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MECHANISM OF MILK SECRETION: MILK COMPOSITION IN RELATION TO POTENTIAL DIFFERENCE ACROSS THE MAMMARY EPITHELIUM

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SUMMARY

1. In conscious lactating goats a significant correlation was found between blood–milk potential difference (p.d.) and milk [lactose] such that in goats with a lower milk [lactose], milk was more negative with respect to blood.

2. When mannose was substituted for glucose in the substrate mixture of isolated perfused goat mammary glands, milk yield and milk [lactose] fell while milk [Na] and [K] increased; in parallel experiments the blood–milk p.d. changed such that milk became more negative with respect to blood. These changes were reversed following the addition of glucose.

3. When milk was made hypertonic by the addition of hyperosmotic sucrose or lactose solutions, water entered milk osmotically and milk became electrically less negative or even positive with respect to blood in goats, cows and guinea-pigs.

4. No effect on p.d. was apparent following the addition of isosmotic sucrose to milk in goats.

5. When milk was held in the teat of goats by a pneumatic cuff around the base of the teat, no effect on p.d. was apparent when hyperosmotic sucrose was introduced into this teat pouch.

6. It is suggested that waterflow-induced potentials (the streaming potential and the transport number effect) can be induced across the mammary epithelium.

7. In goats exogenous oxytocin lowered milk [lactose] and blood–milk p.d. became less negative with respect to blood.

8. In non-lactating and mastitic glands of goats the blood–milk p.d. was within 0·5–2·5 mV of zero.

9. The effects of oxytocin, and the low p.d. in non-lactating and mastitic glands, are compatible with the view that in such circumstances there is a paracellular pathway across the mammary epithelium which partially short-circuits the two sides.

10. It is suggested that, with water being drawn osmotically into milk to dilute newly formed lactose, waterflow-induced potentials may be responsible for establishing the normal p.d. across the apical membrane of the secretary cell, thereby keeping milk [K] and [Na] lower than in intracellular fluid.

INTRODUCTION

The main constituents of the aqueous phase of milk, which is virtually isosmotic to plasma, are lactose, K, Na and Cl. From studies on the electrochemical gradients between milk, intracellular fluid (ICF) and extracellular fluid (ECF), Linzell & Peaker (1971*a*, *b*) proposed a scheme to account for monovalent ion movements across the mammary secretory epithelium in the guinea-pig and goat in full lactation. It was suggested that Na^+ and K^+ are distributed passively across the apical (luminal) membrane of the secretory cell according to the electrical potential difference (p.d.) between ICF and milk. Therefore with milk being electrically positive with respect to the inside of the cell, the concentrations of these ions are lower in milk but the ratio between them is similar at about $3K^+:1Na^+$. This ratio in ICF and milk is believed to be maintained by a typical $Na^+ - K^+$-exchanging pump on the baso-lateral membrane (Fig. 1). For Cl the situation is different and by no means fully understood (Linzell & Peaker, 1975*a*; Peaker, 1977).

It is generally considered that lactose is formed within the Golgi apparatus of the secretory cell and carried to the lumen of the alveolus, along with protein, in secretory vesicles which discharge their contents by exocytosis. Since the secretory vesicle membrane probably then becomes part of the apical membrane (see Keenan, Morré & Huang, 1974) it can be inferred that similar movements of ions occur across the membrane of the secretory vesicle. Moreover, lactose appears to draw water osmotically from the inside of the cell and it is this osmotic passage of water to dilute the concentrated lactose being formed in the Golgi apparatus which is believed to be the main mechanism for water secretion in the mammary gland (see Linzell & Peaker, 1971*a*) (Fig. 1).

The relationships between the main constituents of the aqueous phase in normal day-to-day variation in milk composition in goats are compatible with the scheme outlined, the concentration of lactose ([lactose]) being inversely correlated with both [Na] and [K], the K:Na ratio being maintained at about 3:1. A similar relationship has also been found between species, at least in those apparently lacking a paracellular pathway in full lactation (see below) (Peaker, 1976). In other words, the higher the [lactose], the lower the [Na] and [K] in milk.

In this paper experiments are described which further explore the

relationships between the concentrations of lactose, Na and K in milk, the transepithelial p.d. and water movements, and which were designed to investigate causal relationships in order to attempt to determine the

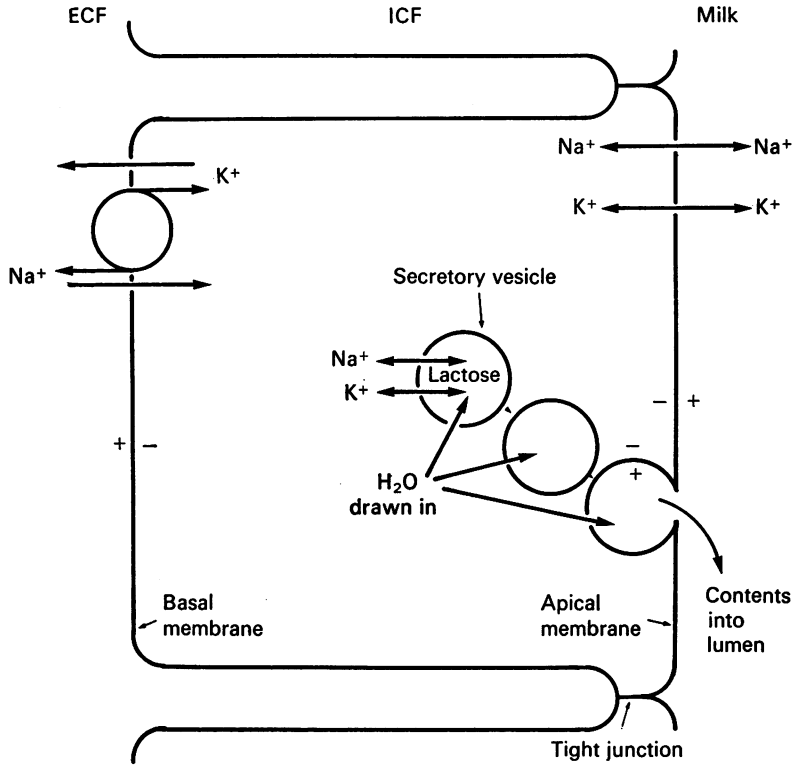

Fig. 1. Suggested scheme for Na and K movements between extra-cellular fluid and milk, and lactose and water secretion. The cellular transport mechanisms, including those into and out of the secretory vesicles from the Golgi apparatus, are those suggested by Linzell & Peaker (1971*a*, *b*).

mechanism by which the composition of the aqueous phase of milk is determined. For the most part the studies concern only transcellular movements across the mammary epithelium but since a paracellular pathway has been proposed which permits substances to pass directly between ECF and milk in some species or in some physiological conditions, the effect of such a pathway will also be considered (see Peaker, 1975).

METHODS

Animals

Goats and cows with a 'milk' (caudal superficial epigastric) vein enclosed in a skin loop (Linzell, 1960) were used in most experiments; the cows were established to be free from subclinical mastitis (Linzell & Peaker, 1975*b*). Guinea-pigs were used on the fourth day of lactation.

Isolated perfused mammary gland

Goat mammary glands were perfused using the procedure described by Linzell, Fleet, Mepham & Peaker (1972). The substrate mixture and artificial kidney fluid were as previously described except in experiments where mannose was substituted for glucose in both fluids.

Measurement of blood–milk p.d.

Conscious goats and cows. Goats stood on a milking stand insulated from the floor by rubber cups under the legs; cows stood on a dry rubber mat. Sterile polyethylene catheters, 15–20 cm long, containing sterile saturated KCl in 3 % (w/v) agar ('KCl-agar bridges') were inserted into a milk vein and into the cistern of the mammary gland via the teat canal. The end of each bridge was immersed in saturated KCl into which was placed a calomel half-cell (< 1 mV difference between the two). The half-cells were connected by screened leads to a millivoltmeter and the p.d. displayed on a chart recorder. The zero point was checked and adjusted by short-circuiting the two pots containing KCl with a KCl-agar bridge.

Guinea-pigs. These were anaesthetized with Na pentobarbitone (27 mg/kg I.P.). KCl-agar bridges were inserted into a jugular vein and into the lumen of a mammary gland via the teat canal.

Perfused gland. KCl-agar bridges were inserted into the gland cistern, and into the cannulated venous outflow from the gland.

Administration of substances into the mammary gland

In all cases sterile solutions at approximately 37° C were injected via the teat canal.

Analytical methods

Na, K, Cl and lactose in milk were determined as described by Fleet, Linzell & Peaker (1972). Milk osmolality was determined using an Advanced Instruments osmometer against NaCl standards; the samples were allowed to stand overnight at 4° C to allow any particulate matter, which may prematurely initiate the formation of ice crystals during supercooling, to settle.

RESULTS

Relation between milk [lactose] and p.d. in normal goats

In seven lactating goats the transepithelial p.d. varied from 6 to 20 mV (milk negative with respect to blood) and [lactose] from 121 to 136 mM. A significant correlation was evident between [lactose] and p.d. ($r = -0{\cdot}88$, $P < 0{\cdot}01$) such that in goats with a lower milk [lactose] milk had a more negative p.d. with respect to blood (Fig. 2).

Although for technical reasons it has not proved possible to measure intracellular p.d. in goat mammary glands it can be inferred from the ionic gradients across the basal membrane that it is likely to be similar in magnitude to that in small animals in which it has been measured *in vivo* (35–40 mV, Evans, Linzell & Peaker, 1971; Linzell & Peaker, 1971*b*; Peaker & Taylor, 1975). Therefore a potential profile across the mammary secretory epithelium has been constructed, and is shown in Fig. 3.

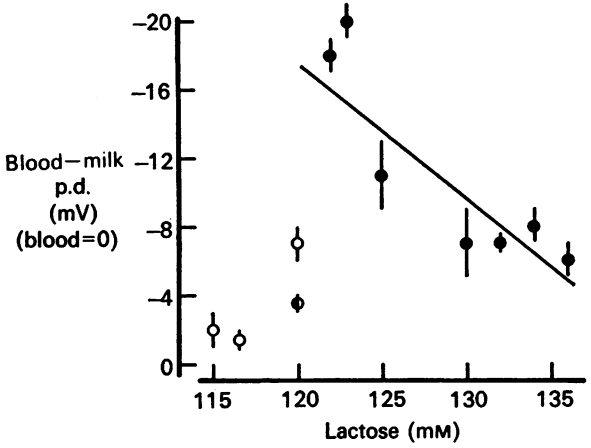

Fig. 2. Blood-milk p.d. in relation to milk [lactose] in normal goats (●), in goats treated with oxytocin (○) (see text) and in an apparently normal goat with a high milk [Na] (◐) (see text). The points shown are the mean ± range recorded over a 5-min period. The calculated regression line is for the seven normal goats ($r = -0{\cdot}88$, $P < 0{\cdot}01$).

If it is argued that the difference in blood–milk p.d. between goats is mainly a reflexion of the p.d. across the apical membrane, i.e. the basal membrane p.d. was similar (which appears likely since a marked difference in permeability or in intracellular ionic composition would be needed to cause a difference of 14 mV), then it is clear that the higher concentrations of lactose in milk were associated with higher p.d.s across the apical membrane. Thus the higher the milk [lactose], the higher the apical membrane p.d. (milk positive with respect to ICF) and the lower the [Na] and [K] in milk.

Effects of omitting glucose from substrates on milk composition and blood–milk p.d. in isolated perfused goat mammary glands

In view of the correlation between milk composition and p.d. it was clearly desirable to investigate whether the secretion of lactose in some way sets up a p.d. across the apical membrane and thereby keeps [Na] and [K] in milk lower than in ICF. Hardwick & Linzell (1960), Hardwick, Linzell & Price (1961) and Linzell *et al.* (1972) showed, using the isolated

perfused goat mammary gland, the necessity of glucose for the secretion of lactose and water. It was also found that other sugars could not substitute for glucose although some, mannose for example, maintained a normal oxygen consumption. Later work (M. Peaker, unpublished) showed that the intracellular ionic composition was maintained during incubation of slices with mannose instead of glucose as a metabolic substrate. In perfusions in which mannose replaced glucose in the substrate

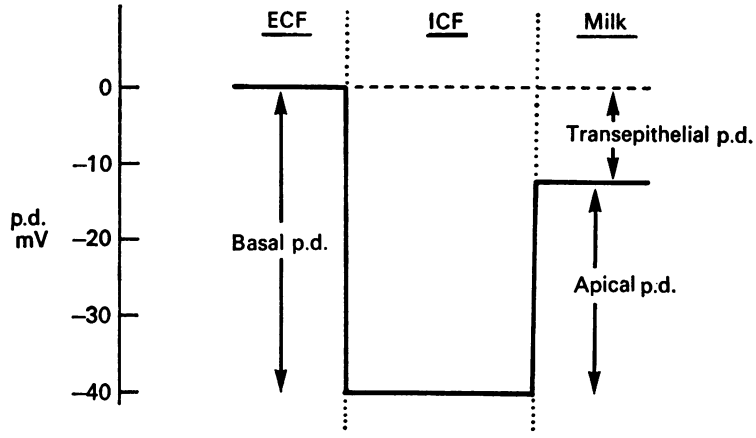

Fig. 3. Suggested p.d. profile across the mammary epithelium of goats (see text for assumptions made).

mixture, milk yield, which is a measure of water secretion since milk in most species is mostly water, fell markedly. When milk yield was low, milk [lactose] also decreased while [K] and [Na] increased, the ratio of approximately 3:1 being maintained (see Fig. 8 in Linzell *et al.* 1972). When glucose was then added to the perfusate the changes in milk composition and in the rate of secretion were reversed.

Since it appeared from these experiments that the secretion of lactose in some way kept milk [K] and [Na] low, a series of perfusion experiments was performed in which transepithelial p.d. was measured. However, there is the complication that during perfusions the mammary duct or secretory epithelia often become disrupted and paracellular movements of ions and lactose supervene. Although oxytocin was not given (see below) a 'leak' still occurred in some experiments. In these the blood–milk p.d. fell to within 1–2 mV of zero during the first hour and the milk showed changes in composition to be expected from disruption of the epithelium, namely a marked decrease in [lactose] and [K] and an increase in [Na] and [Cl] (see below and Linzell *et al.* 1972). In two experiments, however, a transepithelial p.d. in excess of 15 mV was maintained but because the

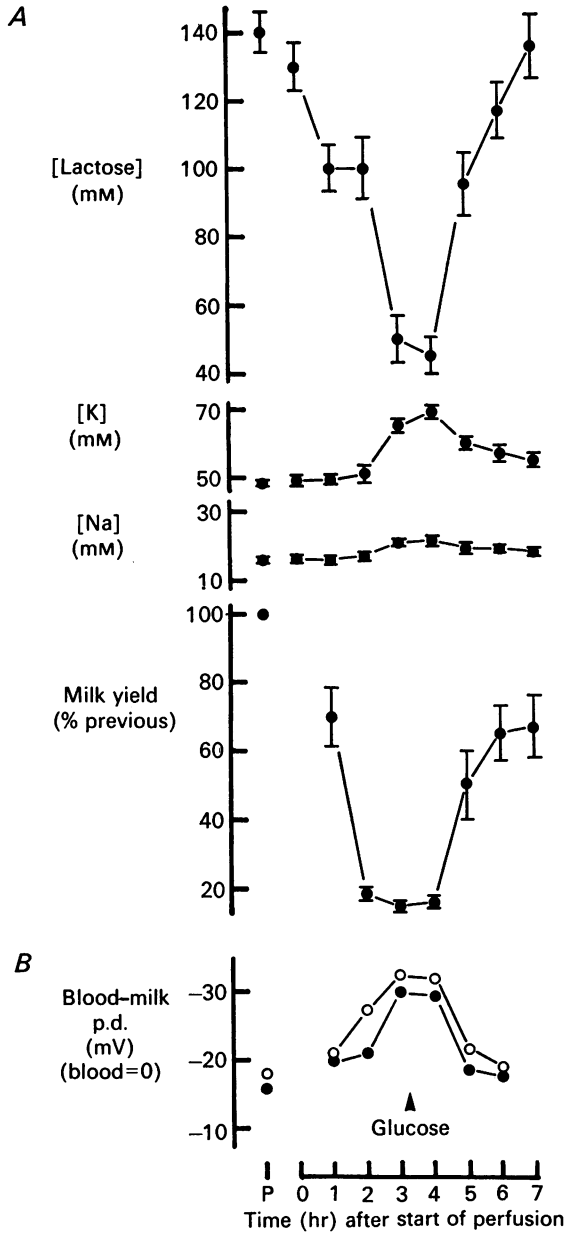

Fig. 4. *A*, changes in milk [lactose], [K], [Na] and yield in isolated perfused glands in which mannose was substituted for glucose in the substrate mixture. Mean ± S.E. of mean in three experiments. *B*, changes in blood–milk p.d. in two similar experiments. P is the day previous to the experiment. Glucose was included in the substrates from the time shown.

glands were not milked (in order to maintain a milk path between the alveoli and the electrode in the cistern of the gland) it was necessary to compare these results with parallel experiments on milk yield and composition in three other glands.

During perfusion with mannose in the substrates instead of glucose, the blood–milk p.d. increased, milk becoming more negative with respect to blood. Before perfusion the p.d. in the two experiments was 16 and 18 mV whereas when milk yield and milk [lactose] were at their lowest, and [K] and [Na] at their highest, p.d. in the parallel experiments reached 29·5 and 32·5 mV. These changes in p.d., like those in milk yield and composition, were reversed when glucose was added to the perfusate (Fig. 4). Therefore it can be suggested that when the rates of lactose secretion and water secretion were very low, the p.d. across the apical membrane fell (i.e. milk became less positive with respect to ICF) and milk [Na] and [K] increased. Thus these results indicate that it is the secretion of lactose and/or water which in some way establishes the normal apical membrane p.d.

Changes in p.d. across the mammary epithelium induced by osmotic water flow

As mentioned in Introduction it is probable that the formation and secretion of lactose is responsible for the bulk movement of water into milk. Although it is difficult to conceive how the secretion of lactose *per se* could establish a p.d. it is possible that a related osmotic flow of water could set up a p.d. across a charged membrane. Experiments were therefore done to investigate whether blood–milk p.d. could be affected by osmotic water movements into milk. Since two mechanisms have been implicated in the establishment of a p.d. by water flow across a charged membrane, namely the *streaming potential* and the *transport number effect* (see Barry & Hope, 1969*a*, *b*) the two electrokinetic phenomena will be referred to collectively as *waterflow-induced potentials*. If such mechanisms are involved in establishing the apical membrane p.d., it follows that if an osmotic gradient is imposed on the mammary epithelium, by introducing hypertonic solutions into milk, then milk should become more positive with respect to both ICF and to blood (Fig. 3).

In four goats, approximately 6 hr after morning milking 20 ml. hypertonic sucrose (2·2 osmole/kg water) were injected into the lumen of one gland via the teat canal; the gland was then massaged. In all there was a rapid and marked decrease in blood–milk p.d. with milk becoming less negative with respect to blood (Fig. 5). During the period of 35–75 min when the p.d. was altered, the gland was observed to swell. When the p.d. had virtually returned to the value obtaining initially, the two glands

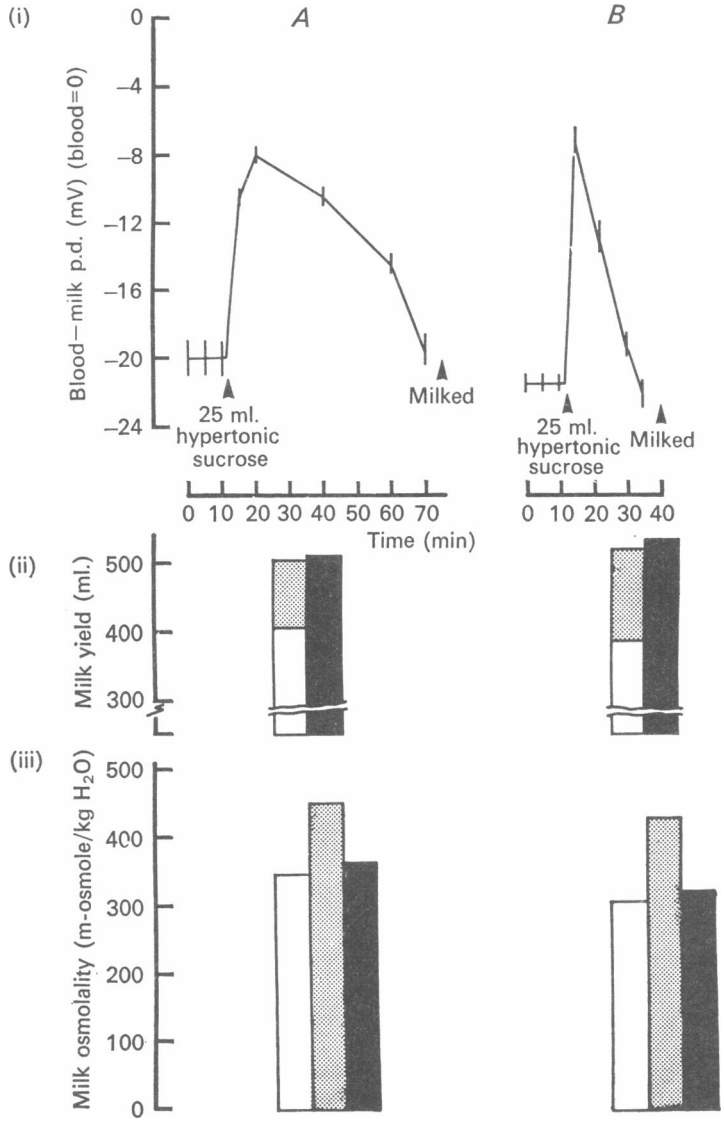

Fig. 5. (i) Changes in blood–milk p.d. induced by the introduction of hypertonic sucrose (2·2 osmole/kg water) into the milk of two goats approximately 6 hr after morning milking. Each point shows the mean ± range over a 1 min period. (ii) The volume of milk obtained at the time of milking (filled columns) compared with the expected milk yield at that time, calculated as described in the text (open columns) plus the theoretical osmotic dilution of the added hypertonic sucrose to the final milk osmolality (stippled columns). (iii) The osmolality of milk before (open column) and after (filled columns) the experiment. The stippled columns show the calculated osmolality of the milk following addition of the hypertonic sucrose (see text).

of each animal were milked. By comparing the volume of milk obtained from the treated and untreated glands and applying the RMQ procedure for detecting unilateral effects on milk yield (Linzell & Peaker, 1971*d*) the amount of milk that would have been expected from the treated gland was calculated. The volume obtained at the end of the experiment was considerably in excess of the volume expected indicating that water had entered milk during the experiment. Furthermore, from the expected milk yield, the volume and osmolality of the sucrose solution and the osmolalities of milk samples taken before and after, the volume of water that must have entered milk osmotically was calculated. When this volume was added to the expected yield close agreement between this total expected volume and the volume obtained was apparent (Fig. 5). It can also be seen in Fig. 5 that by the time blood–milk p.d. had returned virtually to normal, osmotic water flow had been sufficient to restore milk osmolality to almost the pre-treatment value.

Although these results provide *prima facie* evidence for waterflow-induced potentials across the mammary epithelium it could be argued that since the blood–milk p.d. came closer to zero the osmotic gradient imposed may have disrupted the mammary epithelium and short-circuited the two sides. Therefore, hypertonic solutions of sucrose or lactose were administered when there was less milk in the glands, in order to ensure that the concentration of the fluid in the glands was higher, to investigate whether, with higher osmotic gradients the transepithelial p.d. would change from milk negative to milk positive or whether as would be expected from a complete short-circuit, the p.d. would only reach zero.

Following the introduction of hypertonic sucrose (2·2 osmole/kg water) via the teat canal, 1–2 hr after morning milking in three goats (20–30 ml. sucrose) and two cows (60 ml.), the blood–milk p.d. crossed zero to reach 6–16 mV (milk positive) in the goats and 4–8 mV (milk positive) in the cows within the first 5 min. Similar results were obtained with 55 ml. lactose (1 osmole/kg water) in two goats (results for one shown in Fig. 6) and with 100 ml. in two cows. In addition, p.d. changes were recorded across the guinea-pig mammary gland following the introduction of 0·2 or 0·3 ml. sucrose (2·2 osmole/kg water) via the teat canal, in two experiments. In one the p.d. started at 3·5 mV (milk negative) and reached 10 mV (milk positive); in the other the p.d. was initially 2 mV (milk positive) and increased to 10 mV (milk positive) (Fig. 7). Therefore it appears that waterflow-induced potentials can be evoked by the osmotic passage of water into milk with the milk becoming electrically more positive with respect to blood.

In three goats, isosmotic sucrose was introduced into the lumen of the gland; the volume (70–150 ml.) was similar to the total volume of water

drawn into milk in the experiments with hypertonic sucrose. There was no significant effect on p.d. nor, from the calculated expected yield, was there any evidence of significant osmotic water movement across the mammary epithelium (results for two shown in Fig. 8). Therefore it can be concluded that osmotic water movement is necessary for a change in p.d.

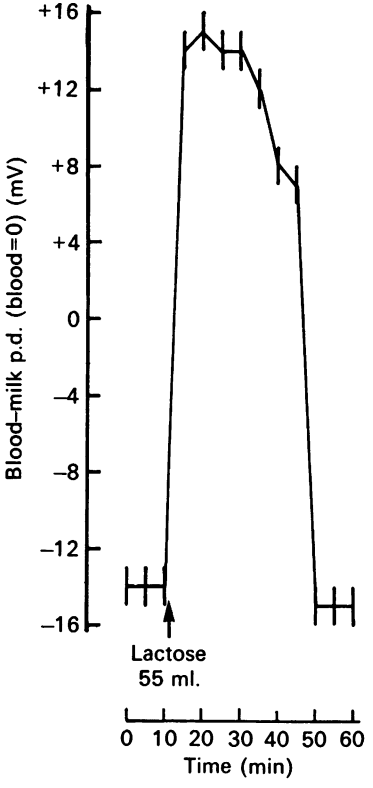

Fig. 6

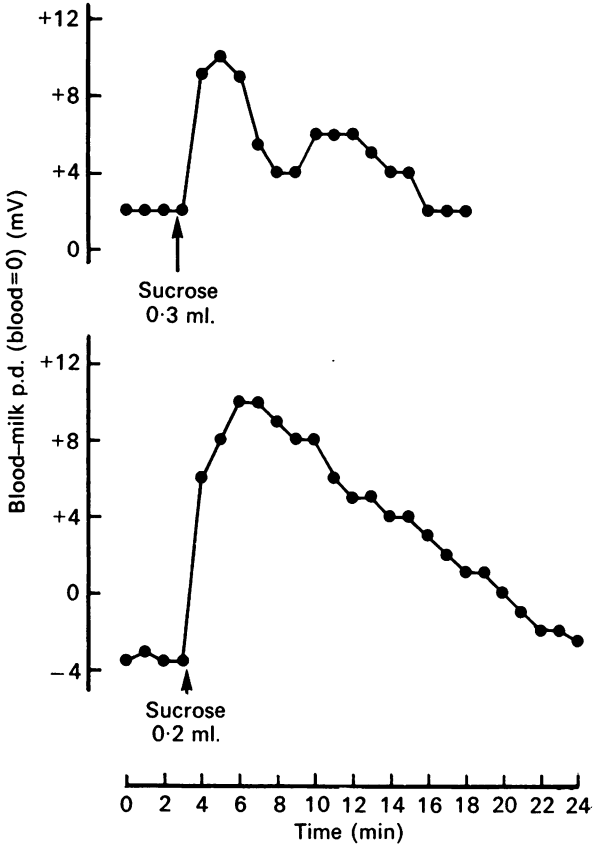

Fig. 7

Fig. 6. Changes in blood–milk p.d. induced by the introduction of hypertonic lactose (1 osmole/kg water) into the milk of a goat. Each point shows the mean ± range over a 1 min period.

Fig. 7. Changes in blood–milk p.d. induced by the introduction of hypertonic sucrose (2·2 osmole/kg water) into the milk of two guinea-pigs.

Since in the above experiments, the p.d. across both the secretory and duct epithelia was being studied, experiments were done to investigate whether waterflow-induced potentials could be developed across the duct

epithelium only. In two goats a light pneumatic cuff was inflated around the base of the teat to isolate the teat from the rest of the gland (Linzell & Peaker, 1971*c*). Milk (7 and 12 ml. respectively) from the same animal was then introduced into the teat pouch. No change in p.d. was apparent when hypertonic sucrose (2·2 osmole/kg water, 1 and 1·5 ml. respectively) was introduced (Fig. 9). The osmolality of the milk was calculated to be 538 and 511 m-osmole/kg water, which is comparable to that in the

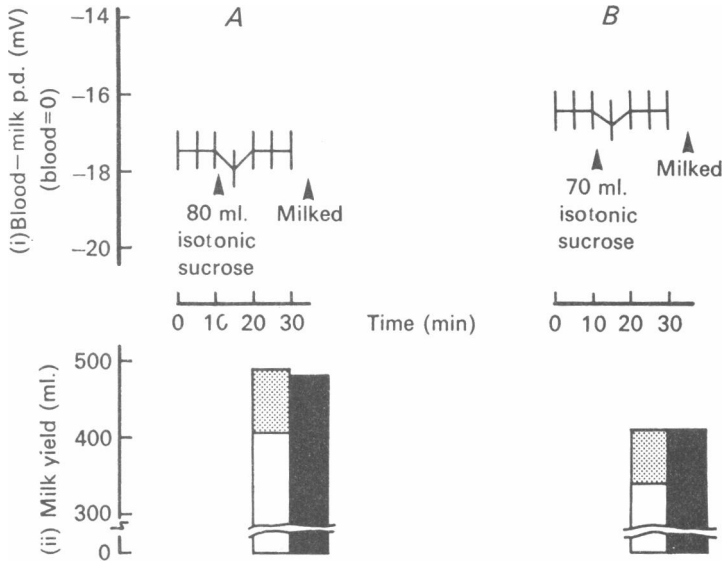

Fig. 8. (i) Effects of addition of isotonic sucrose (300 m-osmole/kg water) to milk on blood–milk p.d. in two goats. Each point shows the mean ± range over a 1 min period. (ii) The volume of milk obtained at the time of milking (filled columns) compared with the expected yield at that time, calculated as described in the text (open columns) plus the volume of isotonic sucrose given (stippled columns).

experiments with hypertonic sucrose in the whole gland. Linzell & Peaker (1971*c*) showed that the duct epithelium, which lines the teat as well as the duct system deeper in the gland, is impermeable to ions during lactation. Since waterflow-induced potentials depend on ion movements it follows that the lack of change in p.d. across the teat in response to an osmotic gradient is in agreement with the earlier findings. Thus it can be concluded that the waterflow-induced potentials obtained in the whole gland were established across the secretory epithelium rather than the duct epithelium.

Effects of oxytocin on blood–milk p.d.

It is well established that treatment with oxytocin increases milk [Na] and [Cl] and decreases [K] and [lactose]. From permeability studies it was concluded that in these circumstances a paracellular pathway exists across the secretory epithelium permitting substances to pass directly between blood and milk down their respective concentration gradients (Linzell & Peaker, 1971*d*; Linzell, Peaker & Taylor, 1975). Therefore the effect of oxytocin on blood–milk p.d. was investigated.

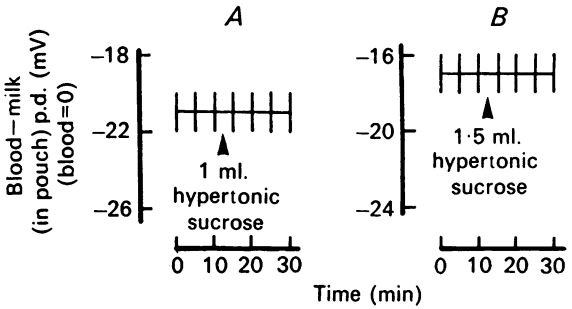

Fig. 9. Effects of the introduction of hypertonic sucrose (2·2 osmole/kg water) into milk held in a teat pouch (see text for details) in two goats on blood–milk in pouch p.d. Each point shows the mean ± range over a 1 min period.

In three goats, oxytocin (500 m-u. I.V.) was given every hour for 4 hr. Thirty min after the last injection blood–milk p.d. was recorded and a sample of milk taken for analysis. At this time p.d. was 1·5, 2 and 7 mV (milk negative) compared with 18, 13 and 20 mV respectively on the previous day. Milk [lactose] was also decreased, and, therefore, the relationship found between [lactose] and p.d. in untreated goats was clearly disrupted by treatment with oxytocin, such that a low milk [lactose] was associated with a low transepithelial p.d., rather than, as from the extrapolated regression line for untreated animals, a high milk-negative p.d. (Fig. 2).

Blood–milk p.d. in non-lactating and mastitic glands

In two goats, suckling one kid each, one gland was well developed and producing milk while the other was small and contained a clear fluid. In the glands producing milk (lactose 124, 127 mM; Na 12·5, 14 mM; K 46·5 45 mM) the p.d. was 18 and 20 mV (milk negative) whereas in the 'dry' glands (lactose < 15 mM; Na 128, 148 mM; K 13, 9·5 mM) the p.d. was 2·5 and 1·5 mV (milk negative).

In a goat with acute mastitis in one gland the blood–milk p.d. was 0·5 mV (milk negative) compared with 18 mV in the uninfected gland.

The results from the non-lactating and mastitic glands are in agreement with the view that there is some continuity between extracellular fluid and the lumen of the mammary gland in these conditions (see Linzell & Peaker, 1971*c*, 1972).

In one goat with a relatively high milk [Na] (21·5 mM) and a [lactose] of 120 mM, the p.d. was 3·5 mV (milk negative) (Fig. 2), which suggests the presence of a 'leak' between blood and milk in one apparently healthy animal.

DISCUSSION

Milk composition and the apical membrane p.d.

The relationship found between milk [lactose] and transepithelial p.d. is in agreement with the scheme for K and Na movements across the apical membrane of the mammary secretory cell suggested by Linzell & Peaker (1971*a*, *b*) (Fig. 1), i.e. the higher the concentration of lactose, the higher the apical membrane p.d., and the lower the concentrations of K Na in milk. The results of the perfusion experiments indicate that it is the secretion of lactose or the associated osmotic movement of water which establishes the p.d. between ICF and milk and thereby keeps the concentrations of K and Na in milk lower than in ICF.

It could be argued that the correlation between apical membrane p.d. and lactose secretion and water movement in the perfusion experiments need not necessarily reflect a causal relationship since the secretion of other components or the exocytosis of secretory vesicles, which possibly might set up a potential, could have been inhibited by the substitution of mannose for glucose. However, earlier studies showed that the secretion of fat, protein, Ca and citrate continues when the rate of lactose and water secretion is very low (Hardwick *et al.* 1961; Linzell *et al.* 1972). Since there is evidence that protein (see Linzell & Peaker, 1971*a*), Ca (Baumrucker & Keenan, 1975) and citrate (Linzell, Mepham & Peaker, 1976) are secreted, like lactose, by exocytosis of vesicles arising from the Golgi apparatus, it is clear that the normal secretory processes of exocytosis and lipid droplet extrusion continue in these circumstances.

The results indicate that waterflow-induced potentials can be established by an imposed osmotic gradient across the mammary secretory epithelium. The polarity of these potentials is such as to be compatible with osmotic water flow into milk, to dilute the newly synthesized lactose, being responsible for the normal apical p.d. One might then conceive that as lactose is formed, water is drawn osmotically across a membrane bearing fixed negative charges, a separation of charge then occurs by the sweeping effect of water flow on the mobile ions (the true streaming potential) and possibly also by the formation of local ionic gradients in

unstirred layers (the transport number effect, see Barry & Hope, 1969*a*, *b*; Wedner & Diamond, 1969), a p.d. is set up and mobile cations are then distributed according to this p.d. as in classical streaming potential theory (Schmid & Schwarz, 1952).

While qualitatively this hypothesis is attractive to account for the composition of the aqueous phase of milk, the question of whether a p.d. of sufficient magnitude to account for the normal apical membrane p.d. could be set up in this way must be considered. Calculations, on the experiments in goats, from the imposed osmotic gradient and the maximum change in p.d. indicate that a 1 m-osmole difference across the epithelium induced a p.d. change of approximately 0·1 mV (0·09–0·11). From this ratio it can be calculated that the concentration of lactose required to establish an apical membrane p.d. of 20–25 mV would be about 3·5 times the concentration in milk (i.e. approximately 500 mM). Since this 'active site' would have to be in contact with milk in the alveolus for a trans-epithelial p.d. to be recorded it can be suggested that the source of the p.d. is the secretory vesicle as it opens into the lumen (Fig. 1). As a corollary it must then be assumed that osmotic equilibration across the membrane of the vesicle is not complete by the time the contents are liberated and that the vesicle carries fluid of up to 3·5 times the final concentration of milk, with further osmotic water movement occurring across the apical membrane to achieve isotonicity.

It should be pointed out that the calculation of the change in p.d. per m-osmole difference is a crude one since there is by no means a guarantee of complete and rapid mixing between the hypertonic solutions added and the milk stored in the gland. Although evidence was obtained that the osmotic gradients imposed did not disrupt the mammary epithelium to completely short-circuit the two sides, an increase in sucrose permeability has been found to occur at this time (M. Peaker, unpublished). A presumed paracellular pathway would have the effect of attenuating the p.d. change (see below) and, therefore, the true change in apical membrane p.d. could have been higher than that recorded (i.e. a ratio of $> 0{\cdot}1$ mV/m-osmole). The estimate of a concentration of 500 mM lactose at the 'active site' should therefore be regarded as a maximum value.

From the evidence available it appears that waterflow-induced potentials could be involved in determining the composition of the aqueous phase of milk. Although this might be regarded as an unusual explanation for the genesis of a p.d. in an animal cell, the mammary gland is, of course, itself unusual in that during lactation it continuously synthesizes and secretes lactose – a highly osmotically active, impermeant molecule. Definitive electrophysiological evidence is difficult to obtain because of the glandular nature of the mammary epithelium but a hope for the future is that a method will be developed for growing sheets of lactating mammary cells in culture and studying transport across them, as has been

done with neoplastic kidney and mammary cells (Misfeldt, Hamamoto & Pitelka, 1974; D. Misfeldt & D. R. Pitelka, personal communication).

If the mechanism suggested does operate then one might envisage that the variation in the composition of the aqueous phase of milk in different species might then be explained by differences in such variables as the rate of lactose synthesis (for which Brew, 1970 provides an enzymological basis) and the properties of the membrane in terms of osmotic water permeability, density of charges etc.

Effects of a paracellular pathway

The effects of exogenous oxytocin on transepithelial p.d. are in agreement with the conclusions of Linzell & Peaker (1971*d*) and Linzell *et al.* (1975), based on changes in milk composition and permeability of the mammary epithelium to disaccharides, that oxytocin disrupts the 'tight junctions' connecting neighbouring cells. Therefore it would appear that such a paracellular or shunt pathway at least partially short-circuits the transepithelial p.d. and results in the dissipation of concentration gradients established across the secretory vesicle and apical membranes (see Schultz, 1972). Similarly, a low blood–milk p.d. has been obtained in other cases where there is believed to be a paracellular pathway, for example throughout lactation in the rabbit (Peaker & Taylor, 1975), and, in the present experiments, in non-lactating glands and in mastitis (see Peaker, 1975).

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