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Short Communications

Milk Glucose as an Index of the Intracellular Glucose Concentration of Rat Mammary Gland

By NICHOLAS J. KUHN and ADRIAN WHITE
Department of Biochemistry, University of Birmingham, P.O. Box 363, *Birmingham B*15 2*TT, U.K.*

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The aqueous glucose concentration of rat milk is about 0.29mM, apparently unmodified by prolonged storage in the gland. Infused 3-*O*-methylglucose reaches a milk concentration similar to its measured mammary intracellular concentration. Milk glucose may therefore reflect its intracellular concentration in the mammary secretory cell.

The glucose concentration within the cells of extrahepatic tissues is exceptionally difficult to measure because its contribution to the total tissue glucose is overshadowed by that of glucose in the extracellular fluid. In mammary tissue, where glucose is a substrate not only for hexokinase (EC 2.7.1.1) but also for lactose synthase (EC 2.4.1.22), knowledge of its concentration is especially desirable; yet it has only been possible to set an upper limit of 1mM to its concentration in the rat mammary gland (Murphy *et al.*, 1973). A new approach to this problem was suggested by recent evidence that the glucose of the mammary cell cytosol passes readily into the Golgi lumen, where it supports the synthesis of lactose (Kuhn & White, 1975), and that therefore the fluid of the Golgi lumen contributes directly to the aqueous phase of milk (Linzell & Peaker, 1971*a*; Saacke & Heald, 1974). According to such a scheme the free glucose concentration of milk water should be similar to that in the cytosol of the secretory cell.

Methods and materials

Animals. Primiparous 13–18-day-lactating rats of Wistar-derived strain were milked in the morning under diethyl ether anaesthesia, after receiving oxytocin (1–2i.u.) intraperitoneally. In some cases the litters had been removed overnight to permit the accumulation of milk. Litters averaged 11.1 ± 0.5 pups (±S.E.M., 35 litters)

Determination of milk glucose and galactose. A known volume of milk (2–4ml) was mixed with [U-^{14}C]glucose (10nCi), was extracted with 19vol. of chloroform–methanol (2:1, v/v) and was then filtered after 30min. The filtrate was mixed with 0.2vol. of water (Folch *et al.*, 1957), and the clear upper phase was evaporated to dryness by rotary evaporation at 45°C. The residue was dissolved in 1ml of water and passed through a column of Amberlite IRC-50 (H^+ form) resin (1ml) above Dowex-1 (formate form) resin (1ml), the column then being washed with water (2ml). The de-ionization step removed an unknown inhibitor of the galactose assay. The pooled eluate was assayed for glucose (Slein, 1963) and galactose [see *Biochemica Catalogue no.* 15095 *EGAA*; Boehringer Corp. (London) Ltd., London W.5, U.K.] by enzymic methods that did not react with lactose, and for ^{14}C by liquid-scintillation counting. The [U-^{14}C]glucose served as an internal standard and its recovery (about 50%) was taken to equal the recovery of endogenous milk glucose, the concentration of which was accordingly corrected. This approach has been widely adopted for the determination of other naturally occurring substances that are isolated in low yield. Portions of the original milk were weighed before and after drying at 70°C to constant weight.

Infusions. Solutions of 3-*O*-[^{14}C]methylglucose (0.25M; 0.55μCi/ml) or [^{3}H]inulin (10mg/ml in 0.9% NaCl; 1.2μCi/ml) were infused at 1–2ml/h for up to 7h with an LKB Varioperspex peristaltic pump through a polyethylene cannula inserted into the right jugular vein (Popovic & Popovic, 1961). A priming dose of 1.0–1.5ml served to raise the blood radioactivity rapidly to the value required. During these procedures rats were maintained under Nembutal or halothane+O_2 anaesthesia. Samples (2h) of milk, obtained after the injection each time of 1–2i.u. of oxytocin, and of tail-vein blood were taken for the determination of radioactivity.

Determination of intracellular 3-*O*-[^{14}*C*]*methylglucose.* 3-*O*-[^{14}C]Methylglucose (24mg; 2μCi) and [^{3}H]inulin (5mg; 3μCi) were injected simultaneously intravenously; 10min later one mammary gland was removed by rapid freezing (Wollenberger *et al.*, 1960), and blood was collected from the trunk. The frozen powdered mammary gland was extracted with 5vol. followed by 2vol. of 5% (w/v) $HClO_4$ at 0°C, and the pooled clear extracts were neutralized with KOH. After removal of $KClO_4$ by centrifugation, the clear extract was assayed for lactose (Reithel, 1963). Duplicate further portions (2ml) were taken for the

separation of [^{3}H]inulin from 3-*O*-[^{14}C]methylglucose by chromatography on a column (2.5cm× 17cm) of Sephadex G-10. The [^{3}H]inulin fraction, recovered in 98% yield, was freeze-dried and the radioactivity was determined by liquid-scintillation counting. Plasma (0.5ml) was treated in the same way. Samples of plasma and of the tissue extract were counted directly for ^{14}C radioactivity, a correction of 4.2% being applied for interference by the [^{3}H]inulin present.

The water space due to retained milk was calculated from the lactose content of the tissue and from the lactose and water content of milk from the same animal; only 78% of the tissue lactose was taken as representing retained milk (Kuhn & White, 1975). From the rates of penetration of inulin and 3-*O*-methylglucose into milk during infusion experiments (results not shown), it was unlikely that significant passage of these substances into the retained-milk compartment had occurred 10min after injection, though it was desirable to avoid a longer time-lapse. On the other hand there appeared to be enough time for their distribution through the interstitial space, which, from the [^{3}H]inulin data, was 0.271±0.035 (7)g/g of tissue after 10min and 0.256±0.010 (6)g/g of tissue after 5min (means±S.E.M. of separate groups of rats; difference not significant). The intracellular water space was calculated as the difference between the total tissue water and the sum of interstitial plus retained milk water. It was assumed that 10min also sufficed to equilibrate 3-*O*-methylglucose into the cell, since lactating rat mammary tissue consumes glucose at a rate (Elkin & Kuhn, 1975) that would generate 5mM-glucose within the cell in less than 1min, were the glucose not metabolized. The intracellular concentration of 3-*O*-methylglucose was therefore calculated from the intracellular water space and the amount of 3-*O*-methylglucose not accounted for by the interstitial fluid of the mammary gland (Table 2).

Results and discussion

Rat milk contained about 0.29mM-glucose in the aqueous phase (Table 1). This value includes a small correction for glucose that might have arisen by the hydrolysis of lactose, and that is assumed to equal the amount of galactose found. The higher concentration of galactose in milk taken from unsuckled glands supports this interpretation of its origin, since much of this milk will have been made many hours previously (Hanwell & Linzell, 1972). On the other hand the essentially identical concentration of glucose in such 'stored' milk suggests that the composition is not changed by prolonged contact with surrounding duct tissue. In an experiment designed to test for possible glucose utilization by cellular elements of the milk, freshly collected milk was reinforced with glucose, to a final concentration of 1.9μmol/ml, and was then incubated under N_2 at 37°C for 4h. At the end of this period it was found to contain 1.8μmol of glucose/ml, which represents an insignificant change. Anaerobic conditions were used partly because freshly drawn milk appears to have a low oxygen partial pressure (Ling *et al.*, 1961) and partly because the operation of a Pasteur effect might elicit a maximum glucose consumption by respiring cells.

To test the idea that the glucose concentration of milk water equals its concentration within the cell, rats were infused with 3-*O*-[^{14}C]methylglucose. This is a non-metabolizable analogue of glucose that, although not hitherto tested on mammary tissue, appears to be recognized by many membrane glucose carriers (Morgan & Whitfield, 1973). Its concentration in the milk was compared with that in the plasma, which, in separate experiments, was then compared with the concentration within mammary cells. During infusion experiments, the 3-*O*-[^{14}C]methylglucose concentration of the plasma was maintained at an approximately constant value. By 2h the concentration in the milk water reached a maximum value that averaged (±S.E.M.) 62±7 (6) % of the plasma value. In separate experiments the intracellular concentration of 3-*O*-[^{14}C]methylglucose in the mammary gland averaged (±S.E.M.) 73±7 (7) % of that in the plasma. The data on which this value rests are shown in Table 2.

From these experiments, and with regard to possible errors and assumptions of the methods, we conclude that the concentration of 3-*O*-[^{14}C]methylglucose in

Table 1. *Concentrations of glucose, galactose and water in rat milk*

Values are given as means±S.E.M., with numbers of rats in parentheses.

	Without prior litter removal	With prior litter removal
Whole milk glucose (μmol/ml)	0.249±0.041 (8)	0.242±0.031 (8)
Whole milk galactose (μmol/ml)	0.008±0.004 (8)	0.042±0.007 (7)
Milk water (g/ml)	0.689±0.022 (8)	0.734±0.021 (8)
Aqueous galactose (mM)	0.013±0.006	0.057±0.009
Aqueous glucese, corrected (mM)*	0.294±0.059	0.276±0.030

* Corrected for an amount of glucose equal to the amount of galactose.

Table 2. *Water spaces of mammary tissue and the intracellular concentrations of* 3-*O*-[^{14}C]*methylglucose relative to that of plasma, 10 min after intravenous injection*

Values are given as means ± S.E.M., with numbers of rats in parentheses.

Total tissue water (g/g of tissue)	0.729 ± 0.005 (9)
Retained milk water (g/g of tissue)	0.113 ± 0.011 (9)
Interstitial water (g/g of tissue)	0.271 ± 0.035 (7)
Intracellular water (g/g of tissue)	0.348 ± 0.026 (7)
Intracellular concentration of 3-*O*-[^{14}C]methylglucose relative to that of plasma (%)	73 ± 7 (7)

the milk water approximates closely to that inside the average mammary cell. That the intracellular concentration is lower than the plasma concentration may reflect the counter-transport phenomenon consequent on the glucose gradient across the plasma membrane (Stein, 1967).

The above results are consistent with the idea that the concentration of milk glucose reflects its derivation from the Golgi lumen and ultimately from the cytosol. That it might also arise directly from the plasma, however, was suggested by experiments in which [^{3}H]inulin was infused for up to 7h into the jugular vein. After 2h its concentration in the milk water reached a value 21–68% of that in the plasma (three rats). The milk radioactivity was not due to low-molecular-weight products of inulin hydrolysis, since it was not excluded by Sephadex G-10. Because Linzell & Peaker (1971*b*) had found that oxytocin and milking increased the permeability of goat mammary gland to disaccharides, we infused [^{3}H]-inulin for 5h into each of two rats before taking one milk sample after a single injection of oxytocin. The concentrations of inulin in the milk water were 28 and 50% respectively of that in plasma. Although some doubt remains as to whether or not this penetration was influenced by injected oxytocin, it is known that prolactin (McMurtry & Malven, 1974) and other plasma proteins (Laurell & Morgan, 1965) normally enter rat milk to an appreciable extent. Insofar as such macromolecules probably diffuse between the secretory cells rather than through them, small molecules such as glucose and 3-*O*-methylglucose must be able to enter milk by the same route.

The above observations are brought into harmony if one accepts that milk glucose, deriving partly from the Golgi secretion and partly by direct transfer from the plasma, equilibrates across the cell membrane with the intracellular glucose. This might imply the presence of a glucose carrier in the apical membrane of the secretory cell. We suggest that the glucose concentration of milk water (here 0.29mM) currently affords the best guide to the concentration within mammary cells, and we note the similarity of this value to the K_m (0.26mM) of the major mammary hexokinase, type II (Gumaa *et al.*, 1971). In contrast, a problem may be posed by the higher K_m reported for the purified lactose synthetase of cow milk (5mM; Fitzgerald *et al.*, 1970) and human milk (3mM; Andrews, 1969) and for the particulate lactose synthase of rat mammary tissue (1.7mM; Murphy *et al.*, 1973).

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