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Metabolic adaptations in goat mammary tissue during pregnancy and lactation

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Summary. Metabolic adaptations of goat mammary tissue during pregnancy and lactation were monitored in serial biopsies of the tissue. Changes in the synthetic capacity of secretory cells were studied by combining measurements of enzyme activities with short-term culture of mammary explants to measure lactose, casein and total protein synthesis. By these criteria, the main phase of mammary differentiation began in late pregnancy and was essentially complete by Week 5 of lactation, coinciding with the achievement of peak milk yield. While milk yield declined after Week 5, the activities of key enzymes expressed per mg DNA and the rates of lactose and casein synthesis in mammary explants were maintained over a considerable period. The results suggest that changes in the synthetic capacity of epithelial cells may account for much of the rise in milk yield in early lactation, but are not responsible for the declining phase of milk production characteristic of lactation in ruminants.

Introduction

Mammary cell proliferation and differentiation prepare the gland for lactation, and although the rate of milk synthesis is influenced by many factors, such as the nutrition of the animal, systemic hormones, and local factors within the gland, ultimately it is the number and activity of secretory cells which determine the milk yield of the lactating animal. The relative importance of these factors in determining peak milk yield and persistence of lactation has rarely been studied even in small animals, for which data are relatively easy to obtain (for recent reviews, see Cowie, Forsyth & Hart, 1980; Knight & Peaker, 1982a). There are a number of practical difficulties associated with making measurements in the udder of ruminant animals, and little information is available to assess the relative importance of changes in secretory cell number and cellular synthetic capacity in controlling the milk yield of these animals. Characteristically, yield rises to a peak early in lactation and declines thereafter for as long as milking is continued. Maintenance of milk production during the later stages of lactation will depend upon the number of secretory cells lost, the extent of cell replacement (if any) and the retention of synthetic capacity by each cell.

In an earlier study, a combination of tissue biopsy (for measurement of nucleic acids) and udder volume estimation was used mainly to assess changes in the size of the mammary secretory cell population in goats (Knight & Peaker, 1984). We now report a study carried out over late pregnancy and 25 weeks of lactation, in which we have measured changes in the degree of epithelial cell differentiation in mammary biopsy samples from goats by using a combination of enzyme activity measurements and short-term tissue culture of mammary explants. Some of the results obtained have been reported briefly elsewhere (Wilde, Henderson & Knight, 1985).

Materials and Methods

Animals. Primigravid British Saanen goats kidding during March or April 1984 were used; measurements in a main group of 7 goats were supplemented at several stages by additional

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measurements in contemporary animals. They were fed hay (~1·6 kg/day) available throughout the day and concentrates (1·25 kg/day; Goat Mix-1, Edinburgh School of Agriculture, Edinburgh, U.K.), half the ration being given at each milking. The animals were milked twice daily at about 0800 and 1600 h, and the yield of each gland was recorded.

Materials. L-[4,5-^{3}H]Leucine (sp. act. 45 Ci/mmol), [U-^{14}C]glucose (sp. act. 270 mCi/mmol), L-[4-^{3}H]phenylalanine (sp. act. 25 Ci/mmol), [6-^{3}H]thymidine (sp. act. 24 Ci/mmol), uridine diphospho-D-[U-^{14}C]galactose (sp. act. 300 mCi/mmol) and sodium [^{14}C]bicarbonate (sp. act. 0·1 mCi/mmol) were purchased from Amersham International, Bucks, U.K. Medium 199 and Fungizone were from Gibco Europe Ltd, Paisley, U.K. Sheep prolactin (NIADDK-oPRL-16) was a gift from the National Institute of Arthritis, Diabetes and Digestive and Kidney Diseases, Bethesda, MD, U.S.A. Other hormones, antibiotics, nucleotides and coupling enzymes were obtained from Sigma Chemical Co., Poole, Dorset, U.K., or the Boehringer Corporation, Lewes, Sussex, U.K. Sodium dodecyl sulphate, ammonium persulphate, acrylamide and N,N,N',N'-tetramethylethylenediamine were from BDH Ltd, Poole, Dorset, U.K. Pentobarbitone sodium anaesthetic (Sagatal) was from May and Baker, Dagenham, Essex, U.K. All other chemicals were of AR grade or the highest grade available.

Surgical procedures. Biopsies were taken under pentobarbitone sodium anaesthesia (22 mg/kg i.v.) using aseptic procedures (Knight & Peaker, 1984) at 9 stages during pregnancy and lactation; to permit biopsy sampling in late pregnancy without precipitating premature lactogenesis, pre-partum fluid was removed and replaced aseptically immediately before and after the biopsy. The biopsy tissue (2–3 g) was trimmed to remove cauterized tissue, fat and connective tissue, and portions were frozen rapidly and stored in liquid nitrogen for subsequent measurement of enzyme activities or placed in cold culture medium (see below) for preparation of mammary explants. The shortest interval between biopsies was 2 weeks, and only biopsies which consisted predominantly of secretory tissue were used in the experiment.

Preparation of tissue fractions. Frozen mammary tissue was ground to a powder in liquid nitrogen and homogenized at 4°C in 9 volumes of an iso-osmotic Tris–sucrose buffer (30 mM-Tris, 0·3 M-sucrose, 1 mM-glutathione, 1 mM-EDTA; pH 7·4) for 15 sec at 80% of maximum speed in an Ultra-turrax homogenizer (type TP18/11). A sample of homogenate was taken for aryl esterase assay, and a particle-free supernatant was then prepared by centrifugation in a microcentrifuge (Eppendorf model 5414) for 5 min. A second homogenate prepared from powdered tissue in 25 volumes of 0·25 M-sucrose (Ultra-Turrax homogenizer, 2 × 25 sec, 80% of maximum speed) was used for assay of galactosyltransferase activity.

Enzyme assays. Enzyme activities were assayed in freshly-prepared particle-free supernatant fractions, with the exceptions of galactosyltransferase and aryl esterase (see below). Enzymes were assayed under conditions in which activity was linearly related to amount of sample and incubation time, and expressed per mg DNA. Total acetyl-CoA carboxylase activity (EC 6.4.1.2) was assayed as described by Ingle, Bauman, Mellenberger & Johnson (1973) over 1·5 min at 37°C following a preincubation of 30 min in the absence of substrates to obtain complete enzyme activation. Fatty acid synthetase activity was assayed by the method of Speake, Dils & Mayer (1975). Lactate dehydrogenase (EC 1.1.1.27), phosphofructokinase (EC 2.7.1.11) and hexokinase (EC 2.7.1.1.) were assayed as described for mammary tissue by Korsrud & Baldwin (1972) and NADP-dependent isocitrate dehydrogenase (EC 1.1.1.42) was measured by the method of Bernt & Bergmeyer (1974). Homogenates for assay of aryl esterase (EC 3.1.1.2; Shephard & Hubscher, 1969) and galactosyltransferase activities (EC 2.4.1.22; Kuhn & White, 1977) were stored for several days at −20°C before assay, without loss of enzyme activity.

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Tissue culture. Tissue was collected in Medium 199 containing Hepes (12·5 mM), antibiotics (1·2 μg penicillin G/ml, 1·0 μg streptomycin/ml), Fungizone (1 μg/ml), sodium acetate (5 mM), insulin (5 μg/ml) and cortisol (10 ng/ml), and mammary explants were cultured in the same medium with or without prolactin (1 μg/ml) in an atmosphere of air/CO_2 (95 : 5 v/v). Preparation of explants (Dils & Forsyth, 1981) and transfer to culture grids (where appropriate) was completed within 40 min of obtaining tissue. Rates of lactose synthesis and casein synthesis in groups of 20 explants were measured over 2 h by the addition of [U-^{14}C]glucose (1 μCi/ml) and L-[4,5-^{3}H]leucine (1 μCi/ml) respectively to the culture medium. Rates of protein synthesis were measured by incubation of groups of 30 explants in a shaking water bath at 37°C for 15 min in medium containing L-[4-^{3}H]phenylalanine (50 μCi/ml). After culture, explants and media were separated and stored frozen at −20°C. Explants were homogenized at 4°C in 1·0 ml 10 mM-Tris–HCl pH 7·0 containing 5 mM-EGTA and 2 mM-phenylmethanesulphonyl fluoride by 10 strokes with a glass–PTFE homogenizer followed by sonication for 30 sec (10 m amplitude, Soniprep 150, MSE Ltd) and a particle-free supernatant was prepared as described above. ^{14}C-Labelled lactose was isolated from explant particle-free supernatant and culture medium by selective precipitation (Kuhn & White, 1975) and ^{3}H-labelled casein was isolated from the particle-free supernatant by isoelectric precipitation and sodium dodecyl sulphate/polyacrylamide gel electrophoresis (Wilde, Razooki Hasan & Mayer, 1984). Incorporation of L-[4-^{3}H]phenylalanine into total protein was measured as trichloroacetic acid precipitable radioactivity in explant homogenates (Speake *et al.*, 1975).

Rates of DNA synthesis were measured in cultured tissue by addition of [6-^{3}H]thymidine (1·0 μCi/ml) to the medium as described previously (Knight & Peaker, 1982b).

DNA determination. DNA was measured in freshly-prepared tissue homogenates by the diphenylamine method (Knight & Peaker, 1982c) and in explant homogenates by a fluorometric method (Labarca & Paigen, 1980) using bovine thymus DNA as a standard. There was no significant difference between the results obtained with the two methods.

Results

Changes in mammary synthetic capacity and milk yield were monitored by taking biopsy samples at particular stages of pregnancy and lactation, thereby dividing the experimental period into a series of phases (termed 'study periods' by Knight & Peaker (1984) in an earlier study).

Milk yield

Individual milk yields of 7 goats which completed at least 14 weeks' lactation are shown in Fig. 1; on 4 occasions when biopsy sampling had resulted in transient decreases in milk yield, weighting was applied to correct the affected values (Knight & Peaker, 1984). While absolute values varied between individual animals, they all showed similar lactation curves (weekly yield plotted against time).

After an initial rapid increase from 6·8 ± 1·0 (kg/gland)/week (mean ± s.e.m.; 60% of individual peak yields) in Week 1 to 10·3 ± 0·9 (kg/gland)/week (92% of peak yields) in Week 3 ($P < 0·01$; analysis by Student's *t* test for paired observations), a peak milk yield of 11·3 ± 0·9 (kg/gland)/week was achieved before Week 5 (Week 4·9 ± 0·9). Thereafter milk yield decreased to Week 8 and Week 15 values of 9·4 ± 1·3 and 9·0 ± 1·1 (kg/gland)/week (82% and 78% respectively of peak milk yields), the differences between Weeks 5 and 15 being significant ($P < 0·001$). By Weeks 18 and 25 milk yields of the two remaining goats had fallen to 83% and 62% respectively of their peak values.

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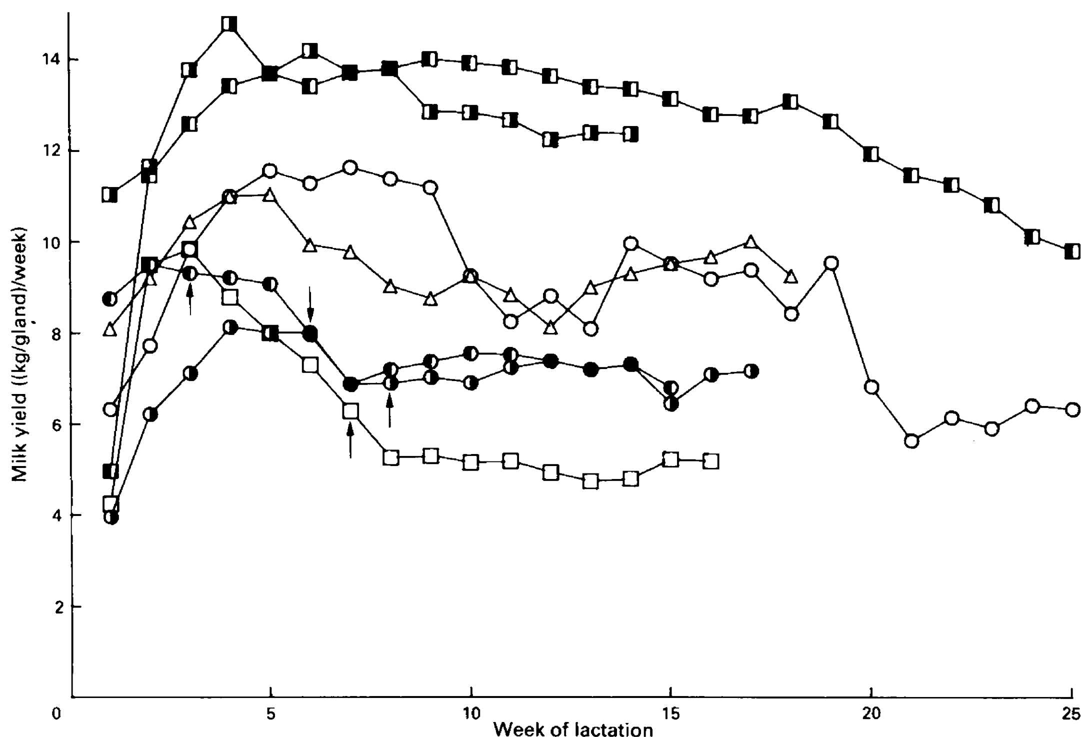

Fig. 1. Milk yields of individual goats. Weekly milk yields from a single gland of seven goats used in the study are shown. Arrows indicate four values that were weighted to correct for transient effects of the biopsy procedure (see Knight & Peaker, 1984).

Enzyme activities

The number of stages at which biopsies were taken and their frequency during late pregnancy and early lactation made sampling of each goat at every stage impractical. Therefore, the statistical significance of changes in enzyme activities and explant synthetic rates between successive stages of pregnancy or lactation were determined by Student's t test for unpaired observations.

The activities of acetyl-CoA carboxylase (Fig. 2a), fatty acid synthetase (Fig. 2b) and galactosyltransferase (Fig. 2c), key enzymes in the synthesis of milk constituents, all increased significantly during early lactation when milk yield was rising. Accumulation of these enzymes was largely complete by Week 5, when peak milk yield was achieved, although galactosyltransferase

Fig. 2. Enzyme activities in goat mammary tissue during pregnancy and lactation. Mammary tissue was obtained by biopsy at stages during pregnancy (denoted at –7 and –1 weeks of lactation) and lactation. Enzyme activities were measured as described in the 'Methods': ACC, acetyl-CoA carboxylase (a); FAS, fatty acid synthetase (b); GT, galactosyltransferase (c); LDH, lactate dehydrogenase (d); HK, hexokinase (e); ICDH, isocitrate dehydrogenase (f); PFK, phosphofructokinase (g); AE, aryl esterase, (h). Results are expressed per mg of DNA in each sample. The number of determinations at each stage is shown; where appropriate, error bars indicate the standard error of the mean. The earliest stage at which the activity of an enzyme showed a significant increase ($P < 0.05$) over that in late pregnancy (Week –1 of lactation) is indicated by a stippled histogram.

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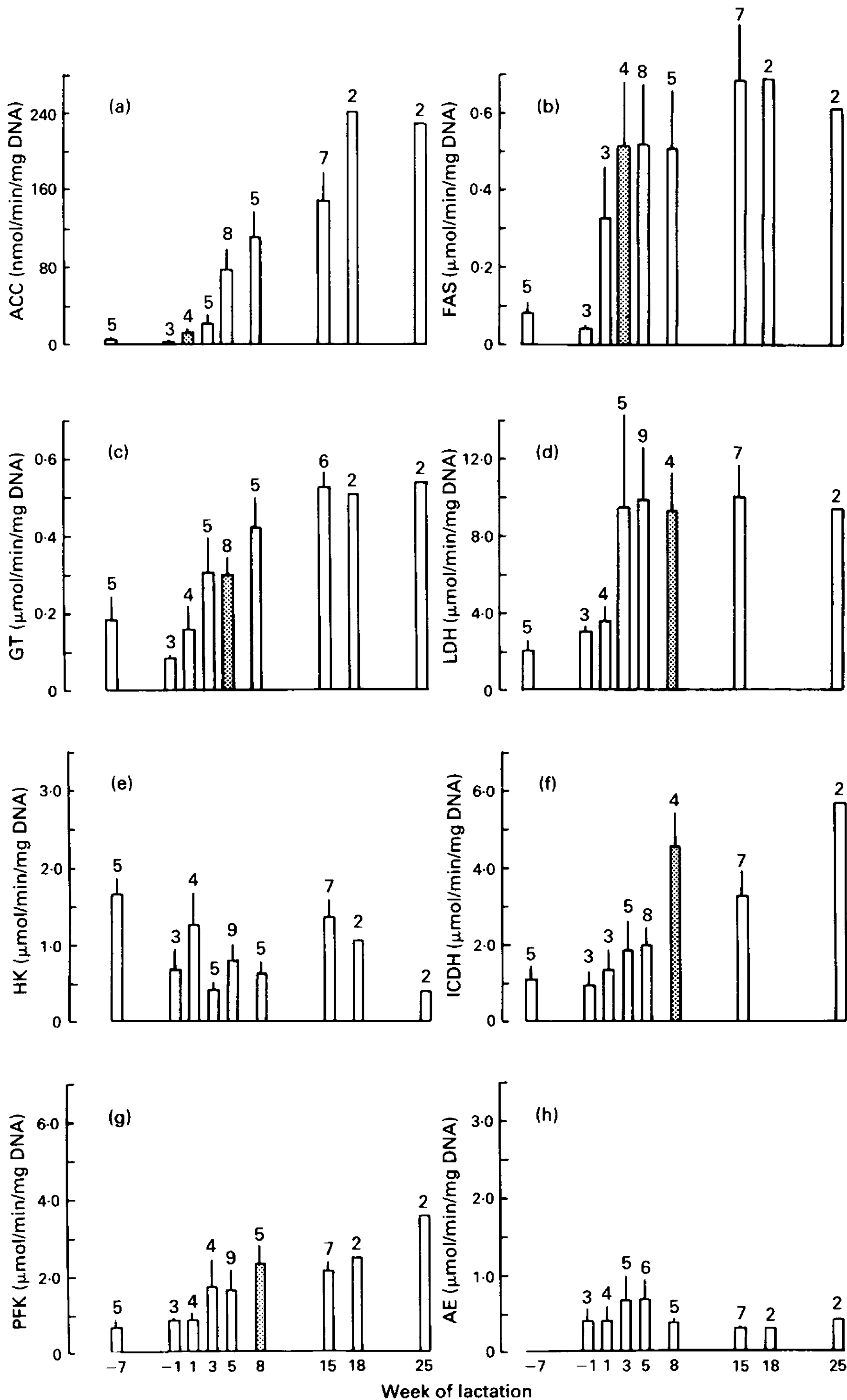

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activity rose by a further 23-fold ($P<0.01$) by Week 15. These changes during early lactation (acetyl-CoA carboxylase by 24-fold, $P<0.05$; fatty acid synthetase by 11-fold, $P<0.05$; galactosyltransferase by 4-fold, $P<0.05$) were markedly greater and occurred more rapidly than those observed for other enzymes. Increases in the activities of lactate dehydrogenase (by 2·8-fold,

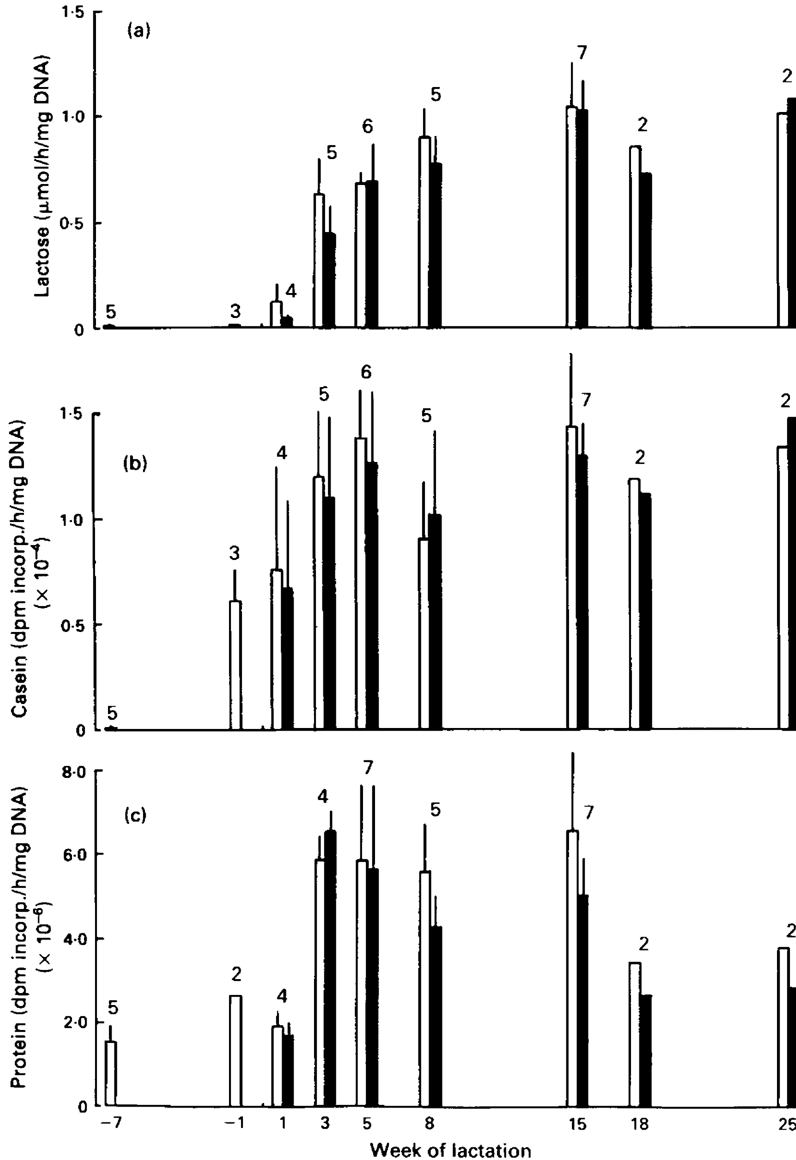

Fig. 3. Rate of lactose, casein and total protein synthesis in goat mammary explants prepared during pregnancy and lactation. Explants were prepared from biopsies taken at stages during pregnancy and lactation and cultured in Medium 199 containing insulin + cortisol (open bars) or insulin + cortisol + prolactin (solid bars). Lactose synthesis (a) and casein synthesis (b) were measured by incorporation of [U-^{14}C]glucose and L-[4,5-^{3}H]leucine and total protein synthesis (c) was measured by incorporation of L-[4,-^{3}H]phenylalanine as described in the 'Methods'. Other details are as described in the legend of Fig. 2.

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$P<0.05$; Fig. 2d), isocitrate dehydrogenase (by 2-fold, $P<0.05$; Fig. 2f) and phosphofructokinase (by 2·7-fold, $P<0.05$; Fig. 2g) over those in late pregnancy became statistically significant only after Week 5, when milk yield had started to fall. Hexokinase (Fig. 2e) and aryl esterase (Fig. 2h) activities showed no significant change throughout the course of the study.

Measurements made at Weeks 18 and 25 were limited, but they indicated that the activities of enzymes at Week 15 were maintained during this period.

Synthetic activity in mammary explants

Short-term culture (2 h) of mammary explants rapidly prepared from biopsy tissue was used to extend the study to direct measurements of lactose, casein and total protein synthesis. In explants prepared from lactating tissue, synthesis rates observed in the presence of insulin + cortisol were not significantly different from those obtained with prolactin also present in the culture medium. Estimates of in-vivo rates of lactose synthesis based on milk yield data (Fig. 1), milk lactose concentrations (Henderson & Peaker, 1984) and udder volumes (Knight & Peaker, 1984) suggest that absolute rates of lactose synthesis in the explants were lower but of the same order as rates measured in intact tissue: an average value of 32% of the in-vivo rate was calculated for explant cultures prepared from 29 biopsies.

Explants obtained at different stages of pregnancy and lactation demonstrated very marked changes in their capacity for both lactose and casein synthesis (Fig. 3a, b). From the low rate observed with explants from late-pregnant goats, lactose synthesis increased 39-fold ($P<0.001$) by Week 5 of lactation, around the time of peak milk yield. However, mammary explants obtained in late pregnancy already showed an increased capacity for casein synthesis; incorporation of [^{3}H]leucine into total casein increased 77-fold ($P<0.01$) between Weeks 14 and 20 of pregnancy, indicating that acquisition of the capacity for synthesis of individual milk constituents occurred at different times in relation to parturition. Synthesis of casein continued to increase until Week 5 of lactation, the overall increase from mid-pregnancy being 170-fold ($P<0.01$). The capacity of the explants for lactose and casein synthesis did not decrease when milk yield started to fall: rates at Weeks 8 and 15 were not significantly different from those at Week 5, and measurements at Weeks 18 and 25 suggested that synthetic capacity was maintained long into the declining phase of lactation.

Table 1. Rates of DNA synthesis in cultured mammary tissue of goats at 2 stages during pregnancy and 7 stages during lactation

Week of lactation	[^{3}H]thymidine incorporation (d.p.m./h/mg DNA)
−7	12433 ± 1104 (5)
−1	4619 ± 565 (3)*
1	308 ± 132 (4)**
3	870 ± 411 (5)
5	608 ± 202 (10)
8	490 ± 105 (7)
15	637 ± 162 (7)
18	332 (2)
25	403 (2)

Values are the mean ± s.e.m. with the number of determinations in parentheses.
*$P<0.01$, **$P<0.001$ for changes between successive stages (Student's unpaired *t* test).

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Total protein synthesis measured in mammary explants increased significantly during early lactation, and remained high until Week 15, but was lower in explants prepared at Weeks 18 and 25 of lactation. These changes in the rate of total protein synthesis did not coincide with the major increase in casein synthesis between mid- and late-pregnancy. Therefore, while total protein synthesis included a contribution attributable to synthesis of caseins and other secreted proteins, under these conditions and using [^{3}H]phenylalanine (one of the less abundant amino acids in goat caseins (Jenness, 1980)), secretory protein synthesis may have accounted for only a small part of the incorporation into total protein.

Rates of protein synthesis observed during pregnancy are due in some part to the processes of cell replication. On the basis of [^{3}H]thymidine incorporation into mammary pieces in suspension (Table 1), cell proliferation was greatest in mid-pregnancy, and fell by 63% ($P < 0.01$) by late pregnancy and by a further 93% ($P < 0.001$) by the first week of lactation. [^{3}H]Thymidine incorporation remained low throughout the rest of the study period.

Discussion

During terminal differentiation the mammary gland rapidly acquires the complement of intracellular enzymes and proteins necessary to meet the prodigious demands of milk synthesis and secretion. Substantial increases in the activities of acetyl-CoA carboxylase and fatty acid synthetase are a characteristic of mammary differentiation in a number of species (Baldwin & Milligan, 1966; Mellenberger, Bauman & Nelson, 1973; Wilde, Paskin, Saxton & Mayer, 1980). The results presented here and those of Sarma & Ray (1984) suggest that these lipogenic enzymes can also act as useful markers of epithelial cell differentiation in goat mammary tissue. As the enzyme activities (and the other characteristics measured) are expressed relative to the DNA concentration of the tissue, the changes observed during pregnancy and lactation can be considered to be due to cell differentiation only, and not to epithelial cell proliferation, which can continue into early lactation in the goat (Knight & Peaker, 1984).

With accumulation of lipogenic enzymes as the main criterion, differentiation is essentially complete by the time of peak milk yield, and is maintained for a considerable period thereafter. However, the activities of other enzymes rose significantly after Week 5 (and non-significant increases of 75% and 31% in the activities of acetyl-CoA carboxylase and fatty acid synthetase respectively were also observed by Week 15), so in some respects the tissue continues to develop, even while milk yield has begun to decline. The sustained but relatively modest changes in these other enzyme activities may represent a background of general cell hypertrophy against which the more strategic changes in key enzyme activities take place. For example, during terminal differentiation of rabbit mammary tissue *in vitro*, a transient reduction in the rate of degradation of fatty acid synthetase (resulting in rapid accumulation of the enzyme) was not limited to this key enzyme, but was a general feature shown by other cytosolic enzymes (Wilde *et al.*, 1980; Wilde, Saxton & Mayer, 1982). The process is not, however, completely unselective, as shown by the constant activity of aryl esterase throughout the study period.

The rising enzyme complement of the tissue and its rate of milk production *in vivo* is accompanied by substantial increases in rates of lactose and casein synthesis by mammary explants prepared during early lactation. Indeed, the increase in casein synthesis between mid-pregnancy and peak lactation was much greater than that previously observed when explants from mid-pregnant goats were stimulated over several days in culture by a combination of insulin, cortisol and prolactin (Skarda, Urbanova, Houdebine, Delouis & Bilek, 1982a, b). The short culture period used here obviates any requirement for exogenous prolactin to maintain synthetic activities *in vitro*: the tissue's requirement is likely to be met by persistence of hormonal effects initiated *in vivo* or by the carry-over of endogenous hormone in the explants, a phenomenon that has been noted in other explant cultures (Rillema & Anderson, 1976; Ganguly, Ganguly, Mehta & Banerjee, 1980). Also, casein synthesis in these short-term cultures increased by late pregnancy, preceding the rise in rates

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of lactose synthesis; sequential appearance of individual milk constituents was observed previously in the goat (Fleet *et al.*, 1975) and also the rat (Kuhn, 1972; Martyn & Hansen, 1980), although this was not necessarily the result of asynchronous changes in their rates of synthesis (see below). The detection of lactose and casein synthesis even in explants prepared at Week 14 of pregnancy is not unexpected, as lactose (Fleet *et al.*, 1975), lactose synthetase activity (Jones, 1979) and other milk constituents (Fleet *et al.*, 1975; Davis *et al.*, 1979) have been found in the udder of goats from mid-pregnancy onwards. Together, the results indicate that the synthetic activities of these short-term cultures are a measure of the synthetic capacity of the tissue *in vivo*.

The reason for the deficit between absolute rates of lactose synthesis in freshly-prepared explants and the estimated rate of synthesis *in vivo* is not clear: there is no rapid loss of capacity for lactose synthesis as initial rates in culture were maintained for at least 24 h, and were not significantly affected by altering the concentration of cortisol in the culture (C. Wilde, unpublished data) as is the case with rat mammary explants (Ono, Perry & Oka, 1981). Conversely, rates of lactose synthesis measured *in vitro* during pregnancy were greater than might be expected from the overall rate of accumulation of lactose in the gland. However, the accumulation of milk constituents during pregnancy is likely to be determined not just by their rates of synthesis, but also by the rate at which they are degraded (see for example Razooki Hasan, White & Mayer, 1982), converted to other products or resorbed in the bloodstream (Kuhn, 1977). These processes will determine the time and rate at which individual milk constituents appear in the gland, and together may prevent excessive accumulation of milk solids before parturition.

Maintenance of the capacity for lactose and casein synthesis in explants prepared after Week 5 of lactation supports the evidence of enzyme measurements that the synthetic capacity of the epithelial cells is retained until at least Week 15 and perhaps longer. Only much later, around Week 40, is there evidence that the cell population is unable to sustain the high synthetic capabilities seen in early lactation. By this stage the mean activities of acetyl-CoA carboxylase and galactosyltransferase measured in a second group of goats had fallen by 61% and 18% respectively, and rates of lactose and casein synthesis in explants were greatly reduced by 93% and 59% respectively (R. Vernon, A. Henderson & C. Wilde, unpublished data). Therefore, the fall in milk yield from the gland is due initially to another factor, and it appears that this may be a decrease in the number of secretory cells (Knight & Peaker, 1984). From [^{3}H]thymidine incorporation into DNA in cultured tissue cells, proliferation had largely ceased by parturition, although it has been found previously that the secretory cell population continued to increase in early lactation (Knight & Peaker, 1984). The low rates of incorporation of [^{3}H]thymidine during lactation may indeed be significant for milk production: there is evidence that the tissue contains a population of stem cells which retain the ability to proliferate (Devore, 1977), and also that differentiation of secretory cells can no longer be considered 'terminal', i.e. that they can temporarily suspend milk synthesis and undergo replication (Franke & Keenan, 1979). Goat mammary gland retains a potential for growth during lactation when stimulated by hemimastectomy (C. Knight, unpublished data) or more frequent milking (Henderson, Blatchford & Peaker, 1985). Therefore, the secretory cell population may turn over during lactation, with cell death (if any) being offset by replacement with new cells. The slow decline in milk yield after peak lactation could represent a shortfall in the rate of cell replacement in the face of an accelerating rate of cell death; at present, however, little is known about the longevity of mammary epithelial cells.

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References

Baldwin, R.L. & Milligan, L.P. (1966) Enzymatic changes associated with the initiation and maintenance of lactation in the rat. *J. biol. Chem.* **241**, 2058–2066.

Bernt, E. & Bergmeyer, H.U. (1974) Isocitrate dehydrogenase. In *Methods of Enzymatic Analysis*, Vol. 2, pp. 624–627. Ed. H. U. Bergmeyer. Academic Press, New York.

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Cowie, A.T., Forsyth, I.A. & Hart, I.C. (1980) *Hormonal Control of Lactation*. Springer-Verlag, Berlin.

Davis, A.J., Fleet, I.R., Goode, J.A., Hamon, M. H., Maule Walker, F.M. & Peaker, M. (1979) Changes in mammary function at the onset of lactation in the goat: correlation with hormonal changes. *J. Physiol., Lond.* **288,** 33–44.

Devore, G. (1977) Ultrastructural evidence for differentiation of mammary gland secretory cells from a population of primitive cells basally located in the epithelium. *J. Cell Biol.* **75,** 33a, Abstr.

Dils, R. & Forsyth, I.A. (1981) Preparation and culture of mammary gland explants. In *Methods in Enzymology*, Vol. 72, pp. 724–742. Eds S. P. Colowick & N. O. Kaplan. Academic Press, New York.

Fleet, I.R., Goode, J.A., Hamon, M.H., Laurie, M.S., Linzell, J.L. & Peaker, M. (1975) Secretory activity of goat mammary glands during pregnancy and the onset of lactation. *J. Physiol., Lond.* **251,** 763–773.

Franke, W.W. & Keenan, T.W. (1979) Mitosis in milk secreting epithelial cells of mammary gland. An ultrastructural study. *Differentiation* **13,** 81–88.

Ganguly, R., Ganguly, N., Mehta, N.M. & Banerjee, M.R. (1980) Absolute requirement of glucocorticoid for expression of the casein gene in the presence of prolactin. *Proc. natn. Acad. Sci. U.S.A.* **77,** 6003–6006.

Henderson, A.J. & Peaker, M. (1984) Feed-back control of milk secretion in the goat by a chemical in milk. *J. Physiol., Lond.* **351,** 39–45.

Henderson, A.J., Blatchford, D.R. & Peaker, M. (1985) The effects of long-term thrice-daily milking on milk secretion in the goat: evidence for mammary growth. *Q. Jl exp. Physiol.*, in press.

Ingle, D.L., Bauman, D.E., Mellenberger, R.W. & Johnson, D.E. (1973) Lipogenesis in the ruminant: effect of fasting and refeeding on fatty acid synthesis and enzymatic activity of sheep adipose tissue. *J. Nutr.* **103,** 1479–1488.

Jenness, R. (1980) Composition and characteristics of goat milk: review 1968–1979. *J. Dairy Sci.* **63,** 1605–1630.

Jones, E.A. (1979) Change in the activity of lactose synthetase in the goat udder during pregnancy. *J. Dairy Res.* **46,** 35–40.

Knight, C.H. & Peaker, M. (1982a) Development of the mammary gland. *J. Reprod. Fert.* **65,** 521–536.

Knight, C.H. & Peaker, M. (1982b) Development of the mammary gland. *Q. Jl exp. Physiol.* **65,** 165–177.

Knight, C.H. & Peaker, M. (1982c) Mammary development in mice: effects of hemihysterectomy in pregnancy and of litter size post partum. *J. Physiol., Lond.* **327,** 17–27.

Knight, C.H. & Peaker, M. (1984) Mammary development and regression during lactation in goats in relation to milk secretion. *Q. Jl exp. Physiol.* **69,** 331–338.

Korsrud, G.O. & Baldwin, R.L. (1972) Effect of adrenalectomy, adrenalectomy–ovariectomy, and cortisol and oestrogen therapies upon enzyme activities in lactating rat mammary glands. *Can. J. Biochem.* **50,** 366–376.

Kuhn, N.J. (1972) Changes in the protein phosphorus content of rat mammary tissue during lactogenesis. *J. Endocr.* **55,** 219–220.

Kuhn, N.J. (1977) Lactogenesis: the search for trigger mechanisms in different species. *Symp. zool. Soc. Lond.* **41,** 165–192.

Kuhn, N.J. & White, A. (1975) The topography of lactose synthesis. *Biochem. J.* **148,** 77–84.

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

Labarca, O. & Paigen, K. (1980) A simple, rapid, and sensitive DNA assay procedure. *Analyt. Biochem.* **102,** 344–352.

Martyn, P. & Hansen, I.A. (1980) Initiation of fatty acid synthesis in rat mammary glands. Biochem J. **190,** 171–175.

Mellenberger, R.W., Bauman, D.E. & Nelson, D.R. (1973) Metabolic adaptations during lactogenesis. *Biochem. J.* **136,** 746–748.

Ono, M., Perry, J.W. & Oka, T. (1981) Concentration-dependent differential effects of cortisol on synthesis of α-lactalbumin and of casein in cultured mouse mammary gland explants: importance of prolactin concentration. *In Vitro* **17,** 121–128.

Razooki Hasan, H., White, D.A. & Mayer, R.J. (1982) Extensive destruction of newly-synthesized casein in mammary explants in organ culture. *Biochem. J.* **202,** 133–138.

Rillema, J.A. & Anderson, L.A. (1976) Rapid interaction of prolactin with mouse mammary gland explants. *Molec. cell. Endocr.* **4,** 131–137.

Sarma, P.V. & Ray, T.K. (1984) Fatty acid synthesizing capacity of mammary gland in relation to plasma hormone levels at different stages of pregnancy and lactation in goats. *Ind. J. Biochem. Biophys.* **21,** 255–259.

Shephard, E.H. & Hubscher, G. (1969) Phosphatidate biosynthesis in mitochondrial subfractions of rat liver. *Biochem. J.* **113,** 429–440.

Skarda, J., Urbanova, E., Houdebine, L-M., Delouis, C. & Bilek, J. (1982a) Hormonal control of casein synthesis in mammary explants from pregnant goats. *Endokrinologie* **79,** 301–307.

Skarda, J., Urbanova, E., Houdebine, L-M., Delouis, C. & Bilek, J. (1982b) Effect of insulin, cortisol and prolactin on lipid, protein and casein synthesis in goat mammary tissue organ culture. *Reprod. Nutr. Develop.* **22,** 379–386.

Speake, B.K., Dils, R. & Mayer, R.J. (1975) Regulation of enzyme turnover during tissue differentiation. *Biochem. J.* **148,** 309–320.

Wilde, C.J., Paskin, N., Saxton, J. & Mayer, R.J. (1980) Protein degradation during terminal cytodifferentiation. *Biochem. J.* **192,** 311–320.

Wilde, C.J., Saxton, J. & Mayer, R.J. (1982) Protein degradation in mammary explants: evidence for limited heterogeneity of protein degradation rates. *Biochim. Biophys. Acta* **714,** 46–57.

Wilde, C.J., Razooki Hasan, H. & Mayer, R.J. (1984) Comparison of collagen gels and mammary extracellular matrix as substrata for study of terminal differentiation in rabbit mammary epithelial cells. *Expl Cell. Res.* **151,** 519–532.

Wilde, C.J., Henderson, A.J. & Knight, C.H. (1985) Lipogenic enzyme activities in goat mammary gland: change with stage of pregnancy and lactation and frequency of milking. *Biochem. Soc. Trans.* **13,** 385–386, Abstr.

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