J. Physiol. (1984), **347**, *pp.* 453–463
With 4 text-figures
Printed in Great Britain

LACTOSE IN PLASMA DURING LACTOGENESIS, ESTABLISHED LACTATION AND WEANING IN SOWS

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(*Received 21 June 1983*)

SUMMARY

1. The concentration of lactose in plasma was determined in different sows at all phases of their reproductive cycle and related to the compositional changes in mammary secretion during lactogenesis, established lactation and weaning.

2. Lactose was present in low concentrations (3–4 μM) in the blood of virgin sows and pregnant sows up to 107 days of gestation.

3. From day 4 pre-partum to day 1 pre-partum circulating lactose rose gradually to $34{\cdot}5 \pm 7{\cdot}7$ μM (mean ± S.E. of mean). Maximal concentrations of $262 \pm 168{\cdot}4$ μM were reached 6 h after parturition. The concentration of lactose in plasma was correlated with the amount of lactose in mammary secretion ($r = 0{\cdot}88$, $P < 0{\cdot}01$) at the beginning of farrowing.

4. During established lactation the concentrations of lactose, Na and K in milk, and of lactose in plasma (72–86 μM), were constant.

5. The concentration of lactose in plasma did not vary significantly during periods of suckling, or after stimulation of milk ejection by oxytocin. However, the amount of lactose in plasma rose significantly ($P < 0{\cdot}02$) after the administration of oxytocin if milk ejection was not accompanied by suckling.

6. The mean plasma concentration of lactose began to rise 36 h after weaning to a peak value of $241{\cdot}8 \pm 53{\cdot}6$ μM at 48 h; thereafter it declined to $10{\cdot}2 \pm 2{\cdot}0$ μM by 6 days.

7. This study has shown that lactose concentrations in the plasma vary according to the secretory activity of the mammary gland. Its plasma concentration provides an earlier temporal measure of lactogenesis in individual sows than is obtained either from observation or analysis of mammary secretion.

INTRODUCTION

The initiation of lactation in rats and rabbits and other small animals has been assessed either by measuring the amount of lactose in mammary slices or by their incorporation of radioactively labelled glucose into lactose (Chadwick, 1962; Shinde, Ota & Yokoyama, 1965; Kuhn, 1969; Nicholas & Hartmann, 1981). However, such

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an invasive procedure is impractical in larger, more expensive animals. Lactogenesis in cows, sheep and women has been related to the concentration of lactose in their mammary secretions (Hartmann, 1973; Hartmann, Trevethan & Shelton, 1973; Kulski, Smith & Hartmann, 1977).

Previous studies have assessed lactogenesis in the sow either subjectively as the earliest time perinatally at which mature milk could be expressed from the teat (Nara & First, 1981; Nara, Welk, Rutherford, Sherwood & First, 1982) or in terms of the lactose concentrations in post-partum milk samples (Martin, Hartmann & Gooneratne, 1978; Gooneratne, Hartmann, McCauley & Martin, 1979). However, studies in this laboratory have shown that the onset of lactation in different sows is rapid but variable, and that lactogenesis is not timed accurately by the subjective observation of milk secretion (Willcox, Arthur, Hartmann & Whitely, 1983).

The blood of male and non-lactating female mammals contains only small amounts of lactose, derived principally from absorption by the gastrointestinal tract and some synthesis by body tissues (Weser & Sleisenger, 1967; Vitek, Vitek & Adams Cowley, 1975). High concentrations of lactose are found in the urine when the mammary glands of female mammals are functionally active. For example, its concentration in cow urine increases at calving and during weaning (Wheelock & Rook, 1966). In the plasma of pregnant goats, lactose concentrations rise from 4 μM at day 4 pre-partum to 33 μM about 12 h before parturition (Kuhn & Linzell, 1970).

In many species the mammary gland does not become actively secretory until shortly before, or just after, parturition (Fulkerson, 1979; Cowie, Forsyth & Hart, 1980). The junctional complexes between the cells of the secretory epithelium in the mammary gland close as lactation begins and open as the gland involutes at weaning (Linzell & Peaker, 1974; Pitelka, Hamamoto, Duafala & Nemanic, 1973; Morgan & Wooding, 1982). Thus at these times the concentration of lactose in blood may reflect changes in its rates of synthesis by the gland.

An enzymic assay coupled with fluorescence spectroscopy, and sensitive to 0·05 nmol, has been used to measure lactose in the medium after culture of mammary epithelial cells (Kulski & Buehring, 1982). We have applied this assay to the measurement of lactose in the plasma of non-pregnant, pregnant, lactating and weaned sows. The concentration of lactose in plasma was assessed in terms of the compositional changes in colostrum and milk which occurred during lactogenesis, established lactation and weaning.

METHODS

Animals

Sows (Landrace, Large White and Landrace × Large White) were housed intensively in a commercial piggery (Baconfield Piggery, Bullsbrook, Western Australia). Non-pregnant gilts (parity 0) were kept untethered in one pen. The mean gestational length of sows in this piggery is 114·0 ± 0·5 days (n = 3682 sows) and the parities and litter sizes of the animals recruited into this study ranged from one to eleven and eight to thirteen, respectively. Sows were kept individually in crates from 1 week pre-partum to weaning 3–4 weeks post partum. They were kept tethered by a neck collar during pregnancy, parturition and lactation. All animals were fed according to rations and given free access to water.

Experimental design

Samples of blood and, where appropriate, mammary secretions were obtained from six different groups of sows as follows.

(1) Virgin sows (gilts).

(2) Pregnant sows, subdivided according to their gestational age: fifteen sows between days 1 and 43 (stage I), seven sows between days 51 and 63 (stage II), and seven sows between days 97 and 107 (stage III).

(3) Parturient sows. In this group blood samples were obtained from day 4 pre-partum to day 4 post partum, and samples of mammary secretion were collected from farrowing (day 0) to day 4 post partum. Three sows were sampled at intervals of 12 h from day 4 to day 1 pre-partum, 6 h from day 1 pre-partum to day 1 post partum, 12 h on day 2 post partum, and daily thereafter to day 4 post partum. Another three sows were sampled daily over this period.

(4*a*) Lactating sows. Blood and milk samples were obtained from six sows once every 5 days from days 5 to 25 post partum.

(4*b*) Lactating sows. During days 4–13 post partum, when lactation was fully established, blood samples were taken from three sows at approximately 20 s intervals during episodes of nuzzling and suckling by their piglets. The sampling periods included spontaneous suckling and suckling after induction of milk ejection by injection of 0·2 i.u. oxytocin (Pitocin; Parke-Davis and Co., Sydney).

(5) Weaned sows. Four blood samples were taken from six sows during the first 12 h following weaning, and daily thereafter to the seventh day of weaning. Over this period, daily milk samples were obtained where possible, according to the rate at which mammary secretion declined in the different sows.

Sampling

Blood was obtained from an ear vein of unrestrained sows by three methods according to the volume required. For 1 ml samples a 26 G needle and 1 ml syringe were used. For volumes up to 15 ml, a 24 G needle was connected to a 2·5 ml syringe via clear vinyl tubing (Dural Plastics, Sydney). Continuous sampling of blood intermittently for up to 2 h was achieved using a Venflow catheter (21 G, Viggo, Sweden). The blood was collected into either 3·5 ml or 10 ml heparinized tubes (Disposal Products, Adelaide) on ice and centrifuged within 30 min to separate the plasma fraction.

Mammary secretion (1–2 ml) was expressed by hand from the four anterior teats and stored separately. From day 2 post partum to the commencement of weaning, milk let-down was induced by the injection of 0·2 i.u. oxytocin. Blood samples were taken before the mammary samples and both were kept at −15 °C until analysed.

Analysis

The concentration of lactose in blood was measured by a modification of the method of Kulski & Buehring (1982). Plasma (200 μl) was deproteinized by equal volumes (80 μl) of 0·15 M-barium hydroxide and 0·15 M-zinc sulphate (Slein, 1963). The mixture was centrifuged (8000 *g* for 4 min) and 100 μl of the supernatant was removed for assay. The assay mixture (550 μl) consisted of 100 mM-potassium phosphate buffer pH 7·5, 1 mM-magnesium sulphate, 0·5 mM-NAD, 0·12 u. β-galactose dehydrogenase S (*P. fluorescens* cloned in *E. coli*; Boehringer, Sydney) and 0·7 u. β-galactosidase (*E. coli*, grade VIII; Sigma, Sydney). After the reaction had incubated for 60 min, the fluorescence due to the NADH produced was measured (Perkin-Elmer 300) using excitation and emission wave-lengths of 340 nm and 460 nm, respectively. The mean intra-assay and inter-assay coefficients of variation for plasma standards with high, medium and low concentrations of lactose were 5·2 % and 8·9 %, respectively.

The concentration of lactose in milk was measured by the method of Kuhn & Lowenstein, (1967). Na and K were measured by atomic absorption spectroscopy (V-T Model 1200, Varian Techtron Pty Ltd). The total protein and fat in the milk was reduced by nitric acid digestion as described by Gunther, Hawkins & Whyley (1965).

Statistical analysis

Results are expressed as mean ±S.E. of mean and analysed by regression analysis (Snedecor & Cochran, 1969) or two-tailed Mann–Whitney *U* tests (Siegel, 1956).

RESULTS

The concentration of lactose in plasma was determined in different sows at all phases of their reproductive cycle (Fig. 1*A*). The highest plasma concentrations of lactose were associated with changes in the secretory activity of the mammary glands (Fig. 1*B*). Lactose was present in low concentrations in the blood of gilts (4·1 ± 0·3 μM) and pregnant sows up to 107 days of gestation (3·5 ± 0·3 μM). From day 4 to day 1

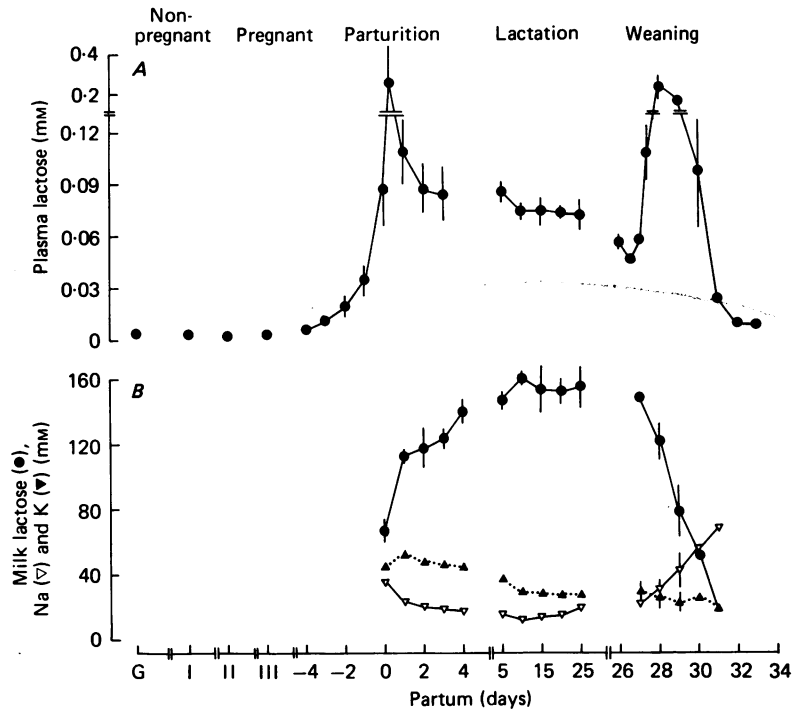

Fig. 1. The concentration in different sows of lactose in the plasma and of lactose, Na and K in mammary secretion during the reproductive cycle. *A*, lactose concentration (●) in plasma. Blood was obtained from non-pregnant gilts (G), and pregnant sows between days 1–43 (I), days 51–63 (II) and days 97–107 (III) of gestation, respectively. *B*, concentrations of lactose (●), sodium (▽) and potassium (▲) in mammary secretion. Mammary constituents were determined separately in the secretions from the four anterior teats of each animal, the mean taken ($n = 4$), and then expressed as the mean ± S.E. of mean of all sows.

pre-partum, lactose concentrations in plasma rose from 7·1 μM (two sows) to 34·5 ± 7·7 μM (six sows) prior to the beginning of mammary secretion on the day of farrowing.

At parturition (day 0) the concentration of lactose in the plasma rose to 262·0 ± 168·4 μM in the three sows for which closely timed samples were obtained. Thereafter it declined to 84·1 ± 15·1 μM by day 3 post partum in all six sows (Fig. 1*A*). Mammary secretion was first obtainable on the day of farrowing. The transition of mammary secretion from colostrum (day 0) to milk (day 4 post partum) was

accompanied by a progressive rise in the mean concentration of mammary lactose from 63·0 ± 5·9 mM to 140·9 ± 6·6 mM (six sows). At the same time, mean Na concentrations decreased from 35·1 ± 2·1 mM to 17·1 ± 1·1 mM and were inversely related to the mean amount of lactose in milk ($r = -0{\cdot}79$, $P < 0{\cdot}001$). K concentrations stabilized at 46·4 ± 1·3 mM by day 4 post partum and were not related to the amount of lactose in milk (Fig. 1*B*).

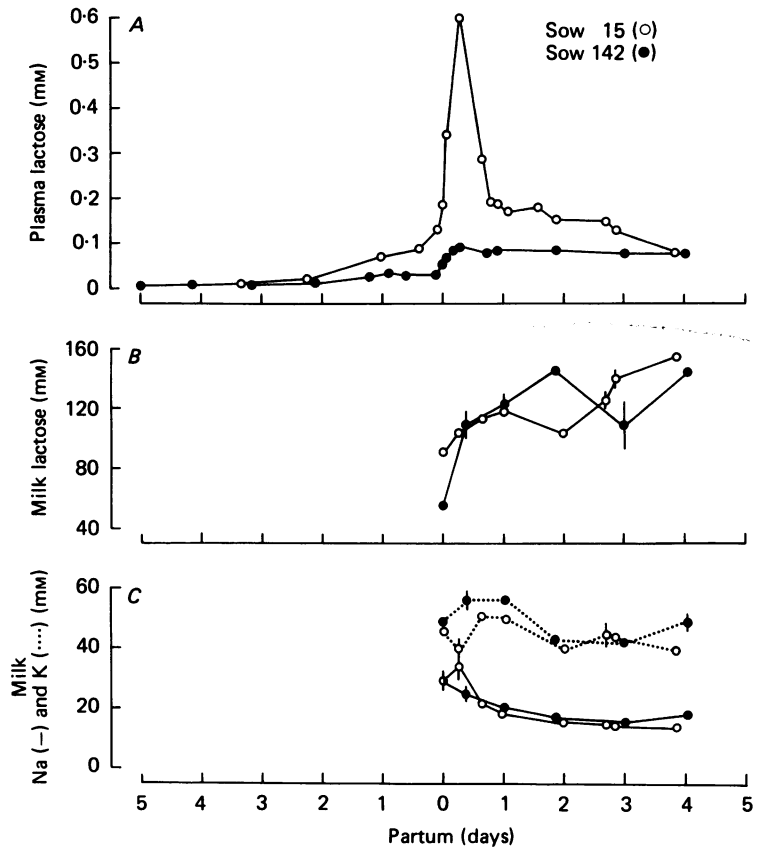

Fig. 2. The concentrations perinatally in two sows of lactose in plasma and mammary secretion, and of Na and K in mammary secretion. *A* and *B*, concentrations of lactose in plasma and milk, respectively. *C*, concentrations of Na (——) and K (.....) in milk. Sow 15 (○), sow 142 (●).

The over-all profiles of lactose in plasma and mammary secretion over the perinatal period were similar in all six sows. At birth (day 0), the concentration of lactose in plasma was correlated with the amount of lactose in mammary secretion ($r = 0{\cdot}88$, $P < 0{\cdot}01$). However, the peak concentration of lactose in plasma was variable between sows (Fig. 2*A*). In general, a high concentration of lactose in mammary secretion at day 0 was associated with a high peak concentration of lactose in plasma (Fig. 2*A* and *B*). In these two sows, the concentrations of Na and K in colostrum and milk were similar and did not vary according to the amount of lactose in either plasma or mammary secretion (Fig. 2*C*).

Blood and milk samples were collected every 5 days from six sows whose lactation was fully established. The mean concentration of lactose in plasma ranged from 72 to 86 μM. Over the same 20-day period, the concentration of milk constituents remained constant also (Fig. 1).

Short-term changes in the plasma concentration of lactose were measured in three sows (Fig. 3). Lactose concentrations fluctuated by 15–30 μM over a 10 min sampling

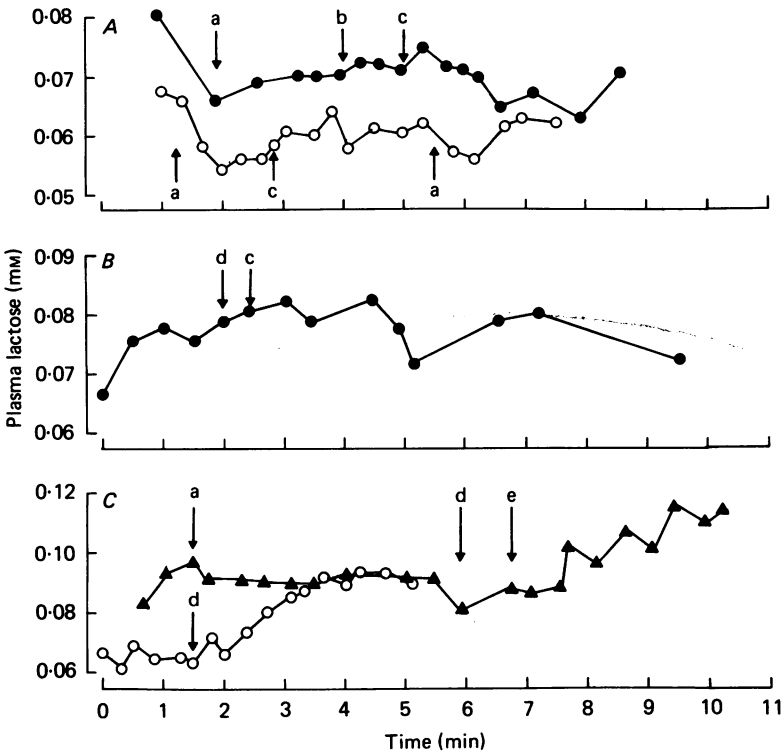

Fig. 3. The short-term changes in the concentration of lactose in maternal plasma during nuzzling and suckling by piglets. *A*, normal suckling behaviour. *B*, suckling preceded by injection of oxytocin into the sow. *C*, lack of suckling behaviour following administration of oxytocin to the sow. Sow 772 (○), sow 915 (●), sow 932 (▲). Key: quiet (a), nuzzling (b), suckling (c), oxytocin (d), milk appears (e).

period but did not vary significantly ($P > 0{\cdot}05$) between or during periods of suckling (Fig. 3*A*), or after stimulation of milk ejection by oxytocin (Fig. 3*B*). However, the amount of lactose in plasma rose significantly ($P < 0{\cdot}02$, sow 932 and $P < 0{\cdot}002$, sow 772) after the administration of oxytocin if milk ejection was not accompanied by suckling (Fig. 3*C*).

Sows were routinely weaned abruptly at 3–4 weeks after farrowing by removal of their piglets. The mean concentration of lactose in the plasma of six sows remained unchanged for 24 h. It began to rise by 36 h and reached a peak value of $241{\cdot}8 \pm 53{\cdot}6$ μM at 48 h, before declining to $10{\cdot}2 \pm 2{\cdot}0$ μM, which was comparable to the amount of lactose in the plasma of pre-parturient sows (Fig. 1). Lactose declined

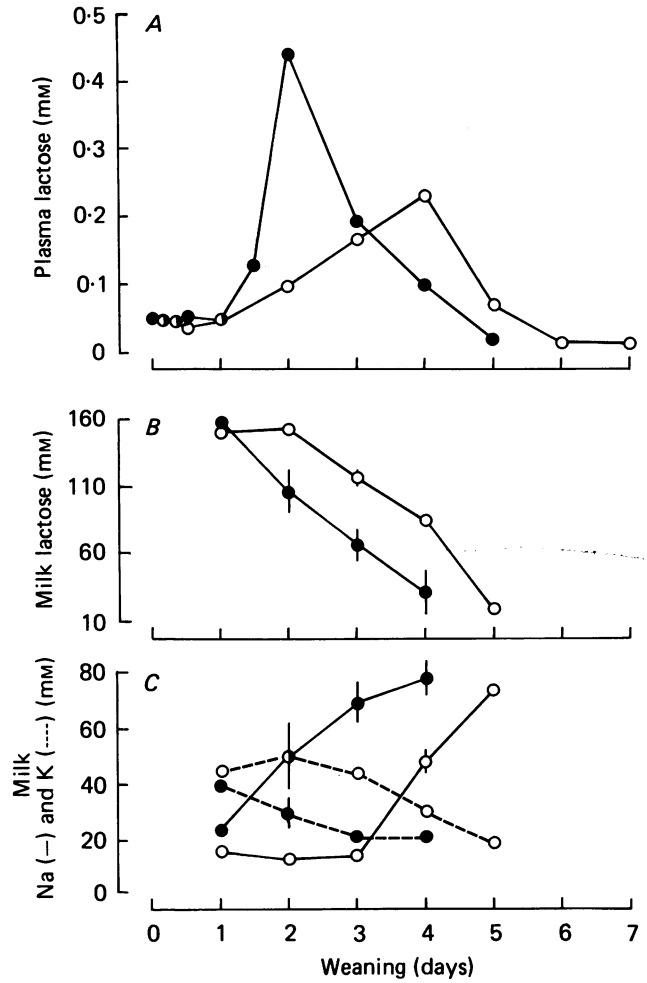

Fig. 4. The concentration of lactose in plasma and milk, and of Na and K in milk in two sows during weaning. *A*, lactose in plasma. *B*, lactose in milk. *C*, Na (——) and K (----). Sow 738 (○) and sow 814 (●).

in the milk of all sows (Fig. 1*B*); the mean concentrations of lactose and Na in milk were inversely related ($r = -0{\cdot}90$, $P < 0{\cdot}001$) over the course of weaning. However, the profiles of lactose in plasma and of Na and K in milk were variable in individual sows. For example, in sow 814 the concentration of lactose in plasma was maximal 2 days after weaning whereas in sow 738 plasma lactose peaked 4 days after weaning (Fig. 4*A*). Lactose and Na and K concentrations in post-weaning milk reflected the timing of the peak of lactose in plasma. Thus, in sow 738 lactose and K in milk started to decline 24 h later than in sow 814 (Fig. 4*B* and *C*). Concomitantly, Na in the milk of sow 814 rose 48 h sooner than it did in the milk of sow 738 (Fig. 4*C*).

DISCUSSION

The combination of enzymic hydrolysis of lactose with the galactose dehydrogenase reaction provided a sensitive and specific assay for lactose in the presence of large amounts of glucose. The concentration of lactose in plasma was only 0·05–0·2 % of its concentration in colostrum and milk during lactation in the sow (Fig. 1). The basal plasma concentrations of lactose in virgin gilts and in sows throughout most of their pregnancy were 10-fold lower at 3–4 μM. The highest plasma concentrations of lactose were associated with the onset of lactation and weaning, which coincided with acute changes in the secretory activity of mammary glands (Fig. 1).

Mature milk is not secreted until 3–6 days post partum in cows, sheep, goats and women (Hartmann, 1973; Hartmann *et al.* 1973; Linzell & Peaker, 1974; Kulski & Hartmann, 1981). However, lactogenesis in the sow occurs rapidly compared with these species. The transition from pre-colostral secretion to mature milk varied between 1 and 2 days post partum in individual sows (Martin *et al.* 1978; Willcox *et al.* 1983). In some sows, mammary secretion was expressible as early as 2 days pre-partum, so that it is difficult to determine precisely when lactogenesis begins in individual sows (Martin *et al.* 1978; Willcox *et al.* 1983). The changes in the concentration of lactose in the plasma of sows during late pregnancy and up to 6 h after farrowing suggest that the initiation of lactation in the sow, as in cows (Hartmann, 1973) and sheep (Hartmann *et al.* 1973), developed in two phases. In the six sows studied over the perinatal period, the mean plasma concentration of lactose increased 37-fold: from 7·1 μM at day 4 pre-partum to 34·5 μM at day 1 pre-partum and then more rapidly to a maximum of 262 μM some 6 h after the start of farrowing (Fig. 1*A*).

Mammary secretion did not begin, at the earliest, until farrowing had begun. The rise in mammary lactose coincided with the second phase of increasing concentration of lactose in plasma (Fig. 1*B*). Thus the plasma concentration of lactose provided a sensitive indicator of the course of lactogenesis. The actual beginning of lactogenesis, in terms of an increase in lactose synthesis by the secretory cells of the mammary gland, may be defined as the time at which lactose begins to increase gradually in plasma (first phase) or the time at which the rate of increase in its plasma concentration changes (transition from first to second phase). Furthermore, the patterns of lactose in plasma and mammary secretion were similar for all the sows, but variable in magnitude (Fig. 2). Thus, relative changes in the amount of plasma lactose in serial samples of individual sows may most accurately reflect the increasing rate of lactose synthesis at this time. The concentration of lactose in plasma began to decline 6 h after the start of farrowing, and by day 4 post partum had stabilized at about 80 μM. Mammary lactose had reached near maximum concentrations of 130–160 mM by this time (Fig. 1*B*).

Lactose is synthesized by the Golgi apparatus of the secretory cells of the mammary epithelium and is secreted by them with other milk constituents into the lumen of the alveoli (Brew, 1969). Peaker (1976) proposed that a paracellular pathway enabled the passage of lactose and K from the alveoli to the adjacent extracellular fluid and the movement of Na and Cl in the reverse direction. Physiological and ultrastructural evidence (Pitelka *et al.* 1973; Linzell & Peaker, 1974) supports the concept that the

junctional complexes of the secretory epithelium change from 'leaky' to 'tight' at parturition. Lactose in milk is inversely related to K during lactogenesis in goats (Linzell & Peaker, 1974) and women (Kulski & Hartmann, 1981). The finding here of lactose in the plasma of sows, together with high concentrations of Na in the mammary secretion around farrowing, is consistent with the existence of a paracellular pathway between secretory cells in the mammary gland of the sow. The removal of mammary secretion from the lumen of the alveoli by suckling piglets (within 1–3 h of birth), together with the closing of the paracellular pathway, as indicated by the ionic changes in milk (Fig. 1*B*), may account for the sudden decrease in the concentration of plasma lactose as lactation becomes established. Once lactation was fully established, the concentrations of lactose in plasma and in milk each remained constant (Fig. 1). It is proposed that during lactation the tight junctions between the secretory cells are closed (Linzell & Peaker, 1974; Peaker, 1975). Our finding in the sow that the plasma concentration of lactose was less than 0·05 % of that in milk, together with the low Na concentration in milk at this time (Fig. 1*B*), supports this view.

The plasma concentration of lactose remained low and constant during suckling, whether or not milk ejection was induced by injection of oxytocin (Fig. 3*A* and *B*). However, significant increases in plasma lactose were observed in sows which were not suckled after administration of physiological doses (0·2 i.u.) of oxytocin (Fig. 3*C*). These acute changes probably result from the leakage of components of milk into the extracellular fluid if milk is not removed from the ducts by suckling. Wheelock, Rook & Dodd (1965) observed changes in the composition of milk after the administration of a pharmacological dose (20 i.u.) of oxytocin. Studies in the rabbit (Linzell, Peaker & Taylor, 1975) and in the goat (Linzell & Peaker, 1971) have shown that physiological doses of oxytocin increased the concentrations of Na and Cl and decreased the concentrations of lactose and K in milk. Experiments with goats, in which electrodes were inserted into a milk vein and into a gland cistern via the streak canal, showed that administration of oxytocin caused a change in the blood–milk potential and a rapid change in milk composition (Peaker, 1977, 1978). These and other effects of oxytocin (Peaker, 1980) have been interpreted as promoting a temporary opening of the tight junctions between the secretory epithelial cells, rather than as movement due to increased intramammary pressure.

The mammary glands of the sows involuted within 6 days of weaning. This process was characterized by a rapid decline in the concentration of lactose and increased concentrations of Na in milk (Figs. 1 and 4). In goats (Fleet & Peaker, 1978), cows (Hartmann, 1973) and women (Hartmann & Kulski, 1978), similar changes at weaning were observed: lactose, glucose and K decreased whilst Na and Cl increased. In lactating goats short-term physiological increases of intramammary pressure failed to rupture the secretory epithelium. This led Peaker (1980) to propose that its loss in integrity was attributed to the failure of neighbouring secretory cells to maintain their junctional complexes. Such a mechanism, together with the possible accumulation of unknown chemical factors in stored milk, was found to increase permeability (Peaker, 1980), so that lactose in the blood rises during involution of the mammary gland. Thus the presence of large amounts of lactose in the plasma of sows after the cessation of milking may be explained similarly by exchange of lactose and ions

between extracellular fluid and milk. Alternatively, lactose concentrations in plasma rise at weaning due to rupture of the alveoli and diffusion of milk constituents into extracellular fluid. Involution of the sow mammary gland is characterized by progressive distension of the alveoli until they burst about 24 h after the start of weaning (Cross, Goodwin & Silver, 1958). These cellular changes are consistent with our finding that lactose did not begin to rise until 24 h after weaning (Fig. 1). Its decline in plasma to pre-lactation levels after 5 days coincides with the cessation of milk synthesis in the gland at this time.

In conclusion, this study has shown that lactose circulates in the blood of sows throughout all phases of their reproductive cycle. Its highest concentrations in plasma occurred at the beginning and end of lactation, and provided measures of the functional changes occurring in the glands at these times. Furthermore, the concentration of lactose in plasma around parturition provided an earlier temporal measure of lactogenesis in individual sows than that obtained by analysis of mammary secretions.

We thank Ian Barker for help and access to the Baconfield Piggery and Simone Prosser, Helen Nottage and Jodi Newton for technical assistance. The work was supported by grants from the Australian Pig Industry Research Committee and the West Australian Pig Industry Compensation Fund. David L. Willcox is a Raine Research Fellow.

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