands of mice by the procedure outlined in Prosser & Topper (1986). Briefly, the tissue was minced and treated with collagenase (1 mg/ml) in medium 199 containing 5 mM-glucose and 3% (w/v) bovine serum albumin for 25 min. The epithelial cells were collected by centrifugation (300 *g* for 3 min; r_{av} , 18 cm), filtered through a 500 μ m nylon mesh and washed three times with medium 199 containing 5 mM-glucose and 1% bovine serum albumin. The final centrifugation was performed at 120 *g* for 45 s (r_{av} , 18 cm). The pellet, consisting of small clusters of epithelial cells (5–25 cells per cluster), was used immediately for the measurement of the uptake of 3-*O*-methylglucose. More than 95% of the cells in suspension excluded Trypan Blue. Similar preparations of epithelial cells contain less than 5% contamination with non-epithelial cells (Voyles & McGrath, 1976; Emerman *et al.*, 1980). The number of cells present was determined by measuring the DNA content of a small sample of the cells by the procedure of Burton (1956).

Protein synthesis

Protein synthesis was measured in explants of the abdominal mammary glands of fasted, then refed, mice. The mice were treated with cycloheximide (0.1 ml of 100 μ M in 0.9% NaCl, given intraperitoneally) 0.5 h before and 2 h after refeeding was initiated. Control animals received 0.1 ml of saline at the same times. The mammary explants were taken 1 h after the last injection. After incubation of the explants for 30 min in medium 199 at 37 °C in the presence of 2 μ Ci of [³H]leucine/ml the explants were dried, weighed, and the ³H-labelled proteins were precipitated with 10% trichloroacetic acid.

Uptake of 3-*O*-methylglucose

The initial rate of carrier-mediated transport of 3-*O*-methylglucose by the mammary epithelial cells was measured by the procedures of Prosser & Topper (1986). The epithelial cells were resuspended in medium 199 without glucose but containing 5 mM-fructose and 1% bovine serum albumin. The uptake of 0.5 mM-3-*O*-methylglucose and L-glucose was measured over a 10 s period. Uptake was terminated by the addition of 20 μ M-cytochalasin B, and the cells were collected by filtration on to glass-fibre filters (GF/C, Whatman).

The 10 s time point was selected since previous studies had shown that this point fell on the linear portion of the uptake curve for 3-*O*-methylglucose. Rates of transport using this time point were confirmed in selected experiments by using the integrated rate equation (Eilam & Stein, 1974), which linearizes the uptake curves for net-

entry experiments as used in this study. The rate of carrier-mediated transport of 3-*O*-methylglucose was calculated by subtracting the rate of uptake of L-glucose from the total rate of uptake of 3-*O*-methylglucose. These rates were also confirmed in some experiments by measuring the uptake of 3-*O*-methylglucose in the presence and absence of cytochalasin B.

Kinetic parameters of transport of 3-*O*-methylglucose

The kinetic parameters for hexose transport in the mammary cells were determined under conditions of equilibrium exchange. The cells were isolated from the mammary tissue of the mice as described above. They were divided into 0.1 ml portions and incubated for 30 min at room temperature with 1–25 mM-3-*O*-methylglucose and for a further 15 min at 37 °C. The assay was initiated by the addition of 3-*O*-[³H]methylglucose (1 μ Ci) and L-[¹⁴C]glucose (0.5 μ Ci) and continued for 10 s. The uptake was stopped by the addition of 0.5 ml of ice-cold 20 μ M-cytochalasin B, and the cells were collected on to glass filters. The carrier-mediated uptake of 3-*O*-methylglucose was calculated by subtracting the rate of uptake of L-glucose from the total rate of uptake of 3-*O*-methylglucose. The assay was performed on three different preparations of cells from each group of mice.

Plasma-membrane preparation

A plasma-membrane-enriched fraction was isolated from the mammary epithelial cells by a scheme similar to that of Shin *et al.* (1975). The cells were homogenized in 10 vol. of buffer (HB: 20 mM-Tris, pH 7.4, 1 mM-EDTA and 0.25 M-sucrose). The homogenate was centrifuged at 750 *g* for 10 min (r_{av} , 18 cm) to pellet any unbroken cells. The pellet was resuspended in approx. 10 vol. of HB and re-homogenized. The homogenate was combined with the supernatant obtained previously, and this was centrifuged at 12000 *g* for 10 min (r_{av} , 75 mm) to pellet the nuclei and mitochondria. Over 95% of the activity of succinate-cytochrome *c* reductase (a marker for the mitochondria; Keenan *et al.*, 1974) and 90% of the DNA present in the homogenate appeared in this pellet. The supernatant was then centrifuged at 110000 *g* for 1 h (r_{av} , 59 mm) to give a crude membrane fraction and cytosolic fraction. The membrane pellet was resuspended in 5 vol. of 45% (w/w) sucrose by ten strokes of a loose-fitting Dounce homogenizer. A discontinuous gradient of 3 ml each of 45% (containing the membrane proteins), 36, 32 and 0.9% (w/w) sucrose was prepared. All sucrose solutions were made up to 20 mM-Tris (pH 7.4)/1 mM-EDTA. The sample was fractionated by flotation through the density gradient at 96000 *g* for 4 h in a Beckman SW 40 rotor. The plasma-membrane-enriched fraction at the interface of the 0.9%- and 32%-sucrose layers was collected, diluted with 20 mM-Tris (pH 7.4)/1 mM-EDTA and centrifuged at 110000 *g* for 1 h (r_{av} , 59 mm). The pellet was resuspended to a final concentration of 1–2 mg of protein/ml of HB and stored at –70 °C until used.

Analysis of the plasma-membrane fraction

Protein was determined by using Coomassie Brilliant Blue as described by Bradford (1976); bovine serum albumin was used as the standard. 5'-Nucleotidase activity was measured by the method described by Avruch & Wallach (1971). UDP-galactose:*N*-acetylglucosamine galactosyltransferase activity was measured

Fig. 1. Effect of the time of refeeding on the rate of carrier-mediated transport of 3-*O*-methylglucose in mammary epithelial cells of the fasted lactating mouse

Mice were fasted for 16 h overnight and refed for the indicated periods of time. The mammary epithelial cells were isolated and the uptake of 0.5 mM-3-*O*-methylglucose was measured. Values are means $\pm$ S.E.M. of four experiments, with the transport activity measured in triplicate.

by the method of Fitzgerald *et al.* (1970). Succinate-cytochrome *c* reductase was assayed by the method of Morre (1970). The (Na⁺-K⁺)ATPase activity was determined in the absence or presence of 1 mM-ouabain as described by Vreeswijk *et al.* (1973). Rotenone-insensitive NADPH-cytochrome *c* reductase activity was determined by the method of Dallner *et al.* (1966).

Cytochalasin B binding

Equilibrium cytochalasin B binding to the plasma-membrane-enriched fraction of the mammary epithelial cells was measured by a procedure similar to that outlined by Cushman & Wardzala (1980) for binding to their microsomal membrane fraction. The binding assays were performed in the presence of 2.5 μ M-cytochalasin B and in the presence or absence of 500 mM-glucose. Cytochalasin B was present at concentrations of 30, 50,

100, 200 and 300 nM. [¹⁴C]Urea instead of [³H]sucrose was used to determine the trapped unbound [³H]-cytochalasin B in the membrane pellets.

RESULTS

The basal rate of transport of 0.5 mM-3-*O*-methylglucose (measured in the absence of any added hormones) by mammary epithelial cells of normally fed lactating mice was 227 ± 9 pmol/min per μ g of DNA (mean $\pm$ S.E.M., $n = 11$). Fasting the donor mouse for 16 h overnight caused a 3.5-fold decrease in the transport rate, to 65 ± 4 pmol/min per μ g of DNA (mean $\pm$ S.E.M., $n = 10$). After refeeding the fasted animals for 3 h, the transport rate was restored at 230 ± 12 pmol/min per μ g of DNA (mean $\pm$ S.E.M., $n = 12$). These effects cannot be attributed to weaning-like effects, since the pups were frequently observed to suckle during the time of fasting. Moreover, little milk was found in the gland of the fasted animal, showing that the accumulation of milk could not be a factor in the decreased transport activity of the cells.

It must be noted that the transport rate measured in this work is that of the carrier-mediated transport of hexose. The component due to non-mediated diffusion, measured by the uptake of L-glucose, has been subtracted from the total uptake. The absolute rate of non-mediated transport of 3-*O*-methylglucose was not affected by fasting or subsequent refeeding, indicating no significant changes in the non-carrier-mediated permeability of the plasma membrane. However, since the total uptake of 3-*O*-methylglucose (carrier-mediated and non-mediated combined) was decreased by starvation, the percentage contribution of the non-mediated uptake to the total uptake increased from 4% before to 10% after fasting.

The time course for the recovery of the transport activity during refeeding of the fasted mouse is shown in Fig. 1. Maximal recovery was reached by 3 h. The rate returned to that seen for the normally fed mice and did not exhibit any over-compensation. To determine the role of protein synthesis in the reversal, cycloheximide was given to some fasted mice 0.5 h before refeeding and 2 h after refeeding. This regimen was found to inhibit protein synthesis in explants of the mammary gland by at

Fig. 2. Concentration-dependence of 3-*O*-methylglucose equilibrium exchange entry by mammary cells from (a) normally lactating, (b) fasted lactating and (c) fasted and then refed lactating mice

Cells were preincubated with 1–25 μ M-3-*O*-methylglucose (s) and the uptake of 3-*O*-[³H]methylglucose was measured over 10 s at 37 °C. The initial carrier-mediated uptake was calculated and the data were plotted according to Hanes' transformation of the Michaelis-Menten equation. Each point represents the mean $\pm$ S.E.M. of triplicate observations from one experiment. Units of v are nmol/min per μ g of DNA.

Table 1. Characteristics of the membrane fraction from mammary epithelial cells used for analysis of cytochalasin B binding

The membrane fraction was prepared from mammary epithelial cells by density-gradient centrifugation. Marker enzyme activities were measured in the original homogenate and the membrane fraction. The enrichment refers to the fold increase in specific enzyme activity (pmol/h per mg of protein) in the membrane fraction over that of the homogenate. The recovery is the enzyme activity in the homogenate. The results are means $\pm$ S.E.M. for three separate preparations; N.D., not determined.

Enzyme	Mice . . .		Fasted		Fasted + refed	
	Normal	Recovery (pmol/h per μ g of DNA)	Enrichment	Recovery (pmol/h per μ g of DNA)	Enrichment	Recovery (pmol/h per μ g of DNA)
5'-Nucleotidase	13.3 $\pm$ 1.4	11.8 $\pm$ 1.5	11.5 $\pm$ 1.2	14.7 $\pm$ 1.1	10 $\pm$ 1	15.7 $\pm$ 0.1
(Na-K)ATPase	7.8 $\pm$ 2.7	15 $\pm$ 1	6 $\pm$ 0.2	13 $\pm$ 2	ND	ND
Galactosyltransferase	1.1 $\pm$ 0.2	32 $\pm$ 2	1.2 $\pm$ 0.5	11.6 $\pm$ 0.7	1.3 $\pm$ 0.2	19.5 $\pm$ 0.1
Cytochrome <i>c</i> reductase	1.6 $\pm$ 0.1	21 $\pm$ 9	1.5 $\pm$ 0.6	18 $\pm$ 3	1.1 $\pm$ 0.1	23 $\pm$ 5

least 75 % after 3 h of refeeding. The transport activity for the cells from the cycloheximide-treated mice was 200 ± 28 pmol/min per μ g of DNA (mean $\pm$ S.E.M., $n = 4$) compared with 184 ± 17 pmol/min per μ g of DNA (mean $\pm$ S.E.M., $n = 4$) for the untreated mice.

To examine the mechanism by which fasting decreases the hexose transport activity in mammary cells, the kinetics of transport were measured. In this instance equilibrium exchange experiments were utilized to eliminate any possible error caused by the change in the rate of back-flux of 3-*O*-methylglucose with increasing concentrations of substrate (Gliemann & Rees, 1983). The kinetic parameters were calculated by using Hanes' transformation of the Michaelis-Menten equation. Fig. 2 shows the data from one experiment on each group of mice. In total, the assay was performed on three different preparations of cells from each group of mice. The $V_{\max}$ and K_m for transport by cells from lactating mice were 6.6 ± 0.4 nmol/min per μ g of DNA and 14 ± 2 mM respectively (means $\pm$ S.E.M.). Overnight fasting caused a dual effect, in that both the $V_{\max}$ and K_m of transport by the mammary cells were decreased, to 0.9 ± 0.2 nmol/min per μ g of DNA ($P < 0.01$) and 6 ± 1 mM ($P < 0.05$) respectively (means $\pm$ S.E.M.). Refeeding the fasted mouse for 3 h resulted in an increase in the $V_{\max}$ and K_m to

6.5 ± 0.1 nmol/min per μ g of DNA and 13 ± 1 mM respectively.

To investigate further the mechanism of the effect of fasting, a plasma-membrane-enriched fraction was prepared from the isolated cells and analysed for D-glucose-inhibitable cytochalasin B binding. The recovery and enrichment of the marker enzymes in this fraction from the original homogenate of the three cell types is shown in Table 1. If it can be assumed that the activity of the 5'-nucleotidase or (Na⁺-K⁺)ATPase in the homogenate represents the amount of plasma membrane recovered, then the data suggest that fasting does not alter the total amount of plasma membrane in the cell. Moreover, fasting does not in any way affect the characteristics of the membrane, so that its enrichment and recovery from the starting homogenate are unchanged.

The number of D-glucose-inhibitable binding sites, representing glucose transporters, was decreased 3.4-fold by fasting (Table 2). Refeeding the fasted mouse for 3 h increased the number of sites, to that normally seen in the lactating mouse.

The dissociation constant (K_d) for cytochalasin B binding to the D-glucose-inhibitable sites (Table 2) is consistent with the K_i of 190 nM for the inhibition of glucose transport activity in the intact cells by cytochalasin B (C. G. Prosser, unpublished work). Moreover, this is of the same order as the K_d for cytochalasin B binding to membranes of the rat adipocyte (Karnieli *et al.*, 1981) and diaphragm (Wardzala & Jeanrenaud, 1983). Fasting resulted in a 33 % decrease in the dissociation constant for the mammary cells, although this appears not to be statistically significant. Refeeding the fasted mouse restored the K_d to that seen in the normally fed mice (Table 2).

DISCUSSION

The results presented here demonstrate that fasting the lactating mouse for 16 h overnight causes a 3.5-fold decrease in the basal carrier-mediated transport of 0.5 mM-3-*O*-methylglucose by the mammary epithelial cells. Refeeding the fasted mouse for a minimum of 3 h results in the full restoration of the transport activity to that of the normally fed mouse (Fig. 1). These results are in agreement with those of Threadgold & Kuhn (1984),

Table 2. D-Glucose-inhibitable cytochalasin-B-binding characteristics of plasma membranes of mammary cells

Equilibrium D-glucose-inhibitable cytochalasin-B-binding assays were performed on the plasma-membrane fractions of the mammary epithelial cells. The differences from the normal values were tested for significance by Student's *t* test (ns, not significant).

Mice	No. of sites (pmol/mg of protein)	K_d (nM)
Normal*	5.7 $\pm$ 1.5	133 $\pm$ 13
Fasted*	1.7 $\pm$ 0.1 ($P < 0.01$)	88 $\pm$ 13 (ns)
Fasted + refed†	6.5 $\pm$ 1.3 (ns)	123 $\pm$ 16 (ns)

* Mean $\pm$ S.E.M. of three experiments.

† Mean $\pm$ range of two experiments.

which showed a decreased mammary uptake of 2-deoxyglucose in the intact rat after an overnight fast and that refeeding reversed this effect.

The present results differ from the results obtained *in vitro* by Threadgold *et al.* (1982), in that they could not demonstrate the same effects of starvation on the isolated rat mammary acini. The reason for this disparity is not immediately apparent, but may relate to differences in the preparation and subsequent incubation of the mammary cells. Although the influence of the use of different species as the source of the cells is also not known, it is perhaps significant that in my hands the mouse mammary gland provides a cell preparation having a greater viability and yield of epithelial cells than the mammary gland from the rat.

It is also of interest that the time course of restoration of the transport activity by refeeding the mouse (Fig. 1) is longer than that observed by Threadgold & Kuhn (1984) or Page & Kuhn (1986) for the rat (3 h for the mouse, compared with less than 1 h for the rat). Although this difference may result from the different experimental approaches used to measure transport activity, it may indicate a larger inhibitory effect in the mouse than in the rat, which would further contribute to the different responses seen *in vitro*.

To determine the mechanism of the decrease in the glucose-transport activity during fasting, the kinetics of transport of 3-*O*-methylglucose in intact cells and the characteristics of cytochalasin B binding in plasma-membrane-enriched fractions were investigated. The use of D-glucose-inhibitable cytochalasin B binding to estimate the number of hexose transporters in the mammary gland has not been documented previously. However, it has been used extensively in a variety of other cell types (Lin & Spudich, 1974; Wardzala *et al.*, 1978; Wardzala & Jeanrenaud, 1983; Axelrod & Pilch, 1983; Ciaraldi *et al.*, 1986). A procedure similar to that outlined by Cushman & Wardzala (1980) for the microsomal membranes of the rat adipocyte was used in the present work to analyse the number of D-glucose inhibitable binding sites in the plasma-membrane-enriched fraction of the mammary epithelial cells. The characteristics of binding in the mammary-derived membrane fractions were very similar to those of the adipocyte, indicating a close similarity between the glucose-transport systems of the two cell types (results not shown).

The results of the kinetic and cytochalasin-B-binding studies suggest that fasting has a dual effect on the glucose-transport system of the lactating mammary epithelial cell. The decrease in $V_{\max}$ of transport and the number of D-glucose-inhibitable binding sites in the plasma membrane (Table 2) are consistent with a decrease in the number of active transporters in the plasma membrane. In contrast, the starvation-induced increase in the affinity of the transporter for hexose (Fig. 2) suggests an enhancement of the intrinsic function/activity of the transporter proteins. This may represent an initial response by the cells to fasting in order to compete more effectively for the lower amount of glucose moieties in the circulation. However, it would seem that the decrease in transporter number predominates in long-term fasting, since the overall effect is a decreased transport rate.

Since the amount of plasma membrane in the cell is unchanged during fasting (Table 1), the loss of transporter from the plasma membrane must be selective and

not due to a general loss of plasma-membrane components. Moreover, the studies by Threadgold & Kuhn (1984) and those here, using cycloheximide to inhibit protein synthesis, suggest that synthesis of new transporters is not required for the reversal of the fasting effect. Thus it may be concluded that the decrease in the number of functional transporters from the plasma membrane during fasting does not arise from the degradation of transporter protein.

There are perhaps two ways in which a selective loss of transporters from the plasma membrane could be achieved. The first involves a change in the orientation and/or conformation of the transport protein in the plasma membrane, such that the apparent number of hexose- and cytochalasin-B-binding sites is decreased. The second involves their translocation to other membrane sites within the cells, as occurs in the adipocyte during the reversal of the insulin stimulation (Karnieli *et al.*, 1981). Unfortunately, the difficulties involved in preparing an intracellular membrane fraction which is free of significant contamination by the plasma membrane precluded the estimation of the intracellular transporter distribution. Moreover, preliminary experiments reveal that the amount of D-glucose-inhibitable cytochalasin B binding to the intracellular membrane fraction of mammary cells from fed mice is too low to allow accurate determination of the number of binding sites (C. G. Prosser, unpublished work).

Clearly, further studies are required to clarify the nature of the decrease in the K_m of transport during fasting. It is of interest that the K_d for cytochalasin B binding to the D-glucose-inhibitable sites also showed a small but not significant decrease (Table 2). A change in either of these two parameters is consistent with a change in the immediate environment of the glucose-carrier or a conformational change in the transport protein itself. However, the present studies do not preclude the possibility that there are two forms of the glucose transporter in the plasma membrane of the mammary epithelial cells, one of which undergoes translocation. Indeed, heterogeneity of the glucose carrier of adipocytes has been documented (Horuk *et al.*, 1986).

The mechanisms that elicit the change in glucose transport activity during fasting should not be confused with those operating during development. In going from the virgin to the 10-day-lactating state, the basal carrier-mediated transport of glucose by the mouse mammary epithelial cells increased almost 40-fold (Prosser & Topper, 1986). Insulin and/or insulin-like growth factor-I appeared to have an essential role in the attainment of the very high basal rate, but the effects required prolonged exposure (2–3 days) to these agents (Prosser & Topper, 1986; Prosser *et al.*, 1987). On this basis it was suggested that the enhanced rate of transport observed did not involve translocation of glucose-transport proteins. In contrast, the effects of fasting described in the present paper are more rapid and seem to involve a conformational change or translocation of the glucose transporters. Moreover, the role that insulin deprivation plays in mediating the effects of fasting on mammary gland function is controversial (Page & Kuhn, 1986; Mercer & Williamson, 1986). It is significant that an acute exposure of mammary epithelial cells from fasted lactating mice to insulin will only increase the transport activity by 60% (C. G. Prosser, unpublished work). It is also not known what function insulin-like growth factor-I, which is

nutritionally regulated (Merimee *et al.*, 1982; Maes *et al.*, 1983), may have in inducing the changes observed.

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