J. Physiol. (1979), **290**, *pp.* 59–67
With 2 text-figures
Printed in Great Britain

THE SECRETION OF CALCIUM AND PHOSPHORUS INTO MILK

BY M. C. NEVILLE† AND M. PEAKER‡*

From the Agricultural Research Council Institute of Animal Physiology, Babraham, Cambridge CB2 4AT

(*Received 4 September 1978*)

SUMMARY

1. The time course of appearance of radioactivity in milk was studied following close-arterial infusion of labelled phosphate, Ca or leucine into the mammary artery of lactating goats. Maximum activities were reached at 1·5 hr in all milk fractions including inorganic soluble phosphate, inorganic colloidal phosphate, casein P, soluble Ca, protein-associated Ca and casein.

2. At 0·5 hr, labelling of the soluble and colloidal phosphate fractions was significantly higher than that of the casein P.

3. Recovery of ^{32}P or ^{47}Ca 3 or more hours after infusion into the cistern of the mammary glands was 98% or greater, indicating that the mammary epithelium is virtually impermeable to [^{32}P]phosphate and ^{47}Ca in the milk to blood direction.

4. Ca and P failed to enter milk in excess of the normal secretion rate when the milk was diluted with isosmotic sucrose given by intraductal injection.

5. These data suggest that milk Ca and phosphate in their various forms are secreted, like protein and lactose, by exocytosis of Golgi vesicles. Unless a paracellular pathway is present, as in oxytocin-treated animals, the milk concentrations are maintained by virtue of the impermeability of the mammary epithelium to these substances.

INTRODUCTION

Three main routes for the secretion of milk components have been established from electron microscopical, biochemical and physiological studies (Linzell & Peaker, 1971*a*; Peaker, 1978). (1) Substances like Na, K, Cl and water appear to pass directly across the apical (luminal) membrane of the secretory cell, establishing an equilibrium between their intracellular activity and that of the milk. (2) Other substances, casein and lactose, for example, are synthesized and/or sequestered within the lumen of the Golgi apparatus, then discharged across the apical membrane by exocytosis of secretory vesicles. It is likely that Na, K, Cl and water also equilibrate across the membrane of the secretory vesicle. (3) Lipids enter the milk in the form of milk fat globules which are extruded from the apical surface of the cell invested in apical membrane. Although solutes may enter milk via the

* Reprint requests to M.P.

† Permanent address: Department of Physiology, University of Colorado Medical Center, Denver, Colorado 80220, U.S.A.

‡ Present address: The Hannah Research Institute, Ayr, Scotland KA6 5HL.

0022-3751/79/3120-0713 $01.50

paracellular route under certain conditions, there is evidence that this route is not used in the normal secretion of milk during established lactation in the goat.

When isotopically-labelled milk components or their precursors were injected into the blood stream of lactating goats, it was possible to distinguish these three secretion routes on the basis of the time of appearance of isotope in milk (Linzell, Mepham & Peaker, 1976). Thus, substances that are thought to enter by permeating the apical membrane reached a maximum activity within 1 hr; those entering by the Golgi route (for example, citrate and lactose), in 2–3 hr and those in milk fat, in 5–7 hr.

In this paper we describe experiments aimed at determining the route of secretion of two other important milk constituents, Ca and P (see Holt & West, 1977). Although there have been several studies on the appearance of milk P compounds and Ca (for example, Aten & Hevesy, 1938; Barry, 1952; Azimov, Orlov & Belugina, 1961; Kronfeld, Ramberg & Delivoria-Papadopoulos, 1971), in none of these was the resolution of the time course adequate for our purpose. Further, since both elements occur in a number of different forms in milk, it was necessary to consider the secretion of aqueous and colloidal fractions separately. The permeability of the mammary epithelium to Ca and phosphate in the milk to blood direction has also been studied in order to determine the mechanism by which the milk concentrations are maintained during storage in the gland.

METHODS

Animals. Experiments were conducted on lactating goats in which a mammary ('milk' or 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 an exteriorized carotid artery and jugular vein respectively (Linzell, 1963*b*); this enabled catheterization and close-arterial infusion to be carried out without further surgery by Seldinger's (1953) technique under local anaesthesia. The goats were milked daily during lactation at approximately 09.00 and 16.00 hr; the time of milking and the yield of each gland was recorded.

Time course of secretion of P, Ca and casein. In three goats, oxytocin (100 m-u. I.V.) was injected after morning milking and residual milk removed. One hr later this process was repeated in order to remove as much as possible of the milk formed before the experiment. A close-arterial infusion of [^{32}P]phosphate (Na[^{32}P]phosphate injection, Radiochemical Centre, Amersham) was then made into the transplanted gland for 14 min at approximately 3 μc/min. Thereafter the infused glands were milked 0·5, 1, 1·5, 2, 3, 4 and 5 hr after the start of infusion. Oxytocin (100 m-u. i.v.) was given immediately before each milking. In four goats, the same procedure was adopted for ^{47}Ca ($^{47}CaCl_2$ injection, Radiochemical Centre, Amersham), 15 μc being given over 15 min in two goats and 5 min in the other two; in the latter two 15 μc L-[U-^{14}C]leucine was included in the infusate.

Permeability to ^{32}P and ^{47}Ca from milk to blood. Approximately 2 hr after morning milking 25 ml. milk were drawn aseptically from one gland of each of five goats and mixed with approximately 10 μc ^{32}P. Two ml. were then taken for determination of radioactivity and the rest infused into the cistern of the same gland via the teat canal; the gland was then gently massaged. Blood samples were taken from the 'milk' vein 1, 2 and 3 hr after the injection. The samples were taken while the external pudic vein was being compressed manually in order to obtain pure mammary venous blood (Linzell, 1960). The glands were then milked, oxytocin (2 × 100 m-u. I.V.) being given to obtain as much of the residual milk as possible. Isotope recovery was calculated from the product of the isotope concentration in the milk and the milk volume. Similar experiments were done in six goats using approximately 15 μc ^{47}Ca.

These experiments were repeated in two goats for ^{32}P and five goats for ^{47}Ca. The animals were given large I.V. doses (1 u.) of oxytocin, 60 min before, and 10 and 100 min after, the infusion of isotope into the teat.

Passage of phosphate and Ca into diluted milk. In five goats for phosphate and four goats for Ca, 100 ml. sterile, isosmotic sucrose (300 mM) were injected into one gland via the teat canal approximately 3 hr after morning milking; the gland was then massaged gently. Three hours later both glands were milked and the milk Ca and phosphate contents determined. In another experiment 250 ml. sterile citrate solution (30 mM-Na citrate, 240 mM-sucrose) were infused in the same manner.

Handling milk samples. Three fractions of milk phosphorus were studied: soluble inorganic (P_s), colloidal inorganic (P_c) and casein phosphorus (P_{cas}). Trichloroacetic acid (TCA) filtrates of milk, which contain P_s plus P_c, were prepared by the method of White & Davies (1958). For P_s, ultracentrifugates were obtained by spinning milk at 10^5 *g* for 1 hr at 20 °C (see also Davies & White, 1960). Following determination of concentration and radioactivity in these preparations, the contribution of P_c was obtained by difference. For P_{cas} casein was precipitated from skim milk and washed by the method of Rowland (1938). After digestion of the precipitate in 60% (w/v) perchloric acid, samples were taken for determination of phosphorus and radioactivity.

Two fractions of milk Ca were studied: protein-associated Ca (Ca_p, i.e. colloidal Ca plus casein Ca) and soluble Ca (Ca_s, i.e. soluble, both non-ionized and ionized). Whole milk, which contains $Ca_p + Ca_s$, and ultracentrifugate, which contains Ca_s (White & Davies, 1958; Davies & White, 1960) were analysed; the contribution of the protein-associated Ca fraction was obtained by difference.

For the determination of ^{14}C in casein containing ^{47}Ca, a washed casein precipitate (see above) was dissolved in 0·25 M-NaOH, reprecipitated with an equal volume of 10% (w/v) TCA and then washed three times with 5% (w/v) TCA. Finally the precipitate was dissolved in 1 M-NaOH for ^{14}C counting.

Analytical methods. Inorganic phosphate in TCA filtrates and ultracentrifugates was determined in an autoanalyser using Technicon method N-4c, and P in casein digests by the method of King as described by Lindberg & Ernster (1956). ^{47}Ca was determined in a well-type solid scintillation counter, and ^{32}P and ^{14}C by liquid scintillation spectrometry.

Calculation of data. Results on the time course of isotope secretion were normalized by expressing the value at each time point as a percentage of the maximum value obtained in that experiment. For P fractions the results were calculated as specific activities. For Ca fractions, we found it difficult to obtain reliable Ca values when the milk was stored for a period sufficient for ^{47}Ca to decay to negligible levels. Therefore the results were calculated from the radioactivity of the milk samples. Because the Ca content of the various milk fractions did not vary significantly during the course of the experiment, the results are similar to those which would have been obtained from a calculation of specific activity. Data from leucine incorporation into protein were handled similarly to those for Ca.

RESULTS

Milk concentrations of P and Ca

Table 1 gives the Ca and P concentrations of normal goat milk. Of the 20 mM-phosphate about one third (7 mM) was in the soluble phase, nearly one half was non-covalently bound to protein and about one fourth was covalently bound to casein. Of the 27 mM Ca about two thirds were associated with protein and one third in the soluble phase. Since the Ca^{2+} activity determined with a Ca^{2+}-electrode is about 1 mM in goat milk (I. R. Fleet & M. Peaker, unpublished), a portion of the soluble Ca must be complexed with citrate and soluble phosphate.

Time course of secretion

Radioactive phosphate, Ca and leucine were infused into the transplant arteries of lactating goats as described in Methods. For each experiment, the value at each time was expressed as a percentage of the maximum obtained in that experiment; means ± S.E. of these percentages are shown in Figs. 1 and 2.

TABLE 1. Ca and P concentrations in normal goat milk. Values represent the mean ± S.E. of mean from eight milk samples from four different goats

		mM
P		
	Total	20·5 ± 0·8
	Inorganic soluble (P_s)	6·7 ± 0·4
	Inorganic colloidal (P_c)	8·9 ± 0·6
	Casein-bound (P_{cas})	4·9 ± 0·2
Ca		
	Total	26·6 ± 0·8
	Soluble (Ca_s)	8·7 ± 0·6
	Protein-associated (Ca_p)	18·0 ± 0·6

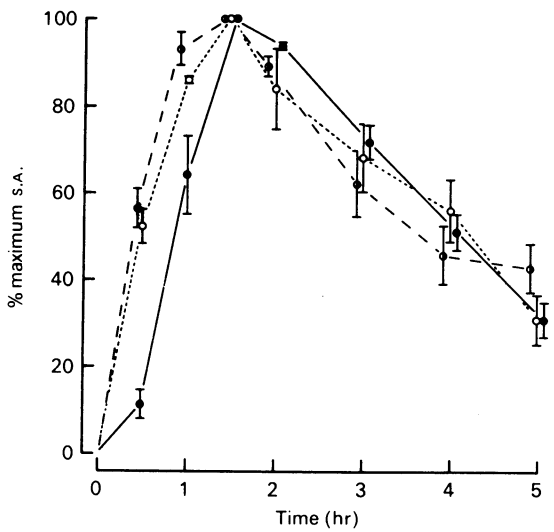

Fig. 1. Time course of the changes in specific activities (S.A.) of inorganic soluble phosphate, P_s (○---○), inorganic colloidal phosphate, P_c (◑---◑) and casein phosphorus, P_{cas} (●—●) in milk following the I.A. administration of [^{32}P]phosphate in three goats, treated and milked as described in the text. In each experiment, the value at each time was expressed as a percentage of the maximum achieved in that experiment; shown are the means ± S.E. of these percentages.

All three fractions of milk P reached their maximum specific activity 1·5 hr after administration of ^{32}P. However, the labelling of the soluble (P_s) and colloidal (P_c) fractions of P increased more rapidly than that of the casein-bound (P_{cas}). Thus at 0·5 hr, P_s and P_c were 52 ± 3·8% and 56 ± 4·7% respectively of the maxima, while P_{cas} was only at 11 ± 3·1% ($P < 0·01$). Maximum radioactivities in the two Ca fractions were also reached at 1·5 hr. While the activity of the protein-associated fraction tended to be higher than that of Ca_s at 0·5 and 1 hr, the difference was not statistically significant (Fig. 2*A*). For [^{14}C]casein from [^{14}C]leucine, the maximum mean for the two experiments was also obtained at 1·5 hr, although the value for 2 hr was very similar (94·8 and 93% respectively) (Fig. 2*B*).

Therefore, the time taken for all the fractions of milk P and Ca studied, as well as for leucine in casein, to reach their maximum radioactivity, was similar.

Permeability to ^{32}P phosphate and ^{47}Ca

Recovery of ^{32}P in the milk 3 hr after the introduction of [^{32}P] phosphate into the cistern of the gland was 98% in five goats (Table 2). The recovery of ^{47}Ca was only 92·8% at the 3 hr milking. However, the remainder of the infused isotope was recovered during the subsequent two milkings bringing the total recovery of ^{47}Ca to 99·7%. Mammary venous blood radioactivity could not be distinguished

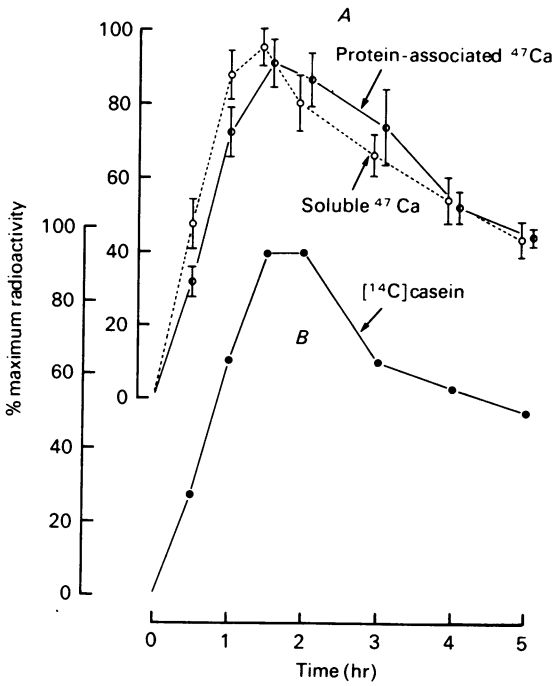

Fig. 2. Time course of the changes in, (*A*) radioactivities of soluble Ca, Ca_s (○---○) and protein-associated Ca, $Ca_c + Ca_{cas}$ (◑—◑) in milk following the I.A. administration of ^{47}Ca in four goats, treated and milked as described in the text, and (*B*) radioactivities of casein (●—●) following the administration of [^{14}C]leucine in two goats. In each experiment, the value at each time was expressed as a percentage of the maximum achieved in that experiment; shown in *A* are the means ± S.E. and in *B* the means, of these percentages.

statistically from background. These results indicate that the mammary epithelium is virtually impermeable to phosphate and Ca passing from milk to blood. Under similar circumstances, ^{24}Na, ^{36}Cl and ^{42}K, which are thought to permeate the secretory epithelium, pass out of milk, as well as ^{3}HOH and [^{14}C]urea which are thought also to permeate the duct epithelium (Linzell & Peaker, 1971*a*, *b*, *c*; Peaker, 1978); by contrast lactose and citrate remain in milk (Linzell & Peaker, 1971*a*, *b*; Linzell *et al.* 1976). The difference between ^{47}Ca and [^{32}P]phosphate recovery at the 3 hr milking could reflect binding of Ca to the luminal surface of the mammary epithelium.

Only 18·2 ± 0·5% of the radioactivity in the milk removed at 3 hr from four goats given ^{47}Ca was present in the soluble fraction. This indicates considerable exchange between soluble and protein-associated fractions and suggests that citrate-bound Ca exchanges very slowly.

By contrast with the results in normal goats, during treatment of goats with large doses of oxytocin (see Methods) in two goats, the recovery of ^{32}P was only 57 and 64% after 3 hr. Such doses of oxytocin are believed to disrupt the junctional complexes of the secretory epithelium, thereby allowing substances to pass directly between extracellular fluid and milk by a paracellular route (Linzell & Peaker, 1971*d*; Linzell, Peaker & Taylor, 1975). In the case of ^{47}Ca in five goats treated in a similar manner, the recovery was 77·3% after 3 hr and 90% after three milkings.

The results in untreated goats are not in agreement with other studies in cows and goats. Kleiber & Black (1956) injected [^{32}P]phosphate into one gland of cows

TABLE 2. Recovery of [^{32}P]phosphate and ^{47}Ca after infusion via the teat canal

	Hours after infusion	Percent recovery: No treatment	Percent recovery: Oxytocin* (1 u. each time)
[^{32}P]phosphate			
First milking		98 ± 1 (5)	60·5 (2)
^{47}Ca			
First milking	3	92·7 ± 0·8 (5)	77·3 ± 4·4 (5)
Second milking	4	3·2 ± 0·4 (5)	6·9 ± 1·2 (5)
Third milking	21	3·9 ± 0·7 (5)	7·4 ± 1·2 (4)
Total		99·7 ± 1·1 (5)	89·7 ± 5·3 (4)

* See text.

and found that [^{32}P]casein was formed by all glands although the specific activity of that formed by the injected gland was higher than that of the other three. Kronfeld *et al.* (1971) found a loss of ^{47}Ca from milk in the cow. However, the movement of these substances would not be unexpected in the dairy cow since the integrity of the epithelium is often disrupted by present or past damage from pathogenic organisms (see Linzell & Peaker, 1972, 1975). Azimov *et al.* (1961) found that ^{32}P and ^{47}Ca appeared in blood following intramammary administration in goats but they made no attempt to measure the losses from milk. However, rough calculations from their data, in which large amounts of radioactivity were given, indicate the losses to be extremely small. Knutsson (1964) found that only 62–74% of ^{32}P was recovered in milk after 1·7–1·9 hr in goats. But, during this period he massaged the udder for 1 min in every 5 with an electrical cosmetic vibrator. From the results of Linzell & Peaker (1971*d*) in which frequent (hourly) milking led to disruption of the epithelium, it can clearly be argued that the very frequent massage in Knutsson's studies had a similar effect, probably by contracting the myoepithelium and compressing the alveoli, and that ^{32}P crossed via a paracellular route.

Passage of phosphate and Ca into diluted milk

Linzell *et al.* (1976) devised a method to investigate whether the movements of substances into milk are influenced by altering the concentration gradient across the apical membrane. When milk in the gland was diluted by adding isosmotic lactose (or sucrose in unpublished experiments) it was established that little or no water crossed the epithelium osmotically and that while Na, K and Cl entered milk

in excess of the secretion rate thus tending to restore milk concentrations to normal, there was no compensatory increase for protein, citrate and total Ca. Thus it was inferred that the rate of extrusion of these latter substances from the secretory cell continued at the previous rate even though the concentrations were lowered in milk, as would be expected of secretion by the Golgi route.

In the present studies, the movements of inorganic phosphate and Ca have been investigated over 3 hr (1 hr longer than previously) with 100 ml. 300 mM-sucrose given 3 hr after morning milking, and milking 6 hr after morning milking. The quantity of a substance entering the diluted milk was calculated in the following manner (Linzell *et al.* 1976). It was assumed that the milk secreted during the 6 hr period between milkings had the same composition as that secreted over the previous day. Therefore the expected amount of the substance was taken as the previous concentration × total volume of milk after 6 hr – 100 ml. (the volume of the diluent). The actual amount obtained was calculated from the concentration and total volume at the end of 6 hr. Had the movement of phosphate and Ca completely compensated for dilution (i.e. the concentration restored to normal) the amount obtained as a percentage of the amount expected would have been $121 \pm 2{\cdot}1$ (S.E.)% for the phosphate experiments (five goats) and $129 \pm 3{\cdot}0$ for the Ca experiments (four goats). In fact the percentages obtained were $99 \pm 4{\cdot}2$ for $P_c + P_s$, $97{\cdot}4 \pm 4{\cdot}6$ for P_s, $105 \pm 2{\cdot}0$ for whole milk Ca and $104 \pm 3{\cdot}1$ for Ca_s. In four goats 30 mM-sodium citrate was substituted for 60 mM-sucrose in the infusate and 250 ml. infused. Although this concentration of citrate should have reduced the free Ca in the milk nearly to zero the amount of Ca secreted was 103 ± 3% of that expected.

DISCUSSION

Linzell *et al.* (1976) suggested that since maximum labelling of citrate and lactose, and casein in other experiments, occur at similar times during frequent milking following the intravascular administration of labelled precursors, and that since, on other evidence, lactose and casein are secreted by the Golgi route, citrate is also secreted by this means. By contrast, maximum labelling of substances which are thought to permeate the apical cell membrane and of these secreted as part of the milk fat globule, occurred earlier and later respectively. In the present experiments (where milking and sampling were, incidentally, more frequent than in earlier studies) maximum labelling of all the P and Ca fractions studied coincided with that of casein from labelled leucine. Therefore, we suggest that milk Ca and P in their various forms are secreted by exocytosis of Golgi vesicles. The failure of Ca and P to enter diluted milk in excess of the normal secretion rate is also compatible with this view.

There is evidence from other studies that at least some fractions of milk Ca and P are secreted by the Golgi apparatus. For example, electron microscopical histochemical localization with microanalysis shows Ca in the cisternae and vacuoles (Wooding, 1977), and Baumrucker & Keenan (1975) have some evidence to suggest that Golgi-rich fractions from mammary homogenates accumulate Ca by an ATP-dependent process. Bingham, Farrell & Basch (1972) have also shown that Golgi fractions contain a kinase for the phosphorylation of casein. Recently, Kuhn & White (1977) have suggested that inorganic phosphate is generated within the Golgi lumen by the hydrolysis of UDP as part of the process of lactose synthesis.

Although maximum labelling occurred at the same time the slower time course of labelling of P_{cas} compared with P_c and P_s should be pointed out. While the reason for this difference is not known, one possible explanation is that inorganic phosphate may be added at a later stage of Golgi vesicle differentiation than phosphorylation of casein.

The failure of labelled Ca and phosphate to permeate the apical membrane, except when paracellular movements supervene, indicates that the Ca and P compounds, once secreted into milk, are held at their concentrations by virtue of the relative impermeability of the alveolar and duct epithelia to these substances. If Ca^{2+} activity in the alveolar cells is, as in other cells, very low, the finding that Ca does not flow down a concentration gradient into milk, is in accord with the hypothesis that active transport mechanisms are necessary for Ca secretion into the Golgi vesicles. The possibility of active Ca extrusion across the apical epithelium cannot be ruled out on the basis of these experiments.

We are most grateful to Jane Goode, I. R. Fleet and A. J. Davis for assistance.

REFERENCES

ATEN, A. H. W. & HEVESY, G. (1938). Formation of milk. *Nature, Lond.* **142**, 111–112.

AZIMOV, G. I., ORLOV, A. F. & BELUGINA, O. P. (1961). Sekretsiya moloka i ee zakonomernosti. *Zhivotnovodstvo* **1**, 40–48.

BARRY, J. M. (1952). The source of lysine, tyrosine and phosphorus for casein synthesis. *J. biol. Chem.* **195**, 795–803.

BAUMRUCKER, C. R. & KEENAN, T. W. (1975). Membranes of the mammary gland. X. ATP dependent calcium accumulation by Golgi apparatus fractions from bovine mammary gland. *Expl Cell Res.* **90**, 253–260.

BINGHAM, E. W., FARRELL, H. M. & BASCH, I. J. (1972). Phosphorylation of casein. Role of the Golgi apparatus. *J. biol. Chem.* **247**, 8193–8194.

DAVIES, D. T. & WHITE, J. C. D. (1960). The use of ultrafiltration and dialysis in isolating the aqueous phase of milk and in determining the partition of milk constituents between the aqueous and disperse phases. *J. Dairy Res.* **27**, 171–189.

HOLT, C. & WEST, D. W. (1977). The salt composition of milk and formation of bovine casein micelles. *Hannah Research Institute, Annual Report*, pp. 57–61.

KLEIBER, M. & BLACK, A. L. (1956). Tracer studies on milk formation in the intact dairy cow. In *Atomic Energy Commission Report*, no. TID 7512. Washington D.C.

KNUTSSON, P-G. (1964). Exchange of sodium, potassium, chloride, and phosphate ions across the mammary epithelium in the goat. *LantbrHögsk. Annlr* **30**, 477–506.

KRONFELD, D. S., RAMBERG, C. F. & DELIVORIA-PAPADOPOULOS, M. (1971). Active transport of calcium across placenta and mammary gland measured *in vivo*. In *Cellular Mechanisms for Calcium Transfer and Homeostasis*, ed. NICHOLS, G., pp. 339–347. New York: Academic.

KUHN, N. J. & WHITE, A. (1977). The role of nucleotide diphosphatase in a uridine nucleotide cycle associated with lactose synthesis in rat mammary-gland Golgi apparatus. *Biochem. J.* **168**, 423–433.

LINDBERG, O. & ERNSTER, L. (1956). Determination of organic phosphorus compounds by phosphate analysis. In *Methods of Biochemical Analysis*, vol. 3, ed. GLICK, D., pp. 1–22. New York: Interscience.

LINZELL, J. L. (1960). Mammary-gland blood flow and oxygen, glucose and volatile fatty acid uptake in the conscious goat. *J. Physiol.* **153**, 492–509.

LINZELL, J. L. (1963*a*). Carotid loops. *Am. J. vet. Res.* **24**, 223–224.

LINZELL, J. L. (1963*b*). Some effects of denervating and transplanting mammary glands. *Q. Jl exp. Physiol.* **48**, 34–60.

LINZELL, J. L., MEPHAM, T. B. & PEAKER, M. (1976). The secretion of citrate into milk. *J. Physiol.* **260**, 739–750.

LINZELL, J. L. & PEAKER, M. (1971*a*). Mechanism of milk secretion. *Physiol. Rev.* **51**, 564–597.

LINZELL, J. L. & PEAKER, M. (1971*b*). Intracellular concentrations of sodium, potassium and chloride in the lactating mammary gland and their relation to the secretory mechanism. *J. Physiol.* **216**, 663–700.

LINZELL, J. L. & PEAKER, M. (1971*c*). The permeability of mammary ducts. *J. Physiol.* **216**, 701–716.

LINZELL, J. L. & PEAKER, M. (1971*d*). The effects of oxytocin and milk removal on milk secretion in the goat. *J. Physiol.* **216**, 717–734.

LINZELL, J. L. & PEAKER, M. (1972). Day-to-day variations in milk composition in the goat and cow as a guide to the detection of subclinical mastitis. *Br. vet. J.* **128**, 284–295.

LINZELL, J. L. & PEAKER, M. (1975). Efficacy of the measurement of the electrical conductivity of milk for the detection of subclinical mastitis in cows: detection of infected cows at a single visit. *Br. vet. J.* **131**, 447–461.

LINZELL, J. L., PEAKER, M. & TAYLOR, J. C. (1975). The effects of prolactin and oxytocin on milk secretion and on the permeability of the mammary epithelium in the rabbit. *J. Physiol.* **253**, 547–563.

PEAKER, M. (1978). Ion and water transport in the mammary gland. In *Lactation*, vol. 4, ed. LARSON, B. L., pp. 437–462. New York: Academic.

ROWLAND, S. J. (1938). The precipitation of milk protein and the determination of the nitrogen distribution of milk. *J. Dairy Res.* **9**, 30–46.

SELDINGER, S. I. (1953). Catheter replacement of the needle in percutaneous arteriography. A new technique. *Acta radiol.* **39**, 368–376.

WHITE, J. C. D. & DAVIES, D. T. (1958). The relation between the chemical composition of milk and the stability of the caseinate complex. 1. General introduction, description of samples, methods and chemical composition of samples. *J. Dairy Res.* **25**, 236–255.

WOODING, F. B. P. (1977). Comparative mammary fine structure. In *Comparative Aspects of Lactation*, ed. PEAKER, M. *Symp. zool. Soc. Lond.* **41**, pp. 1–41. London: Academic.