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MAMMARY FUNCTION AND ITS CONTROL AT THE CESSATION OF LACTATION IN THE GOAT

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SUMMARY

1. The changes in mammary function following cessation of milking during declining lactation have been studied in conscious goats.

2. No significant changes in the rate of milk secretion, mammary blood flow or metabolism occurred in the first 24 h after cessation of milking. After then, secretory rate, mammary blood flow, oxygen consumption, glucose uptake and acetate uptake decreased markedly over the next 3 days. Up to the time of maximum udder distension on day 3, there were no major changes in milk composition.

3. It was found that the rate of milk secretion declined when the calculated pressure within the alveoli became positive.

4. After 3 days, mammary volume and intramammary pressure decreased, and the composition of milk changed slowly to resemble that of extracellular fluid, i.e. [Na^+], [Cl^-], [HCO_3^-] and pH increased while [K^+], [lactose] and [citrate] decreased. During this time [lactose] and [K^+] were positively correlated, and [lactose] and [Na^+], and [lactose] and [Cl^-] negatively correlated.

5. It is suggested that the changes in milk composition, the decreases in mammary volume and in intramammary pressure after day 3 are due to the loss of integrity of the mammary epithelium.

6. By about 7 weeks after the cessation of milking the udder volume was less than the empty udder volume before milking was stopped, indicating a loss of mammary tissue as well as the resorption of fluid.

7. When milking of an autotransplanted gland was stopped, while milking of the control gland *in situ* was continued, the rate of secretion in the transplant fell while that of the control did not change.

8. In goats milked normally but in which a volume of isosmotic lactose equal to the volume of milk removed at that milking was injected into the lumen of one gland at each milking, the rate of secretion of that gland, but not that of the other, decreased.

INTRODUCTION

When milk removal ceases during lactation, milk accumulates within the gland, the rate of secretion falls and changes in composition supervene (see Turner, 1952). In many animals this is the usual process by which lactation is brought to an end; for example, in dairy cows and goats, milking is usually stopped when the milk yield

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is relatively low, laboratory rats are often weaned when the female is still yielding appreciable quantities of milk (Hanwell, 1972) and in women cessation of suckling is often a sudden process. This type of cessation of lactation has to be contrasted with that occurring during self-weaning, when the young continue to be suckled until the mammary glands are producing only very little milk (see Cowie & Tindal, 1971).

The mechanism by which the rate of secretion is reduced during the accumulation of milk has been little studied. It is often assumed, following the early work of Petersen & Rigor (1932) in the cow, that the pressure of milk in the alveoli inhibits secretion, but this does not normally appear to be the case in the rat. Hanwell (1972) and Hanwell & Linzell (1973 and unpublished) concluded that the decrease in the rate of secretion, which occurs in the rat within 4–8 hr of removal of the young, is hormonally mediated and due to the withdrawal of the suckling stimulus, rather than a direct effect of milk accumulation within the mammary glands. Another possible mechanism, mentioned by Tucker, Reece & Mather (1961), is the accumulation of a chemical inhibitor of milk secretion, and there is evidence that such an inhibitor may in part be involved in regulating the rate of milk secretion (Linzell & Peaker, 1971*a*). Therefore, in the present paper, the time course of physiological changes in mammary function after the cessation of milking in the goat are described and the results of studies on the mechanism by which secretion is arrested are presented.

METHODS

Animals. The experiments were conducted on goats in which a 'milk' (caudal superficial epigastric) vein and a carotid artery were exteriorized in loops of skin (Linzell, 1960, 1963*a*). Some of the animals also had one mammary gland transplanted to the neck with the artery and vein anastomosed to the exteriorized carotid artery and jugular vein respectively (Linzell, 1963*b*). These surgical preparations enabled catheterization to be carried out without further surgery by Seldinger's (1953) technique under local anaesthesia.

Prior to the cessation of milking, goats were milked twice daily at approximately 09.00 and 16.30; the yields of the two glands and the time of milking were recorded, and yield expressed in ml. hr^{-1}.

Mammary blood flow and arterio-venous samples. Mammary blood flow was determined by Linzell's (1966*a*) thermodilution technique in the exteriorized milk vein (see also Linzell, 1974). Carotid arterial and mammary venous blood samples were taken at the same time; coagulation was prevented by the addition of a small drop of heparin solution (5000 u./ml.). Blood flow was measured, and venous samples taken, while the animals were standing and while the external pudic vein at the base of the udder was being compressed manually; this was done to obtain true mammary venous blood (Linzell, 1960). Mammary uptake or output of substances was calculated as the product of blood flow and arteriovenous (A – V) difference in blood concentration, or as plasma flow (blood flow corrected by packed cell volume) and A – V difference in plasma concentration.

Milk samples. Milk samples were taken at the time of milking; if HCO_3^- was to be determined they were taken anaerobically (Linzell & Peaker, 1975). After the cessation of milking, 5–10 ml. samples were taken aseptically from the teat; the teat was then sealed with pliable plastic film (Nobecutane spray, B.D.H.)

Measurement of udder volume. Udder volumes were determined by displacement of water (Linzell, 1966*b*), in some cases following the injection of 100 m-u. oxytocin I.V. and removal of the residual milk (i.e. empty udder volume).

The volume of accumulated milk was calculated from udder volume in one of two ways. When on the first occasion the empty udder volume was determined (V_{e1}) and on the second occasion the udder contained milk (V_2), an approximate correction for retained milk was applied if the

animal had been milked after the initial measurement was made. A volume equal to 10% of the milk yield on the last occasion the goat was milked (R) was added to the initial value; the second value was then subtracted, i.e. volume of milk accumulated = $V_{e1}+R-V_2$. When for both measurements milk was present in the glands, the calculation was simply V_1-V_2. Obviously it is necessary to assume in these calculations that the changes in udder volume reflect changes in the volume of accumulated secretion and not changes in tissue volume. Preliminary experiments indicated that over several days the calculated volume was within 10% of that actually recovered by milking.

Measurement of intramammary (milk) pressure. A sterile catheter was inserted via the teat canal, allowed to fill with milk and connected to a pressure transducer. Initially the transducer was held level with the tip of the teat to record the pressure at that point. The pressure measured at the tip of the teat comprises, when the gland contains sufficient milk, the hydrostatic pressure

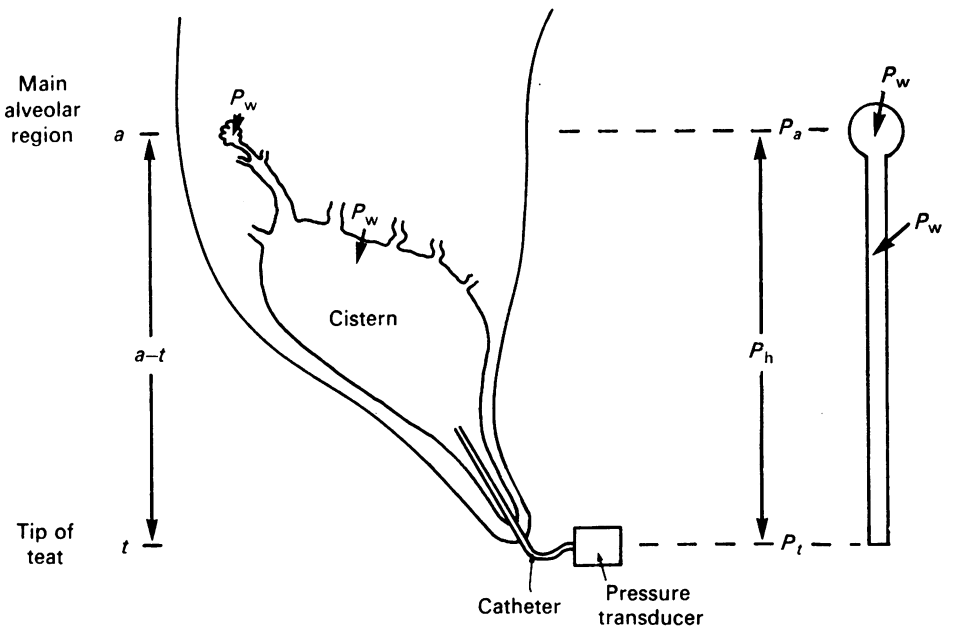

Fig. 1. Diagram showing the basis of intramammary pressure measurements (see text for details). On the left a diagram of a goat mammary gland is shown, and on the right a simple hydraulic analogy.

of a column of milk in the gland (P_h) plus the pressure exerted by the walls of the gland, which we are calling wall pressure (P_w). Therefore the recorded pressure at the tip of the teat (P_t) is the sum of P_h and P_w. When the gland contains sufficient milk, P_h is the pressure exerted by a column of milk between the main alveolar region (a) and the tip of the teat (t) (Fig. 1). In glands containing relatively little milk, we have found that $P_t < P_h$, probably because there is not a continuous column of milk and the ducts are collapsed (see also Schmidt, 1971). Therefore intramammary pressure cannot be assessed until the gland contains sufficient milk, i.e. $P_t \geqslant P_h$. Although it is not possible to determine intra-alveolar pressure directly, it was assumed that when $P_t > P_h$, milk in the alveoli was in continuity with that in the ducts. Therefore, intra-alveolar pressure (P_a) was calculated as follows:

$$P_h = (a-t)/d, \quad (1)$$

$$P_a = P_t - P_h \quad (2)$$

where P_a = intra-alveolar pressure (mmHg), P_t = pressure at tip of teat (mmHg), P_h = hydrostatic pressure (mmHg), $a-t$ = vertical distance between main alveolar region of gland and tip of teat (mm), d = sp.gr. of milk (1·034, Macy, Kelly & Sloan, 1953) × sp.gr. of Hg (13·55).

In some experiments a long catheter was passed into the teat and the transducer held at the level of the alveolar region, i.e. P_h was subtracted as part of the measurement; virtually identical values for P_a were obtained by the two methods.

It should be appreciated that the determination of P_a is an approximation of the probable mean value since it is a matter of judgment to choose the point a. However, the same point of reference was used on different occasions in the same animal, although the distance $a-t$ varied when different amounts of milk were present in the gland.

Analytical methods

Milk. The methods used to determine pH and HCO_3^- were as described by Linzell & Peaker (1975). Samples were analysed for fat (Fleet & Linzell, 1964), protein (Udy, 1956, using Orange G dye in a Technicon autoanalyser), citrate (White & Davies, 1963), phosphate (Technicon method N-4c for dialysable phosphate), lactose, Na^+, K^+, Cl^- (Fleet, Linzell & Peaker, 1972) and for total Ca and total Mg (atomic absorption spectrophotometry).

Blood and plasma. Blood oxygen and carbon dioxide contents, and packed cell volumes, were determined as described by Linzell & Peaker (1975). Plasma samples were analysed for glucose (glucose oxidase-perid method, Boehringer Mannheim, in a Technicon autoanalyser), acetate (Lindsay & Setchell, 1976), lactate (Boehringer Mannheim kit) and Ca (atomic absorption spectrophotometry).

RESULTS

Mammary function after cessation of milking

The time course of changes in mammary function following the cessation of milking during declining lactation was studied in four goats. At the start of the experiment, the animals were in their second to fourth lactation, had been in lactation for more than 7 months, and were yielding, in their last week, 1330–1990 ml. milk/day; they were in early pregnancy (3–10 weeks). The changes, and their time courses appeared similar in the four goats, and the data were bulked. Because the size of the mammary glands was variable, the results are expressed, for the most part, as percentages of the initial value or change from initial value in Figs. 2 and 3. Actual initial values are given in Table 1.

Milk secretion and mammary metabolism. The rate of milk secretion after cessation of milking was calculated from the changes in udder volume as described in the Methods. By day 1 after cessation of milking there had been no appreciable change in the rate of secretion from that obtaining previously, i.e. the glands at this time contained a volume of milk equal to that of the daily milk yield. However, the rate of secretion then declined markedly, to a mean of 43% of the previous yield during day 2, and to 22% during day 3.

The decrease in the rate of secretion after day 1 was matched by decreases in the mammary uptake of oxygen, glucose and acetate, and in mammary blood flow, over the next 3 days; there were no significant further changes after day 4 (Fig. 2). The relative decreases in glucose and acetate uptakes were greater than the decrease in blood flow, whereas the relative decrease in oxygen consumption was similar to that of blood flow (Figs. 2 and 4); for example, on day 3, mean glucose uptake was 26%, acetate uptake 36%, oxygen consumption 41% and blood flow 42% of the values

Fig. 2. Changes in the rate of milk secretion, mammary blood flow and metabolism following cessation of milking in four goats (mean ± S.E. of mean). The rate of milk secretion is expressed as % of the mean milk yield over the 7 days prior to stopping milking, and the uptakes as % of the values on day 0.

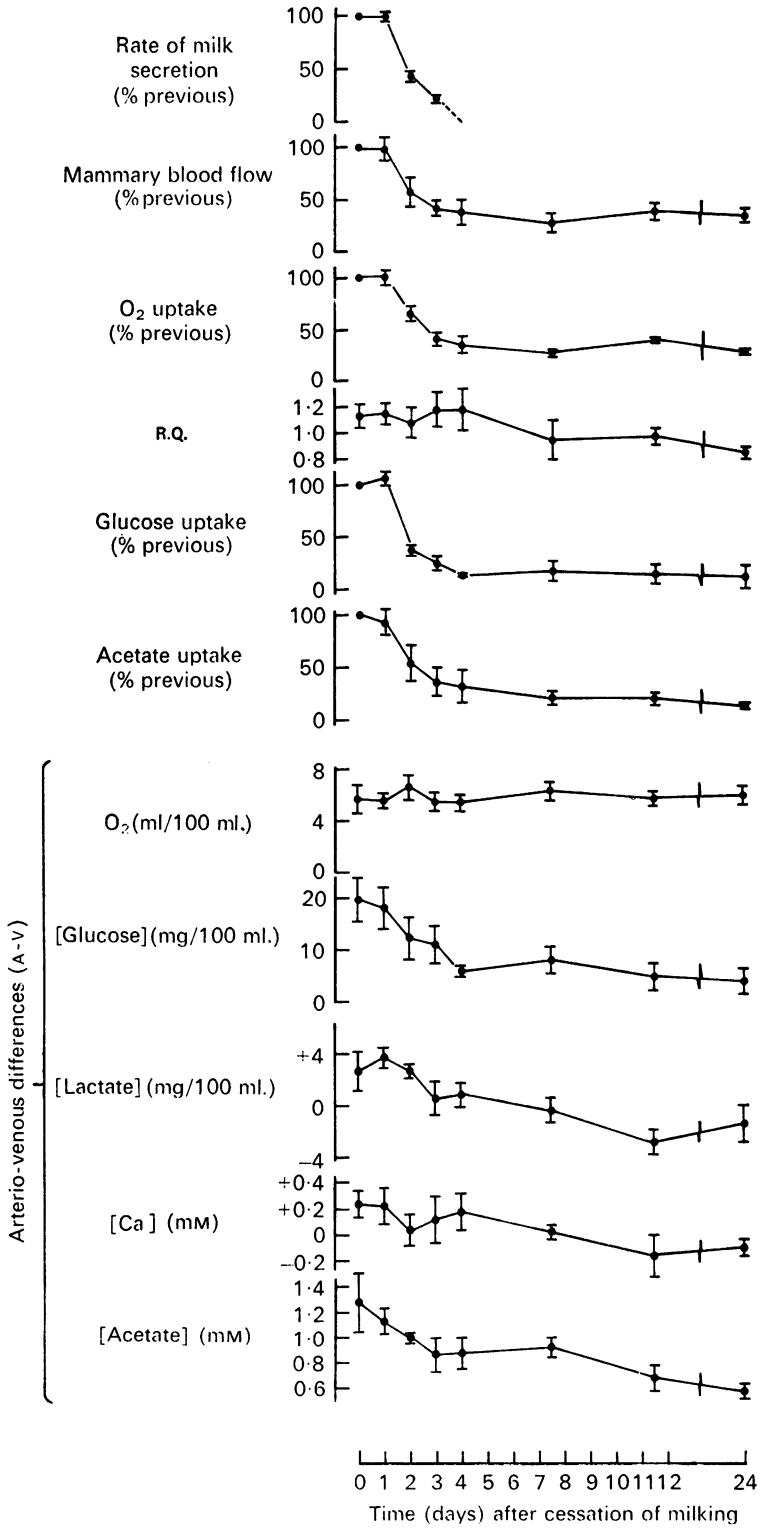

Fig. 2. For legend see facing page.

obtained before cessation of milking. These differences are due to a fall in the A – V difference for glucose and acetate but not for oxygen (Fig. 2). In other words, the lower uptakes of glucose and acetate after day 1 can be attributed to decreases both

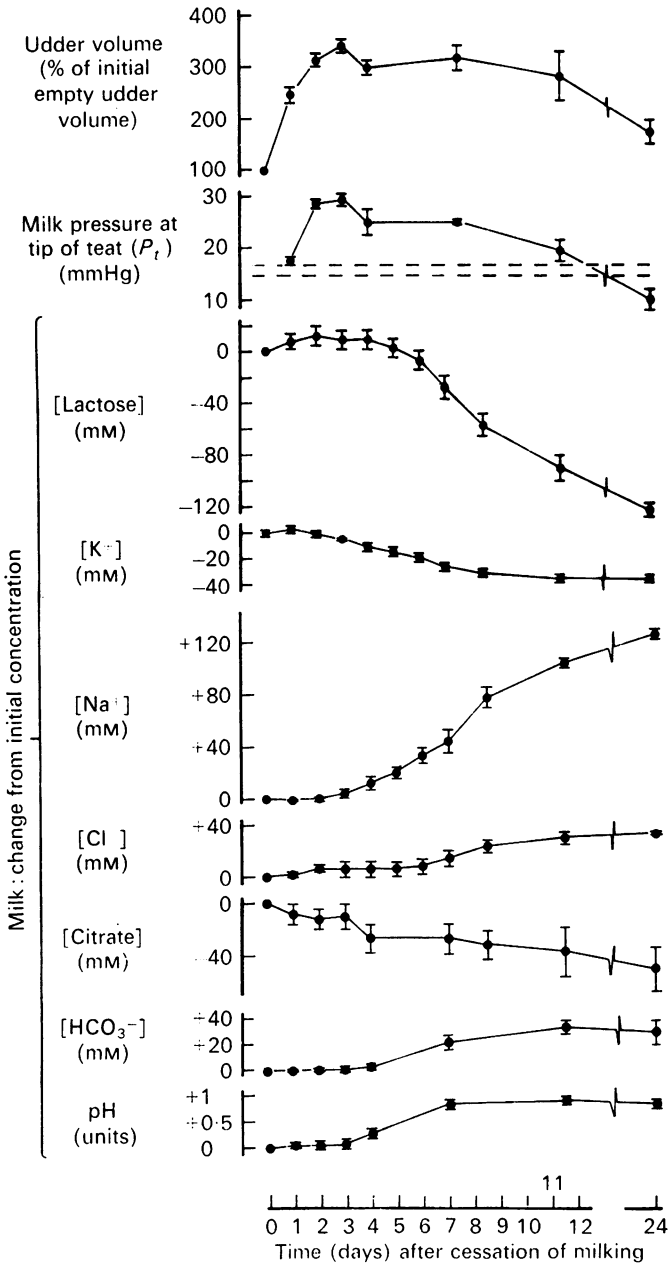

Fig. 3. Changes in udder volume, intramammary pressure and milk composition following cessation of milking in four goats (mean ± S.E. of mean). The dashed lines on the pressure panel indicate the range of the contribution of the hydrostatic pressure (P_h) to the pressure recorded at the tip of the teat (P_t).

in mammary blood flow and in A – V difference, while the fall in oxygen consumption was related to the decrease in blood flow alone.

It is evident from Fig. 4 that there was a close similarity between the relative decrease in the rate of milk secretion and the relative decrease in glucose uptake. In Fig. 5 individual values for glucose uptake (y) are plotted against milk yield over the preceding 24 hr (x), and it can be seen that the calculated regression line ($y = 1{\cdot}1x - 3{\cdot}65$) falls very close to the 45° line (i.e. $y = 1x$). It can be seen that the data for day 4,

TABLE 1. Initial determinations of mammary function made before cessation of milking in four goats (see text for details)

	Mean ± S.E.
Both glands	
Udder volume (empty) (l.)	1·44 ± 0·12
Milk yield* (ml./day)	1759 ± 148
Milk yield (ml./day. ml. tissue)	1·24 ± 0·12
Gland studied†	
Blood flow (ml./min)	309 ± 95
O_2 uptake (ml./min)	15·2 ± 2·9
R.Q.	1·13 ± 0·18
Glucose uptake (mg/min)	34·7 ± 7·9
Acetate uptake (mg/min)	19·6 ± 9·5
A–V differences	
O_2 (ml./100 ml.)	5·7 ± 1·1
Glucose (mg/100 ml.)	19·8 ± 4·8
Lactate (mg/100 ml.)	2·7 ± 1·5
Ca (m-mole/l.)	0·24 ± 0·10
Acetate (mg/100 ml.)	7·7 ± 1·4
Milk	
Yield* (ml./day)	904 ± 100
Lactose (mM)	124 ± 3·1
K^+ (mM)	43·5 ± 2·8
Na^+ (mM)	16·6 ± 1·4
Cl^- (mM)	51·9 ± 2·8
Citrate (mg/100 ml.)	73·3 ± 15·0
Phosphate (inorganic dialysable) (mg P/100 ml.)	70·0 ± 9·7
Ca (mM)	31·2 ± 0·9
Mg (mM)	7·2 ± 0·9
HCO_3^- (mM)	3·5 ± 0·2
pH	6·48 ± 0·02
Protein (g/100 ml.)	4·08 ± 0·23
Fat (g/100 ml.)	4·56 ± 1·24

* Average over previous week.
† One gland in each animal was prepared with a milk vein loop.

when fluid resorption had begun and secretory rate was taken as zero, fall above the calculated regression line for days 1–3. This suggests that there was a basal uptake of glucose in the absence of secretion and/or that secretion was continuing at a low rate as fluid was absorbed. Also superimposed on Fig. 5 are the calculated regression lines between milk yield and oxygen consumption, and between milk yield and blood

flow; clearly the fit of these lines to the 45° line is less good than that between milk yield and glucose uptake.

The A – V differences for lactate, although variable, indicated that lactate was taken up by the glands before the cessation of milking, as found previously (see Linzell, 1974). After cessation, however, in some goats, the mammary venous concentration was higher than arterial, and the glands were producing lactate. The A – V differences for Ca were also variable but the tendency was for uptake to fall (Fig. 2).

Milk composition. In contrast to the marked changes in the rate of milk secretion and in mammary metabolism between days 1 and 2 following cessation of milking, changes in milk composition were not apparent until day 3 or 4, and then only as

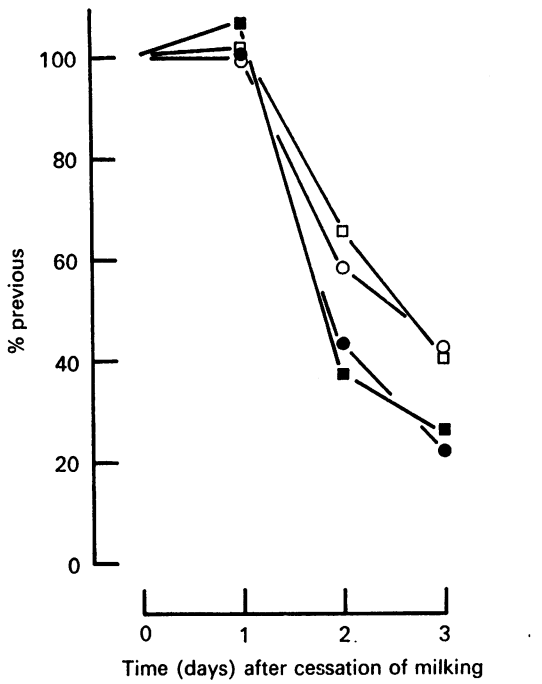

Fig. 4. Changes in mammary blood flow (○), oxygen uptake (□), glucose uptake (■) and milk yield (●) after cessation of milking in four goats. Mean data from Fig. 2.

a slight rise in [Na^+] and a fall in [K^+]. After the [Na^+] continued to increase, and [K^+] to decrease, while [Cl^-], [HCO_3^-], pH and [protein] also increased, and [lactose] and [citrate] decreased (Fig. 3); there was little change in [Mg] while [Ca] tended to increase and [dialysable phosphate] to decrease. The concentrations of fat are not given since the values obtained from small samples after cessation of milking are not necessarily representative of the fat concentration throughout the milk in the gland.

The changes from the initial concentrations of Na^+, K^+ and Cl^- after day 4 are plotted against those of lactose in Fig. 6. There were significant negative correlations between [lactose] and [Na^+] and between [lactose] and [Cl^-], and a significant positive correlation between [lactose] and [K^+].

Udder volume and intramammary pressure. As can be inferred from the calculated

rates of secretion, the mean udder volume reached a maximum on day 3; after that time it declined. The start of the decline coincided with the changes in milk composition (Fig. 3).

Intramammary pressure also reached a maximum on day 3, reaching a mean of 29 mmHg when recorded at the tip of the teat. In Fig. 3 dashed lines indicate the contribution of the hydrostatic pressure (P_h) to that recorded at the tip of the teat (P_t). From day 1 to day 11–12 it can be seen that $P_t > P_h$, and, therefore, that the wall pressure (P_w) was positive.

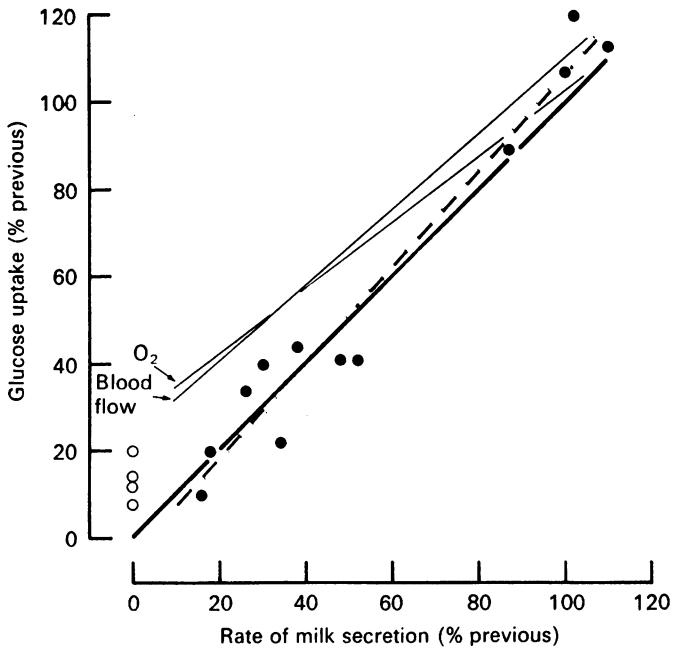

Fig. 5. Relation between rate of milk secretion and mammary glucose uptake 1–3 days after cessation of milking in four goats (filled circles). The calculated regression line (dashed) is shown, and the 45° line (thick, solid). The open circles show values obtained on day 4 when the rate of secretion was taken to be zero (see text); these values were not included in the regression analysis. Also shown as labelled thin solid lines are the calculated regression lines between the rate of milk secretion and oxygen uptake, and the rate of milk secretion and mammary blood flow. Details of the relationships, where glucose uptake is y_1; oxygen uptake, y_2; blood flow, y_3; and rate of secretion, x; in all cases as % of value obtaining before cessation of milking:

$$y_1 = \underset{\pm 0{\cdot}07}{1{\cdot}10x} \underset{\pm 4{\cdot}91}{-3{\cdot}65}, \quad r = 0{\cdot}977, \quad P < 0{\cdot}001$$

$$y_2 = \underset{\pm 5{\cdot}03}{27{\cdot}14} + \underset{\pm 0{\cdot}08}{0{\cdot}76x}, \quad r = 0{\cdot}951, \quad P < 0{\cdot}001$$

$$y_3 = \underset{\pm 9{\cdot}62}{21{\cdot}45} + \underset{\pm 0{\cdot}16}{0{\cdot}88x}, \quad r = 0{\cdot}876, \quad P < 0{\cdot}001$$

In Fig. 7 the calculated intra-alveolar pressure (P_a) is plotted with the calculated rate of milk secretion during the previous 24 hr. The fall in secretory rate clearly coincides with the appearance of a positive P_a.

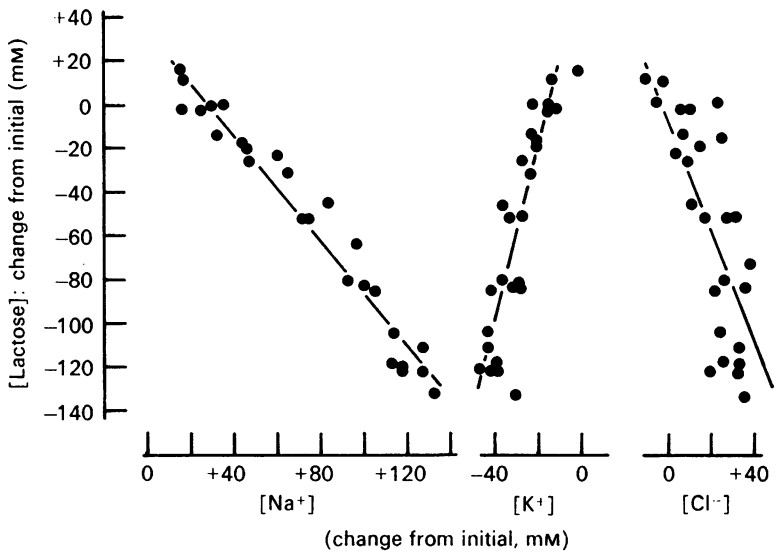

Fig. 6. Relationships between changes in milk [lactose], [Na^+], [K^+] and [Cl^-] from days 5 to 24 inclusive after cessation of milking in four goats; the calculated regression lines are shown. [Na^+]: $r = -0{\cdot}982$, $P < 0{\cdot}001$, [K^+]: $r = +0{\cdot}833$, $P < 0{\cdot}001$, [Cl^-]: $r = -0{\cdot}754$, $P < 0{\cdot}001$).

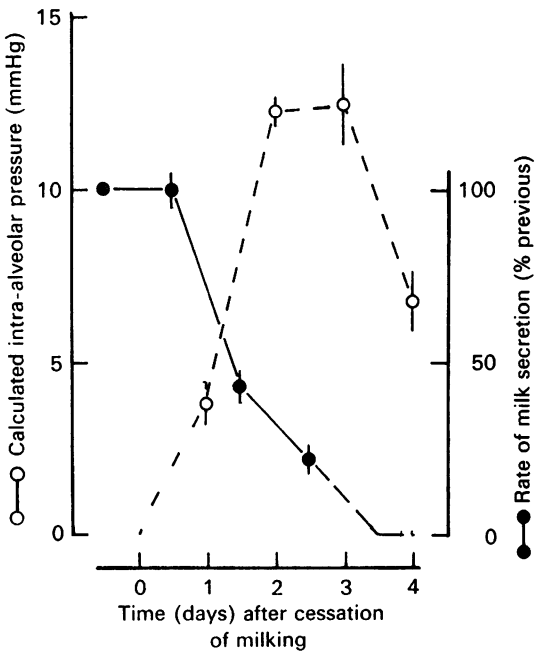

Fig. 7. Calculated intra-alveolar pressure (P_a) and rate of milk secretion after cessation of milking in four goats (mean ± S.E. of mean). The rates of secretion are shown in the middle of the 24 hr period over which they were determined.

Long-term changes in udder volume

In another five goats, details of which are given in Table 1 of Fleet, Goode, Hamon, Laurie, Linzell & Peaker (1975), changes in udder volume were determined for 80 days after cessation of milking (Fig. 8). The initial distension was similar to that in the experiments described above. There was then a fall in udder volume and by about 4 weeks the glands had returned to the size of the empty glands before milking was stopped; palpation of the glands confirmed that most of the retained fluid had been re-absorbed. After this time udder volume decreased still further and there appeared to be little fluid in the glands; indeed in two of the goats no fluid could be obtained from the teats. Thus it is clear that the volume of mammary tissue in this 'dry period' was less than during lactation.

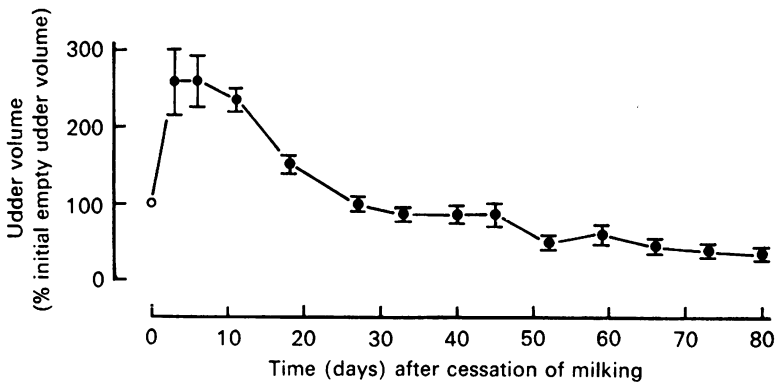

Fig. 8. Changes in udder volume after cessation of milking in five goats (mean ± S.E. of mean). Except before cessation, when empty udder volume was determined (open circle), fluid was not removed from the glands.

Factors responsible for the decrease in milk secretion after cessation of milking

The decrease in the rate of milk secretion might be attributed to either one or a combination of three factors, namely (i) the accumulation of milk causing distension of the gland and a rise in intramammary pressure, (ii) the accumulation of chemical factors in milk which may inhibit secretion (see Linzell & Peaker, 1971*a*), and (iii) the lack of hormones normally released by the milking stimulus. The following experiments were designed to distinguish between these possibilities.

Unilateral cessation of milking of autotransplanted glands. In goats with one gland autotransplanted to the neck, and therefore denervated (Linzell, 1963*b*), the normal hormonal response to suckling is maintained by the gland remaining *in situ*. Milking the transplant does not lead to milk ejection (Linzell, 1963*b*) or prolactin release (Hart & Linzell, 1977) although the response of growth hormone is variable (Hart & Linzell, 1977). Thus, if milking the transplant, but not the control gland *in situ*, is stopped, the normal hormonal milieu should be maintained. Therefore, if the rate of secretion of the transplant falls relative to that of the control gland *in situ*, local rather than systemic factors are clearly responsible.

In four goats, milking of the transplanted gland was stopped during declining lactation, approximately 8 months after parturition; twice-daily milking of the

control gland was continued. The rate of milk secretion of the transplants, calculated from the rise in udder volume, fell with a similar time course to that of the normal animals described above, while the milk yield of the controls was apparently unaffected (Fig. 9). The fact that the yield of the control glands remained normal indicates that the hormonal events in the animals were not appreciably affected by stopping milking the transplant. Thus it appears that withdrawal of the milking stimulus acting via hormonal factors is unlikely to be the mechanism by which the rate of milk secretion is reduced after cessation of milking. Similar changes in milk composition to those described above supervened in the transplants but not in the control glands.

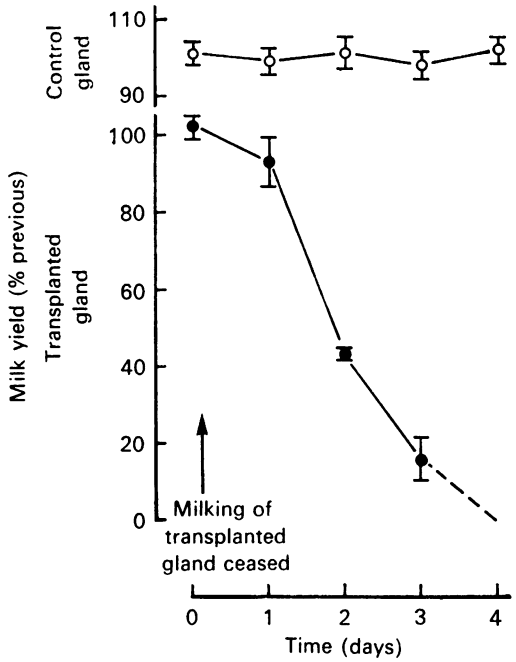

Fig. 9. Effect of stopping milking the autotransplanted glands of four goats on the rate of milk secretion; the control gland *in situ* was milked normally throughout (mean ± S.E. of mean).

Effects of replacement of accumulated milk by isosmotic lactose. In four goats in established lactation, twice-daily milking of both glands was continued normally. However, in one gland of each animal a volume of sterile, isosmotic (300 mM) lactose, equal in volume to the volume obtained from the gland at that milking, was injected back into the lumen of the gland via the teat canal. In other words, the milk which normally would have accumulated after cessation of milking was replaced by lactose solution, and normal milking was continued. This procedure was repeated at each milking until the volume obtained was less than at the previous milking (i.e. the point of maximum distension had been passed). In all four goats, the rate of secretion of the gland so treated, calculated as the difference between the amounts of fluid removed (and replaced as fresh lactose solution) at one milking and the next, fell to zero within 1–2 days, while that of the untreated gland remained apparently unchanged (Fig. 10). Therefore, since the normal milking stimulus was applied and

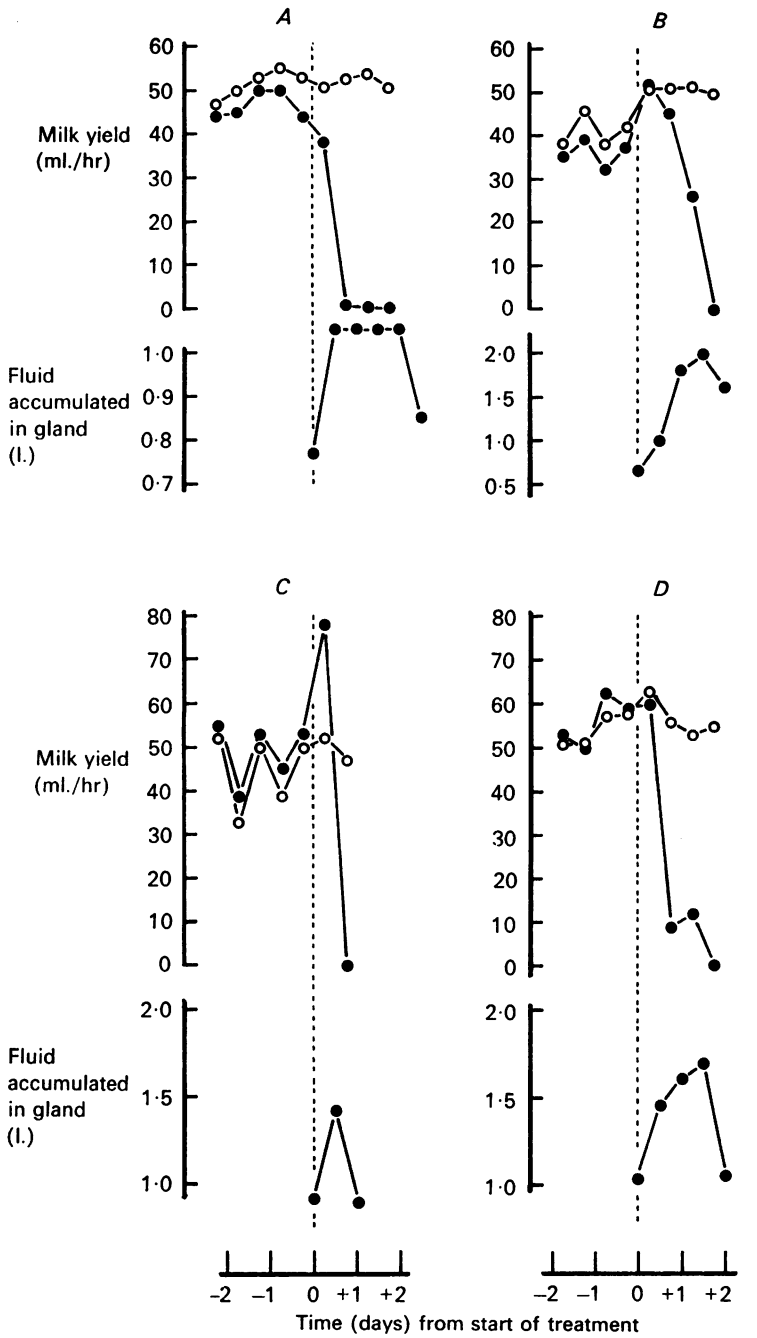

Fig. 10. Effect of replacing accumulated milk by lactose solution in four goats (*A–D*). Both glands were milked twice daily but starting on day 0, a volume of 300 mM-lactose, equal to the volume of milk removed at each milking, was injected back into the gland through the teat canal (see text for details). Therefore, fluid but not milk accumulated in the gland. In the upper panel for each animal are shown the calculated rates of secretion for the treated gland (filled circles) and the untreated gland (open circles). In the lower panel the volume of fluid accumulated in the gland is shown.

since milk was not allowed to accumulate for more than the normal interval between milkings, it can be concluded that mammary distension mimicked by lactose replacement of equal volume was responsible for the arrest of milk secretion in this experiment, and that withdrawal of the milking stimulus or the accumulation of chemical factors in milk are not the major factors in the decrease in the rate of secretion following cessation of milking.

That lactose itself does not act as a feed-back inhibitor of milk secretion seems fairly certain. It does not normally permeate the apical membrane of the cells (see Linzell & Peaker, 1971*b*; Peaker, 1978), and when infused close-arterially does not affect the rate of secretion (the late J. L. Linzell & M. Peaker, unpublished observations). Moreover, there is evidence that the proposed substance(s) in milk which can influence secretory rate (Linzell & Peaker, 1971*a*) is lipid-soluble (Sala, Cannata, Luther, Arballo & Tramezzani, 1973). Furthermore, similar experiments to those described above have been done using sucrose solution, with identical results.

It should be noted that in two of the glands, the rate of secretion increased initially after the start of treatment. Because there is evidence that 300 mM-lactose or sucrose does not induce osmotic water movements into milk (Linzell, Mepham & Peaker, 1976; Peaker 1977, and unpublished observations), it seems possible that this transient stimulatory effect could have been mediated by dilution of a feed-back inhibitor normally present in milk with the large volumes of lactose solution given.

DISCUSSION

Changes in mammary function after cessation of milking

Following the cessation of milking in declining lactation, two phases of mammary activity can be distinguished: *distension*, up to the point of maximum udder distension and intramammary pressure, and *involution* thereafter. In this particular experiment, maximum distension of the glands occurred three days after cessation of milking, although it might be expected to vary according to the milk yield at the time.

During the distension phase intramammary pressure increased while after day 1 the rate of milk secretion decreased markedly. The mammary uptake of substances required for the formation of milk components and for oxidative metabolism (glucose, acetate, oxygen) also decreased, as did mammary blood flow. Therefore, the rate of synthesis of milk constituents, as well as the rate of secretion, fell. The decrease in glucose uptake was closely correlated with the decline in the rate of secretion. This is as would be expected since glucose is required for the secretion of lactose and water, and water accounts for approximately 90% of milk (see Linzell & Peaker, 1971*b*; Peaker, 1978). However, the decrease in glucose uptake was brought about by a fall in the A – V difference across the gland as well as by a reduction in blood flow. Since, in lactation, the reduction of blood flow by, say, adrenaline, is accompanied by an increase in the A – V difference for glucose (see Linzell, 1974), the fact that after cessation of milking both blood flow and the A – V difference decreased, suggests that the 'affinity' of the secretory cells for glucose fell; a similar argument can be applied to acetate.

The mechanism by which mammary blood flow is controlled, or reduced in the present series, is not known. Two possibilities must be considered to account for the present findings: (i) a direct effect of raised intramammary pressure on capillary flow and (ii) a secondary effect of a reduction in activity by the gland. We have preliminary unpublished findings which suggest that at the pressures reached after cessation of milking mammary blood flow is not affected directly. In actation there are strong indications that mammary blood flow is controlled by vasodilator

substances produced by the active gland, and milk yield and blood flow are positively correlated (see Linzell, 1974). A similar mechanism could apply after cessation of milking, but in this case the primary effect would be on activity of the secretory cells, with blood flow falling as activity decreased. If that is so, then the closer correlation between oxygen uptake and blood flow than between glucose uptake (and milk yield) and blood flow might suggest that blood flow is more closely related to metabolic (or oxidative) activity than to secretory activity in these circumstances.

Except on day 3, when there were very slight changes, milk composition remained essentially unchanged during the distension phase. After the time of maximum distension, during the involution phase, there was a slow resorption of fluid, and the fluid in the glands came to resemble extracellular fluid rather than milk; as the long-term study of udder volume showed, there was also a loss of tissue during this phase, as is well documented (Cowie & Tindal, 1971).

The changes in milk composition that occur following cessation of milking are well known, and are the reverse of those that occur in late pregnancy and the onset of lactation (Linzell & Peaker, 1974; Fleet *et al.* 1975). In non-lactating pregnant goats it was argued that paracellular movements of ions and small molecules occur through 'leaky' tight junctions but that at about the time of parturition the junctions become truly 'tight' and milk of normal composition is then secreted during lactation. Permeability and electrophysiological studies have indicated that in non-lactating glands the mammary secretion is at least in partial equilibrium with plasma (Linzell & Peaker, 1974; Peaker, 1977, 1978), and the rise in milk [Na^+], [Cl^-], [HCO_3^-], and the fall in [K^+] and [lactose] in the present experiments during involution indicates a gradual loss of integrity of the epithelium. In other situations where the epithelium is believed to be leaky, a positive correlation between milk [lactose] and [K^+] has been found, in contrast to the negative correlation during lactation (Linzell & Peaker, 1971*a*, 1974; Peaker, 1978). The positive correlation evident in the present experiments also suggests that the epithelium becomes leaky with K^+ and lactose moving out of milk down their concentration gradients, and Na^+ and Cl^- passing in the reverse direction.

The loss in intramammary pressure at the time fluid begins to be reabsorbed and changes in milk composition supervene also indicates that the epithelium loses its integrity and cannot maintain the raised pressure. However, whether the raised pressure literally bursts the epithelium or whether loss of integrity is a more subtle process following loss of cellular activity is not known and is being investigated.

Factors responsible for the arrest of secretion

The experiments in which milking of autotransplanted glands was stopped and those in which glands were allowed to accumulate lactose solution rather than milk while milking was continued, clearly show that it is mammary distension, rather than the withdrawal of the milking stimulus or the accumulation of chemical feed-back inhibitors in milk, which is responsible for the arrest of secretion after the cessation of milking in goats.

For many years a rise in intramammary pressure has been thought to be a major factor in arresting milk secretion. Petersen & Rigor (1932) showed in cows that intramammary pressure increased to a maximum of 39 mmHg at 5 days after cessation of milking and then fell; by blowing air into a gland they deduced that the

rate of secretion was proportional to pressure and ceased at 25 mmHg. However, previous experiments on milk pressure are difficult to interpret because the contribution made by hydrostatic pressure was not stated and the point of reference (for example, tip of teat or elsewhere) often not given. These are particularly important in the cow with a long column of milk between the teat and the main alveolar region of the gland.

Although intramammary pressure appears to be the major factor in the goat, withdrawal of the suckling stimulus is clearly more important in the rat. Following separation from the young, the rate of milk accumulation in the glands decreases. However, when the young are not removed but removal of milk is prevented by sealing the teats with adhesive, the rate of milk secretion remains high (Hanwell, 1972; Hanwell & Linzell, 1973; see also Selye, 1934). Eventually of course the rate of secretion falls in the rat with sealed teats but clearly the rise in milk pressure is of secondary importance to the hormonally mediated withdrawal of suckling at sudden weaning. The rise in pressure might be responsible for the involution of unsuckled glands of suckling rats. The mechanism in other species is unknown but the difference between rats and goats may be related to the frequency of suckling, with mammary activity being dependent upon the very frequent release of lactogenic hormones in the rat. Clearly the investigation of species like the rabbit and tree-shrew which lack a capacious duct system for milk storage but which only suckle every 24 or 48 hr respectively (Zarrow, Denenberg & Anderson, 1965; D'Sousa & Martin, 1974) would be of interest.

Our studies indicate that the rate of milk secretion is reduced when the wall pressure of the alveoli becomes positive, i.e. the alveoli are distended and the pressure is due to the elasticity of the secretory epithelium and supporting tissue. The mechanism by which pressure acts to reduce the rate of secretion is now being investigated.

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