The Utilization of Glucose for the Synthesis of Milk Components in the Fed and Starved Lactating Goat *in vivo*

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1. [$U\text{-}^{14}\text{C}$]Glucose and [$3\text{-}^3\text{H}$]glucose were infused into fed and starved lactating goats in order to study glucose metabolism in the mammary gland. 2. Glucose carbon was oxidized and metabolized to milk lactose, citrate and triacylglycerol in the lactating goat udder. 3. Recycling of glucose carbon in the lactating animal accounted for 10–20% of the total glucose turnover in the whole animal. Recycling of glucose 6-phosphate in the udder accounted for about 25% of the glucose 6-phosphate metabolized. 4. Flux of glucose 6-phosphate through the pentose phosphate pathway was sufficient to account for 34% of the NADPH required for fatty acid synthesis in the gland in the fed animal. 5. Net metabolism of glucose 6-phosphate via the pentose phosphate pathway accounted for 17.8 and 1.2% of the glucose phosphorylated by the mammary gland in the fed and starved animal respectively. Metabolism of glucose 6-phosphate via the pentose phosphate pathway was sufficient to account for all the CO_2 produced from glucose in the fed animal, but only 17% of the CO_2 produced from glucose in the starved animal.

The utilization of glucose by the mammary gland has been studied in the lactating goat *in vivo* (Annison & Linzell, 1964; Annison *et al.*, 1968). Glucose is oxidized by the mammary gland, and glucose carbon has been shown to be used for the synthesis of lactose, triacylglycerol glycerol and citrate in the isolated perfused goat udder (Hardwick *et al.*, 1963). Metabolism of glucose may also be important in providing the reducing equivalents required for the synthesis of fatty acids *de novo* in the mammary gland (Bauman & Davis, 1975).

The synthesis of fatty acids in the udder requires a source of reducing equivalents in the form of NADPH. In the rat mammary gland there is evidence that the oxidation of glucose via the pentose phosphate pathway provides the major portion of the NADPH required for fatty acid synthesis (Katz *et al.*, 1974b). However, ruminant mammary tissue appears to differ from that of the rat. Studies *in vitro* using both sheep mammary-tissue slices (Balmain *et al.*, 1952) and the perfused goat udder (Hardwick *et al.*, 1963) have shown that large amounts of acetate can be incorporated into fatty acids when glucose is absent. Bauman *et al.* (1970) have postulated that the NADPH-dependent isocitrate dehydrogenase reaction of the cytosol is an important source of NADPH in ruminant mammary-tissue metabolism. From studies performed *in vitro* it has been postu-

lated that about 40% of the NADPH required for fatty acid biosynthesis in the cow and sheep mammary gland might be derived from glucose metabolism via the pentose phosphate pathway (Gumaa *et al.*, 1973; Bauman *et al.*, 1973).

Very few data are available on fatty acid synthesis and NADPH production in the udders of lactating ruminants *in vivo*. The maximum contribution of glucose to NADPH production has been calculated on the assumption that all glucose oxidation to CO_2 occurs via the pentose phosphate pathway in the udder. Bauman & Davis (1975) have calculated, from the results of Bickerstaffe *et al.* (1974) and Annison *et al.* (1974), that, on this assumption, glucose would provide approx. 50% of the NADPH required for the incorporation of acetate into fatty acids in the cow's udder. Similar calculations on data obtained from the lactating goat (Annison & Linzell, 1964) suggest that glucose metabolism via the pentose phosphate pathway would provide all the NADPH required for fatty acid biosynthesis in the lactating goat udder (Bauman & Davis, 1975).

The present study adopts the more direct approach to the problem of glucose metabolism and NADPH production in the goat udder by following the metabolism and transfer of ^3H from [$3\text{-}^3\text{H}$]glucose to milk fat in conditions of high and low rates of fatty acid synthesis *de novo* (i.e. in the fed

and starved lactating goat). The results presented here are part of a more extensive study of glucose metabolism in the goat udder during pregnancy and lactation.

Experimental

Materials

Goats. Six female British Saanen goats aged from 3 to 6 years, in their third to ninth lactation and weighing 40–60 kg, were used. They were housed in an insulated byre and fed on hay *ad libitum* and 1700 g of concentrates (BOCM Silcock Ltd., Basingstoke, Hants., U.K.) per day, given in two meals at the times of milking (08:00 and 16:00 h). Starved animals had food withdrawn for 48 h before the experiment. Before lactation started each animal had its right common carotid artery exteriorized into a skin-covered loop, and blood vessels crossing between the two halves of the udder were ligated (Linzell, 1960). Experiments were carried out between weeks 6 and 12 of lactation, when animals were accustomed to experimental procedures and surroundings.

Chemicals and biochemicals. All enzymes were obtained from Boehringer Corp. (London), Lewes, Sussex, U.K. All coenzymes were obtained from Sigma (London) Chemical Co., Poole, Dorset, U.K. All other chemicals were obtained from British Drug Houses, Poole, Dorset, U.K.

Radiochemicals. [U-¹⁴C]Glucose and [3-³H]glucose were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. The specific radioactivity of both was over 230 mCi/mmol.

Methods

On the day before the experiment catheters were placed in a jugular vein and in the exteriorized carotid artery, and two catheters were placed in the left subcutaneous abdominal vein (Thompson & Thomson, 1977). At about 08:00 h a priming dose of radioactive glucose (5 μ Ci of [3-³H]glucose + 3 μ Ci of [U-¹⁴C]glucose) in sterile 0.9% (w/v) NaCl was injected into the jugular vein, followed by an infusion (0.4 μ Ci of [3-³H]glucose/min, 0.2 μ Ci of [U-¹⁴C]glucose/min) which continued for 4 h. This procedure produced a constant specific radioactivity of plasma glucose throughout the final 1 h of infusion. During the final 1 h, three sets of blood samples were taken from the carotid artery and subcutaneous abdominal vein for measurements of mammary-gland blood flow, production of ¹⁴CO₂ by the mammary gland and plasma glucose concentration and specific radioactivity (Jones, 1965). All data for mammary-gland metabolism refer to half the udder (i.e. one mammary gland). Milk was collected for 40 h after the end of the infusion for measurement of radioactive glucose incorporation into lactose, citrate, lipid and triacylglycerol glycerol.

Blood flow through half the udder was determined by measuring the dilution of Indocyanine Green dye (Thompson & Thomson, 1977). Plasma glucose concentrations were measured enzymically (Slein, 1963). The radioactivity in blood bicarbonate was measured by acidification of a known volume of blood with an equal volume of 1 M-HClO₄ and trapping the ¹⁴CO₂ evolved in 10 M-NaOH in an airtight vessel (Lin & Fritz, 1972). Lactose was isolated from milk (Wood *et al.*, 1965) and a sample assayed for radioactivity; a further sample was hydrolysed (Wood *et al.*, 1965) and the concentration of glucose in the hydrolysate assayed enzymically (Slein, 1963). Milk fat was isolated by centrifugation (Wood *et al.*, 1965), saponified, and radioactivity in non-esterified fatty acids and triacylglycerol glycerol determined (Vernon, 1977). Milk fat was determined gravimetrically and triacylglycerol glycerol was determined as free glycerol after saponification (Garland & Randle, 1962). Citrate from milk was isolated by anion-exchange chromatography (Hardwick *et al.*, 1963) and the concentration determined enzymically (Dagley, 1963). Radioactivity in plasma water was determined by equilibration with a second pool of water (Ashcroft *et al.*, 1972). The fatty acid composition of milk fat was determined as described by Thomson *et al.* (1979).

Calculations

Uptake (*U*) of glucose by the mammary gland, expressed in μ mol/min, was calculated from the equation:

$$U = Q_p \times (G_A - G_v) \quad (1)$$

where Q_p = mammary plasma flow (ml/min), G_A = arterial plasma glucose concentration (μ mol/ml) and G_v = mammary-venous plasma glucose concentration (μ mol/ml).

Glucose turnover in the whole animal (*T*), expressed as μ mol/min, was calculated from the equation:

$$T = I / *G_A \quad (2)$$

where *I* = rate of infusion of [U-¹⁴C]- or [3-³H]-glucose (μ Ci/min) and $*G_A$ = specific radioactivity of [U-¹⁴C]- or [3-³H]-glucose in arterial plasma at equilibrium (μ Ci/ μ mol).

Recycling of glucose carbon (*C*) in the whole animal, expressed as percentage glucose turnover, was calculated from the equation:

$$C = 100 \times (T_3 - T_{14}) / T_3 \quad (\text{Katz } et al., 1974a) \quad (3)$$

where T_3 = turnover of glucose calculated from [3-³H]glucose and T_{14} = turnover of glucose calculated from [U-¹⁴C]glucose.

Release (*R*) of ¹⁴CO₂ into mammary-venous blood, expressed as μ mol of glucose incorporated

into CO_2/min , was calculated from the equation:

$$R = Q_b \times ({}^{14}\text{CO}_{2v} - {}^{14}\text{CO}_{2a}) / {}^*G_A \quad (4)$$

where Q_b = mammary blood flow (ml/min), ${}^{14}\text{CO}_{2a}$ = arterial-blood ${}^{14}\text{CO}_2$ and ${}^{14}\text{CO}_{2v}$ = mammary-venous-blood ${}^{14}\text{CO}_2$ ($\mu\text{Ci}/\text{ml}$).

Incorporation (A) of radioactivity into milk components, expressed as μmol of glucose incorporated/min, was calculated from the equation:

$$A = {}^*M / (t \times {}^*G_A) \quad (5)$$

where *M = total ${}^3\text{H}$ or ${}^{14}\text{C}$ in milk component (μCi) and t = time of infusion (min). This value of A probably underestimates incorporation of radioactivity from glucose into milk constituents by using *G_A . During the early part of the infusion, the specific radioactivity of plasma glucose is likely to be below that determined at equilibrium.

Requirement (P) for NADPH for fatty acid synthesis, expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation:

$$P = \sum [\text{FFA}_n \times (n-2)] \quad (6)$$

where n = chain length of the fatty acid (6 to 16) and FFA_n = output in milk of fatty acid of chain length n ($\mu\text{mol}/\text{min}$).

Net metabolism of glucose (B) to the galactose or glucose moiety of lactose, expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation:

$$B = L \quad (7)$$

where L = output of lactose in milk ($\mu\text{mol}/\text{min}$).

Metabolism of glucose via the pentose phosphate pathway (PC) can be calculated from the equation:

$$Y = 3PC / (1 + 2PC) \quad (8)$$

where Y = specific yield of ${}^{14}\text{CO}_2$ from $[1-$

${}^{14}\text{C}]$ glucose via the pentose phosphate pathway (Katz & Wood, 1963). If the NADPH formed via the pentose phosphate pathway were used exclusively for reductive biosynthesis of fatty acids, the ${}^3\text{H}$ incorporation from $[3-{}^3\text{H}]$ glucose into fatty acids would equal the ${}^{14}\text{CO}_2$ released from $[1-{}^{14}\text{C}]$ glucose via the pentose phosphate pathway (Katz *et al.*, 1974b).

Metabolism of glucose via the pentose phosphate pathway was therefore calculated from the equation:

$$Z = 3PC / (1 + 2PC) \quad (9)$$

where Z = (total ${}^3\text{H}$ in milk fatty acid / $[t \times {}^*G_A \times (U - B)]$).

Net metabolism of glucose 6-phosphate (E) via the Embden-Meyerhof pathway, expressed as $\mu\text{mol}/\text{min}$, was calculated from the equation:

$$E = C - (B + PC) \quad (10)$$

where C = rate of glucose phosphorylation by the gland, i.e. $(U - B)$.

Statistics. Results were compared by using Student's t test for paired observations. Data are presented as means $\pm$ S.E.M., with the numbers of observations in parentheses.

Results

Utilization of glucose carbon in the mammary gland

Glucose uptake by one half of the udder decreased during starvation from 363 ± 63 (6) $\mu\text{mol}/\text{min}$ to 92 ± 16 (6) $\mu\text{mol}/\text{min}$ (Table 1). This decrease in glucose uptake was primarily due to a fall in mammary blood flow from 469.8 ± 37.9 (6) to 145 ± 28.2 (6) ($P < 0.001$) ml/min. During this time milk production also fell significantly from

Table 1. Utilization of glucose carbon in half the udder of the lactating goat

Glucose uptake and incorporation into related products was determined as described in the Experimental section. Results are means $\pm$ S.E.M. for six animals. Values for the starved animals which are significantly different from those of the fed animal are shown by * $P < 0.05$, ** $P < 0.02$, *** $P < 0.01$.

	Fed	48 h starved
Glucose uptake ($\mu\text{mol}/\text{min}$)	363 ± 63	$92 \pm 16^{***}$
Lactose in milk ($\mu\text{mol}/100\text{ml}$)	13.77 ± 1.18	$9.85 \pm 1.43^*$
Citrate in milk ($\mu\text{mol}/100\text{ml}$)	0.69 ± 0.11	0.71 ± 0.115
Triacylglycerol in milk (mg/100ml)	3.43 ± 0.78	$5.29 \pm 1.85^*$
[${}^{14}\text{C}$]Glucose incorporation ($\mu\text{mol}/\text{min}$) into:		
Milk lactose	243 ± 50	$48 \pm 5.4^{**}$
Milk citrate	1.49 ± 0.26	1.02 ± 2.22
Milk triacylglycerol glycerol	14.7 ± 3.6	$3.92 \pm 0.98^{***}$
Venous-blood CO_2	12.5 ± 2.0	$1.76 \pm 0.4^{***}$
Percentage of glucose appearing as:		
Milk lactose	75.3 ± 23.3	68.9 ± 33.4
Milk citrate	0.49 ± 0.13	$1.11 \pm 0.23^*$
Milk triacylglycerol glycerol	4.47 ± 1.25	4.45 ± 1.36
Venous-blood CO_2	4.31 ± 0.80	2.20 ± 0.71

1.07 ± 0.07 (6) to 0.30 ± 0.03 (6) ml/min as a result of starvation ($P < 0.001$). The utilization of glucose for synthesis of milk lactose, triacylglycerol and citrate decreased during starvation, as did the rate of oxidation of glucose in the udder (Table 1). However, with the exception of milk citrate, there was no significant difference in the percentage of glucose taken up by the udder that was utilized for the synthesis of milk components (Table 1). The ratio of the specific radioactivity of milk [^{14}C]citrate relative to that of plasma glucose increased significantly during starvation from 0.228 ± 0.034 (6) to 0.488 ± 0.090 (6) ($P < 0.002$). No radioactive carbon was found in the fatty acid fraction of saponified milk fat in the starved state.

$^3\text{H}/^{14}\text{C}$ ratios in glucose and related products

The $^3\text{H}/^{14}\text{C}$ ratio in arterial plasma glucose was lower than that of the infusion, indicating some recycling of glucose carbon in the whole animal (Table 2). This recycling accounted for 10.2 ± 3.4 (6)% and 21.1 ± 3.7 (6)% ($P < 0.05$) of the glucose turnover in the fed and starved animal respectively. A further decrease in the $^3\text{H}/^{14}\text{C}$ ratio was seen in milk lactose (Table 2). As the glucose moiety of lactose arises directly from plasma glucose (Wood *et al.*, 1965), this decrease in the ratio must be due to metabolism of glucose 6-phosphate within the udder before incorporation into lactose as galactose. From the decrease in the $^3\text{H}/^{14}\text{C}$ ratio in lactose it can be calculated that 26.6 ± 3.9 (6)% and 21.4 ± 2.9 (6)% of the galactose moiety of lactose in fed and starved animals respectively arose from glucose 6-phosphate, which had been metabolized in the mammary gland such that its ^3H was removed before incorporation into lactose. ^3H was also detected in milk triacylglycerol glycerol, but the $^3\text{H}/^{14}\text{C}$ ratio was much lower than that of plasma glucose (Table 2). There was a significantly higher $^3\text{H}/^{14}\text{C}$ ratio in triacylglycerol glycerol isolated from milk obtained from the starved animals ($P < 0.001$) (Table 2).

NADPH production from glucose

^3H from C-3 of glucose was recovered in milk fat. The major portion of this ^3H was associated with the fatty acid fraction of the saponified triacylglycerol, less than 2% being present in triacylglycerol gly-

cerol in the fed animal and about 20% in the starved animal. The percentage of fat in milk increased significantly during starvation from 3.43 ± 0.32 (6)% to 5.29 ± 0.75 (6)% (g/100 ml) ($P < 0.05$). The composition of the fatty acids in milk triacylglycerol also changed as a result of starvation (Table 3). There was a decrease in the concentration of medium-chain-length fatty acids and an increase in the long-chain fatty acids as a result of starvation. Annison & Linzell (1964) and Annison *et al.* (1968) demonstrated that all medium-chain-length fatty acids and about 30% of C_{16} fatty acids are synthesized from acetate within the goat mammary gland in the fed state, but very little fatty acid synthesis *de novo* occurs during starvation. From these data it can be calculated from the milk fat composition and output in the present experiment that the requirement for NADPH for fatty acid synthesis was 537.7 ± 97 (6) $\mu\text{mol}/\text{min}$ in the fed animal. Incorporation of ^3H from [$3\text{-}^3\text{H}$]glucose into milk fatty acids was equivalent to 72.9 ± 26.9 (6) and 1.19 ± 0.34 (6) μmol of glucose/min in the fed and starved animal respectively. Metabolism of glucose via the pentose phosphate pathway yields 2 mole-

Table 3. Fatty acid composition of milk fat in the fed and starved animal

The fatty acid composition of milk triacylglycerol was determined by g.l.c. after saponification as described in the Experimental section. Values are the means of six animals. Values for the starved animals which are significantly different from those of the fed animals are shown by *** $P < 0.01$, ** $P < 0.02$, * $P < 0.05$.

Fatty acid chain length	Fatty acid output in milk ($\mu\text{mol}/\text{ml}$ of milk)	
	Fed	48 h starved
C_6	7.7 ± 0.73	$3.9 \pm 0.61^{***}$
C_8	14.0 ± 0.99	$3.3 \pm 0.52^{***}$
C_{10}	19.7 ± 3.7	$5.8 \pm 1.4^{***}$
C_{12}	7.1 ± 1.5	$2.7 \pm 0.7^{***}$
C_{14}	14.2 ± 1.4	$6.0 \pm 0.8^{***}$
C_{16}	33.4 ± 1.9	$44.1 \pm 3.6^*$
$\text{C}_{16:1}$	3.2 ± 0.7	$6.2 \pm 0.9^{**}$
C_{18}	18.8 ± 2.1	$39.5 \pm 2.6^{***}$
$\text{C}_{18:1}$	34.5 ± 2.3	$81.6 \pm 2.5^{***}$
$\text{C}_{18:2}$	4.7 ± 0.9	6.5 ± 0.1

Table 2. $^3\text{H}/^{14}\text{C}$ ratios in plasma glucose and related products

Determinations of radioactivity and specific radioactivity were performed as described in the Experimental section. Values given are means $\pm$ s.e.m. ($n=6$) relative to a $^3\text{H}/^{14}\text{C}$ ratio of 1 in the infusion, and results from the starved animals which are significantly different from those of the fed animals are shown by *** $P < 0.01$.

	Plasma glucose	Milk lactose	Milk galactose	Milk triacylglycerol glycerol	Milk citrate
Fed	0.888 ± 0.084	0.741 ± 0.115	0.642 ± 0.182	0.070 ± 0.056	0.537 ± 0.061
48 h starved	0.808 ± 0.086	0.747 ± 0.080	0.652 ± 0.105	$0.209 \pm 0.045^{***}$	0.537 ± 0.052

cules of NADPH/molecule, only one of which would be labelled with ^3H in the present experiments. Thus NADPH formation from glucose accounted for 26.7 ± 10.1 (6)% of that required for fatty acid synthesis *de novo* in the mammary gland of the fed goat. If there is a common pool of glucose 6-phosphate which is available for both lactose synthesis and pentose phosphate-pathway metabolism, then the recycling of glucose 6-phosphate within the udder (see the previous section on $^3\text{H}/^{14}\text{C}$ ratios, and Table 2) would result in too low a value for NADPH production from glucose. Correction of the calculated values for the lower $^3\text{H}/^{14}\text{C}$ ratio in the galactose moiety of lactose (Table 2) gave a revised estimate of 33.7 ± 7.2 (6)% for the contribution of NADPH formed in the pentose phosphate pathway to fatty acid synthesis in the fed animal. ^3H from $[3\text{-}^3\text{H}]\text{glucose}$ was also incorporated into milk citrate (Table 2). As this ^3H is lost to NADPH or water in metabolism via the pentose phosphate pathway or glycolysis, it is likely that ^3H incorporation into milk citrate was also via NADP^3H . The ratio of the ^3H specific radioactivity of milk citrate to that of plasma glucose was 0.126 ± 0.016 (6) and 0.21 ± 0.07 (6) in the fed and starved animals respectively.

The ^3H in plasma water was present at 0.0101 ± 0.0015 (6) and 0.0317 ± 0.0040 (6) $\mu\text{Ci/mol}$ in the fed and starved animals respectively. It was assumed that this value was too low to interfere in any significant way with the calculations.

Glucose metabolism via the pentose phosphate pathway

From the incorporation of ^3H from $[3\text{-}^3\text{H}]\text{glucose}$ into fatty acids the flux through the pentose

phosphate pathway was calculated to be 72.9 ± 26.9 (6) and 1.19 ± 0.34 (6) $\mu\text{mol/min}$ in the fed and starved animal respectively (Table 4). Correction for the lower $^3\text{H}/^{14}\text{C}$ ratio likely to be present in intracellular glucose 6-phosphate gave flux values of 96.2 and 2.0 $\mu\text{mol/min}$ for the fed and starved animals respectively.

Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (PC) has been defined as glucose 6-phosphate metabolized according to the equation:

Complete metabolism of one molecule of glucose 6-phosphate according to this equation would require three cycles of the pentose phosphate pathway; therefore flux through the pathway should be three times the net rate of glucose metabolized in the pentose phosphate pathway.

From the results of individual animals, mean values of 17.8% and 1.2% of the glucose phosphorylated by the mammary gland were calculated to be completely metabolized via the pentose phosphate pathway in the fed and starved animal respectively (Table 4). In the fed animal this rate $[30.4 \pm 10.6$ (6) $\mu\text{mol/min}]$ was adequate to account for all of the $^{14}\text{CO}_2$ $[75.6 \pm 10.4$ (6) $\mu\text{mol/min}]$ and all of the milk triacylglycerol $[^{14}\text{C}]\text{glycerol}$ $[29.4 \pm 7.2$ (6) $\mu\text{mol/min}]$ produced from glucose by the gland. In the starved animal, the rate $[0.59 \pm 0.11$ (6) $\mu\text{mol/min}]$ of the glucose metabolism via the pentose phosphate pathway was too low to supply either the $^{14}\text{CO}_2$ $[10.7 \pm 5.1$ (6) $\mu\text{mol/min}]$ or the triacylglycerol glycerol $[7.8 \pm 1.9$ (6) $\mu\text{mol/min}]$ produced from glu-

Table 4. *Rates of pathways of glucose metabolism in half the udder of the lactating goat*

Determinations and calculations were performed as described in the Experimental section. Some values have been corrected for recycling of glucose 6-phosphate in the udder by correcting for the lower $^3\text{H}/^{14}\text{C}$ ratio in the galactose moiety of milk lactose compared with plasma glucose. Results are means $\pm$ s.e.m. for six animals. Values for the starved animals which were significantly different from those of the fed animals are shown by *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

	Fed	48h starved
Flux through the pentose phosphate pathway calculated as ^3H incorporation into milk fatty acid (equivalent μmol of glucose/min)	72.9 ± 26.9	$1.19 \pm 0.34^{***}$
Corrected ^3H incorporation into milk fatty acid (equivalent μmol of glucose/min)	96.2 ± 28.8	$2.0 \pm 0.5^{***}$
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway (%)†	17.8 ± 5.6	$1.2 \pm 0.5^{**}$
Net metabolism of glucose 6-phosphate via the pentose phosphate pathway ($\mu\text{mol/min}$)†	30.4 ± 10.6	$0.59 \pm 0.11^{***}$
Metabolism of glucose 6-phosphate to the galactose moiety of lactose (%)	77.2 ± 6.9	60.0 ± 7.1
Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway (%)	4.9 ± 6.3	$39.1 \pm 7.2^{**}$
Metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway ($\mu\text{mol/min}$)	14.8 ± 14.3	25.8 ± 7.9

† Glucose 6-phosphate $\rightarrow$ glyceraldehyde 3-phosphate + 3 CO_2 calculated according to Katz *et al.* (1974b).

cose by the mammary gland (Tables 2 and 4). Metabolism of glucose via the Embden-Meyerhof pathway was calculated and the proportion of glucose metabolized this way increased significantly during starvation (Table 4). The absolute rate of metabolism of glucose via the Embden-Meyerhof pathway also appeared to increase on starvation, but the increase was not statistically significant (Table 4).

Discussion

Glucose carbon was used by the mammary gland to produce lactose, citrate and triacylglycerol for milk secretion. The data obtained for the utilization of glucose carbon for the synthesis of milk components are similar to those described previously for the fed and starved lactating goat (Annison & Linzell, 1964; Annison *et al.*, 1968). However, the rate of oxidation of glucose by the udder in the present study was much lower than that reported by Annison and co-workers (Annison & Linzell, 1964; Annison *et al.*, 1968) for both fed and starved goats. In the present study 82.8 ± 12.0 (6)% and 76.9 ± 13.1 (6)% of the [U- ^{14}C]glucose taken up by the mammary gland can be accounted for as lactose, citrate, triacylglycerol glycerol and CO_2 in the fed and starved animal respectively. Some glucose may also be converted into amino acids, which can then be used for milk-protein synthesis (Linzell & Mepharm, 1968) or lost as venous-plasma lactate.

The use of both [^3H]- and [^{14}C]-glucose in the present study has allowed the recycling of glucose in the whole animal and in the mammary gland of the lactating goat to be calculated. Recycling of glucose carbon in the whole animal accounted for 10 and 21% of the glucose synthesized in the fed and starved animal respectively. These values are comparable with the data of Judson & Leng (1972), who reported values of 13% recycling in the non-lactating fed sheep. Extensive recycling of glucose 6-phosphate was observed in the mammary gland itself. In the fed and starved animal respectively, 27 and 21% of the galactose moiety of milk lactose appeared to arise from recycled glucose 6-phosphate in the udder. In the fed animal, metabolism of glucose 6-phosphate in the pentose phosphate pathway would account for this observation. In the starved animal it is possible that recycling around phosphofructokinase in a futile cycle may be occurring (Katz & Rognstad, 1976).

In addition to the use of glucose carbon for milk synthesis, the hydrogen from glucose has been shown to be incorporated into milk fat. Studies *in vitro* have shown that glucose metabolism via the pentose phosphate pathway may not be as important for NADPH production as in the rat. Fatty acid synthesis from acetate can occur in the absence of glucose in sheep mammary-tissue slices (Balmain *et*

al., 1952) and the perfused goat udder (Hardwick *et al.*, 1963). Moreover, when glycerol is present in the incubation medium, the addition of glucose results in only a 40% increase in the incorporation of acetate into fatty acids by cow mammary-tissue slices (Bauman *et al.*, 1972), as opposed to a 100-fold increase in the rat (Balmain *et al.*, 1952). To date, estimates of the contribution of the pentose phosphate pathway in providing NADPH for fatty acid synthesis *in vivo* have been based on the assumption that all the glucose that was oxidized to CO_2 was metabolized via the pentose phosphate pathway. However, few data have been available from which to calculate the relative metabolism of glucose 6-phosphate via the Embden-Meyerhof pathway or the pentose phosphate pathway in the ruminant udder *in vivo*. Black *et al.* (1957), using [1- ^{14}C]glucose and [6- ^{14}C]glucose, estimated that 50–70% of the glucose catabolized in the whole body of the lactating cow had entered the pentose phosphate pathway. In the isolated perfused cow's udder 23–30% of the glucose was estimated to be metabolized via the pentose phosphate pathway (Wood *et al.*, 1965). The data presented here provide evidence that at least 27% of the NADPH required for fatty acid synthesis *de novo* in the udder of the fed goat arose from glucose metabolism. These data probably underestimate the actual values because of the recycling of glucose 6-phosphate within the udder, with the consequent loss of ^3H from glucose 6-phosphate. Correction for this recycling based on the lower $^3\text{H}/^{14}\text{C}$ ratio present in the galactose moiety of milk lactose resulted in a value of 34% for the contribution of glucose metabolism to NADPH production in the udder of the fed animal.

Katz *et al.* (1974b) have shown that net metabolism of glucose in the pentose phosphate pathway (i.e. glucose 6-phosphate $\rightarrow 3\text{CO}_2$ + glyceraldehyde 3-phosphate) can be calculated from the incorporation of ^3H from [3- ^3H]glucose into fatty acids by assuming that the NADPH formed is used exclusively for reductive biosynthesis of fatty acids. This technique has mainly been used to study the metabolism *in vitro* of rat mammary and adipose tissue (Katz & Wals, 1970, 1972; Katz *et al.*, 1966). However, as the fatty acids synthesized in the lactating udder are readily available as milk fat, the technique is particularly appropriate for the study of the pentose phosphate pathway in the lactating animal *in vivo*. On the basis of the techniques and calculations of Katz *et al.* (1974b), and by assuming that cytosolic NADPH is used solely for fatty acid synthesis, it has been shown that 17.8 and 1.2% of the glucose phosphorylated by the udder of the fed and starved goat respectively was metabolized via the pentose phosphate pathway. In the fed goat this was sufficient to account for all the CO_2 produced from glucose in the gland and indicates that, within

experimental error, all the glucose oxidized in the udder of the fed goat is oxidized via the pentose phosphate pathway. Smith (1971) has postulated that this might be so for the lactating ruminant mammary gland. In the udder of the starved goat, metabolism via the pentose phosphate pathway could account for only 17% of the CO_2 produced from glucose, and the proportion of the glucose taken up by the udder that is oxidized in the tri-carboxylic acid cycle must increase during starvation. The increase in the specific radioactivity of milk citrate on starvation is further evidence of this. In addition, calculations showed that there was an increase in both the proportion and absolute amount of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway during starvation. Evidence supporting an increased proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway compared with the pentose phosphate pathway is the 3-fold increase in the $^3\text{H}/^{14}\text{C}$ ratio in milk triacylglycerol glycerol in the starved animal. Metabolism of glucose 6-phosphate by the Embden-Meyerhof pathway can result in ^3H being retained in glycerol if the triose phosphate isomerase reaction is not completely at equilibrium (Katz & Rognstad, 1976). Metabolism of glucose 6-phosphate by the pentose phosphate pathway results in the loss of all ^3H from $[3\text{-}^3\text{H}]\text{glucose}$. Thus an increased $^3\text{H}/^{14}\text{C}$ ratio in milk triacylglycerol glycerol during starvation could indicate increased disequilibrium at the triose phosphate isomerase reaction and/or an increased proportion of glucose 6-phosphate metabolized via the Embden-Meyerhof pathway. Williamson & Robinson (1977) have also noted a decreased uptake of glucose during starvation in the rat mammary gland. This has been attributed to a decreased rate of glycolysis resulting from inhibition of phosphofructokinase by the elevated cytosolic citrate concentrations. In the ruminant the decreased metabolism of glucose 6-phosphate seen during starvation appeared to result in a decreased flux through the pentose phosphate pathway and a decreased rate of lactose synthesis.

^3H and carbon from glucose were also shown to be incorporated into milk citrate. Glucose carbon provided 22 and 48% of the carbon skeleton of citrate in the fed and starved animal respectively. These values are comparable with those reported by Hardwick *et al.* (1963), using the isolated perfused goat udder. Hardwick (1965) had postulated that milk citrate could be synthesized from 2-oxoglutarate via the NADP-dependent isocitrate dehydrogenase reaction. The presence of ^3H in milk citrate in the present experiments would appear to support this hypothesis. However, net synthesis of citrate via the cytosolic NADP-dependent isocitrate dehydrogenase reaction is unlikely, as net flux through the enzyme is thought to be in the opposite direction

(Bauman & Davis, 1975). Incorporation of ^3H into milk citrate probably occurred by an exchange reaction at this enzyme, which is thought to be at equilibrium. However, from the specific radioactivity of the ^3H , and assuming that all the ^3H is positioned on C-2 of milk citrate, glucose metabolism via the pentose phosphate pathway could account for 33 and 56% of the NADPH-exchangeable hydrogen in citrate in the fed and starved animal respectively. This value for the fed goat is in close agreement with the value of 34% obtained for the contribution of NADPH generated in the pentose phosphate pathway to the NADPH requirement for fatty acid synthesis in the udder of the fed goat, and would indicate that both fatty acid synthesis and the NADP-dependent isocitrate dehydrogenase reaction share a common pool of cytosolic NADPH.

In conclusion, the data presented here represent the first estimations *in vivo* of the pentose phosphate pathway and its contribution to NADPH production in the lactating ruminant udder and allow estimations to be made of the relative contributions of the different pathways to glucose metabolism. Of the glucose taken up by the gland, 8.4 and 0.6% are metabolized completely in the pentose phosphate pathway in the fed and starved animal respectively. This represents a flux through the pentose phosphate pathway of 26.2 and 2.2%. This rate of metabolism accounts for 34% of the NADPH required for fatty acid synthesis *de novo* in the udder in the fed animal and represents all the glucose oxidized to CO_2 in the fed animal, but only 17% of the CO_2 produced from glucose in the starved animal. During starvation it would appear that a larger proportion of the glucose 6-phosphate is metabolized via the Embden-Meyerhof pathway.

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