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Monosaccharide transport in the mammary gland of the intact lactating rat

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1. The Michaelis–Menten equation for the utilization of competing substrates was applied to the uptake of 2-deoxy[^{3}H]glucose into the mammary gland of anaesthetized lactating rats. Intracellular water was calculated from total tissue water and sucrose space. 2. Fed rats had a mean transport capacity of 2.2 μmol/min per g of tissue, giving an actual glucose transport *in vivo* of 1.1 μmol/min per g. 3. Transport decreased by 90% on overnight starvation and returned to normal by 2 h of re-feeding. Similar changes were observed in the 1 min or 5 min transport of circulating 3-*O*-methylglucose. 4. Transport of 3-*O*-methylglucose in starved rats was restored towards normal by insulin. In fed rats it increased between parturition and day 12 of lactation. 5. The findings support the proposal that transport is a rate-limiting factor in the mammary utilization of carbohydrate.

The lactating mammary gland consumes most of the available glucose in the body, and is in turn completely dependent on this supply for its function, yet only recently has interest in carbohydrate metabolism in this gland focused on monosaccharide transport across the plasma membrane. Amato & Loizzi (1979) have studied the uptake of low concentrations of 2-deoxyglucose by slices of tissue from lactating guinea pigs and shown it to be inhibited by cytochalasin B. Threadgold *et al.* (1982) have used acini of rat mammary gland to characterize the transport of both 3-*O*-methylglucose and 2-deoxyglucose. Transport was inhibited by cytochalasin B, $HgCl_2$, *N*-ethylmaleimide and phloretin and by a range of structurally related sugars. Uptake of 2-deoxyglucose exhibited an apparent K_m of 16 mM and was inhibited by D-glucose with an apparent K_i of 6.8 mM. Assuming $K_i = K_m$, plasma glucose appears to half-saturate the carrier *in vivo*.

Recent experiments in our laboratory have attempted without success to demonstrate effects of insulin, corticosterone, dexamethasone, acetoacetate, theophylline, dibutyryl cyclic AMP or adenosine on transport of 2-deoxyglucose in the presence either of glucose or of pyruvate plus lactate as energy source *in vitro*. Further, transport appeared to be unaffected by previous starvation of the animal from which the acini were prepared. Some of these conditions have been reported to affect glucose uptake by rat mammary tissue *in vitro*. However, it has generally been found that the glucose uptake by acini *in vitro* is little influenced by the simple addition of insulin or by the nutritional state of the donor animal (Williamson *et al.*, 1975; Robinson & Williamson, 1977*c*; Agius & Williamson, 1980; Wilde & Kuhn, 1981).

In view of these difficulties, we have now explored the possibility of monitoring monosaccharide transport into the lactating mammary gland of the intact, lightly anaesthetized, rat. Procedures using both 2-deoxyglucose and 3-*O*-methylglucose are described, and large changes in transport are reported for starved, re-fed and insulin-treated rats and for animals at different stages of lactation.

Materials and methods

Primiparous Wistar-derived rats from the Departmental colony were maintained on a 14 h-light/10 h-dark cycle, with food and water given *ad lib.* except where stated. Anaesthesia was induced with 8% (v/v), and maintained with approx. 2.5% (v/v), halothane in O_2. Where indicated, insulin ('Lente'; Wellcome Foundation) was injected (2 units/kg body wt.) subcutaneously in 0.9% NaCl 2 h before measurement of 3-*O*-methylglucose uptake.

Measurement of 2-deoxyglucose transport

Each rat received 2-deoxy[^{3}H]glucose (0.63 μmol, 4.8 μCi) and [^{14}C]sucrose (1.5 μmol, 2.5 μCi) by a single injection into the femoral vein. Blood samples (approx. 0.3 ml) were subsequently taken from the tail at 1, 2, 3, 5, 10, 20 and 30 min

into heparinized tubes that were then centrifuged to sediment erythrocytes. A portion (50 μl) of each plasma was taken for determination of radioactivity, and another portion (50 μl) was deproteinized with 5% (w/v) $HClO_4$ (2.45 ml) for determination of glucose with a glucose oxidase/peroxidase kit (Boehringer). At 30 min one posterior mammary gland was excised and frozen in liquid N_2. This was subsequently powdered by grinding under liquid N_2, and a weighed portion (100–200 mg) was dried at 50°C to constant weight to obtain total tissue water. Further duplicate, weighed, portions (approx. 1 g) were extracted with 5 ml, followed by 3 ml, of 5% $HClO_4$. The pooled extracts were neutralized with KOH, their volumes were recorded, and portions (1 ml) were taken for determination of radioactivity. Further duplicate portions (2 ml) were passed through columns of Dowex-1 (formate form) resin, which were then washed with water (2 ml) to elute 2-deoxyglucose (Threadgold *et al.*, 1982). Phosphorylated 2-deoxyglucose was calculated as the difference between loaded and eluted radioactivity.

The [^{14}C]sucrose contents of plasma and tissue allowed the calculation of extracellular tissue water, which, subtracted from total tissue water, gave a value for intracellular water. No attempt was made to correct this for retained milk water. It was assumed that all the 2-deoxy[^{3}H]glucose 6-phosphate was intracellular, and the amount of intracellular 2-deoxy[^{3}H]glucose was calculated as the difference between total tissue ^{3}H and extracellular tissue ^{3}H.

Measurement of 3-O-methylglucose transport

Anaesthetized rats were dissected to expose one posterior mammary gland without disturbing its vascular supply. NaCl (0.25 ml; 0.9%) containing 3-*O*-methyl[^{3}H]glucose (0.8 μmol, 10 μCi) and [^{14}C]sucrose (0.8 μmol, 5 μCi) was injected into the contralateral femoral vein, and the mammary gland was excised at 1 or 5 min and transferred to liquid N_2. A sample (0.5 ml) of blood was immediately taken, from the severed neck, into a chilled heparinized tube and centrifuged to obtain plasma. The frozen tissue was treated as described above to measure total water, ^{14}C and ^{3}H, whereas the plasma was used for determination of glucose, ^{14}C and ^{3}H.

Counting procedures and sources of materials have been described (Threadgold *et al.*, 1982). [^{14}C]Sucrose and 2-deoxy[^{3}H]glucose were purified by paper chromatography before use.

Results

Use of 2-deoxyglucose to estimate transport capacity

Fig. 1 shows a typical curve describing the change in plasma concentration of 2-deoxyglucose during 30 min after its injection, and the relatively constant plasma concentration of glucose. The shapes of such curves were similar for rats in different nutritional states, apart from the longer half-life of 2-deoxyglucose in starved rats. The 30 min was arbitrarily divided into successive 2 min periods, during each of which 2-deoxyglucose was regarded as entering the mammary cells at a constant rate corresponding to its mean concentration in the plasma in that period. Entry was assumed to occur in competition with plasma glucose via a common carrier of apparent K_m 15.6 mM for 2-deoxyglucose and apparent K_i 6.75 mM for glucose. The assumptions, which ignore any efflux of 2-deoxyglucose and therefore may underestimate transport by up to 10%, arise from the previous study of 2-deoxyglucose transport into mammary acini *in vitro* (Threadgold *et al.*, 1982) and from estimates of the intracellular concentration of glucose in mammary cells of the lactating rat, goat and other species (Kuhn & White, 1975; Wilde & Kuhn, 1981; Faulkner *et al.*, 1981). Applying the equation for competitive inhibition between two substrates of an enzyme:

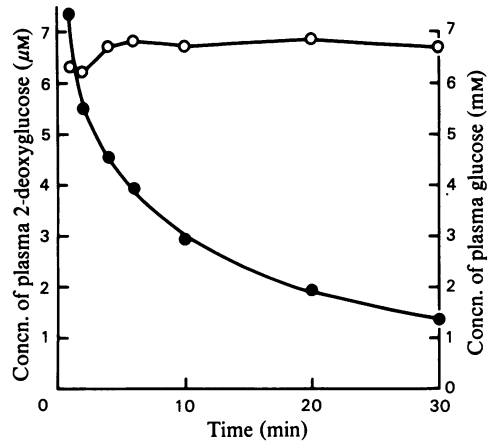

Fig. 1. *Concentrations of glucose* (○) *and 2-deoxyglucose* (●) *in plasma of a lactating rat*
For details see the text.

$$v = \frac{s \cdot V_{max.}}{s + K_m\left(1 + \frac{I}{K_i}\right)}$$

where v = rate of 2-deoxyglucose transport (μmol/min per g fresh wt.), $V_{max.}$ = maximum transport capacity, s = concentration of plasma 2-deoxyglucose (mM), I = mean concentration of plasma glucose (mM) during the 30 min of measurement, and K_m and K_i are as given above, $V_{max.}$ can be calculated in terms of v for each 2 min period.

Successive values of v (i.e. $2v_1 + 2v_2 + \ldots 2v_{15}$) can be summed over 30 min and equated with the total 2-deoxyglucose (free plus phosphorylated) present in the gland at this time:

$$\int_0^{30\,\text{min}} v = \frac{2V_{\text{max.}} \int_0^{30\,\text{min}} s}{K_m\left(1+\frac{I}{K_i}\right)} = \text{tissue 2-deoxyglucose}$$

where s in the denominator has been discarded as being very small compared with K_m. Therefore

$$V_{\text{max.}} = \frac{\text{tissue [2-deoxyglucose]} \times K_m\left(1+\frac{I}{K_i}\right)}{2\int_0^{30\,\text{min}} s}$$

For example, the values from one rat gave:

$$V_{\text{max.}} = \frac{0.0078 \times 15.6\left(1+\frac{6.66}{6.75}\right)}{2 \times 0.042}$$

$$= 2.87\,\mu\text{mol/min per g of tissue}$$

Measurement of extra- and intra-cellular spaces

In preliminary experiments sucrose and inulin were compared for use in measuring extracellular space by the procedure detailed in the Materials and methods section. The mean values (±S.E.M.) for five rats each, obtained with sucrose and inulin respectively, were 0.297±0.029 ml/g of tissue and 0.274±0.04 ml/g of tissue at 10 min, and 0.265±0.008 ml/g of tissue and 0.254±0.013 ml/g of tissue at 30 min after injection. These values do not differ significantly among themselves, by Student's t test, and agree closely with the value of 0.271±0.035 ml/g of tissue obtained previously with inulin (Kuhn & White, 1975). Sucrose was adopted for use in subsequent experiments. The mean extracellular space (±S.E.M.) in the mammary glands of five rats starved overnight was 0.210±0.008 ml/g of tissue. The mean total tissue water was 0.728±0.007 ml/g in fed rats and 0.684±0.020 ml/g in rats starved overnight, also in agreement with a previous report (Kuhn & White, 1975).

Values of transport capacity measured with 2-deoxyglucose

Tables 1 and 2 show the results of applying the 2-deoxyglucose technique to rats that were fed, starved overnight, or starved and then re-fed for various times up to 270 min. Both the amount and concentration of intracellular 2-deoxyglucose (free plus phosphorylated) decreased greatly on starvation and returned rapidly to normal on re-feeding. The extent of phosphorylation of the sugar (about 70%) was unaffected by starvation. The calculated mean transport capacity ($V_{\text{max.}}$) was 2.22±0.25 μmol/min per g of tissue in the fed rats, falling by 90% on starvation and recovering to about 60% of normal already within 15 min of giving food. Cycloheximide (approx. 4 mg/kg body wt.) given 1 h previously to inhibit protein synthesis did not impair either the transport capacity in fed rats (2.91 μmol/min per g) or the recovery of this process in starved/re-fed rats (2.21 μmol/min per g; mean for two rats each).

The question arises whether the concentrations of glucose and 2-deoxyglucose in tail blood appropriately reflect those to which the mammary gland is exposed. In a separate group of six fed rats the mean (±S.E.M.) concentration of glucose in whole blood collected successively from the tail, the inguinal mammary vein and the aorta were respectively 7.46±0.19, 4.00±0.28 and 8.25±0.22 μmol/ml. In a group of starved rats examined over the same period, the corresponding glucose concentrations were 6.12±0.11, 5.76±0.07 and 6.40±0.09 μmol/ml. The mean

Table 1. *Effect of starvation and re-feeding on the mammary uptake of 2-deoxyglucose in vivo*

The 2-deoxyglucose values shown are the sums of free and phosphorylated forms of the sugar. Reported values are means±S.E.M., with the numbers of animals in parentheses.

Treatment of the animal	Intracellular 2-deoxyglucose at 30 min (nmol/g of tissue)	Intracellular concn. of 2-deoxyglucose at 30 min (μM)	$V_{\text{max.}}$ of sugar uptake (μmol/min per g of tissue)
Fed (12)	4.23±0.48	8.83±0.90	2.22±0.25
Starved (7)	0.79±0.05	1.64±0.12	0.21±0.02
Re-fed for 15 min (6)	3.07±0.57	6.94±0.80	1.24±0.07
Re-fed for 30 min (5)	4.45±0.62	8.88±1.56	1.60±0.15
Re-fed for 60 min (7)	3.91±0.56	7.45±1.12	1.88±0.10
Re-fed for 120 min (6)	3.75±0.49	7.69±0.97	1.98±0.25
Re-fed for 270 min (6)	4.16±0.21	9.54±0.84	1.84±0.12

Table 2. *Effect of starvation and re-feeding on the plasma concentrations of glucose and 2-deoxyglucose, the intracellular mammary concentration of free 2-deoxyglucose and the extent of phosphorylation of intracellular 2-deoxyglucose*
Reported values are means ± S.E.M., with the numbers of animals in parentheses.

Treatment of the animal	Concn. of plasma glucose (mM)	Concn. of plasma 2-deoxyglucose (μM)	Concn. of free intracellular 2-deoxyglucose (μM)	Extent of phosphorylation of intracellular 2-deoxyglucose (%)
Fed (12)	6.45 ± 0.22	1.15 ± 0.14	2.49 ± 0.28	72.1 ± 1.8
Starved (7)	5.82 ± 0.24	2.12 ± 0.20	0.49 ± 0.14	72.4 ± 7.3
Re-fed for 15 min (6)	5.61 ± 0.11	1.25 ± 0.16	2.14 ± 0.24	68.4 ± 2.8
Re-fed for 30 min (5)	6.52 ± 0.20	1.34 ± 0.11	2.39 ± 0.31	71.9 ± 1.9
Re-fed for 60 min (7)	6.05 ± 0.29	1.13 ± 0.14	1.96 ± 0.27	72.5 ± 2.7
Re-fed for 120 min (6)	6.74 ± 0.38	1.18 ± 0.09	2.21 ± 0.25	68.3 ± 2.3
Re-fed for 270 min (6)	6.15 ± 0.29	1.24 ± 0.06	3.44 ± 0.33	63.6 ± 1.9

Table 3. *Effect of starvation and re-feeding on the mammary uptake of 3-O-methylglucose in vivo*

Nutritional state	Time of sample ...	Amount of intracellular 3-O-methylglucose (nmol/g of tissue)		Concn. of plasma 3-O-methylglucose (μM)	
		1 min	5 min	1 min	5 min
Fed		0.60 ± 0.16 (9)	0.87 ± 0.09 (6)	8.46 ± 0.28 (9)	5.71 ± 0.52 (6)
Starved (15 h)		0.03 ± 0.02 (6)	0.04 ± 0.02 (6)	9.97 ± 0.73 (6)	6.98 ± 0.61 (6)
Re-fed (2 h)		0.59 ± 0.13 (8)	–	10.34 ± 0.48 (8)	–

duration of blood collection for a given animal was about 2.1 min. It is not unreasonable to accept a glucose concentration midway between its arterial and its mammary-venous values as reflecting that to which an average mammary epithelial cell is exposed. The above data show that such a value is about 82% of the tail-blood glucose concentration in fed rats and about 99% of that in starved rats. Assuming that blood sampled from different sites exhibits similar differences in concentration of 2-deoxyglucose, the use of tail blood may lead to an 18% underestimate of $V_{max.}$ in fed and in re-fed rats.

From the blood glucose concentrations given above, the arteriovenous difference across the mammary gland decreased by 85% on starvation. Even without allowance for a probable retrenchment of mammary blood flow, these data imply a decrease in mammary glucose consumption comparable with the apparent decrease in transport.

Unexpectedly, the concentration of free 2-deoxyglucose within the cells of fed and re-fed rats apparently exceeded that in the plasma at the same time. This was not observed with starved rats nor with 3-*O*-methylglucose as the transported sugar, and may indicate some irreversible compartmentation associated with partial hydrolysis of sugar phosphate. The effect remains to be investigated.

Use of 3-O-methylglucose to monitor transport capacity

The use of 2-deoxyglucose to measure monosaccharide transport is open to the criticism that phosphorylation, rather than transport, is actually being measured (Foley *et al.*, 1980), although in that case a lower percentage phosphorylation of the sugar might have been expected in the tissue of starved rats. Therefore the mammary uptake of 3-*O*-methylglucose and sucrose from the plasma over 1 min or 5 min was examined in fed, starved and re-fed animals, the preferential uptake of 3-*O*-methylglucose being ascribed to its penetration of the cells. Table 3 shows that the amount of intracellular 3-*O*-methylglucose taken up fell by about 95% on starvation. Recovery, measured only at 1 min, was complete within 2 h of re-feeding. These values have not been adjusted for any variation in concentration of plasma 3-*O*-methylglucose between groups of animals, data for which are, however, included in Table 3.

The calculated mean intracellular concentration of 3-*O*-methylglucose at 5 min after injection did not exceed 52% of that in plasma, whereas in two rats examined at 30 min the intracellular and extracellular concentrations were equal (results not shown). Thus, unlike the situation with 2-deoxyglucose, there was no apparent concentration of

3-*O*-methylglucose in the cell. The identity of [^{3}H]-3-*O*-methylglucose extracted from the tissue was confirmed by paper chromatography, which exhibited a single peak of radioactivity in material isolated with 92% yield.

Effect of insulin on 3-O-methylglucose transport in starved rats

Insulin (2units/kg body wt., subcutaneously) was administered to rats starved overnight, in an attempt to restore the transport of 3-*O*-methylglucose to normal. Because of the large variations in plasma glucose in these rats, it was essential to correct for its varying competition for the common monosaccharide carrier. The fraction of carrier sites occupied by glucose (x) was calculated for each rat from the Michaelis–Menten relationship

$$x = \frac{v}{V_{max.}} = \frac{s}{s + K_m}$$

where s=plasma glucose concentration and K_m=6.75 (Threadgold *et al.*, 1982). Each value of 3-*O*-methylglucose uptake was then divided by $1-x$ to give the uptake expected in the absence of glucose.

Table 4 shows that insulin increased the quantity and apparent concentration of intracellular 3-*O*-methylglucose in both fed and starved rats. In the fed rats, however, this increase disappeared when the glucose correction was applied, showing that it had been due solely to decreased competition by the greatly depressed concentration of plasma glucose. But in the starved rats application of the correction left a large increase in intracellular 3-*O*-methylglucose over that in the control starved rats. In fact, the uptake of 3-*O*-methylglucose in insulin-treated rats was underestimated by ignoring the 29–30% decrease in its plasma concentration caused by insulin itself. Thus insulin did appear to restore the transport capacity of starved rats towards the value seen in fed rats.

Transport of 3-O-methylglucose at different stages of lactation

Fig. 2 shows that the uptake of 3-*O*-methylglucose over 1min increased substantially between parturition and day 10 of lactation, thereafter remaining constant up to day 20. In Fig. 2 the data are depicted as the ratio of 3-*O*-methylglucose/sucrose in the tissue relative to that in the plasma. In general such a ratio should range between unity, where no transport of 3-*O*-methylglucose occurs at all, and about 2.6 (the ratio of total tissue water to extracellular water), where 3-*O*-methylglucose has equilibrated throughout the tissue. Insofar as one attempts to measure initial transport rates, it is desirable that measured ratios should not extend too far up this range. Consistent with this was the finding that 5min uptake ratios (not shown) were greater, but showed less change with stage of lactation.

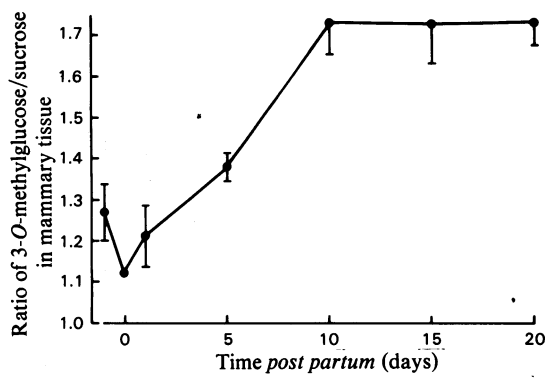

Fig. 2. *Transport of 3-O-methylglucose into mammary-gland cells at different stages of lactation*
Results are shown as 3-*O*-methylglucose/sucrose ratios for whole tissue. Each point is the mean ± S.E.M. for eight or nine rats.

Table 4. *Effect of insulin on plasma glucose, and the mammary uptake of 3-O-methylglucose in fed and starved rats in vivo*
The uptake of tracer [^{3}H]3-*O*-methylglucose by mammary gland was measured for 5min. The total 3-*O*-methylglucose taken up was corrected for extracellular sugar with the aid of a [^{14}C]sucrose marker. The values are reported as means ± S.E.M. for seven animals.

	Treatment of animal			
	Fed control	Fed + insulin	Starved control	Starved + insulin
Concn. of plasma glucose (mM)	7.36±0.11	3.14±0.59	6.11±0.09	1.62±0.17
Intracellular 3-*O*-methylglucose at 5min (nmol/g of tissue)	1.04±0.14	1.39±0.06	−0.10±0.02	1.29±0.04
Intracellular concn. of 3-*O*-methylglucose at 5min (μM)	2.24±0.33	3.11±0.13	−0.24±0.06	2.75±0.11
Intracellular 3-*O*-methylglucose corrected for carrier occupancy by glucose (nmol/g of tissue)	2.28±0.25	2.09±0.18	−0.19±0.04	1.60±0.05

Discussion

There have been rather few previous attempts to determine monosaccharide transport into particular tissues *in vivo*. The single-injection 'indicator diffusion' technique developed by Crone (1963) has been applied to studies of transport across the blood/brain barrier (Crone, 1965) and across the hepatocyte membrane in dogs (Goresky & Nadeau, 1974). A similar approach was employed with perfused rat liver (Williams *et al.*, 1968). The relative abilities of different sugars to penetrate the blood/brain barrier over longer times have been explored in dogs by Fishman (1964) and in cats by Eidelberg *et al.* (1967). The measurement of 2-deoxyglucose uptake by brain (Sokoloff *et al.*, 1977), which originally prompted the present work, is believed to reflect metabolism rather than transport.

We have rather used acini *in vitro* to study the basic characteristics of monosaccharide transport into mammary epithelial cells (Threadgold *et al.*, 1982) and have explored the present techniques *in vivo* to obtain a measure of capacity and of changes in different physiological states. The use of 2-deoxyglucose leads to an apparent absolute value for $V_{max.}$ of 2.22 μmol/min per g of tissue in the fed rat. Half-saturation of the carrier by plasma glucose yields an actual glucose transport of 1.11 μmol/min per g of tissue. As pointed out in the Results section, this may underestimate the true value by up to 10% because no allowance for sugar efflux was made, and by about 18% owing to inadequacies and uncertainties in blood sampling. Therefore actual rates of glucose transport might be in the range 1.1–1.5 μmol/min per g of tissue according to this technique. Rates of glucose uptake by rat mammary tissue can also be derived from data on mammary blood flow and arteriovenous glucose concentration differences. The former has been measured as 0.62 ml/min per g of tissue by Chatwin *et al.* (1969). For the latter, values range from about -1.68 μmol/ml of blood (Robinson & Williamson, 1977*a*,*b*) to -3.64 μmol/ml of blood (Elkin & Kuhn, 1975) or -4.25 μmol/ml of blood (the present paper), giving apparent uptakes of 1.04–2.64 μmol/min per g of tissue. These discrepancies remain to be resolved by combined measurements of blood flow and glucose concentrations in the same laboratory.

Starvation greatly decreases the uptake of 2-deoxyglucose, but probably not by impairment of its phosphorylation, which remains unchanged. The effect is ascribed above to a decrease in the $V_{max.}$ of transport, by analogy with the effect of withdrawing insulin from adipose tissue. Perhaps less likely is an approx. 24-fold rise in K_m, which, applied equally to 2-deoxyglucose and glucose, can be calculated to yield the same decrease in 2-deoxyglucose uptake.

The use of 3-*O*-methylglucose does not lead to absolute rates of monosaccharide transport, but does avoid possible criticism that rates of uptake are determined less by transport than by phosphorylation. Its uptake can be expressed variously as intracellular amount or concentration, with or without correction for competition by plasma glucose, or as a ratio relative to extracellular marker. From the close agreement between the two methods it may be concluded that monosaccharide transport is greatly impaired by starvation, and recovers on re-feeding with a rapidity that appears to exceed the recovery of glucose incorporation into either fatty acids or lactose (L. E. Bussmann, S. Ward & N. J. Kuhn, unpublished work). This is likely to afford one important mechanism for arresting the drain on body glucose in times of carbohydrate shortage. Table 4 constitutes the first evidence that transport may be under the control of insulin, but this evidently requires further substantiation under conditions where plasma glucose concentrations are better controlled. The apparent decrease in transport observed at parturition (Fig. 2) might also reflect the fall in concentration of plasma insulin that occurs at this time (Sutter-Dub *et al.*, 1974; Wilde & Kuhn, 1979). It remains to identify the factors responsible for the subsequent increase in transport as lactation develops.

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