THE MOVEMENT OF SOLUTES ACROSS THE EPITHELIUM OF THE DUCTS AND CISTERNS IN THE MAMMARY GLAND OF THE EWE

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Summary

The role of the cisterns and large ducts in the overall process of milk secretion has been studied in experiments which have been carried out to determine the change in composition of solutions injected into the mammary gland cisterns of lactating and dry ewes. These solutions contained electrolytes, lactose, and a marker substance and were allowed to remain in the gland for periods of up to 4 hr. The concentration of sodium, potassium, chloride, and lactose in the solution injected into lactating glands approached with time that in milk. The data indicated that the rate of change in concentration of sodium, potassium, and chloride was more rapid than could be accounted for by the mixing of the injected solution with milk from the ducts. Sodium, potassium, and chloride ions were secreted into solutions in which the concentrations of these ions were lower than those in milk and sodium and chloride ions were absorbed from solutions in which their concentration was higher than that in milk. Water absorption occurred from the solutions containing sodium and chloride at a higher concentration than that in milk. In the dry gland the composition of the solutions injected approached with time that of involution secretion which has a higher sodium and chloride and lower potassium and lactose concentration than normal milk. Lactose was absorbed from dry glands but not from the lactating glands.

I. INTRODUCTION

Experiments have been described previously in which the composition of milk was determined in samples obtained from cows during the milking period and following successive injections of oxytocin (Mackenzie and Lascelles 1965). The concentration of sodium and chloride in whey increased with successive samples while the concentration of lactose in whey, and protein in skim milk decreased. It was postulated on the basis of these results that as fluid from the alveoli passes through the ducts water, sodium, potassium, and chloride are absorbed. Although this interpretation appeared the most likely, other interpretations could not be eliminated. The role of the cisterns and ducts in the overall process of milk secretion has been studied further in experiments which have been carried out to determine the change in composition of solutions injected into the cisterns of lactating and dry ewes. These solutions contained electrolytes, lactose, and a marker substance and were allowed to remain in the gland for periods of up to 4 hr.

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TABLE 1

CHANGES IN COMPOSITION OF SOLUTIONS CONTAINING LACTOSE AND A MARKER, AFTER INJECTION INTO THE MAMMARY GLAND CISTERNS OF THREE LACTATING EWES TOGETHER WITH THE COMPOSITION OF THE MILK AND THAT OF THE INJECTED SOLUTION

Samples were collected at 5 and 30 min, and at 1, 2, and 4 hr, and at 4 hr after an injection of oxytocin. The estimated volumes of the mixture of milk and solution have been computed from the concentration of the marker. The values presented are means of the two sides over the three sheep together with the respective standard errors. The results are also expressed as a percentage of the theoretical values estimated from the mixing of the solution with milk. The mean recovery of the marker was 82%

Sample	Estimated Volume (ml)	Chloride		Sodium		Potassium		Lactose		Protein	
		(m-equiv/l)	(% of theoretical)	(m-equiv/l)	(% of theoretical)	(m-equiv/l)	(% of theoretical)	(mM)	(% of theoretical)	(g/100 ml)	(% of theoretical)
Milk		30·1±4·4		16·3±2·7		32·0±3·2		152·5±9·0		4·68±0·56	
Solution injected	46·0± 2·8	0·5±0·3		1·4±0·7		0·4±0·2		247·9±7·9		0·99±0·50	
5 min	58·7± 3·2	8·2±1·9	133	7·3±1·9	181	8·3±1·6	111	228·9±4·3	99	1·56±0·17	93
30 min	63·5± 3·0	11·7±1·0	120	9·6±1·2	166	13·7±2·4	116	220·1±1·5	101	1·87±0·05	86
1 hr	63·6± 4·4	14·7±1·0	131	11·4±0·9	177	16·9±2·5	125	212·8±2·2	99	2·03±0·08	88
2 hr	70·8±10·0	20·3±2·0	133	12·7±0·3	163	17·6±3·1	118	202·7±2·5	99	2·36±0·08	89
4 hr	111·6±10·4	27·6±1·2	138	17·9±2·0	166	27·1±2·4	116	180·7±0·6	97	3·20±0·25	94
4 hr after oxytocin	137·5±40·0	28·6±1·3	126	17·4±1·8	142	29·8±2·5	113	174·3±1·2	99	3·55±0·38	93

II. Methods

(*a*) *Experimental Procedure*

Experiments were carried out on six lactating and four dry multiparous ewes of mixed breeds. Each animal was used only once.

(*b*) *Lactating Ewes*

Immediately prior to the experimental period the ewes were milked by hand and the strippings were obtained after an intravenous injection of 1 i.u. of oxytocin (Syntocinon Sandoz Ltd.) in 5 ml of saline. A milk sample was kept for analysis. A polythene cannula was passed into the teat cistern via the streak canal. The free end of the cannula was attached to a hypodermic syringe containing a solution of known composition and a measured volume was injected into the cistern. The solution was injected until the mammary gland was filled but not distended as judged visually and by palpation. Thus, the volume injected was partly dependent on the size of the mammary gland cisterns. The solution in the cistern was sampled at intervals by reintroduction of the cannula through the streak canal and then collecting the required volume of sample (3–4 ml) by gentle suction with the syringe. Generally, sampling was carried out at 5 min, 30 min, 1 hr, 2 hr, and 4 hr after the initial injection. Most of the fluid was milked from the gland at the 4-hr sampling. An intravenous injection of oxytocin was then given and the remaining milk collected, its volume measured, and a sample kept for analysis.

(*c*) *Dry Ewes*

The procedure was similar to that for lactating ewes. Involution secretion, if present in the glands was milked out and kept for analysis. None of the dry ewes received an injection of oxytocin.

(*d*) *Estimation of Fluid Volume*

Milk is continuously produced in the alveoli of the lactating mammary gland and a portion of the alveolar milk passes down the ducts into the cisterns. It was necessary to estimate the quantity of each solute and the volume of fluid which could be accounted for by passage of milk into the cisterns during the experimental period. The changes in the fluid volume of the mixture in the cistern were estimated by changes in concentration of a marker—either albumin-bound T-1824 or ^{131}I-labelled human serum albumin (^{131}I HSA). This measured total volume changes only. It did not distinguish between the flow of milk from the ducts and the possible movement of water across the epithelium.

Albumin-bound T-1824 was prepared by adding 10 mg T-1824 (G. T. Gurr Ltd.) to 25 ml of ovine serum. The serum was then dialysed for 12 hr in a dialysis bag (Visking, H. B. Selby & Co.) against three 1-l. changes of distilled water. The dialysed serum was used to make up 100 ml of final solution. ^{131}I HSA of high specific activity was obtained from the Radiochemical Centre, Amersham, England. Tracer amounts of this preparation were added to the solutions to give approximately 5000 counts/ml/min.

TABLE 2

CHANGES IN COMPOSITION OF SOLUTIONS CONTAINING SODIUM CHLORIDE, LACTOSE, AND A MARKER, AFTER INJECTION INTO THE MAMMARY GLAND CISTERNS OF THREE LACTATING EWES TOGETHER WITH THE COMPOSITION OF THE MILK AND THAT OF THE INJECTED SOLUTION

Samples were collected at 5 and 30 min, and at 1, 2, and 4 hr, and at 4 hr after an injection of oxytocin. The estimated volumes of the mixture of milk and solution have been computed from the concentration of the marker. The values presented are means of the two sides over the three sheep together with the respective standard errors. The results are also expressed as a percentage of the theoretical values estimated from the mixing of the solution with milk. The recovery of the marker was 90%

Sample	Estimated Volume (ml)	Chloride		Sodium		Potassium		Lactose		Protein	
		(m-equiv/l)	(% of theoretical)	(m-equiv/l)	(% of theoretical)	(m-equiv/l)	(% of theoretical)	(mM)	(% of theoretical)	(g/100 ml)	(% of theoretical)
Milk		27·1±2·6		14·2±2·4		35·4±0·7		140·2±8·8		4·39±0·15	
Solution injected	43·3±3·0	65·4±4·6		66·5±8·3		0·8±0·1		145·4±2·4		1·34±0·68	
5 min	51·2±2·5	58·6±1·9	98	56·6±3·1	96	11·3±1·7	185	147·2±0·9	102	1·88±0·32	106
30 min	49·8±3·6	53·4±2·9	94	50·9±2·3	87	18·1±0·3	175	147·5±0·9	103	2·17±0·38	116
1 hr	59·2±2·4	45·3±1·0	91	36·5±0·7	81	23·0±1·1	157	149·0±2·7	104	2·64±0·19	102
2 hr	58·8±2·7	37·4±2·6	80	26·5±1·2	64	28·3±1·0	167	156·1±0·8	108	2·95±0·09	104
4 hr	99·6±8·5	29·6±1·7	79	17·3±1·0	60	33·1±0·7	132	157·1±3·1	110	3·65±0·13	102
4 hr after oxytocin	109·4±9·6	26·6±1·7	80	15·4±1·0	65	34·3±0·8	119	153·6±4·2	108	3·86±0·12	110

(e) *Chemical Analyses*

The samples obtained from lactating ewes were centrifuged to remove most of the fat and the skim milk was kept in the deep-freeze until analysed. Sodium, potassium, chloride, and lactose were determined as previously described by Mackenzie and Lascelles (1965). It was not possible to estimate chloride in the presence of T-1824. The T-1824 was removed from the solutions containing the dye by protein precipitation with tungstic acid (Harrison 1957). The precipitate was centrifuged and traces of blue in the supernatant were adsorbed by passing it through filter paper. Total protein was estimated by a microKjeldahl procedure. The ammonia was collected in boric acid and titrated with 0·01N HCl to the end point with a mixed indicator (0·1% bromocresol green–0·1% methyl red in 95% ethanol). T-1824 was estimated by the method described by Lascelles (1962).

Radioactivity measurements were made at the completion of each experiment. These were carried out on 0·2-ml samples using a thin mica end-window Geiger–Müller tube. The samples were spread over metal planchets 5 cm^2 in area and covered with a lens tissue disk.

III. Results

The changes in composition after intramammary injection of 250 mM lactose solutions containing labelled albumin are presented in Table 1. The estimated volumes have been computed from changes in the concentration of the marker. The amount of milk passing into the cistern has been computed from the estimated volumes assuming that there had not been any water movement across the duct epithelium. The computed changes in concentration of the solutes in the mixture in the cisterns due to the addition of milk has been termed the theoretical concentration. The derivation of the theoretical concentration is presented in the Appendix.

The composition of the mixture approached that of the original milk with time. The actual concentrations expressed as percentages of the theoretical concentrations indicated that potassium, sodium, and chloride ions were moving into the solution more rapidly than could be attributed to the passage of milk alone. The actual concentration of lactose was the same as the theoretical concentration indicating that changes in fluid volume and lactose concentration were due entirely to passage of milk into the cisterns. Unfortunately in a number of samples the protein precipitated in the deep-freeze before they were analysed for protein. It is suspected that this increased the sampling error which may account for the discrepancy between the percentage of the theoretical concentration for lactose and that for protein.

The changes in composition after intramammary injection of a solution containing 65 mM sodium chloride, 145 mM lactose, and labelled albumin are presented in Table 2, which has been compiled in a similar manner to the previous table. It may be seen that as time passed the composition of the mixture in the cisterns approached that of the original milk. The actual values expressed as percentages of the theoretical concentrations indicated that sodium and chloride ions were being absorbed from the cisterns. Potassium concentration rose more rapidly than could be accounted for by the passage of milk into the cisterns. There was better agreement between the lactose and protein results than in the previous table.

The changes in composition of milk after intramammary injection into the dry gland are presented in Table 3. The concentration of sodium and chloride increased with time and that of potassium and lactose decreased. A marker of fluid volume was not used in these experiments. However, the total volume of fluid recovered was slightly less than that added, indicating little fluid movement.

TABLE 3

CHANGES IN COMPOSITION OF MILK AFTER INJECTION INTO THE MAMMARY GLAND CISTERNS OF THREE DRY EWES TOGETHER WITH THE COMPOSITION OF THE INJECTED MILK

Samples were collected at 5 min, and at 1, 2, and 4 hr. The values presented are means of the two sides over the three sheep together with the respective standard errors. The volume of fluid recovered was 92% of that injected

Sample	Chloride (m-equiv/l)	Lactose (mM)	Sodium (m-equiv/l)	Potassium (m-equiv/l)
Milk injected	42·2±8·5	123·7± 9·2	34·5±8·2	40·8±2·5
5 min	45·0±5·4	122·8± 8·6	35·0±5·1	34·6±2·0
1 hr	52·7±5·8	116·2± 9·2	50·5±7·2	32·6±2·2
2 hr	58·3±6·4	109·8± 9·3	59·2±7·2	29·7±2·5
4 hr	64·7±6·1	101·4±10·0	72·2±8·3	27·6±2·9

The changes in composition of a solution containing 90 mM sodium chloride, 80 mM lactose, and 1 m-equiv/l potassium after injection into the dry gland, are presented in Table 4. The concentration of sodium, potassium, and chloride increased

TABLE 4

CHANGES IN COMPOSITION OF A SOLUTION CONTAINING SODIUM CHLORIDE AND LACTOSE AFTER INJECTION INTO THE MAMMARY GLAND CISTERNS OF A DRY EWE TOGETHER WITH THE COMPOSITION OF THE INJECTED SOLUTION

Samples were collected at 1, 2, and 3 hr after the injection. The values are the means for the two sides. 12 ml of solution were injected into each side and 10·6 ml were recovered

Sample	Chloride (m-equiv/l)	Lactose (mM)	Sodium (m-equiv/l)	Potassium (m-equiv/l)
Solution injected	93·6	81·9	92·6	0·9
1 hr	96·1	78·2	98·0	1·6
2 hr	95·3	73·8	98·0	2·7
3 hr	94·4	59·7	102·4	4·3

slightly during the experiment while that of lactose decreased markedly. After 4 hr the concentration of potassium was similar to, while that of sodium and chloride were less than plasma concentrations.

IV. Discussion

In our attempts to demonstrate ion exchange in the lactating mammary gland, it has been necessary to estimate the amount of milk mixing with the injected solution. The calculations were based on changes in volume estimated from changes in concentration of a marker in the solution injected into the cisterns. This method of estimation of volume change is valid providing the marker is neither absorbed nor adsorbed and its distribution is the same as that of the other constituents in the injected solution. The recoveries of the marker were from 69 to 97%, with an average of 86%, which suggests that some of the marker may have been absorbed or adsorbed in some of the experiments. However, it is more likely that recovery of the contents of the gland was incomplete in these cases since the glands were only washed by the small amount of milk ejected after the injection of oxytocin at the end of the experiment. In any case, if absorption or adsorption had occurred, an overestimation of the quantity of milk in the cisterns would be obtained and this would have resulted in an underestimation of the movement of ions across the epithelium.

It is even more difficult to tell whether the distribution of the marker was the same as that of the other constituents in the injected solution. It is probable that in the relatively large volume of the cisterns and larger ducts the distribution of the marker and the other solutes was similar since purely mechanical factors would be expected to be more important than diffusion in the mixing of solutes. Thus any discrepancy between the distribution of the marker and the other constituents of the injected solution would probably have been small and confined to the smaller ducts. The calculations were based on the analyses of subsamples drawn from the mixture in the cisterns. The concentration of marker in the mixture drawn after the intravenous injection of oxytocin was always lower than that in the mixture obtained immediately prior to the injection. This indicated that the marker was not distributed evenly throughout the duct system. Similar results have been obtained with vegetable dyes (McFarlane 1952; Rasmussen 1964). Thus, neither the labelled albumin nor the much smaller molecules of the vegetable dyes were distributed evenly throughout the mammary gland after their injection into the cisterns. It is probable that factors other than diffusion, such as partial collapse of the duct system after milking, inhibited the distribution of these markers.

It has been reported by several authors (Jackson and Rothera 1914; Brown, Petersen, and Gortner 1926; Petersen 1944) that the injection of any solution into the cistern of the mammary gland results in the production of an abnormal secretion. The reasons for the abnormal function of the gland are not clear although the results of Brown, Petersen, and Gortner (1936) could have been due to a distension of the epithelium allowing some direct communication between the extracellular fluid and the milk. The composition of the milk collected after 4 hr in the present experiments was very similar to that of the original milk. It is considered therefore that the secretory process remained essentially unchanged over the experimental period.

The composition of the original milk in the lactating gland has been approached from solutions containing higher and from those containing lower concentrations of sodium and chloride. Sodium, potassium, and chloride moved from the epithelial

cells into the mixture in the cisterns following the injection of a solution containing a low concentration of sodium, chloride, and potassium (Table 1). The experiments in which solutions containing 65 mM sodium chloride and 145 mM lactose were injected indicated that sodium and chloride ions were moving from the mixture into the epithelial cells and that potassium ions were moving in the opposite direction (Table 2). Although it was considered unlikely that lactose would be absorbed from the cistern of the lactating gland, the protein concentration was determined to check the possibility. The probable inaccuracy of some of these determinations has been mentioned earlier and this is thought to be responsible for the discrepancy between the results for the protein and lactose (Table 1). It is considered that lactose was neither absorbed nor secreted in the cisterns and ducts and that the results in Table 2 indicate water absorption from the cisterns. This was not taken into account in the calculations of the movement of sodium, potassium, and chloride ions. Thus the values for sodium and chloride absorption would be underestimated while those for potassium overestimated. The decrease in concentration of sodium and chloride ions in the mixture must ultimately result in the movement of either sodium or chloride ions against an electrochemical gradient from the milk to the blood. In these circumstances the active transport of either ion would be expected to result in the passive movement of the other ion in the same direction.

Although the solutions infused into the lactating gland were hypotonic to plasma and milk, there was little water movement from solutions containing either high or low concentrations of sodium chloride. The low sodium chloride solution had an estimated osmotic pressure of 250 m-osmoles/l but water absorption was not detected. Some water absorption probably occurred from the high sodium chloride solutions either as a result of the further lowering of the osmotic pressure by the absorption of sodium chloride or as a result of a closer link between the absorption of water and that of sodium. The failure to demonstrate significant absorption of water was unexpected since normal milk is isotonic with blood (Jenness and Patton 1959) and the results of the experiments by Mackenzie and Lascelles (1965) had indicated that the absorption of water from alveolar milk occurred as it passed through the ducts and cisterns.

The active transfer of sodium ions across a number of epithelial membranes has been demonstrated (Ussing 1958). It appears almost certain that a similar mechanism is functioning in the epithelium of the larger ducts and cisterns of the mammary gland. The present data are not adequate, however, to give a detailed hypothesis on the movement of ions across the epithelium. More precise data are required on the relationship between sodium, potassium, chloride, and water fluxes. Information is also required on the potential difference across the epithelium and the concentration of the ions within the epithelial cells. The hypothesis would need to explain the movement of sodium and chloride ions from the milk to the blood against a concentration gradient and the maintenance of the potassium ion concentration in the milk.

The mammary glands of some dry ewes contains a small quantity of involution secretion which does not resemble milk either physically or chemically. The involution secretion taken from one of the ewes in the present experiments contained

82 m-equiv/l chloride, 130 m-equiv/l sodium, 9 m-equiv/l potassium, and less than 1 mM lactose. Thus, the electrolyte content of the secretion was between that of plasma and normal milk. The results obtained after the injection of solutions into the mammary gland of dry ewes indicated that the concentration of the constituents changed very slowly towards the composition of involution secretion (Tables 3 and 4). The data suggest that the ducts either have a decreased capacity to absorb sodium and chloride or the amount of sodium and chloride passing into the ducts is greatly increased during the dry period.

The possible effect of oxytocin on the composition of the milk secreted was discussed by Mackenzie and Lascelles (1965). It is not possible from the present data to attribute any specific effect of oxytocin on milk secretion. The changes in ionic composition occurred in the dry gland in the absence of oxytocin and similar changes were measured in the lactating gland in the 2–4 hr period after the injection of oxytocin.

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Appendix

Derivation of the theoretical concentration of a solute in the mixture in the mammary gland at the second sampling period

$$M_{\text{remaining}} = M_{\text{injected}} - M_{\text{sample(1)}},$$

$$V_{\text{total}} = M_{\text{remaining}}/[M_{\text{sample(2)}}],$$

$$V_{\text{remaining}} = M_{\text{remaining}}/[M_{\text{injected}}],$$

$$V_{\text{milk}} = V_{\text{total}} - V_{\text{remaining}},$$

$$[S_{\text{theoretical}}] = \frac{(V_{\text{milk}} \times [S_{\text{milk}}]) + (V_{\text{remaining}} \times [S_{\text{injected}}])}{V_{\text{total}}},$$

where

$M_{\text{remaining}}$ = Quantity of marker remaining in the gland after the previous sampling,

M_{injected} = Quantity of marker injected into the gland,

$M_{\text{sample(1)}}$ = Quantity of marker removed in the previous sample,

V_{total} = Total volume of fluid in the gland at sampling,

$[M_{\text{sample(2)}}]$ = Concentration of marker in the gland at sampling,

$V_{\text{remaining}}$ = Volume of the injected solution remaining in the gland after the previous sampling,

$[M_{\text{injected}}]$ = Concentration of the marker in the injected solution,

V_{milk} = Volume of milk in the gland at sampling,

$[S_{\text{theoretical}}]$ = Theoretical concentration of a solute in the gland at sampling due to mixing of milk with the injected solution remaining in the gland,

$[S_{\text{milk}}]$ = Concentration of the solute in the milk,

$[S_{\text{injected}}]$ = Concentration of the solute in the solution injected.