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Monosaccharide transport into lactating-rat mammary acini

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The uptake and release of 3-*O*-methyl-D-[^{3}H]glucose at 37°C by acini, prepared from lactating-rat mammary gland with collagenase, was inhibited by glucose, phloretin, cytochalasin B, $HgCl_2$ and low temperature. Uptake and phosphorylation of 2-deoxy-D-[^{3}H]glucose, studied in greater detail, could be ascribed to a specific, saturable, inhibitable, process of apparent K_m 16 mM and $V_{max.}$ approx. 56 nmol/min per mg of protein, plus a non-specific, non-inhibitable process that was monitored with [^{14}C]fructose. The mean rate of uptake of 5 mM-2-deoxyglucose (16 nmol/min per mg of protein) was similar to the rate of consumption of 5 mM-glucose, suggesting that transport was a rate-limiting step in the overall metabolism of glucose. This accords with evidence for a glucose gradient across the plasma membrane.

The provision of glucose to lactating mammary cells plays a central role in milk production, since it is both an oxidizable fuel and a precursor of fat, lactose and some amino acids. At the same time the activity and size of the fully lactating gland is such that its consumption of glucose dominates the fate of body glucose as a whole (Kuhn, 1978).

Recent attempts to measure intracellular mammary glucose, both by direct analysis of acini isolated from the rat (Wilde & Kuhn, 1981) and by a study of free glucose concentrations in the milk of various species (Kuhn & White, 1975; Faulkner *et al.*, 1981) have indicated concentrations of less than 0.5 mM. Compared with plasma glucose concentrations (6–7 mM), such a value clearly implies that the transport of glucose across the plasma membrane is a rate-limiting step in its overall consumption.

In this paper the transport of monosaccharide into rat mammary acini *in vitro* is characterized, and compared with the rate of net glucose utilization under similar conditions. A preliminary account of this work has appeared (Threadgold *et al.*, 1981).

Methods

Preparation of lactating-rat mammary acini

Primiparous rats in mid lactation (11–15 days) were exsanguinated under light ether anaesthesia. The abdominal mammary glands were excised, trimmed of connective tissue, and chopped mechanically with a razor blade at 3 mm intervals, first in one direct and then again at 90°. The mince (7–8 g) was washed twice with portions (100 ml) of chilled 0.9% (w/v) NaCl solution, and then once with Krebs–Henseleit–Ringer bicarbonate buffer (100 ml) previously equilibrated to pH 7.4 with O_2/CO_2 (19:1) (Krebs & Henseleit, 1932). All glassware subsequently used in the preparation was siliconized.

The tissue was transferred to an Ehrlenmeyer flask with digestion medium (40 ml) consisting of Krebs–Henseleit–Ringer bicarbonate buffer containing collagenase (0.1%, w/v), glucose (10 mM), bovine serum albumin (2%, w/v) and Ficoll-400 (5%, w/v). The flask was then reciprocally shaken at 200 strokes/min at 37°C, while being gassed continuously with O_2/CO_2 (19:1), for 45–65 min.

The digested tissue was filtered through two layers of surgical gauze to remove any undigested material. The filtrate was carefully layered on a cushion (5 ml) of NaCl (0.9%, w/v) containing sodium phosphate buffer, pH 7.4, (10 mM) and Ficoll-400 (35%, w/v) in polycarbonate centrifuge tubes. The acini were then sedimented at 1000 rev./min for 90 s at 5°C in an MSE Major centrifuge. The supernatant fluid was aspirated and replaced by Krebs–Henseleit–Ringer bicarbonate (15 ml) containing Ficoll-400 (2%, w/v), bovine serum albumin (1%, w/v), lithium L-lactate (9.4 mM) and sodium pyruvate (0.6 mM). The acini were resuspended in the same medium and again sedimented as before. The entire washing procedure was then repeated. Finally the acini were harvested from above the Ficoll cushion and resuspended in a suitable volume of Krebs–Henseleit–Ringer bicarbonate buffer containing Ficoll-400 (2%, w/v) and

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bovine serum albumin (1%, w/v), and either lactate and pyruvate as above when sugar transport was to be measured, no substrate where glucose oxidation was to be measured, or 5mM-glucose where glucose consumption was to be measured. The concentration of filterable acini was 0.5–2mg of protein/ml. The acini were then distributed into appropriate flasks, gassed with O_2/CO_2 (19:1), stoppered, and stored on ice until required.

Determination of glucose uptake

Portions (3ml) of acini resuspended in glucose were distributed among several 25ml flasks, from each of which a sample (100μl) was taken and placed in a tube maintained at 100°C for 90s. The flasks were incubated with gentle shaking for 1h at 37°C with periodic gassing and further samples (100μl) were withdrawn every 15–20min. Glucose was determined in these samples with glucose oxidase and peroxidase (Bergmeyer & Bernt, 1965).

Conversion of [^{14}C]glucose to $^{14}CO_2$

Portions (2.7ml) of acini in substrate-free medium were pipetted into 25ml flasks each containing a small centre well. Subaseal stoppers were inserted and the flasks were briefly gassed. Medium (300μl) containing glucose (0.1M, 4mCi/mol) was then injected into each flask and the acini were shaken at 37°C for 30min. Reactions were terminated by the injection of phenylethylamine/methanol (1:1, v/v; 0.5ml) into the centre well and $HClO_4$ (5%, w/v; 0.8mol) into the medium. The flasks were then shaken for a further 1h at room temperature to collect the displaced $^{14}CO_2$. The content of each centre well was then quantitatively transferred to a scintillation vial and mixed with 10ml of toluene scintillation fluid.

Uptake of radioactive sugars

All uptakes were measured at 37°C except for those reported in Fig. 2. Samples (0.9ml) of freshly prepared acini were distributed among 10ml conical glass flasks. In turn each flask was preincubated for 4min at 37°C in a water bath with reciprocal shaking (65strokes/min). Sugar transport was initiated by the addition of 0.9% (w/v) NaCl (0.1ml) containing the appropriate amount of carrier sugar mixed with [^{3}H]- and/or [^{14}C]sugar (1–5μCi each). Sugars being tested for competitive inhibition (100μmol) were also added at this stage (see Table 2). Solid α- and β-glucose were dissolved 15s before required. The inhibitors cytochalasin B, phloretin and $HgCl_2$, however, were included in a preincubation extended to 10min. Incubation in the water bath at 37°C was continued, except in the case of 5s and 10s uptakes, for the appropriate time. Sugar uptake was arrested by the addition of 0.9% (w/v) NaCl (10ml) chilled to 0°C, and the suspension was immediately filtered through a glass fibre filter under the suction of a water pump. The filter was washed with further NaCl solution (40ml) at 0°C. The entire filtration and washing took about 15–20s. The effectiveness of low temperature in stopping transport is shown below (Fig. 2). Filters were soaked in 1% (w/v) Triton X-100 (1ml) for 3h and then xylene/Triton scintillation fluid (10ml) was added. Incubations were usually carried out in triplicate and were routinely corrected for blank values due to adsorption of radioactivity to the filters themselves.

Analysis of radioactive sugar from filtered acini

Filters were immediately extracted four times with 4ml of 50% (v/v) aqueous ethanol at 70°C for 10min. Carrier 2-deoxyglucose and glucose 6-phosphate (100μg each) were included. The pool extracts from several filters were dried at 50°C and redissolved in water (3ml). A sample (1ml) was passed over a column (400μl) of Dowex-1 resin (8×, 200 mesh, formate form). The column was washed with water (4ml) and the total eluate collected. The column was then eluted with 1M-HCl (6ml) which was separately collected. The amounts of free and phosphorylated sugar were determined from the radioactive contents of the respective eluates. Total radioactive recoveries were 90–98%. In a separate experiment added glucose 6-phosphate was recovered in 98% yield.

In one experiment the extract was also subjected to descending chromatography on Whatman no. 4 paper developed with 1M-ammonium acetate (pH3.5)/95% (v/v) aqueous ethanol (1:2, v/v). 2-Deoxyglucose and glucose 6-phosphate markers were run alongside, and the paper was finally cut into 1cm sections for the determination of radioactivity. Only two regions of radioactivity were located, one migrating with authentic 2-deoxyglucose and the other migrating ahead of glucose 6-phosphate in the position expected for 2-deoxyglucose 6-phosphate.

Efflux of 3-O-methyl[^{3}H]glucose

Acini were preincubated with 3-*O*-methyl[^{3}H]-glucose (5mM, 0.2Ci/mol) for 20min at 37°C to allow equilibration of the sugar into the cells. Portions (1ml) of the acinar suspension were then transferred to 15ml conical polycarbonate centrifuge tubes stored in ice. The tubes were centrifuged, in small batches at a time, for 90s in a bench centrifuge to pellet the acini, and the supernatant fluids were discarded. After preincubation for 3min at 37°C each pellet (about 100–200μl) was rapidly resuspended in Krebs–Henseleit–Ringer bicarbonate/Ficoll buffer (5ml), containing 10mM-Hepes [4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid] buffer at pH7.4, to dilute out extracellular sugar and

initiate efflux of the intracellular sugar. Efflux was terminated at the appropriate time by filtration and washing of the filter as described above.

Assay of hexokinase (EC 2.7.1.1)

Mammary tissue from lactating rats was chopped, homogenized with 3 vol. of 0.25 M-sucrose at 5°C and centrifuged for 1 h at 105000 *g* to obtain a clear supernatant fluid. Hexokinase activity with 2-deoxy[^{3}H]glucose was measured by a radiochemical method (Newsholme *et al.*, 1967) modified only in that 2-deoxy[^{3}H]glucose 6-phosphate was extracted from the filters with 1 M-phosphoric acid (1 ml) before the addition of xylene/Triton scintillation fluid (10 ml).

Protein determination

In each experiment several filters, with filtered acini, were digested singly with 1 M-NaOH (1 ml) at 70°C for 30 min. Portions (25 μl) of each digest were assayed for protein (Lowry *et al.*, 1951), comparable amounts of NaOH being included with the standards.

Materials

Rats were of a Wistar-derived strain bred in the Department and given free access to food and water.

Collagenase type 1 and bovine serum albumin, Cohn fraction V, were from Sigma. Ficoll-400 was supplied by Pharmacia. Glucose oxidase and peroxidase were supplied as a kit by Boehringer. Sugars and related substances were from Sigma and BDH. Various sources of 2-deoxyglucose were examined for purity by paper chromatography, and that from BDH was selected for use.

2-Deoxy-D-[1-^{3}H]glucose, 3-*O*-methyl-D-[1-^{3}H]-glucose, D-[U-^{14}C]fructose, D-[U-^{14}C]sorbitol, D-[U-^{14}C]glucose and [U-^{14}C]sucrose were from The Radiochemical Centre (Amersham). It was necessary to repurify the 3-*O*-methyl[^{3}H]glucose by paper chromatography from some trace contaminant that became incorporated into lipid during longer incubations with acini. Labilized ^{3}H was periodically removed by evaporation.

Whatman glass fibre discs (GF/C, 25 mm) were supplied by Fisons (Loughborough, U.K.). The discs were supported on sintered glass discs in the filtration apparatus supplied by Millipore.

All radioactive aqueous samples were mixed with 10 ml of xylene/Triton scintillation fluid, whereas radioactive mixtures of phenylethylamine/methanol were added to 10 ml of toluene scintillation fluid (Kuhn & White, 1977). Radioactive samples were counted for ^{14}C, ^{3}H or for both isotopes in a suitably programmed Philips liquid-scintillation analyser. Quenching was corrected for with the aid of an external standard.

Experimental results in the text and figures that are means of several values are accompanied by S.E.M. values, with numbers of values (*n*) in parentheses.

Results

Viability of the acinar preparation

The preparation of mammary acini was a modification of the procedure of Wilde & Kuhn (1979) who showed that such acini consumed glucose and synthesized lactose at constant rates for at least 1 h (Wilde & Kuhn, 1981). Following Greenbaum *et al.* (1978), however, we also included Ficoll-400 to reduce the tendency to clumping. Although this aided the uniform distribution of acini among different flasks, lack of complete uniformity remained a limitation on the design of experiments.

Three parameters of cellular viability were examined, especially to check that acini stored at 0°C did not change their characteristics before use in transport studies. Firstly, glucose uptake was observed to remain constant for at least 1 h (results not shown) at a mean rate of 18.2 ± 4.4 nmol/min per mg of protein ($n = 5$). This compares well with other reported values (Katz *et al.*, 1974; Robinson & Williamson, 1977; Wilde & Kuhn, 1979). Secondly, the conversion of [U-^{14}C]glucose to $^{14}CO_2$ over a 10 min period at 37°C remained constant during storage of acini at 0°C for 90 min (Table 1). Finally, the uptake of 5 mM-2-deoxy[^{3}H]glucose over 1 min at 37°C, and the extent of its phosphorylation, were both unchanged by storage of the acini at 0°C for up to 2 h (Table 1). It was therefore concluded that the method of preparation and storage of the acini

Table 1. *Ability of acini, stored at 0°C, to oxidize [U-^{14}C]glucose and to take up and phosphorylate 2-deoxyglucose*
Uptake of 5 mM-2-deoxyglucose was measured over 1 min. All other details are given in the Methods section.

Time of storage of acini at 0°C (h)	$^{14}CO_2$ production (nmol/min per mg of protein)	Uptake of 2-deoxyglucose (nmol/min per mg of protein)	Phosphorylation (%)
0	9.05	12.9	84
0.5	8.51	12.8	79
1	8.79	12.8	79
1.5	8.01	12.4	83
2	–	12.3	86

provided suitably viable material for the transport studies described below.

Uptake and efflux of 3-O-methyl[^{3}H]glucose

Fig. 1 shows that 5mM-3-*O*-methylglucose was very rapidly taken up by acini at 37°C. Uptake ceased after about 2min, by which time the calculated intracellular concentration of the sugar was about 4.8mM. For this calculation the intracellular water content was taken as being 2.76μl/mg of protein (Wilde & Kuhn, 1981). It proved difficult to observe initial uptake rates since they clearly did not extend beyond 10–20s.

Efflux, studied in acini previously loaded with 3-*O*-methyl[^{3}H]glucose, was extremely rapid and about 50% complete in 10s (Fig. 1). These observations demonstrate the reversibility of transport.

Inhibition of 3-O-methyl[^{3}H]glucose transport by low temperature, phloretin, cytochalasin B and $HgCl_2$

Uptake of this sugar varied linearly with temperature, with an extrapolated rate of nearly zero at 0°C (Fig. 2). This relationship appears to justify our reliance on low temperature to arrest transport rapidly in the studies reported here. In several experiments, which varied in the details of incubation, the uptake of 3-*O*-methylglucose was approximately halved by phloretin (1mM), cytochalasin B (10μg/ml) and $HgCl_2$ (2.5mM). However their inclusion in the cold saline used to stop transport and to wash the filtered acini did not enhance the amount of radioactivity retained.

Uptake of 2-deoxy[^{3}H]glucose

Although 3-*O*-methylglucose was useful to initiate the present study, the rapidity of its transport made it unsuitable for initial rate measurements at 37°C. It was therefore decided to use 2-deoxyglucose which, being rapidly phosphorylated within the cell, can enter over a longer period before rising intracellular concentrations of the free sugar contribute significantly to efflux. The greater sugar uptake that could thus be permitted presumably accounted for the relatively lower blanks (about 10–20% of total uptake) observed in the absence of acini. This was useful since high blanks limited the accuracy of measurement with 3-*O*-methylglucose.

Following a preliminary experiment that indicated only very low uptake of 2-deoxyglucose at 0°C, we investigated the effect of delaying the filtration of acini by up to 2min after their dilution by 0.9% (w/v) NaCl at 0°C. After the exposure of the acini to 5mM-2-deoxyglucose for 1min at 37°C, the mean amounts of sugar subsequently retained on filtration were 5.8, 5.5, 4.8 and 4.8nmol after delays of 0, 30, 60 and 120s respectively. The greater part of the sugar lost by delaying filtration was accounted

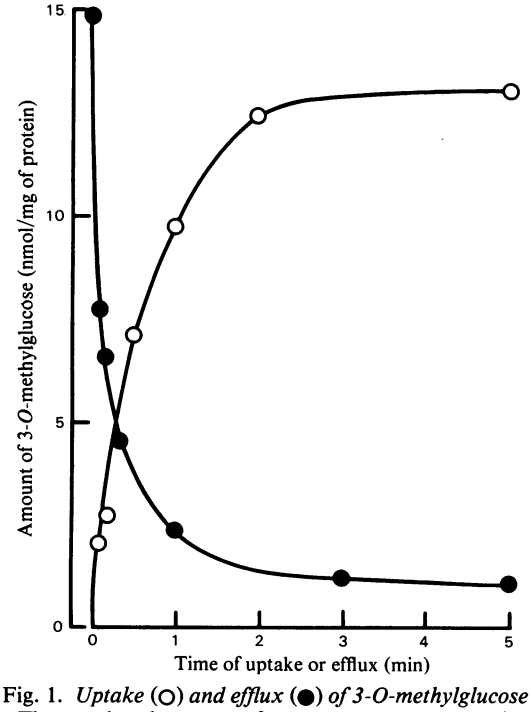

Fig. 1. *Uptake* (○) *and efflux* (●) *of 3-O-methylglucose*
The results shown are from two separate experiments. The concentration of 3-*O*-methylglucose in the medium during uptake was 5mM.

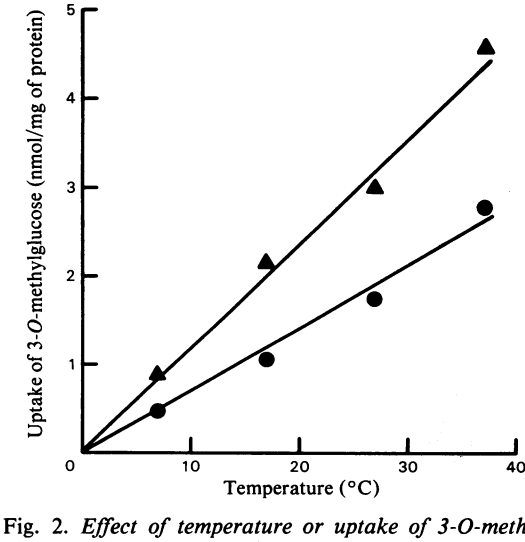

Fig. 2. *Effect of temperature or uptake of 3-O-methylglucose*
Uptakes of sugar at 10s (●) and 20s (▲) are shown.

for by the non-specific element, the definition and measurement of which is described below. It was therefore concluded that cold saline alone provides a satisfactory medium for the arrest of transport and the washing of the acini.

Fig. 3. shows the time course of 2-deoxy[^{3}H]-glucose uptake at different sugar concentrations. At 1 and 5mM concentration, uptake rates were constant for 30–60s. At 15 and 20mM concentration, however, constancy of uptake lasted less long. In subsequently described experiments, therefore, conditions were chosen such that initial rates were achieved as closely as possible. Transport times less than 10s were not considered reliable.

Analysis of the 2-deoxy[^{3}H]glucose taken up by filtered acini that had been exposed to 1–15mM sugar for 1min showed a mean degree of phosphorylation of $80.5 \pm 1.5\%$ ($n = 9$). In one experiment analysis by both ion exchange resin and paper chromatography gave 70% and 68% phosphorylation respectively. Although the extent of phosphorylation appeared to be unaffected by the amount of 2-deoxyglucose taken up, it tended to decrease after 2min incubation. The total uptake of 2-deoxyglucose ceased after 10min, for unknown reasons. In several experiments, however, the calculated intracellular concentration of free sugar that was reached approximated closely to its concentration in the external medium, suggesting that phosphorylation had now become limiting and had actually stopped.

The mean rate of uptake of 5mM-deoxyglucose, measured in 62 preparations, was 16.0 ± 0.7nmol/min per mg of protein.

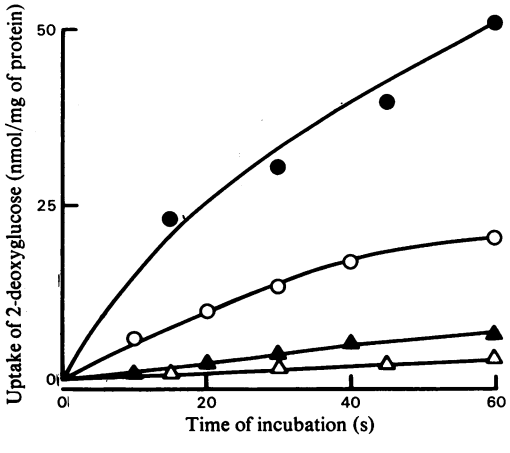

Fig. 3. *Time course of 2-deoxyglucose uptake*
The uptakes of 1mM (△), 5mM (▲), 15mM (○) and 20mM (●) 2-deoxyglucose were measured on separate preparations of acini, so that absolute rates of uptake are not comparable.

Specificity of 2-deoxyglucose uptake

Table 2 shows the abilities of various sugars (100mM) to inhibit the uptake of 2-deoxyglucose (5mM). The best inhibitor was D-glucose, with its α- and β-anomers being equally effective, whereas no inhibition was given by L-glucose. Other good inhibitors were 6-deoxy-D-glucose, D-mannose, D-xylose and 3-*O*-methylglucose. It is clear that inhibition of 2-deoxyglucose uptake is not an osmotic phenomenon but requires specific stereochemical groupings in the inhibitor molecule.

Uptake of D-[^{14}C]fructose, D-[^{14}C]sorbitol and [^{14}C]sucrose

Since fructose and sorbitol appeared not to interact with the monosaccharide carrier, their uptake by acini was examined as affording a possible measure of non-specific monosaccharide uptake. Sucrose was also tested since another disaccharide, lactose, had previously proved useful as an extracellular marker for acini (Wilde & Kuhn, 1981). In an experiment to compare the effects and possible interactions of these different sugars the uptake of 5mM-2-deoxy[^{3}H]glucose over 30s (10.5nmol/min per mg of protein) was found to be essentially unchanged (10.9–12.0nmol/min per mg of protein) by the simultaneous inclusion of 5mM-[^{14}C]fructose, -[^{14}C]sorbitol or -[^{14}C]sucrose. In turn the amounts of these sugars taken up in the incubations were 1.02, 0.94 and 1.20nmol/min per mg of protein respectively. Table 3 shows that whereas the uptake

Table 2. *Inhibition of 2-deoxyglucose uptake by other sugars or sugar analogues*
Uptake of 5mM-2-deoxyglucose over 30s was studied in the absence or presence of 100mM competing sugar or sugar analogue added simultaneously. Numbers of experiments are given in parentheses.

Sugar or sugar analogue	Inhibition (%)
D-Glucose (at anomeric equilibrium)	75 ± 4 (10)
α-D-Glucose	72 ± 6 (6)
β-D-Glucose	67 ± 3 (5)
6-Deoxy-D-glucose	62 ± 2 (5)
D-Mannose	57 ± 3 (5)
3-*O*-Methyl-D-glucose	44 ± 7 (6)
D-Xylose	44 ± 5 (6)
D-Galactose	40 ± 7 (6)
Maltose	31 ± 8 (6)
Lactose	12 ± 12 (5)
D-Sorbitol	7 ± 10 (5)
α-Methyl-D-glucoside	6 ± 5 (5)
β-Methyl-D-glucoside	5 ± 5 (5)
L-Glucose	4 ± 7 (5)
D-Fructose	1 ± 8 (5)
N-Acetyl-D-glucosamine	0 ± 10 (5)
myo-Inositol	−11 ± 7 (7)

Table 3. *Effects of cytochalasin B, phloretin, $HgCl_2$ and glucose on the uptakes of 2-deoxyglucose, fructose and sorbitol by acini*

The results, which are given as means ± S.E.M. of several experiments, are expressed as a percentage of the sugar uptake (5 mM for 30 s) observed in the absence of added inhibitor. Numbers of experiments are given in parentheses.

Addition	Uptake (%) of: 2-Deoxyglucose	Fructose	Sorbitol
None	100	100	100
Cytochalasin B (10 μg/ml)	33.6 ± 4.9 (7)	108 ± 5 (9)	97 ± 4 (9)
Phloretin (1 mM)	44.7 ± 9.0 (3)	95 ± 9 (6)	104 ± 7 (6)
$HgCl_2$ (2.5 mM)	38.4 ± 4.3 (5)	144 ± 13 (15)	167 ± 58 (6)
Glucose (100 mM)	–	115 ± 0 (12)	104 ± 10 (9)

of 2-deoxyglucose was much inhibited by cytochalasin B, phloretin and $HgCl_2$, the uptakes of fructose and sorbitol (sucrose was not tried) were not inhibited at all. In fact their uptake was increased by $HgCl_2$, which probably damages the plasma membrane. Similarly, although glucose did inhibit the uptake of 2-deoxyglucose (Table 2), it did not affect uptake of fructose or sorbitol (Table 3). In a single experiment 2-deoxyglucose (100 mM) was also found not to affect the uptake of these two sugars (1 mM).

Given these differences in the uptake of 2-deoxyglucose on the one hand and of fructose, sorbitol and sucrose on the other hand, and with regard to the very differing molecular structures shown by the last three substances, it is evident that the latter are taken up by a non-specific mechanism that does not interfere with, but must contribute to, the overall uptake of 2-deoxyglucose. It is even possible that this non-specific uptake is due to damaged cells, and that it accounts for the non-phosphorylated component of the 2-deoxyglucose that is taken up. Therefore to correct for this in the experiments described below, [^{14}C]fructose was included with 2-deoxy[^{3}H]glucose at equal concentration and the uptakes of both sugars were measured simultaneously.

Variation of 2-deoxyglucose uptake with concentration

The uptake of 2-deoxyglucose and fructose was measured at varying sugar concentrations. Fig. 4(*a*), which contains the results of nine experiments normalized to the mean rate at 5 mM, shows that the transport process was not saturated by 20 mM-2-deoxyglucose, the highest concentration used. The apparent K_m for uptake of 2-deoxyglucose, corrected for that of fructose, determined by different graphical methods was 16.4 mM (Hanes, 1932), 13.1 mM (Hofstee, 1952) and 17.4 mM (Eisenthal & Cornish-Bowden, 1974). The respective $V_{max.}$ values were 59, 50 and 59 nmol/min per mg of protein. The results from the last-named plot are depicted in Fig. 4(*b*).

Inhibition of 2-deoxyglucose uptake by glucose

In the presence of various concentrations of D-glucose the uptake of 1 mM-fructose was unaffected whereas that of 1 mM-2-deoxyglucose decreased progressively at higher concentrations of glucose (Fig. 5*a*). Inhibition was nearly maximal at 100 mM-glucose, leaving a residual uptake of 2-deoxyglucose nearly equal to that of fructose. Half-maximal inhibition of specific 2-deoxyglucose uptake occurred at 6.5 mM-glucose and, in another experiment with 0.1 mM-2-deoxyglucose, at 7.0 mM-glucose. Since the concentrations of 2-deoxyglucose used in these experiments were low compared with its apparent K_m, the apparent K_i for glucose is 6.5–7.0 mM (Neame & Richards, 1972).

Inhibition of 2-deoxyglucose uptake by cytochalasin B and $HgCl_2$

Increasing concentrations of cytochalasin B and of $HgCl_2$ progressively decreased the uptake of 5 mM-2-deoxyglucose to reach a value close to that of fructose uptake (Figs. 5*b* and 5*c*). Cytochalasin B did not affect fructose uptake, but $HgCl_2$ caused some increase, the magnitude of which was however usually smaller than in the experiment shown. This increase probably represents some non-specific leakiness induced in the plasma membrane.

Hexokinase studies

After it had been established that the phosphorylation of 2-deoxyglucose increased linearly with time and amount of supernatant protein, the activity of hexokinase was measured at different concentrations of substrate. Fig. 6(*a*) shows the increase in activity up to 4 mM-2-deoxyglucose and Fig. 6(*b*) shows a plot of s/v against s (Hanes, 1932). The enzyme activity appeared to follow Michaelis–Menten kinetics, with an apparent K_m in

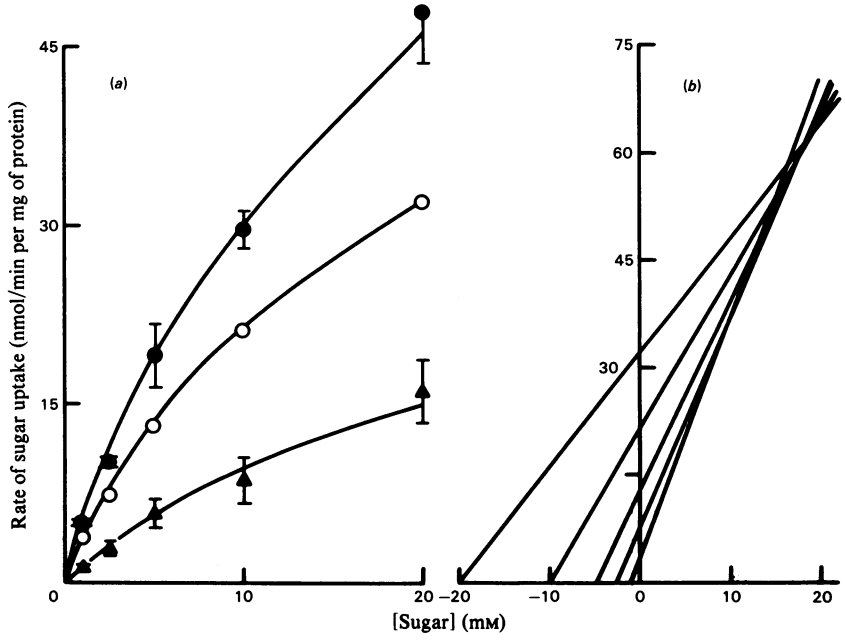

Fig. 4. *Variation of 2-deoxyglucose and fructose uptake with concentration*
Sugar uptake was measured over 10s at each concentration. (*a*) Actual rates of uptake of 2-deoxyglucose (●), fructose (▲) or the difference between them (○). (*b*) Plot of corrected 2-deoxyglucose uptake according to Eisenthal & Cornish-Bowden (1974).

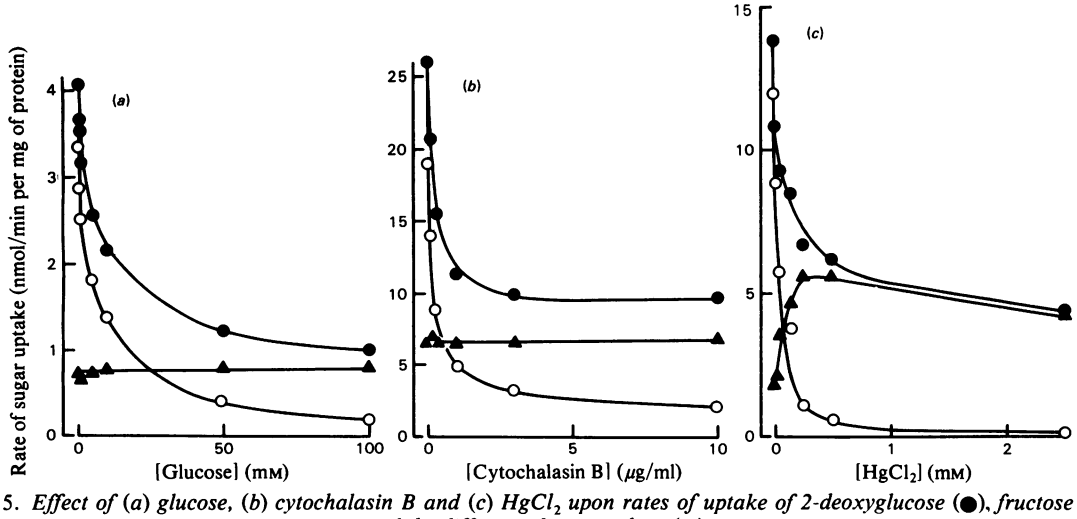

Fig. 5. *Effect of* (*a*) *glucose*, (*b*) *cytochalasin B and* (*c*) $HgCl_2$ *upon rates of uptake of 2-deoxyglucose* (●), *fructose* (▲) *and the difference between them* (○)
The results with each inhibitor were obtained with a separate preparation of acini. Bovine serum albumin was omitted from incubations testing the effect of $HgCl_2$.

two separate experiments of 0.9 and 1.0mM respectively. It was not inhibited by cytochalasin B.

Discussion

The ability of the mammary cell membrane to transport glucose is ideally studied by measuring initial rates of uptake of a non-metabolized, radioactively labelled, analogue. Under such conditions the rate of efflux is still negligible and losses due to metabolism to CO_2 and water are avoided. The use of 3-*O*-methyl[^{3}H]glucose, a popular analogue for such studies, has enabled us to demonstrate both influx and efflux by a system that is inhibited by

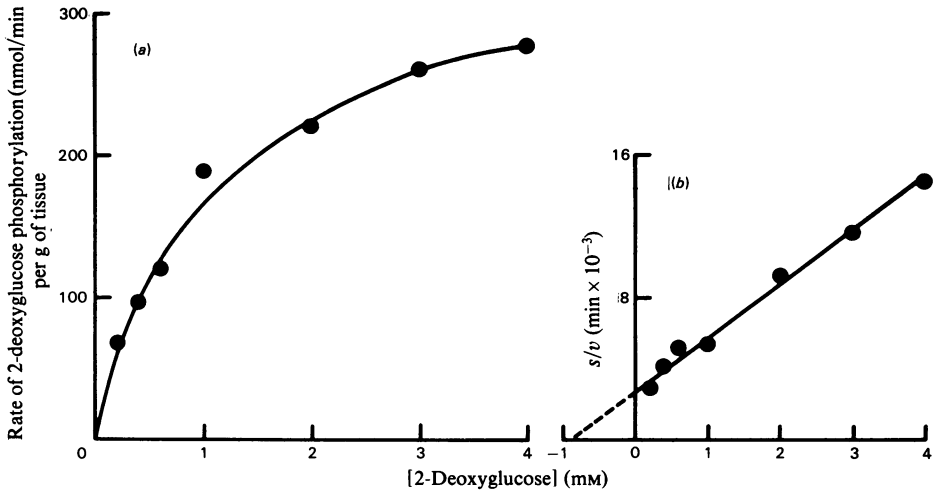

Fig. 6. *Variation of hexokinase activity* (v) *with concentration of 2-deoxyglucose* (s)
(a) Substrate–velocity curve; (b) plot of s/v against s.

cytochalasin B, phloretin and $HgCl_2$. The activity of this system, however, is such that initial rate studies would have demanded transport to be measured over 5 s or less, which was not considered feasible with the acinar preparations available. It was also clear that at such short uptake times the magnitude of the blank correction, due to retention of sugar by the filter, would have been unacceptable.

2-Deoxyglucose, adopted as an alternative, is rapidly metabolized to the 6-phosphate by mammalian cells but is not significantly metabolized further. Such trapping of intracellular sugar prolongs the initial rate of uptake and reduces the relative magnitude of the blank correction. The extensive phosphorylation of the 2-deoxyglucose taken up suggested that phosphorylation was nevertheless not rate-limiting. This was confirmed by the apparent K_m for 2-deoxyglucose uptake (about 16 mM) which clearly differed from the apparent K_m for 2-deoxyglucose phosphorylation by hexokinase (about 1 mM) and by the inhibition that cytochalasin B exerted on uptake by the acini but not on phosphorylation by hexokinase. Further, the specificity of inhibition of 2-deoxyglucose by a variety of sugars added in excess appeared different from the binding specificity of hexokinase (Crane, 1962). It was therefore concluded that 2-deoxyglucose is suitable for studying monosaccharide transport into the acinar cells.

Rate-limiting nature of glucose transport

Given the above conclusion, the close similarity of the rate of glucose uptake to the separately measured rate of 2-deoxyglucose transport appears to establish transport as a rate-limiting step for overall consumption of glucose. This accords with evidence that the intracellular glucose concentration is less than 0.5 mM (Wilde & Kuhn, 1981; Kuhn & White, 1975; Faulkner *et al.*, 1981) and that a substantial concentration gradient of glucose therefore occurs across the plasma membrane.

Specificity of monosaccharide transport

Most of the transport activity can be ascribed to a process that shows clear structural specificity; it accepts D-glucose, 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose and is inhibited by 6-deoxy-D-glucose, D-xylose and various other sugars. However there appears to be little affinity for sugar alcohols, inositol, fructose or L-glucose. There may be a small affinity for maltose. This specificity is not unlike that established for the red blood cell (LeFevre, 1961). The susceptibility of the present process to cytochalasin B, to phloretin and to $HgCl_2$ also resembles that of sugar transport into other mammalian cells. In particular, cells of the R3230 AC mammary adenocarcinoma appear to transport monosaccharides by a system not unlike the present one (Harmon & Hilf, 1976). On the other hand the acini do not distinguish between α- and β-glucose, as do red blood cells (Faust, 1960; Barnett *et al.*, 1973), ascites tumour cells (Fishman & Bailey, 1973) and pancreatic β-cells (Miwa *et al.*, 1975).

Proposed nature of the transport process and its importance to the mammary gland

The above results suggest the involvement of a protein carrier to which the sugar must bind in order to be carried across the membrane. The characteristics of this process differ radically from those of

the process by which glucose crosses the Golgi membrane of the mammary secretory cell and for which a water-filled pore or channel appears to be a better model (White *et al.*, 1980, 1981). From the apparent K_m for 2-deoxyglucose (about 16mM) and the apparent K_i for glucose (about 7mM) it is clear that plasma glucose (6–7mM) (Wilde & Kuhn, 1979) by no means saturates the carrier and that variations in the concentration of plasma glucose can be expected to affect the rate of glucose transport into the mammary gland and its steady state intracellular concentration. This explains the linear increase in glucose consumption and lactose synthesis shown by acini exposed to increasing concentrations of glucose *in vitro* (Wilde & Kuhn, 1981). Major variations in plasma glucose concentration probably do not occur under physiological conditions, but a temporary rise has been noted in the post-parturient rat (Wilde & Kuhn, 1979; Nicholas, 1977).

A minor, but not negligible, contribution to monosaccharide uptake by the acini appeared to be due to a non-specific-process that was not inhibited by any of the above agents. This process, which admitted fructose, sorbitol and sucrose, can probably not be ascribed to the apical membrane which is unlikely to admit disaccharides. It may represent a fraction of damaged or leaky cells in the acinar preparation, but a possible physiological connection cannot be ruled out.

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