Changes in the concentrations of the minor constituents of goat's milk during starvation and on refeeding of the lactating animal and their relationship to mammary gland metabolism

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1. Changes in the concentrations of the minor constituents of goat's milk were observed during 48 h starvation and on refeeding.

2. The concentrations of hexose phosphate and UDP-hexoses increased during starvation and decreased on refeeding.

3. The concentrations of phosphoenolpyruvate and glycerate 3-phosphate decreased during starvation and increased on refeeding.

4. Isocitrate:2-oxoglutarate increased during starvation and decreased on refeeding.

5. Changes in the minor constituents of milk can be explained in terms of the metabolic changes occurring in the mammary gland during starvation. It is proposed that changes in the concentrations of these metabolites in milk reflect changes in their concentrations in the cytosol or Golgi vesicles of the mammary gland.

Previous studies in vivo have shown that major changes occur in the metabolism of the mammary gland of the goat during starvation. There is a reduction in the flux through the pentose phosphate pathway (Annison *et al.* 1968; Chaiyabutr *et al.* 1980). The rate of lactose synthesis decreases and milk yield falls; at the same time glucose uptake by the gland decreases (Annison & Linzell, 1964; Annison *et al.* 1968; Chaiyabutr *et al.* 1980). These findings were obtained in vivo using radiotracer techniques and by measuring arterio-venous concentration differences of metabolites across the gland. However, detailed information on the changes in the concentrations of metabolites within the mammary gland corresponding to these metabolic changes is not available in the ruminant.

Cellular constituents have previously been identified as minor components of milk (Jenness, 1974; Johke, 1978) and it has been proposed that the concentrations of some of these metabolites may reflect mammary tissue concentrations (Kuhn & White, 1975). Changes in the concentrations of carnitine and acetylcarnitine in milk have been shown to occur under different physiological conditions in the cow (Erfle *et al.* 1970). It is possible that the concentrations of metabolites in milk may provide information on metabolism within the mammary secretory cell.

This paper reports changes in the concentrations of some metabolites in goat's milk during starvation and on refeeding and compares these with the known metabolic changes which occur in the mammary gland at these times.

MATERIALS AND METHODS

Materials

Goats. British-Saanen goats aged from 3–6 years and weighing 40–60 kg were used. They were milked twice daily at 08.00 and 16.00 hours. Starved animals had food withdrawn for 48 h. Before lactation started each animal was surgically prepared for the sampling of arterial and mammary-venous blood as described by Linzell (1960).

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Chemicals and biochemicals. All coenzymes and the enzymes, citrate lyase (*EC* 4.1.3.6), UDP-glucose pyrophosphorylase (*EC* 2.7.7.9) and UDP-glucose 4-epimerase (*EC* 5.1.3.2), were obtained from Sigma (London) Chemical Co. Ltd, Poole, Dorset. All other enzymes were obtained from Boehringer Corporation (London) Ltd, Lewes, Sussex. All other chemicals were obtained from British Drug House, Poole, Dorset.

Methods

Milk (10 ml) was centrifuged at 50000 *g* for 45 min at 2° within 15 min of milking. The aqueous phase below the solidified fat layer was removed and deproteinized with 1 ml 5 M-perchloric acid. The precipitated protein was removed by centrifugation at 2500 *g* for 10 min. The supernatant fraction was neutralized with 5 M-potassium hydroxide at 0° and precipitated potassium perchlorate was removed by centrifugation. The resulting supernatant fraction was used directly for the spectrophotometric determination of metabolite concentrations. Glucose 6-phosphate was determined as described by Lang & Michal (1974); glucose 1-phosphate as described by Bergmeyer & Michal (1974); isocitrate as described by Siebert (1974); citrate as described by Dagley (1974); 2-oxoglutarate as described by Bergmeyer & Bernt (1974); L-malate as described by Gutmann & Wahlefeld (1974); nucleoside diphosphate as described by Jaworek *et al.* (1974); nucleoside monophosphate as described by Grassel (1974); phosphoenolpyruvate as described by Czok & Lamprecht (1974); glycerate 3-phosphate as described by Czok (1974); AMP as described by Jaworek *et al.* (1974); ATP as described by Lamprecht & Trautschold (1974). UDP-Glucose and UDP-galactose were determined by successive additions of the enzymes UDP-glucose pyrophosphorylase and UDP-galactose 4-epimerase; the resulting glucose 1-phosphate was determined as described previously. Inorganic phosphate and lactose were determined colorimetrically as described by Weil-Malherbe & Green (1951) and Teles *et al.* (1978) respectively. The rate of lactose synthesis was determined from the milk yields and the lactose concentrations in milk.

The uptake of glucose by the lactating mammary gland was calculated from the mammary blood flow and the arterio-venous concentration difference of glucose (Chaiyabutr *et al.* 1980). The fatty acid composition of milk was determined as described by Thomson *et al.* (1979). The rate of fatty acid synthesis in the mammary gland was calculated from the milk yields and the concentrations of the medium-chain fatty acids (up to C_{14}); in addition approximately 30% of the palmitate is synthesized *de novo* in the mammary gland of the fed animal (Annison *et al.* 1967; Annison *et al.* 1968) and this was taken into account in the calculations.

Statistics. Results obtained from animals during starvation were compared with those obtained from fed animals using the Student's paired *t* test.

RESULTS

Changes in the concentrations of hexose phosphates and UDP-hexoses in milk. The concentrations of glucose 6-phosphate, glucose 1-phosphate, UDP-glucose and UDP-galactose in milk increased during starvation and decreased on refeeding (Figs. 1 and 2; Table 1). The concentrations of UDP-glucose and UDP-galactose in milk appeared to decrease after 40 h of starvation when milk synthesis was constant. A further decrease in the concentrations of the UDP-hexoses was seen on refeeding. More prolonged periods of starvation would be required to determine whether the changes in the concentrations of the UDP-hexoses seen on starvation are transient or a more permanent feature. There was a negative correlation between the rate of lactose synthesis and the concentration of glucose 6-phosphate in milk (r −0·847; $P < 0·01$). Glucose uptake by the mammary gland also

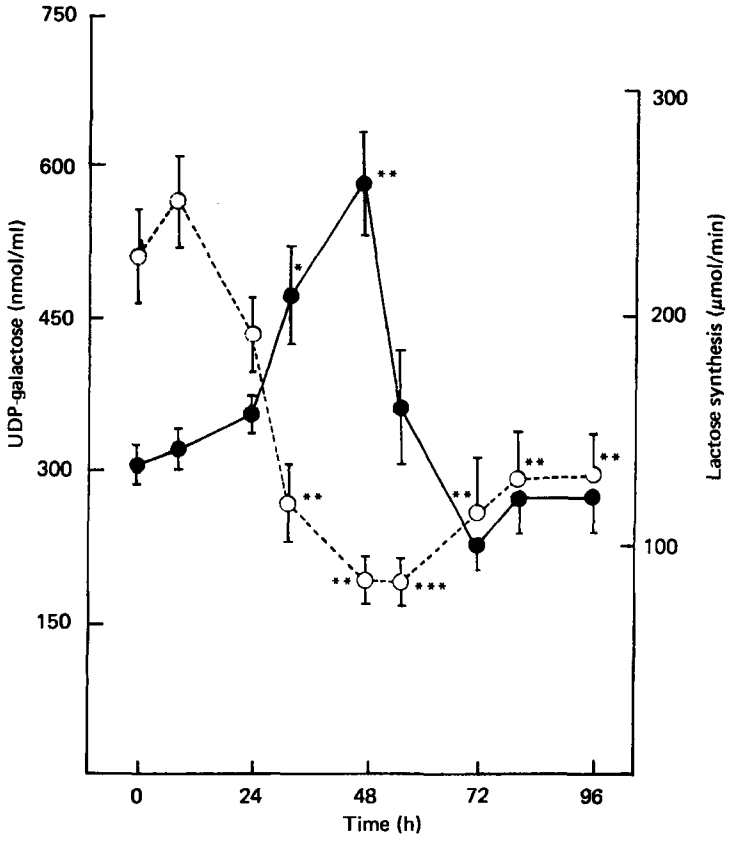

Fig. 1. Changes in the concentration of UDP-galactose (nmol/ml) in milk (●) and the rate of lactose synthesis (μmol/min) in the mammary gland (○). Goats had food withdrawn 8 h after the start of the experiment and food was offered again 57 h after the start of the experiment. Points are the mean values with their standard errors represented by vertical bars for five animals. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}005$.

decreased during starvation (Fig. 2). There was a negative correlation between the rate of glucose uptake by the mammary gland and the concentration of glucose 6-phosphate in milk (r −0·845; $P < 0{\cdot}01$). UDP-Glucose:UDP-galactose was constant at approximately 0·5 in the fed animal and during starvation and refeeding. Glucose 6-phosphate:UDP-glucose was constant at approximately 0·6 in the fed animal and during the early period of starvation but increased to approximately 1·6 after 48 h starvation and remained high for 24 h after refeeding.

Changes in nucleoside phosphate and inorganic phosphate concentrations in milk. A nucleoside diphosphate was present in milk; this reacted with phosphoenolpyruvate to form pyruvate catalyzed by the enzyme, pyruvate kinase (*EC* 2.7.1.40). This nucleoside diphosphate did not react with myokinase (*EC* 2.7.4.3) to form ATP; hence it was not ADP. As UDP has been identified in milk previously (Johke, 1978), it was assumed that this nucleoside diphosphate was UDP. The concentration of the nucleoside diphosphate increased during starvation and decreased on refeeding (Table 1). A nucleoside monophosphate, assumed to be UMP, was also present in milk and the changes in its concentration were similar to those of UDP (Table 1). The concentration of inorganic phosphate decreased during starvation and increased on refeeding (Table 1).

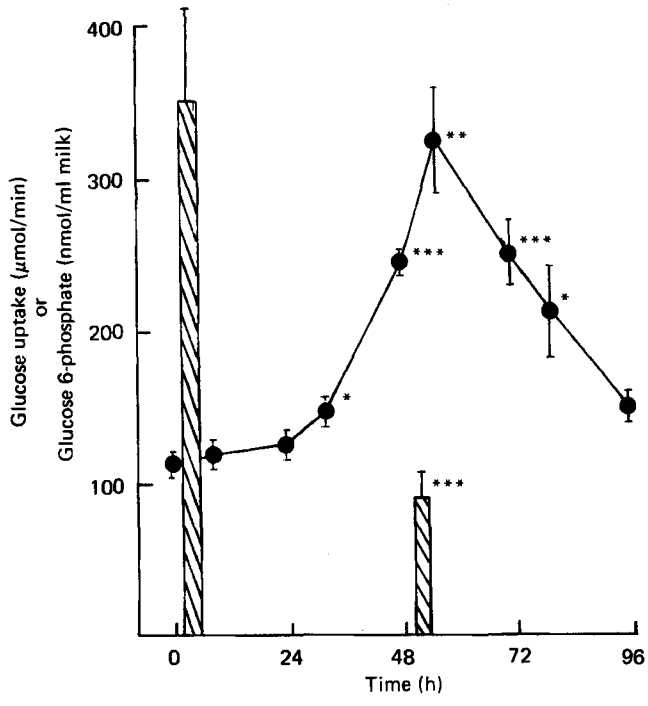

Fig. 2. Changes in the concentration of glucose 6-phosphate (nmol/ml milk) in milk (●) and the rate of glucose uptake (μmol/min) by the lactating mammary gland (▨). Goats had food withdrawn 8 h after the start of the experiment and food was offered again 57 h after the start of the experiment. Points are the mean values with their standard errors represented by vertical bars for five animals. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

Changes in the concentrations of glycerate 3-phosphate and phosphoenolpyruvate in milk. The concentrations of glycerate 3-phosphate and phosphoenolpyruvate decreased during starvation and increased on refeeding (Fig. 3). This decrease during starvation corresponded to a decreased flux through the pentose phosphate pathway as described previously (Chaiyabutr *et al.* 1980). There was a positive correlation between the concentration of phosphoenolpyruvate in milk and the estimated rate of the pentose phosphate pathway (r 0·859; $P < 0.01$). Glycerate 3-phosphate:phosphoenolpyruvate was relatively constant at approximately 1·7 in the fed, starved and refed goat.

Changes in the concentrations of tricarboxylic acid cycle intermediates in milk. There was a positive correlation between the concentrations of citrate and isocitrate in milk (r 0·98; $P < 0.01$). Both increased during starvation and decreased on refeeding; isocitrate:citrate remaining constant at approximately 0·022 (Table 1). The concentrations of 2-oxoglutarate and L-malate decreased during starvation and increased on refeeding (Table 1). Isocitrate:2-oxoglutarate increased several fold during starvation and fell on refeeding (Fig. 4). At the same time there was a large decrease in the rate of fatty acid synthesis *de novo* in the mammary gland during starvation (Fig. 4). There was a negative correlation between isocitrate:2-oxoglutarate in milk and the rate of fatty acid synthesis in the mammary gland (r −0·814; $P < 0.01$).

Table 1. *Changes in the concentrations of metabolites in goat's milk during starvation and refeeding*

(Mean values with standard errors for five animals; goats had food withdrawn 8 h after the start of the experiment and food was offered again 57 h after the start of the experiment)

	Metabolite concentrations in milk (nmol/ml)																			
Time (h)	Glucose-1-phosphate		UDP-Glucose		Nucleoside diphosphate		Nucleoside monophosphate		AMP		Citrate		Isocitrate		2-Oxoglutarate		L-Malate		Inorganic phosphate		Milk yield (ml/min)	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	12·5	2	163	10	101	14	50	12	36	2	3160	330	68	8	122	2	64	12	17900	3000	1·47	0·06
8	14	2	186	10	163	23	68	15	55	6	2760	230	74	5	140	10	66	8	18000	2000	1·66	0·08
24	20	2*	186	11	119	6	68	17	40	6	3250	550	71	3	87	12*	34	3*	13400	3000	1·23	0·09
32	26	2**	224	31	306	33***	164	25*	61	11	4180	120	90	12	74	8**	32	5*	12700	2000*	0·77	0·12**
48	53	12*	285	36**	257	70*	170	19**	16	3*	5750	1450**	117	9*	67	13**	33	4*	8600	2000*	0·57	0·07**
56	32	7*	188	42	280	25**	196	21**	22	8	7270	1480***	165	23**	90	15**	34	6*	6600	2500**	0·58	0·05***
72	28	7*	120	14	147	46	39	16	9	3**	4950	930**	115	13*	109	13	96	24	8400	3000*	0·74	0·13**
80	23	6	156	12	129	19	84	22	20	10	4850	720**	99	27	116	19	73	15	13000	4000	0·89	0·12**
96	13	2	152	16	90	12	42	11	20	5	2170	326	55	4	89	14	51	9	11700	3000	1·03	0·13*

*$P < 0·05$, **$P < 0·01$, ***$P < 0·005$.

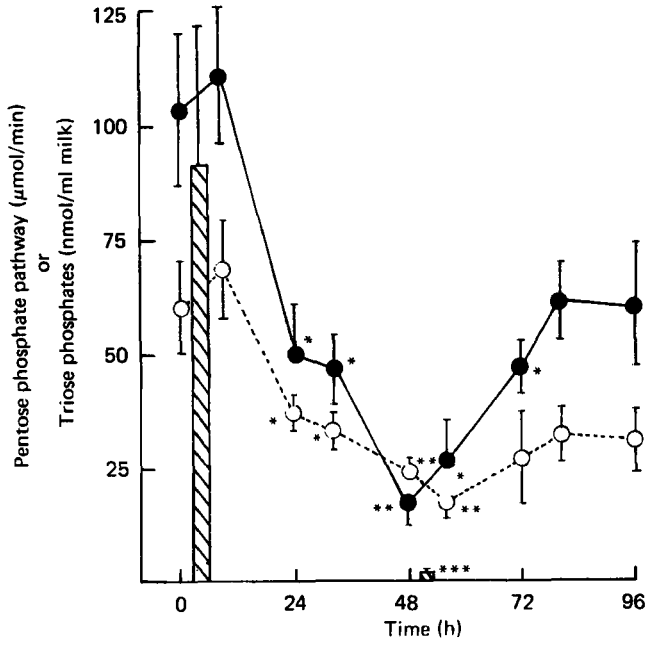

Fig. 3. Changes in the concentrations of phosphoenolpyruvate (nmol/ml milk; ○) and glycerate 3-phosphate (nmol/ml milk; ●) in milk and the flux through the pentose phosphate pathway (μmol/min) in the mammary gland. Goats had food withdrawn 8 h after the start of the experiment and food was offered again 57 h after the start of the experiment. The values for the fluxes through the pentose phosphate pathway (▨) were taken from Chaiyabutr *et al.* (1980). Points are mean values with their standard errors represented by vertical bars for five animals. $*P < 0.05$, $**P < 0.01$, $***P < 0.005$.

DISCUSSION

The results demonstrate that the concentrations of certain minor constituents of milk change during starvation and refeeding of the lactating goat. It is possible that, as milk yield decreases during starvation, increases in the concentration of some metabolites may represent a constant rate of secretion of the metabolite into a reduced volume. However, of the metabolites that increase in concentration during starvation, only glucose 6-phosphate concentrations show a significant negative correlation with milk yield and decreases in the concentrations of metabolites in milk cannot be explained in terms of changes in milk yield. Since these changes can be interpreted in terms of alterations in the metabolic activity of the mammary secretory cell, it seems likely that the concentrations of these substances in milk reflect their concentrations in the cytosol. Therefore, analysis of milk for such minor constituents may provide an insight into biochemical processes that can normally only be gained by obtaining tissue samples.

The rate of milk secretion decreases during starvation in goats (Annison *et al.* 1968) and since milk volume is thought to be determined primarily by lactose secretion (Linzell & Peaker, 1971) the decrease in milk yield can be attributed to a decrease in the rate of lactose synthesis. The increased concentrations of UDP-galactose in milk during starvation may represent a build-up of this substrate of lactose synthetase (*EC* 2.4.1.22) in the cytosol and Golgi vesicles following decreased enzyme activity. Increases in the concentrations of UDP-glucose and glucose 6-phosphate would then follow as the relevant enzymes established new equilibria. Hence, the observed decrease in the rate of glucose uptake by the mammary

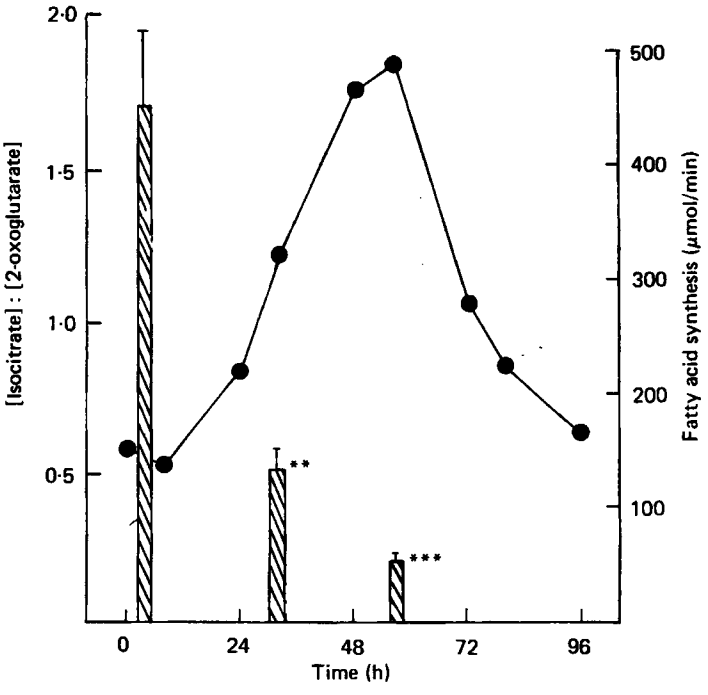

Fig. 4. Changes in isocitrate:2-oxoglutarate in goat's milk (●) and the rate of fatty acid synthesis *de novo* in the mammary gland (▨). Goats had food withdrawn 8 h after the start of the experiment. Isocitrate:2-oxoglutarate was calculated from the means of the concentrations of isocitrate and 2-oxoglutarate in milk (see Table 1). The points are mean values with their standard errors represented by bars for five animals. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}005$.

gland may result from a decrease in the rate of phosphorylation of glucose as hexokinase (*EC* 2.7.1.1) becomes inhibited by high cytosolic concentrations of glucose 6-phosphate (Gummaa *et al.* 1971). Alternatively the elevated UDP-galactose concentrations may reflect an initial increase in the cytosolic glucose 6-phosphate concentration if regulation of glucose uptake by the mammary gland precedes the decreased rate of lactose synthesis. However, during prolonged starvation and on refeeding, the concentration of UDP-galactose in milk fell more rapidly than that of glucose 6-phosphate, and it is possible that the equilibrium between these two metabolites is not maintained in the cytosol under these conditions. UDP-Glucose:UDP-galactose in milk is constant at approximately 0·5, but this is far from the equilibrium constant of 3·5 for the enzyme UDP-galactose 4-epimerase (Wilson & Hogness, 1968). If the aqueous phase of milk is derived mainly from the fluid of the Golgi vesicles (Linzell & Peaker, 1971), the high concentration of UDP-galactose in milk may reflect its concentration in the Golgi vesicles rather than in the cytosol; this could be evidence of a specific transport of UDP-galactose into the Golgi vesicles as has been shown in the rat (Kuhn & White, 1976).

The concentrations of nucleoside mono- and diphosphate (assumed to be UMP and UDP) in milk increased during starvation and decreased on refeeding. Since UDP is a product of lactose synthesis, it must be present in the Golgi vesicles. Hence its concentration in milk may reflect its vesicular rather than cytosolic concentration. It is known that UDP is a potent inhibitor of lactose synthetase (Khatra *et al.* 1974). If its increased concentration in milk

during starvation reflects an increased concentration in the Golgi vesicles, UDP may be one of the factors responsible for the decreased rate of lactose synthesis seen at this time. However, Kuhn & White (1977) have demonstrated the presence of a UDPase (*EC* 3.6.1.6) in the Golgi vesicles of the rat mammary gland. They argue that this enzyme effectively removes UDP, thus preventing inhibition of lactose synthesis. The presence of UMP in goat's milk may be evidence of a similar mechanism operating in the ruminant.

At the same time as the decrease in the rate of lactose synthesis there is a large decrease in the rate of fatty acid synthesis *de novo* in the mammary gland during starvation (Annison *et al.* 1968). This decrease would be expected to result in a decreased rate of utilization of NADPH and a rise in NADPH:NADP in the mammary gland. If the concentration of carbon dioxide remains relatively constant, isocitrate: 2-oxoglutarate can be used to follow changes in NADPH:NADP in the cytosol (Veech *et al.* 1969). In milk isocitrate:2-oxoglutarate increased during starvation and fell on refeeding, and was related inversely to the rate of fatty acid synthesis in the mammary gland (Fig. 4). Therefore, changes in isocitrate:2-oxoglutarate in milk appear to reflect the expected changes in the corresponding cytosolic values. The activity of the enzyme, isocitrate dehydrogenase (*EC* 1.1.1.42), is high in ruminant mammary gland and the reaction catalyzed by this enzyme is thought to be important in the generation of NADPH for fatty acid synthesis (Bauman *et al.* 1970). Changes in the concentrations of isocitrate and 2-oxoglutarate may also alter cytosolic citrate concentrations by interconversion of citrate and isocitrate and thus provide an explanation for the observed changes in the citrate content of milk reported previously (Linzell, 1967; Konar *et al.* 1971).

An increase in NADPH:NADP in the mammary gland may bring about the decrease in flux through the pentose phosphate pathway observed previously during starvation (Chaiyabutr *et al.* 1980). A decreased flux through the pentose phosphate pathway when the rate of glycolysis is low reduces the rate of triose phosphate production and is consistent with the decrease in the concentrations of glycerate 3-phosphate and phosphoenolpyruvate in milk seen during starvation (Fig. 3). This is further evidence that the concentrations of these metabolites in milk reflect their cytosolic concentrations.

There appears to be little change in the concentration of the constituents in milk after secretion. We have obtained samples by milking at 4, 8 and 16 h intervals and detected no major changes in the concentrations of intermediates at these times (A. Faulkner, 1980; unpublished observations). Linzell (1967) found only a small increase in milk citrate and decrease in milk lactose in fed goats milked at 15 min and 1 h intervals when compared with normal milk, and these changes may have been caused by the use of oxytocin to obtain milk samples over these shorter time intervals. Of the intermediates studied only the concentration of 2-oxoglutarate decreased slightly but significantly when goats were milked at 16 h intervals (Faulkner, 1980). Although cytosolic enzymes have been detected in milk, their activity is low and there seems to be little metabolic interconversion of the milk constituents.

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