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THE EFFECT OF VERY FREQUENT MILKING AND OF OXYTOCIN ON THE YIELD AND COMPOSITION OF MILK IN FED AND FASTED GOATS

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SUMMARY

1. The effect of milking goats 1–4 times an hour for 3–12 hr on the yield and composition of milk has been studied in fed and fasted animals at all stages of lactation.

2. It was essential to inject oxytocin (50–400 m-u. I.V.) just before each milking to remove all the milk already in the udder and then the yield was similar to that obtained on twice daily milking ($105 \pm 2{\cdot}1$ S.E. %). There were no significant differences between goats or between the two glands of one goat, even if one had been denervated by autotransplantation. However, the variation from hour to hour was 1·5 times greater than from day to day.

3. The claim of Zaks (1964) and Zaks, Natochin, Sokolova, Tanasichuk & Tverskoy (1965) that milking every 15 min always produces a large rise in milk Na and a fall in K and lactose, which is characteristic of alveolar milk, is not substantiated. In high yielding goats milking gently by hand or with a cannula caused a small change in K only, but vigorous hand milking exacerbated this fall and also caused a fall in lactose and a rise in Na and Cl. Still larger changes were produced by using excessively large doses of oxytocin (2500 m-u.) when there was also a rise in citrate and total nitrogen. Hourly milking in goats fasted for 24 hr had the same effect.

4. In fasted goats the milk yield fell to 90 % within 8 hr and to $56 \pm 2{\cdot}1$ % of the previous level by 24 hr. It remained at this level for a further 10–12 hr on twice daily or on hourly milking. The yields of autotransplanted glands usually fell slightly but significantly more than that of the glands *in situ*. In most goats mammary blood flow was halved but in all animals there were large falls in mammary uptake of glucose, acetate and amino acids and greatly increased uptake of free fatty acids. There were significant differences between fasted goats on hourly milking.

5. It is concluded that, in spite of changes in milk composition, milking

hourly can be a useful technique for studying milk secretion. The striking effects of a short fast in a lactating animal are emphasized.

INTRODUCTION

Milk secretion is believed to be a continuous process but little is known of the factors controlling it over short periods. This is because milk accumulates in the distensible alveoli and ducts and the glands are not completely emptied at each suckling or milking, leaving behind 13% of the milk and 32% of the fat in a cow that is milked twice daily (Johansson, 1952). In early studies of the effect of milking cows every 1–2 hr the milk yield was extremely variable (Brown, Petersen & Gortner, 1936), but, once it was discovered that oxytocin aids mammary emptying by squeezing the alveoli and widening the ducts, far more uniform yields of milk were obtained on frequent milking by injecting intravenously extra oxytocin just before each milking to remove the residual milk (Shaw, 1942; Smith, 1947; Donker, Koshi & Petersen, 1954; Lakshmanan, Shaw, McDowell, Ellmore & Fohrman, 1958). In general these workers found that the milk yield was similar to that on twice daily milking, although the yield of milk fat was at first lowered and then raised about 50% in some experiments.

Isolated perfused goat mammary glands that are milked hourly with the aid of oxytocin show significant responses to variations in blood flow and the availability of substrates within 1 hr (Hardwick, Linzell & Price, 1961). Furthermore, studies in many laboratories with isotopically labelled milk precursors in conscious cows and goats show that the time taken for synthesized components to appear at the teat is of the order of 1–6 hr, but in very few cases was the rate of milk secretion measured at the time of administering the isotope in such experiments.

In the present work the feasibility has been investigated of studying milk secretion in conscious goats by milking them frequently with the aid of injected oxytocin.

METHODS

All goats in this herd are routinely milked by hand twice daily at about 8 and 16 hr intervals and the yields of the two glands recorded separately. The milk yields on frequent milking are expressed as a percentage of the mean yield for the same interval calculated from the mean daily yield 3–10 days before the experiment. Milk for analysis was a sample of the total milk produced by each gland 1–3 days before the experiment stored at 4 °C.

The experiments were done at all stages of three consecutive lactations, of 9–10 months duration, when the milk yields were within the range 25–100 ml./hr/gland. They were conducted on twelve Saanen and two Welsh goats in the animal house under conditions that caused the minimum of disturbance, generally with the animal in its own pen or on the milking stand (Linzell, 1966*b*). Some of the animals were surgically prepared for arterial and mammary venous blood sampling and for mammary blood flow measurement (Linzell,

1960*a*, 1963, 1966*a*). Plastic catheters were inserted into arterial loops and/or into the right heart by Seldinger's (1953) technique under local anaesthesia and attached to a leather harness worn by the animal so that blood sampling and injections could be carried out with the minimum of restraint. Hourly milking was carried out for 8–11 hr in twenty-six experiments on twelve goats and half or quarter hourly milking for 3–4 hr in thirteen experiments on nine goats.

Experiments started at morning milking, when the mammary glands were emptied as thoroughly as possible after an intravenous injection of oxytocin and thereafter in the same way before each milking. It is usual to inject a very large dose to remove the residual milk (10–25 u. in cows, 1–5 u. in goats). However, an effective dose for a rise in milk pressure in goats is 1 m-u. (Denamur & Martinet, 1953) and 120–250 m-u. in cows (Donker, 1958). There are a number of recent reports that high concentrations of oxytocin have an insulin-like activity. Therefore in this work the minimum effective dose needed to empty the glands, determined at the start of each experiment, was employed. The range was 50–400 m-u., with 100–200 m-u. being used in most experiments (2–4 m-u./kg).

The effect of fasting was studied in thirty-two experiments in ten of the goats. Food, but not water, was removed immediately after morning milking, and the animals milked as usual 8 and 24 hr later, after which hourly milking was started.

Analytical methods

Milk. Fat was measured by the rapid method of Fleet & Linzell (1964). The freezing point was determined at once on 2 ml. of milk in a Fiske osmometer, using the precautions recommended by England & Neff (1963). Cell count was estimated by the method of Blackburn (1965). Proteins were precipitated by diluting 0·1–1·0 ml. of milk to 10 ml. with Grimbleby's precipitant (Biggs & Szijarto, 1963) diluted 1:80. After standing, lactose was determined in the supernatant at a final dilution of 1:1000 by the method of Marier & Boulet (1959) taking a standard through with each batch and periodically checking the recovery by adding extra lactose to one milk sample. Total N was determined by Keldahl on 1–2 ml.of diluted milk (1:20) and Na and K on a 1:400 dilution by flame photometry, against a standard containing 0·1 m-equiv/l. Na and 0·3 m-equiv/l. K and Ca, Mg and PO_4 in the proportions present in milk. Chloride was determined by the method of Van Slyke & Hiller (1947), on 2 ml. of supernatant after diluting 1 ml. of milk with 9 ml. of precipitant. Citrate was determined by the method of White & Davies (1963), using 0·5 ml. of milk.

In some experiments milk protein tended to be spun down when milk was centrifuged to remove the fat. Therefore all analyses were done on whole milk. The concentrations of Na, K, Cl, N, lactose and citrate are expressed on a fat free basis.

Blood. Glucose was estimated by the glucose oxidase method of Huggett & Nixon (1957) and later by Hultman's toluidine method (Hyvarinen & Nikkila, 1962), which gave identical results to the glucose oxidase. Volatile fatty acids were measured by the method of Annison (1954); free fatty acids by the method of Dole (1956), and amino acids by the method of Hamilton & Van Slyke (1943). Mammary blood flow was measured by the continuous thermodilution method of Linzell (1966*a*).

RESULTS

As in the cow (Shaw, 1942; Smith, 1947) when the goats were milked at frequent intervals it was necessary to inject oxytocin I.V. just before each milking in order to remove most of the milk already present in the udder. Without it 50–90 % of the milk was left behind.

Milking every hour. Like the total daily yield on twice daily milking the yield from hour to hour appeared to vary randomly, although this vari-

ability was greater. In twenty-four experiments on twelve goats the coefficient of variation of daily yield on twice daily milking for 10 days before the experiment was 6·3 ± 2·3 (S.D.) and of the yield on hourly milking was 9·3 ± 3·6 (P = 0·01). The mean yield on hourly milking for 10 hr expressed as a percentage of the yield immediately before the experiment

TABLE 1. The effect of hourly milking on the composition of milk in fed and 24 hr-fasted goats. Mean ± S.E. of mean (no. of observations). The bulked milk sample was an aliquot of the total milk produced during the 3 days before the experiment. The hourly samples are mean figures for the 3rd to 5th hr inclusive

	Fed			Fasted	
	Bulked	Morning	Hourly	Morning	Hourly
Osmolality (m-osmole/kg H_2O)	297 ± 2·5 (11)	295 ± 4·4 (3)	304 ± 5·5 (3)	294 ± 1·8 (10)	298 ± 2·1 (10)
Lactose (m-equiv./l.)	124 ± 2·5 (16)	129 ± 2·5 (23)	123 ± 2·9 (22)	128 ± 2·5 (28)	103 ± 3·0 (28)
Na (m-equiv./l.)	19 ± 2·9 (17)	18 ± 1·5 (10)	25 ± 3·5 (11)	17 ± 2·0 (20)	29 ± 3·3 (19)
K (m-equiv./l.)	46 ± 1·5 (14)	47 ± 1·4 (10)	42 ± 1·3 (11)	40 ± 1·5 (18)	30 ± 1·5 (20)
Cl (m-equiv./l.)	43 ± 1·9 (11)	49 ± 4·0 (5)	54 ± 4·7 (5)	46 ± 1·7 (17)	62 ± 2·4 (17)
Fat (g/l.)	39·6 ± 1·2 (21)	37·9 ± 1·5 (12)	37·6 ± 2·2 (17)	45·1 ± 2·4 (22)	54·3 ± 2·6 (28)
Total N (g/l.).	4·34 ± 0·25 (23)	4·84 ± 0·33 (7)	4·46 ± 0·18 (18)	5·48 ± 0·17 (11)	6·41 ± 0·12 (13)
Citrate (g/l.)	0·98 ± 0·12 (14)	1·03 ± 0·14 (7)	1·39 ± 0·26 (7)	1·08 ± 0·20 (10)	1·36 ± 0·22 (11)
Milk yield (%)	100	104 ± 1·1 (24)	105 ± 2·5 (18)	56 ± 2·1 (37)	48 ± 2·3 (28)

TABLE 2. Changes in milk composition on hourly milking in fed and 24-hr-fasted goats. The mean figure for the 3rd to 5th hr inclusive is expressed as a percentage of the concentration of the component in the morning milk

	Fed			Fasted		
Component	Mean ± S.E.	n	P	Mean ± S.E.	n	P
Osmolality	103 ± 1·1	3	N.S.	101·4 ± 0·4	10	0·01
Lactose	97 ± 1·4	19	0·05	81 ± 2·2	25	0·001
Na	140 ± 8·5	10	0·001	175 ± 11·7	17	0·001
K	92 ± 2·6	10	0·05	75 ± 2·5	17	0·001
Cl	110 ± 4·1	5	0·07	133 ± 6·0	11	0·001
Fat	102 ± 6·2	15	N.S.	127 ± 5·6	22	0·001
Total N	102 ± 2·1	18	0·2	127 ± 4·1	9	0·001
Citrate	134 ± 14·4	6	0·07	130 ± 6·4	6	0·005

on twice daily milking ranged from 92 ± 4 to 132 ± 15 (S.D.) %. Analyses of variance confirmed that the variation on hourly milking between different goats was not significantly different from the variation within goats. This applied when experiments were compared in early and late lactation and even in three different lactations. There were no significant differences between the two glands of one goat even if one had been transplanted to another site on the body (Linzell, 1963), the ratio of yields being the same on hourly milking as on twice daily milking. The mean yield on hourly milking for 10 hr was 105 ± 2·1 (S.E.) % of the previous yield on hourly milking (P = 0·02) (see also Fig. 1 and Table 1). A similar small increase was seen when one gland was milked hourly as compared with the other milked once at the end of 8 hr.

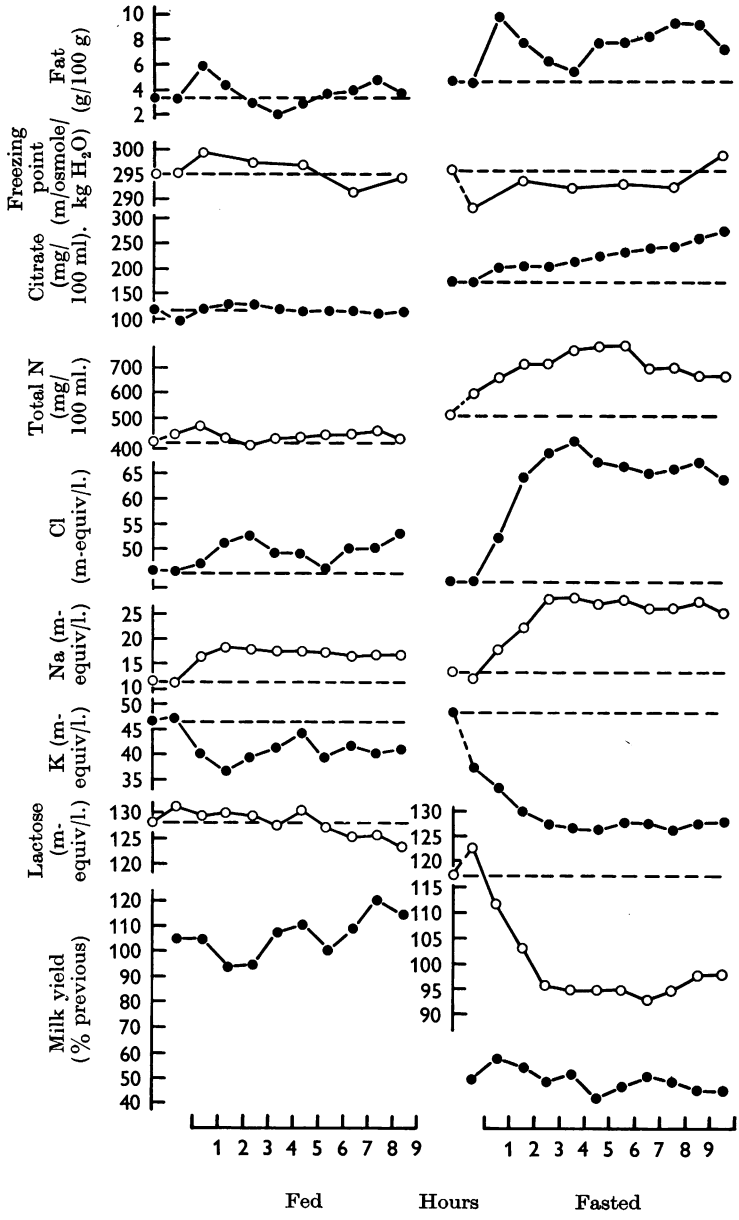

Fig. 1. The yield and composition of milk of two goats milked hourly, one fed and the other fasted for 24 hr. The horizontal lines represent the value before the experiment (3 days yield combined) and the first point the morning milk.

Table 1 shows the changes in mean milk composition on hourly milking. Analyses of variance for all components showed that the variation between goats was not significantly different from the variation within goats. It will be seen that there are rises in the concentration of Na, Cl and citrate and a fall in K. However, changes are more accurately detected by comparing

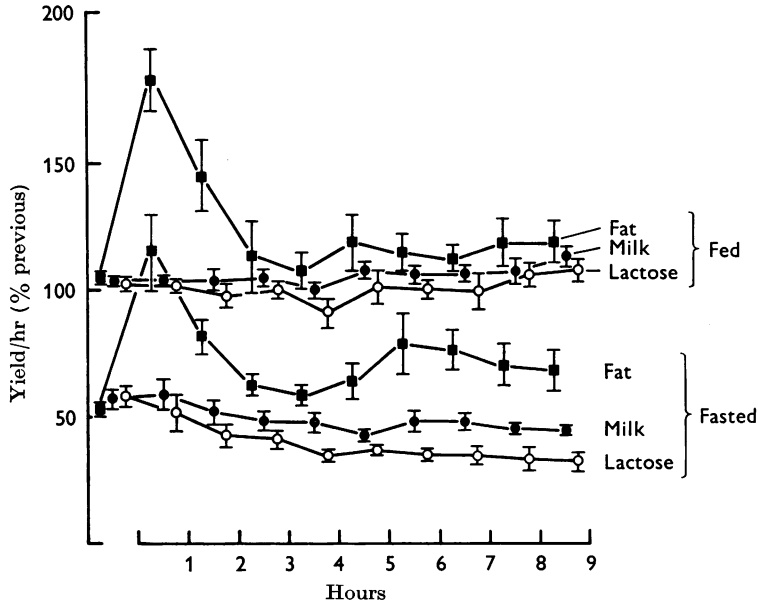

Fig. 2. Mean and S.E. yields of milk, lactose and fat of six fed and nine fasted goats milked hourly, expressed as a percentage of the quantities secreted before the experiment on twice daily milking (milk yield mean of 7 days and composition from 3 days yield combined). The first point is the milk obtained at morning milking on the day of the experiment.

the composition with that of milk obtained from the same animal immediately before the experiment (Table 2). This shows a significant rise in Na, significant falls in K and lactose, and barely significant rises in Cl and citrate concentrations.

In Fig. 2 the quantities of fat and lactose secreted each hour are compared with the yield of milk. As was to be expected from the well known fact that residual milk has a high concentration of fat it was 4 hr before the fat yield levelled out. Thereafter the amount of fat and total milk formed each hour were not significantly different, but the amount of lactose was significantly lowered ($P = 0{\cdot}025$).

Effect of fasting. After 8 hr of fasting the milk yield in thirty-seven experiments on ten goats was 90 ± 3 % (S.E. of mean) of the yield 3–7 days

before the experiment. By 24 hr the yield had fallen to $56 \pm 2{\cdot}1$ % and it remained at this level or slightly lower for a further 8–12 hr whether the animal was milked in the evening as usual or at every hour (Figs. 1, 2). As in fed goats the two glands of each goat responded similarly, even if

TABLE 3. Effect of 24 hr fast on blood composition and mammary uptake in goats. Mean ± S.E. of mean (no. of observations)

	Fed		Fasted	
	Arterial	Mammary arteriovenous difference	Arterial	Mammary arteriovenous difference
Blood glucose (mg/100 ml.)	$44 \pm 1{\cdot}5$ (17)	$11{\cdot}8 \pm 0{\cdot}9$ (14)	$30 \pm 1{\cdot}1$ (29)	$6{\cdot}9 \pm 0{\cdot}9$ (20)
Blood volatile fatty acids (m-equiv/l.)	$1{\cdot}32 \pm 0{\cdot}1$ (14)	$0{\cdot}90 \pm 0{\cdot}10$ (10)	$0{\cdot}33 \pm 0{\cdot}09$ (13)	$0{\cdot}14 \pm 0{\cdot}03$ (9)
Plasma free fatty acids (m-equiv/l.)	$0{\cdot}32 \pm 0{\cdot}015$ (14)	$0{\cdot}01 \pm 0{\cdot}023$ (10)	$1{\cdot}18 \pm 0{\cdot}05$ (12)	$0{\cdot}35 \pm 0{\cdot}04$ (11)
α amino N (mg/100 ml.)	$4{\cdot}42 \pm 0{\cdot}27$ (6)	1·07, 0·59 (2)	$3{\cdot}5 \pm 0{\cdot}34$ (5)	0·1, −0·1 (2)
Mammary blood flow	100		55 ± 6 (13)	
Mammary uptake (blood flow × arteriovenous difference)				
Glucose	100		31 ± 4 (9)	
Acetate	100		10 ± 2 (9)	

autotransplanted (gland *in situ* $52{\cdot}4 \pm 2{\cdot}8$ %, transplant $47{\cdot}3 \pm 3{\cdot}0$ %). This difference, although small, was consistent (seventeen out of twenty-three experiments on six goats) and was significant ($P = 0{\cdot}01$). Analysis of variance of the yield at the 3rd–5th hr of hourly milking showed that the variation between goats was significantly ($P = 0{\cdot}01$) greater than the within goat variation (repeated experiments on the same goat). This result was largely influenced by one goat in which the mean for four experiments was 71 ± 5 %, whereas in twenty-four experiments on seven other goats the mean was $44 \pm 1{\cdot}5$ %.

On refeeding there was no change in milk yield for 2–3 hr, but then it began to rise steadily. In animals at the height of lactation a few weeks after parturition the yield could reach 90 % of the prefasting level within 8 hr, but at later stages of lactation, on the declining part of the lactation curve, recovery was delayed. At 16 hr after refeeding the mean yield was $59 \pm 2{\cdot}4$ % of prefasting and at 24 hr $86 \pm 2{\cdot}2$ % (thirty-one experiments on nine goats). Analyses of variance showed no difference between goats.

The milk secreted between 8 and 24 hr of fasting showed a rise in total nitrogen ($P = 0{\cdot}02$), fat, and a fall in K concentration, but on hourly milking there were larger and significant changes in all the components investigated compared with milk secreted overnight (Tables 1, 2). The greatest change was a rise in Na concentration, but there was also a rise in fat, total N, Cl, and citrate and a fall in lactose, K and freezing point.

Analyses of variance for all components showed no significant differences between goats as compared to within goat variation.

In Fig. 2 the quantities of fat and lactose secreted each hour in fasted goats milked hourly are plotted. The hourly lactose yield was significantly

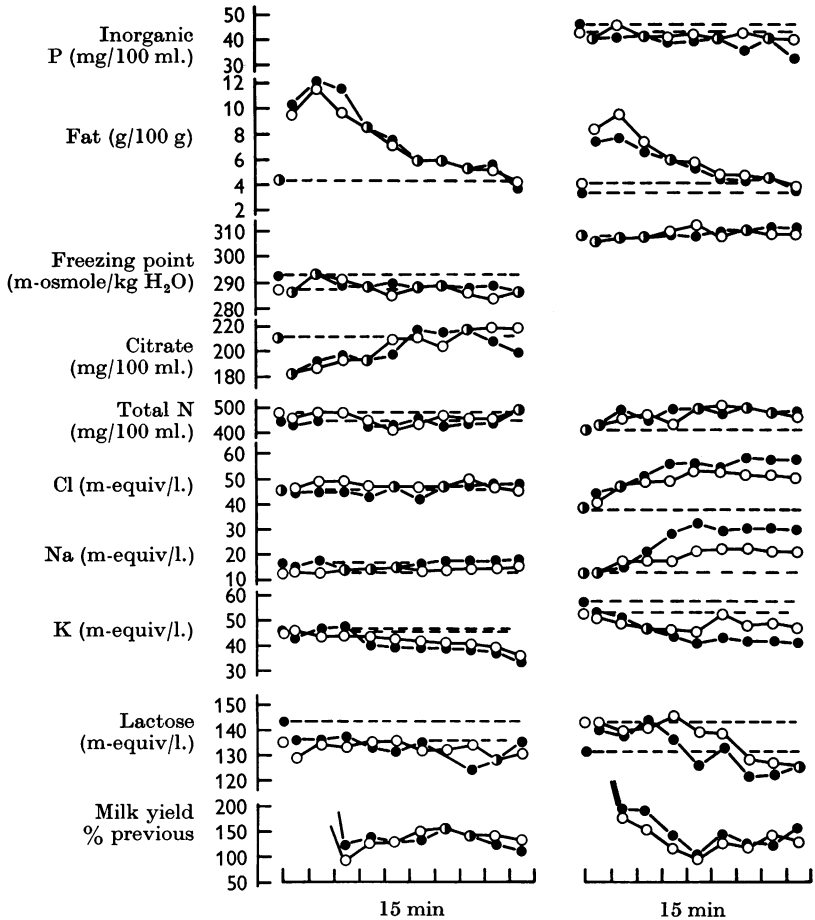

Fig. 3. The yield and composition of milk of two goats milked every 15 min. ● gland milked by hand: ○ gland cannulated. Left, Saanen goat lactating 2 weeks, daily yield 4·74 l. Right, Saanen goat lactating 48 weeks, daily yield 2·25 l. The horizontal line represents the composition of total milk secreted 2 days before the experiment.

lower ($P = 0{\cdot}01$) than the yield of milk and the yield of fat higher ($P = 0{\cdot}05 - 0{\cdot}01$) and consequently the yield of lactose very significantly lower than the yield of fat.

Fasting lactating goats produced significant changes in the blood and plasma concentrations and mammary arteriovenous differences of some

milk precursors (Table 3). The nearly 4-fold rise in plasma free fatty acids (FFA) is in line with the same result in all other species studied, including ruminants, and, in confirmation of the finding of Kronfeld (1965) in the cow, there was a significant net mammary arteriovenous difference only with a raised arterial concentration. The next greatest change was a fall in arterial volatile fatty acid level to 25%, which was accompanied by a

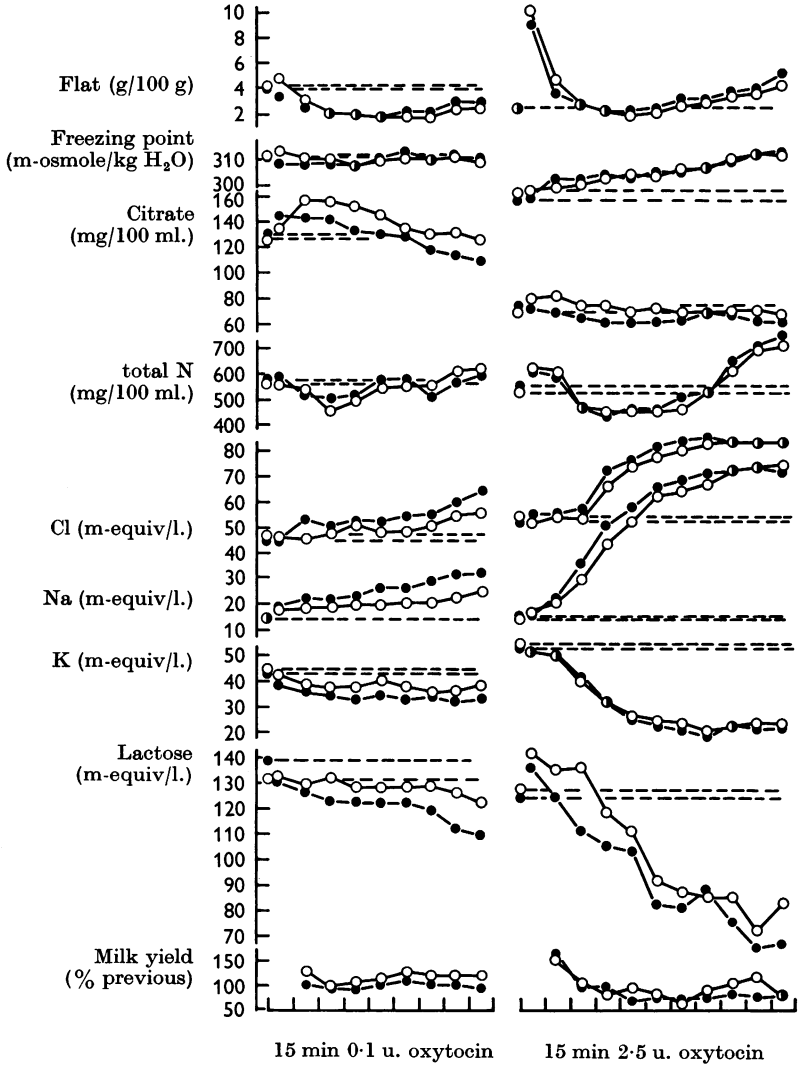

Fig. 4. The yield and composition of milk of a Welsh × Saanen goat, lactating 27 days, daily yield 1·3 l. milked every 15 min 2 days apart. Left, 100 m-u. synthetic oxytocin injected I.V. before each milking. Right, 2500 m-u. oxytocin. ●, Left gland milked by hand. ○, right gland cannulated.

fall in the mammary arteriovenous difference from 68 to 42% of the arterial level. Arterial blood glucose fell to 68% on fasting and the arterio-venous difference from 27 to 23% of the arterial level. Plasma arterial levels of α-amino-N fell to 80% and in two goats the arterio-venous difference fell to zero and 15% of prefasting.

One goat showed no change in mammary blood flow on fasting but five others showed a fall in twelve experiments to 49 ± 2·7 (S.E.M.) %. Therefore it seemed preferable to measure mammary uptake (arterio-venous difference × blood flow). In nine experiments the uptake of glucose fell to 31 ± 4% (S.E.M.) on fasting and of acetate to 10 ± 2% of the prefasting level.

Milking 2–4 times an hour. The results of milking at half-hourly intervals were similar to those of milking hourly but after the work was started Zaks (1964), and Zaks *et al.* (1965) published a theory of milk secretion largely based on the striking finding that when goats were milked every 10–60 min the concentration of milk lactose fell from 124 to 71 m-equiv/l., K from 43 to 16 m-equiv/l. and Na rose from 18 to 90 m-equiv/l. whilst retaining its isosmolality with plasma. To account for this they proposed that milk is secreted in the alveoli with plasma concentrations of Na and K, that, as it lies in the ducts between milkings, there is an exchange of Na for K and lactose and that by milking very frequently one can obtain a solution approaching the primary secretion. Although the changes they reported had been seen in the present work only on hourly milking in fasted animals, their claim was investigated in fed animals milked every 15 min. In most experiments the changes in milk composition were similar to those seen in hourly milking (Fig. 3) and the large changes reported by Zaks (1964) and Zaks *et al.* (1965) were not seen. These were seen only when the milk yield was very low and when a very large dose of oxytocin (2500 m-u. as used by the Russian workers) was employed (Fig. 4). However, in all experiments the changes were greater in the milk from the gland milked by hand as compared with the opposite gland of the same animal, which was cannulated and either emptied at the end of the period of frequent milking or from which small samples were taken for analysis. This suggested that the rise in Na and Cl could be partly due to contamination of the milk already in the teat by tissue fluid expressed from the tissues by compression during manual milking. This idea was supported by the finding that, in three goats with circulating ^{131}I labelled albumin, more protein-bound iodine was found during frequent milking in the milk of the milked gland than in the milk from the cannulated one.

DISCUSSION

The experiments show that milking hourly is a practicable procedure, and that using exogenous oxytocin the quantity of milk, fat and lactose obtained each hour for 10–12 hr is equal to or slightly more than the mean hourly yield calculated from the yield on twice daily milking. Without injecting additional oxytocin the residual milk is an unduly large proportion (50–90 %) of the yield and it is now shown that it is not necessary, and indeed is undesirable, to inject grossly unphysiological doses to remove the residual milk, since the mammary myoepithelium is able to respond fully to small doses of oxytocin at even quarter hourly intervals. This suggests that the yield obtained at the end of each hour is a valid estimate of the mean rate of secretion during the period. An additional point of interest is that expressing milk yields as a percentage of the immediately previous level on twice daily milking removes a major source of variability between goats and between different periods of lactation due to greatly differing milk yields.

Further evidence of the value of frequent milking in studies of milk secretion is seen during a short fast, when there is a concomitant fall in milk secretion and changes in the mammary uptake of milk precursors. This is to be expected because recent studies have shown that, in the lactating goat, the udder itself removes a large proportion of the circulating glucose (Annison & Linzell, 1964), fatty acids (Barry, Bartley, Linzell & Robinson, 1963; Lascelles, Hardwick, Linzell & Mepham, 1964; Annison, Linzell, Fazakerley & Nichols, 1967), and amino acids (Mepham & Linzell, 1966). The fall in milk yield, lactose and blood glucose concentration during fasting has been known for a long time in both goats (Hammond & Hawk, 1917; Taylor & Husband, 1922) and cows (Overman & Wright, 1927; Gowen & Tobey, 1932). It is now shown that, of the synthesized components of milk, the rate of secretion of lactose falls most on fasting and that this fall is similar in magnitude to the fall in mammary glucose uptake and can be rapidly reversed by infusing glucose (Linzell, 1967). By contrast the fall in the rate of fat secretion on fasting is less than that of lactose and this may be explained by the fact that the very large fall in mammary acetate uptake is counterbalanced by a large increase in the uptake of long-chain free fatty acids from the plasma.

The changes in milk composition that may occur on frequent milking are of interest in view of (i) the current speculation as to whether there is active reabsorption of synthesized and ionic components as the milk lies in the gland waiting expulsion towards the teat during milk ejection; (ii) the physiological role of oxytocin and the milking act itself.

The most constant change detected on frequent milking is a fall in

K concentration, but this is often accompanied by a rise in Na and Cl and a fall in lactose. In cows similar changes have been produced by the distension, due to leaving the milk in the udder for long periods (Wheelock, Rook & Dodd, 1965*a*), and by oxytocin (Mackenzie & Lascelles, 1965; Wheelock, Rook & Dodd, 1965*b*), a finding which the present results suggest is due to the magnitude of the doses usually employed. It is also well known that the same changes occur in the milk secreted by diseased glands, and in very low yielding glands in late lactation. Early workers also showed that after 2–3 days of fasting there was a marked fall in lactose concentration and a compensatory rise in Na and Cl (Overman & Wright, 1927; Gowen & Tobey, 1932). The present work shows that the same changes occur earlier in fasting if hourly milking is carried out.

It is probable that a small change in milk composition is produced by exudation of tissue fluid into milk, caused by the act of milking. Evidence for this is, (1) that more labelled plasma protein appears in milk from a gland that is hand milked as compared with the other gland of the same animal drained with a cannula, (2) frequent milking causes a large rise in milk cell count, (3) in some experiments, both the rise in Na and Cl and the fall in lactose and K concentrations could be accounted for by the assumption that 0·5–2·0 ml. of extracellular fluid (assumed to have the composition of goat mammary lymph, Linzell, 1960*b*), had mixed with the milk during removal. However, in most experiments this was not the case and some other explanation must be sought.

Zaks (1964) and Zaks *et al.* (1965) reported a large rise in milk Na and large falls in lactose and K concentrations on frequent milking and concluded that this was characteristic of freshly secreted alveolar milk. However, it is now shown that these changes are chiefly due to the very large doses of oxytocin used by them and most other workers; if their theory is correct then the present experiments suggest that alveolar milk is little different from teat milk and thus that only minor changes in composition occur during storage in the ducts. Further experiments are needed to determine whether the small changes seen on frequent milking in fed goats and the large changes in fasted goats are due to the small doses of oxytocin that have to be used to get all the milk out and if so whether the effect is on the ducts, the alveoli or both.

Note added in proof

Cells in milk. During frequent milking there was usually a rise in the number of epithelial cells, leucocytes and cell fragments in the milk, sometimes to as many as 5×10^6 cells/ml. It is well known that a raised milk cell count occurs during inflammation and this is usually considered diagnostic of microbial mastitis, in which there are also falls in the milk concentrations of lactose and K and rises in Na, Cl and HCO_3. However, there are no reasons for believing that the similar changes encountered in this work were

due to pathological processes. Veterinary examination detected no clinical signs of mastitis, bacteriological examination of the milk revealed no microorganisms and on normal twice daily milking the milk cell counts were within the normal range, usually less than 1×10^5 cells/ml.

Further observations showed that the cell count was also raised in the milk of a gland emptied by cannula at the end of a period of frequent milking the other gland, and that the rise on frequent milking was greater in milked than in cannulated glands, suggesting that contraction of the alveoli in response to oxytocin and teat massage increase the loss of cells into milk. However the concentration of cells in milk is probably influenced by the rate of milk secretion because the rise in cell count was greater in fasted animals milked hourly, where the yield is lowered.

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