Variation Among Species in the Endocrine Control of Mammary Growth and Function: The Roles of Prolactin, Growth Hormone, and Placental Lactogen

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ABSTRACT

Prolactin, growth hormone, and placental lactogen form a family of structurally related hormones, which may have evolved from a common ancestral peptide. Prolactin and growth hormone are present in all mammals, but the biological activity associated with placental lactogen has been detected in only some groups. Attempts to detect placental lactogen using bioassay and radioreceptor assay are reported and have been unsuccessful in an insectivore (the shrew), a bat, an edentate (the armadillo), a lagomorph (the rabbit), several carnivores (dog, cat, ferret), perissodactyls (horse, zebra, rhino), and, within the artiodactyls, pigs. Placental lactogenic activity has been detected in primates (chimpanzee, orangutan), rodents (voles, Pinon mouse, guinea-pig, mara), and in numerous artiodactyls (llama, giraffe, several species of deer, antelope, gnu, gazelle, musk ox, cape buffalo, Barbary sheep, several sheep of the genus Ovis, goat, and cow). These results confirm and extend the work of others and are discussed in relation to the evolution of these hormones. In synergism with steroid and thyroid hormones, protein hormones of the prolactin and growth hormone family play a crucial role in stimulating the development of the mammary gland, the differentiation and function of mammary cells to secrete milk, and in the systemic adjustments in maternal metabolism in pregnancy and lactation. Studies in vitro have shown that mammary tissues from several species synthesize milk components in response to insulin plus adrenal corticoid plus prolactin. However, there are also species differences in minimal hormonal requirements for lactogenesis. In vivo, for example, rabbits will initiate or sustain lactation in response to prolactin alone, whereas sheep and goats require prolactin plus growth hormone plus adrenal corticoid plus thyroid hormone. Measurement of hormone concentrations in the plasma of pregnant animals shows considerable differences among species in the pattern of secretion of lactogenic hormones to bring about mammary development. A surge of prolactin secretion occurs at parturition but may not be essential in the initiation of lactation. The timing of progesterone withdrawal correlates well with lactogenesis in eutherian mammals, but species differ in the mechanisms at parturition which bring this about. Marsupials show a quite different pattern of suckling-induced lactation. In maintaining lactation the greatest contrast is between ruminants, in which growth hormone is of particular importance, and other mammals, in which reduction of prolactin secretion with bromocriptine rapidly suppresses milk synthesis and secretion. Factors acting locally within the mammary gland, and still largely unidentified, are also important.

It is suggested that the requirement to integrate the endocrine control of lactation with other aspects of reproduction may have provided some, at least, of the selection pressures leading to the adoption of different endocrine strategies.

INTRODUCTION

In recent years, the use of in vitro methods, especially organ culture, has greatly improved understanding of the hormonal synergisms involved in the initiation of secretory activity in the mammary gland and of how hormones
affect synthesis of specific milk components (114). Nevertheless, the very success of insulin plus cortisol plus prolactin (usually sheep prolactin) in initiating secretory activity in organ cultures of the mammary glands from such a wide range of species [mice (110), rats, hamsters, rabbits, dogs (8) cows (25), and goats (54)] has tended to divert attention from the variation among species in their response to and requirements for mammotrophic hormones. In earlier studies this variation was well-recognized. Cowie (26) reviewed the increasing complexity of the hormone combinations needed to initiate or maintain lactation after hypophysectomy in rabbits (prolactin), rats [adrenocorticotropic hormone (ACTH) plus prolactin], mice [ACTH plus prolactin or growth hormone (GH) in some strains] and ruminants (ACTH plus prolactin plus GH plus triiodothyronine). The in vivo species differences are reflected in vitro in requirements to stimulate secretory activity (35), casein, and α-lactalbumin synthesis. Indeed, organ culture of mouse, rat, and rabbit mammary gland has revealed complex differences in hormone requirements, requirements for deoxyribonucleic acid (DNA) synthesis for gene expression, and the extent to which the polyamine spermidine can substitute for cortisol in synergism with prolactin (13, 103, 115, 116).

It is a familiar concept that mammals vary widely in their reproductive patterns and that many different strategies are used to achieve perpetuation of the species (145). Lactation is the final phase of reproduction and is essential for breeding success in natural environments. Different physiological strategies are used by mammals for lactation in habitats ranging from temporal to tropic and from aquatic to desert (105). It is increasingly clear that different endocrine strategies are used by different groups of mammals to develop the mammary gland and to sustain lactation. A problem is that so few of the 4000 species of mammals have been studied in any detail. We have no idea of what selective pressures have led to the adoption of different endocrine strategies, although it seems probable that an important factor could be the need to integrate control of lactation with other aspects of reproduction, including parturition in all species (63), a simultaneous pregnancy and lactation in some, and the spacing of pregnancies via the contraceptive effects of lactation in others (122).

Scientists are still heavily dependent on the classic work of Lyons (86) on the rat and of Nandi (99) on the mouse for concepts of which hormones are involved in the various phases of mammary development. This review will consider particularly the structurally related protein hormones prolactin, GH, and placental lactogen (PL) as well as the roles they play — in synergism with ovarian, adrenal, and placental steroids — in mammogenesis and lactation in different species.

The Growth Hormone Gene Family: Occurrence of Placental Lactogens

Niall et al. (102) advanced the hypothesis, based on comparisons of amino acid sequences, that prolactin, GH, and human PL arose by duplication and subsequent divergence from a common ancestral gene. With the techniques of molecular biology, it is now possible to study the messenger ribonucleic acids (mRNA) coding for these hormones and the structure of the genes themselves. So far such studies have been applied to only a few species, but already considerable complexity is apparent. In the human, there appears to be a single copy of the prolactin gene per haploid genome, which is on chromosome 6, whereas there are at least two GH and five PL genes linked on the long arm of chromosome 17 (95). Only one of the human GH genes and at least two of the five human PL genes are expressed. In the rat and cow, there appear to be only single copies of the prolactin and GH genes per haploid genome. Nothing is yet published on their PL genes, although the rat (111, 112) and the mouse (126, 127) are known to have two forms of this hormone, which may be separate gene products.

Prolactins and GH with distinct properties have been isolated from the pituitaries of all vertebrates, including fish, so that the separation of these two hormones was probably an early event in vertebrate evolution. Calculations based on the length of time required to accumulate the number of changes observed in amino acid or coding nucleotide sequences between the two hormones in cows, humans, and rats suggest their existence as separate genes from more than 350 million years ago. This is consistent with the vertebrate fossil record indicating divergence of fish and amphibians 400 million years ago. However, other
calculations based on such an evolutionary clock hypothesis are not consistent with paleoantology (95, 96).

The evolution of PL (also known as chorionic somatomammotropin) is especially problematic. Human GH and human PL show remarkable homology in their amino acid sequence and at the level of the genes. This suggests either a recent origin, well after the mammalian radiation, or a form of evolution different from point mutation (95, 96). A further problem is the apparently very patchy occurrence of PL in mammals. Prolactin-like biological activity has been detected in placentas of rather few orders of placental mammals, primates, rodents, and only some artiodactyls. It appears to be absent from six other orders and seven orders have not yet been studied.

Table 1 summarizes our own results, obtained using either coculture of mammary gland and placenta or radioreceptor assays for lactogenic hormones (Figure 1) based on receptors prepared from rabbit liver (104) or rabbit mammary gland (121). Similarly, using coculture with mouse mammary gland, Talamantes (131) detected lactogenic activity in placenta from baboon, sheep, rat, mouse, hamster, chinchilla, and guinea-pig but not from rabbit or dog. Kelly et al. (75) used receptor assays with specificities directed toward both prolactin-like and GH-like activities to examine placental extracts or serum. The ratio of the two activities varied between species, but using either assay the conclusion was the same, that placental prolactin and growth hormone-like activity is present in man, rhesus monkey, rat, mouse, hamster, guinea-pig, sheep, goat, and cow but not in rabbit, dog, or pig.

It is, of course, possible that with the precedence of the situation for human PL, a gene or genes are present but not expressed (pseudogenes) in groups such as carnivores, perissodactyls, and suiformes (pigs) or that the gene product is not recognized by any of the assay methods used to date. The possibility that PL have evolved more than once must also be considered (50). Limited structural evidence on ovine PL (70) and indications of immunological crossreaction between rat PL II and antisera to human, bovine, and ovine prolactins (111) may indicate that these hormones arose from the prolactin rather than the GH gene. Further, a complementary deoxyribonucleic acid (cDNA) probe for rat GH will not detect a rat PL gene(s) in contrast to the close similarity of the genes for the corresponding hormones in man (96).

In evolutionary terms, immunological crossreactions among PL in different ruminants are also of interest. Immunologically prolactins of sheep, goats, and cows are virtually identical and GH of the same species are similar. A radioimmunoassay for sheep prolactin has also been used to measure the hormone in white-tailed deer (119). By contrast, there is in general relatively little immunological crossreaction between the PL of ruminants, even goats and sheep [Figure 2; see also (23)]. Goats and sheep are thought descended from a common ancestor with a diploid chromosome number, 2N, of 60. The diploid chromosome number is 60 in goats, but in mouflon, bighorn, and domestic sheep it is 54. The reduction in chromosome number has resulted from fusions of identified chromosomes, and several species are known with intermediate chromosome numbers. In one of these, the Barbary sheep, *Ammotragus lervia* (2N = 58), a placental extract shows extensive crossreaction and parallel depletion in an ovine PL radioimmunoassay (Figure 2). It has recently been shown that, like the domestic sheep and unlike the goat, the Barbary sheep secretes substantial quantities of progesterone from the placenta (47). Alteration in the immunological properties of PL, reflecting structural change, and acquisition of placental progesterone synthesis may both be early events in the evolution of the genus *Ovis*.

**MAMMOGENESIS**

During pregnancy, there is a substantial increase in numbers of epithelial cells in the mammary gland. Increase in cell number continues into early lactation in most species, although the extent of lactational mammary growth varies from less than 10% in ruminants to as much as 50% in rats (2, 4, 51, 141). Little is known about its hormonal control. Mammary epithelial cells also complete their differentiation during pregnancy. Small amounts of mRNA for milk proteins are already present in the mammary glands of virgin females (69, 98, 123). A marked increase in gene expression takes place during pregnancy with increased concentrations.
TABLE 1. Species tested for the presence of a placental prolactin-like hormone.¹

<table>
<thead>
<tr>
<th>Order</th>
<th>Positive</th>
<th>Order</th>
<th>Negative</th>
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<tbody>
<tr>
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<td>Pongo pygmaeus</td>
<td>Insectivora</td>
<td>Sorex araneus</td>
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<td>Pan troglodytes</td>
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<td>Crocidura suaveoleus</td>
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<td>Peromyscus truei</td>
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<td>Common shrew</td>
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<td>Cetibromyns glareolus</td>
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<td>Lsr. white-toothed shrew</td>
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<td>Microtus agrestis</td>
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<td>Rodentia</td>
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<td>Dolichotis patagonum</td>
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<td>Pipistrellus pipistrellus</td>
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<td>Cavia porcellus</td>
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<td>Pipistrelle bat</td>
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<td>Artiodactyla</td>
<td>Lama glama</td>
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<td>Cervus dama</td>
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<td>Cervus elaphus</td>
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<td>Ovis moschatus</td>
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<td>Ammotragus lervia</td>
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<td>Ovis canadensis</td>
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<td>Perissodactyla</td>
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<td>Hippotigris burchelli</td>
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<td>Ceratotherium simum</td>
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<td>Rhinoceros unicornis</td>
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<td>Pig</td>
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¹ Includes unpublished results. The assistance of suppliers of tissue, and especially the Zoological Society of London, is gratefully acknowledged.
yield occurs postpartum by adjustment to the demands of the young. However, more recent evidence suggests that prepartum factors exert an important influence on potential milk yield and can even set an upper limit to it.

Mammary development in pregnancy occurs in response to steroid hormones (estrogens plus progesterone plus adrenal corticoids) in synergism with protein hormones of the prolactin and GH family. How do the secretion patterns of the latter relate to mammary development?

Species with Placental Lactogen

The effect of hypophysectomy in pregnancy on mammary development provided the earliest evidence that the placenta might secrete a mammogenic hormone. Continued mammary development and a transient lactation at parturition were observed as long ago as the

of mRNA and increased rates of synthesis of milk components. This can be considered the onset of lactogenesis stage I, the accumulation of precolostrum in the mammary gland (46). It should be noted that there is variation among species in the onset of this phase and that, within a given species, initiation of the synthesis of different milk components is not necessarily coincidental. For example, in rabbits, synthesis of casein (123), lactose (34), and milk fat (130) occurs by d 18 to 24 of pregnancy (30 d gestation), whereas in guinea pigs expression of casein and α-lactalbumin genes occurs only during the onset of lactation (15). In rats, mRNA for six different milk proteins were each shown to have characteristic and different patterns of accumulation during pregnancy (69).

Milk yield is determined by epithelial cell number and by secretory activity per cell. Blaxter (11) concluded that regulation of milk

Figure 1. Radioreceptor assay for prolactin-like activity in crude extracts of placenta from six species of artiodactyl. Placentae were extracted in four volumes (wt/vol) of 0.1 M ammonium bicarbonate, pH 9.5. (I.A. Forsyth and S. Iley, unpublished results.)


Figure 2. Double antibody radioimmunoassay for ovine placental lactogen. Serial dilutions of crude extracts of the placentae of Barbary, Bighorn, and Soay sheep were parallel with the standard curve. Placental extracts from other artiodactyls, including goat and cow, show little immunological crossreaction. (I.A. Forsyth and G. Thordarson, unpublished results.)
1930's in hypophysectomized pregnant mice, rats, and guinea pigs [see (50)]. More recently, extensive mammary development has been observed after removal of the pituitary in ewes (37) and goats (17). The latter is one of the best controlled studies so far with careful examination for the possibility of pituitary remnants by sectioning the sella turcica and by measuring prolactin in basal states and after a stimulation test. Nevertheless, what can happen in an experimental situation and what does happen in normal pregnancy cannot be equated with total confidence. Progress has been hampered by the lack of knowledge of the mechanisms controlling PL synthesis and secretion. It has not been possible, therefore, to manipulate concentrations of the hormone artificially. Recent studies have implicated ovarian and fetal factors in influencing rat PL (113, 140). There is, however, a positive correlation between placental mass (influenced both by stage of gestation and number of fetuses) and circulating PL in several species and this provides additional evidence for the involvement of PL in mammary development.

**Rats and Mice.** Levels of GH are reported to be low in pregnant rats (117). Studies of the secretion of prolactin and PL show a pattern of considerable complexity. The stimulus of coitus initiates a pattern of twice daily surges of prolactin release, one nocturnal (0100 to 0900 h) and one diurnal (1500 to 2100 h, lights on 0600 to 1800 h). This pattern of prolactin release continues until midpregnancy when first the diurnal (d 8) and then the nocturnal (d 10) surges cease. Rising PL may be responsible for terminating the prolactin surges (124). From d 8 to 14, with maximum concentrations on d 12, a form of PL, rat PL-I, is observed in the circulation. This is of 40,000 M<sub>r</sub>, has an isoelectric point of 4.5, and a ratio of prolactin to GH-like activity in radioreceptor assays of .35 (112). From d 12, with maximum concentrations on d 20, a second and immunologically distinct form of PL (rat PL-II) is found. This has 20,000 M<sub>r</sub>, isoelectric point of 6.2, and a ratio of prolactin to GH-like activity of 4.2 (112). On d 21 of pregnancy, rat PL-II increased exponentially with number of fetuses between 1 and 9, with plateaus of about 1,000 ng/ml for fetal numbers between 10 and 20 (111). A relationship between fetal number and mammotropic activity in serum has also been reported on d 13 of pregnancy in rat (108) and mouse (88). The mouse appears to be very similar to the rat in showing different forms of PL in early and late pregnancy, and there are differences between mouse strains in PL concentrations (126, 127).

Although early mouse PL has lactogenic activity (126), the late form of the hormone seems more likely to be involved in the relationship between the number of fetuses and placentae and the development of the mammary gland in mice (97). In hypophysectomized rats, a minimum of three fetoplacental units is required to maintain normal mammary development (3). Suckling intensity also affects mammary growth. The less developed mammary glands of mice, which were hemihysterectomized to reduce litter size on d 8 of pregnancy, showed compensatory mammary growth if suckled from birth by a litter of 9 but not 4 pups (78). Increasing litter size from 9 to 18 pups on d 6 of lactation, when lactational mammary growth is over, does not stimulate milk yield (80).

**Guinea-Pigs.** In guinea pigs, mammary gland weight and milk yield postpartum are positively correlated with number of young born (range 1 to 5) and cannot be enhanced by increasing the number of pups suckling (31). The potential milk yield in guinea pigs appears to be a function of prepregnant factors, even though mammary DNA increases slowly in pregnancy with a rapid rise only at parturition (101).

To examine the relationship of mammary development to PL, we have used a radioreceptor assay (104) to measure lactogenic activity in the plasma of pregnant guinea pigs and related it to mammary development observed within 12 h postpartum (138). Lactogenic activity was first detected after midpregnancy (d 30), reached a peak of 687 ± 78 ng/ml (mean ± SEM, n = 7) between d 50 and 60, and then declined markedly toward term (d 65). Kelly et al. (75) reported a similar pattern of secretion. The area under the curve was taken as a measure of total PL secreted, and this was correlated (r = .80, P < .05, df 5) with the weight of the litter. Both PL and litter weight were in turn correlated with mammary development (wet weight of mammary tissue, total DNA content, or the activity of lactose synthetase in the gland) (Table 2). This suggests a role for PL even though the peak of PL secretion occurs well before the rapid phase of
TABLE 2. Correlation between litter weight or placental lactogen and mammary development in guinea pigs.¹

<table>
<thead>
<tr>
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<th>Correlation coefficient (df = 5)</th>
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<tr>
<td>Total weight of litter</td>
<td></td>
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<tr>
<td>Wet weight of mammary gland</td>
<td>.81*</td>
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<tr>
<td>Total mammary gland DNA ²</td>
<td>.81*</td>
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<tr>
<td>Lactose synthetase/g tissue</td>
<td>.72</td>
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<tr>
<td>Placental lactogen</td>
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<tr>
<td>Wet weight of mammary gland</td>
<td>.80*</td>
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<tr>
<td>Total mammary gland DNA ²</td>
<td>.89**</td>
</tr>
<tr>
<td>Lactose synthetase/g tissue</td>
<td>.77*</td>
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</table>

¹Data from Thordarson and Forsyth (138).
²Deoxyribonucleic acid.
*P<.05.
**P<.02.

mammary growth (101) and differentiation (15) at parturition.

Ruminants. A relationship between number of kids born and milk yield in goats has been reported by ourselves (61) and others [(55, 128), Table 3]. The only hormone we have observed to correlate with milk yield postpartum is PL measured in late pregnancy, wk 16 to 20 (Figure 3). This provides strong circumstantial evidence that PL is involved in udder development. Sheep similarly show a relationship between fetal number and mammary development (109), fetal number and milk yield, and fetal number and concentrations of PL in late pregnancy [(16), Table 4].

Earlier studies, in which the occurrence of a PL in ruminants was first discovered, used bioassays based on the response of mouse or rabbit mammary gland (19, 49) and subsequently radioreceptor assays based on rabbit mammary gland prolactin receptors (74). In these tests, ruminant PL are equipotent to ovine prolactin. However, when tested on sheep mammary tissue, ovine PL is less potent than ovine prolactin in stimulating accumulation of α-lactalbumin [(20), Figure 4] or messenger RNA for casein (120). Preliminary tests with goat PL and goat mammary gland indicate a similar result (20). We have confirmed a difference in potency in an in vivo study (52) by comparing the effects on lactogenesis stage I of suppressing prolactin secretion to less than 2 ng/ml with bromocriptine in primigravid goats carrying single and twin kids, thus differing in amounts of PL (Figure 3). The occurrence of lactogenesis stage I was determined by seeing whether secretion could be expressed from the mammary gland, measuring lactose in expressed secretion, and by measuring milk protein α-lactalbumin in plasma. Goats with twin kids showed no effect of bromocriptine treatment on lactogenesis stage I, which was observed in wk 11 of pregnancy. However, in goats with single kids, bromocriptine treatment delayed lactogenesis stage I from wk 11 to 13 to wk 17 when PL rose above 200 ng/ml. Prolactin concentrations in control goats were unaffected by number of kids and averaged 12 ± 2 ng/ml (mean ± SEM). Nevertheless, despite the delay in mammary differentiation in goats with single kids, udder size on d 140 of pregnancy and milk yield postpartum were not significantly affected by bromocriptine treatment, regardless of the number of kids born. Cumulative milk yields to 50 d were correlated with concentrations of PL in wk 16 to 20 of pregnancy. They were not correlated with amounts of prolactin, estrone sulphate, or GH.

We have also carried out studies in vitro that indicate that triiodothyronine (T₃) synergises with low prolactin to stimulate α-lactalbumin synthesis by sheep mammary explants. However, PL-stimulated α-lactalbumin synthesis is not enhanced by T₃ (20). Bhattacharya and Vonderhaar (10) have suggested that T₃ increases

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TABLE 3. Effect of litter size on milk yield in goats.¹

<table>
<thead>
<tr>
<th>Number of kids born</th>
<th>Milk yield (kg)</th>
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<tbody>
<tr>
<td>1</td>
<td>191¹</td>
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<td>2</td>
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<td>3</td>
<td>191</td>
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¹Data from Hayden et al. (61) based on 50-d milk yield in 61 lactations, corrected for effects of lactation number (1 to 4).
²Data from Steine (128) based on at least 200 d of lactation within the observation period. Number of observations 22,038. Age of goats (1 to 9 yr) and season of kidding held constant.
the effectiveness of prolactin in stimulating \(\alpha\)-lactalbumin synthesis by mouse mammary gland by enhancing the binding of prolactin to its receptor.

In vitro, high prolactin concentrations (1 to 20 \(\mu\)g/ml) induced a refractory state in rabbit mammary gland, indicated by depression of casein and DNA synthesis (41). Concentrations of PL may approach this high range in late pregnancy in sheep and goats (Figure 3). The lower potency of PL as a stimulator of secretory activity in ruminant mammary tissue and the lack of synergism between PL and T\(_3\) may together prevent the development of a refractory state in the udder in late pregnancy in the face of high concentrations of PL.

Serveley and coworkers (120) have suggested that the prolactin-like activity of sheep PL may have little physiological significance by comparison with its GH-like properties. It is, however, clear that when prolactin is absent or suppressed in sheep or goats, PL can fulfill a role as a stimulator of mammary epithelial cell differentiation (17, 37, 90). In normal pregnancies, there is a temporal relationship between the onset of lactogenesis stage I and rising PL concentrations. In goats, concentrations of PL rise and lactogenesis stage I begins on d 70 to 80 of pregnancy (62). In sheep both events are delayed to d 100 (23, 89). The relationships among concentrations of PL in late pregnancy, udder size prepartum, and milk yield postpartum

![Figure 3. Concentrations of prolactin and placental lactogen in the plasma of primigravid British Saanen goats. Prolactin was measured by radioimmunoassay in goats carrying one or two kids. Placental lactogen was measured by radioreceptor assay. Difference between goats carrying one and two kids are significant \((P<.02)\). Number of goats in parentheses. Data from Forsyth et al. (52).](image)

![Figure 4. Dose-response curves for the effect of ovine prolactin or ovine placental lactogen, in the presence of insulin plus cortisol, on accumulation of \(\alpha\)-lactalbumin in medium plus tissue of mammary gland explants from a pregnant sheep. Data from Byatt (20).](image)

<table>
<thead>
<tr>
<th>No. of lambs</th>
<th>d 107</th>
<th>d 138</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\bar{X})</td>
<td>(\text{SE})</td>
<td>(\bar{X})</td>
</tr>
<tr>
<td>1</td>
<td>66</td>
<td>7 (18)</td>
</tr>
<tr>
<td>2</td>
<td>153</td>
<td>12 (27)*</td>
</tr>
<tr>
<td>3</td>
<td>250</td>
<td>16 (32)*</td>
</tr>
<tr>
<td>4</td>
<td>301</td>
<td>37 (6)</td>
</tr>
</tbody>
</table>

*Values are means ± standard errors; number of animals in parentheses. Unpublished data of G. Thordarson, I. A. Forsyth, and T. T. Treacher.

\(P<.001\) compared with ewes with one less lamb.
implicate goat and sheep PL in the increase in cell number in the gland. It remains to be seen whether this effect is direct or indirect and brought about via interaction with a prolactin or a GH receptor, sited locally or in another tissue such as liver. However, in goats (60) and sheep (68) with intact pituitaries, udder growth, induced by the injection of estrogen and progesterone, is prevented if prolactin secretion is inhibited using bromocriptine. This strongly suggests involvement of a prolactin-like hormone in udder growth. Anderson et al. (5) discussed the possibility that mitogenic actions of prolactin are brought about by synerism with a prolactin-stimulated somatomedin-like molecule.

Although the presence of a PL in the cow is now well-established (6, 18), little is known about its physiological role, especially as circulating concentrations of the hormone appear to be so low (9, 118). Prolactin concentrations in cows, as in other ruminants, are dependent on day length and temperature and, thus, on season. Secretion of prolactin is apparently little affected by stage of gestation (18) so that in temperate climates the pattern of prolactin in pregnancy is quite different in animals calving in autumn versus spring. Cows that calve in autumn will experience higher basal prolactin concentrations in the second half of pregnancy. The reverse will apply to cows calving in spring. The secretion of a hormone independent of day length and temperature changes would therefore appear to be of advantage. It is clearly important to know what the potency of bovine PL is by comparison with bovine prolactin in stimulating secretory activity and, directly or indirectly, mitosis in bovine mammary gland.

Evidence relating to the effects of twinning on milk yields in dairy cows is conflicting and may be influenced by the level of nutrition in pregnancy and by the tendency to a shorter gestation in twin-bearing cows (24). In several studies, depression of milk yield has been observed in the lactation following the birth of twins. However, calf birth weight and milk yield are positively correlated in singleton pregnancies (45, 137), suggesting an effect of some function relating to the conceptus, although it is not known which hormone, if any, is involved. Kay (73) reported a higher milk yield in the lactation concurrent with a twin pregnancy, but this effect was not observed by Chapin and Van Vleck (24).

**Species Without Placental Lactogen**

Prolactin of concentrations during pregnancy have recently been measured by radioimmunoassay in four species that appear not to have a PL (Table 1), namely, rabbit (93), pig (43), cat (7), and dog (32). There is no quantitative information about mammary development in the last two species, but histological studies indicate that lobulo-alveolar growth is well-advanced with initiation of secretory activity between 40 and 50 d after mating (143). This appears to coincide with a period of rising prolactin concentrations from about midpregnancy, d 35, in a gestation length of 63 d.

By contrast, prolactin is very low and even falling in rabbits and pigs during the phase of rapid mammary development (34, 77) that occurs after d 18 in rabbits (gestation length 30 d) or d 75 in pigs (gestation length 115 d). A requirement for prolactin in rabbit mammary development was, nevertheless, clearly shown by Denamur and Delouis (36). Rabbits hypophysectomized on d 14 of pregnancy showed normal mammary development only if prolactin, as well as progesterone, was injected. Initiation of secretory activity occurs in rabbits in vivo in response to prolactin in the absence of the ovaries and adrenals (28). In vitro, mammary explants from pseudopregnant or midpregnant rabbits synthesize casein (38), medium-chain fatty acids (53), and show induction of &alpha;lactalbumin activity (116) in response to prolactin without requiring the presence of exogenous insulin or glucocorticoid. All these findings point to a requirement for prolactin. However, this may be met, not by rising concentrations of the hormone, but by the modest increase in prolactin receptors in the gland at midpregnancy (40) together with a changed balance of hormone secretion with progesterone concentrations falling and glucocorticoids rising (42). In pigs, mammary development may be promoted by estrogen production by the fetoplacental unit with the declining progesterone concentrations after d 90, allowing differentiation as in rabbits (77).
gilts carrying 4 to 7 or 8 to 11 fetuses. Pregnant gilts showed greater development of the mammary glands, suggesting an effect of the conceptuses. However, higher fetal number was not associated with any further increase in mammary development (76).

LACTOGENESIS AT PARTURITION

The onset of copious milk secretion at parturition, lactogenesis stage II (46), is a feature of lactation in all eutherian mammals, that meets the nutritional requirements of their relatively well-developed neonates. There have been two main theories to account for this phenomenon, either an increased positive stimulus from lactogenic hormones, especially prolactin and glucocorticoids, or the release from inhibition by progesterone. Theories of positive stimulation arose in part from the effects of giving exogenous hormones in pregnancy. Rabbits are the only species known to initiate substantial milk secretion in pregnancy in response to exogenous prolactin (94). Exogenous glucocorticoids are effective in rabbits (94, 132), rats (132), mice (142), and sheep (33), but the interpretation is difficult as corticoids may also promote premature delivery. If the surge of prolactin secretion that occurs at parturition is blocked by giving the dopamine agonist bromocriptine, normal onset of lactogenesis stage II is prevented in rats (12), rabbits (39), and pigs (133). However, bromocriptine is a long acting drug and in nonruminants, which require prolactin for the maintenance of lactation (see next section), effects on lactogenesis and on galactopoiesis could become confused. In ruminants, carefully controlled experiments indicate that prolactin inhibition at parturition delays rather than totally prevents lactogenesis stage II when milking is continued (1, 29), although milk yield in cows is partially correlated with plasma prolactin concentrations in the period from 48 h before to 2 h after parturition (45). A strong case has been made by Kuhn (81, 82) to regard progesterone withdrawal. There are differences in the site of control (at the fetal hypothalamus in sheep or at the maternal hypothalamus in rabbits (63)) and in the tissues and enzymes involved (induction of placental 17α-hydroxylase in the sheep or luteal 20α-hydroxysteroid dehydrogenase in the rat (82).

Understanding of how a progesterone block on the rate of milk synthesis in pregnancy operates is far from complete, but a number of different mechanisms may be involved, some local and some systemic (82, 136). Progesterone inhibits prolactin stimulation of its own receptor (39). It also inhibits many other actions of prolactin, including transcription, stabilization, and translation of messenger RNA for casein in rabbits (133, 136) and rats (114). It may in part act in competition with glucocorticoids, but this does not explain all its effects, since, at least in rabbits, not all of prolactin’s stimulatory actions require a glucocorticoid (135). Metabolism or transport of progesterone by the mammary gland itself may be involved (83, 129). The difficulty generally experienced in demonstrating inhibition in vitro with physiological concentrations of progesterone (22, 135) may be explained in part by instability of the progesterone receptor but also if some of progesterone’s inhibitory actions are by indirect or systemic mechanisms.

The progesterone block on lactogenesis is not, however, absolute. If it were, then simultaneous pregnancy and lactation would be impossible. Once lactation is established, it cannot be inhibited by progesterone alone. Thoroughbred horses quite commonly show premature onset of lactation and leakage of milk prepartum. Premature lactation can be readily initiated in ruminants by milking, although the effect is a local one, occurring only in the milked gland. Experiments in goats (91) have suggested that prostaglandin F2α (PGF2α) synthesized by the mammary gland, acts as a local inhibitor of lactogenesis. Output of PGF2α into mammary venous blood ceases near term, associated both with metabolism to dehydroxy-PGF2α and an increased output into milk. Milking in late pregnancy would remove or dilute the inhibitor.

GALACTOPOIESIS

It is common to all species so far examined, including marsupials (64), that the pituitary is

required to maintain lactation and milk yields fall dramatically within a few days after hypophysectomy.

An increase in circulating prolactin in response to suckling is also a general observation. Basal prolactin tends to be high in early lactation and later declines. The rise in prolactin induced by suckling also becomes smaller, although there is no clear evidence that diminished prolactin release is responsible for declining milk yields (27). Indeed, in rats only a small proportion of the prolactin released at suckling appears to be required to maintain milk secretion (57).

The different minimum requirements for anterior pituitary hormones that will restore milk yields to preoperative amounts in hypophysectomized animals have been mentioned previously. Treatment with bromocriptine to prevent the suckling-induced release of prolactin and to depress basal concentrations of the hormone similarly has a rapid effect in inhibiting milk secretion in rabbits, pigs, dogs, rats (48), and humans (14). In some species [e.g., rabbits (134) and rats (12)], exogenous prolactin administration has reversed this effect. However, in mice (21, 146) and notably in cows (72, 125), goats (58), and sheep (68), suppression of prolactin secretion either has no effect (cows and goats) or only partially (sheep and mice) inhibits lactation. The probable importance of GH for lactation in ruminants was shown in replacement experiments in hypophysectomized goats and sheep (26, 35). It has also been known for many years that GH administration has a dose-dependent effect in stimulating milk yield in dairy cows (71). Commercial exploitation of such an effect was not practicable until the advent of recombinant DNA technology stimulated such interest in this topic (107). It is, however, increasingly likely that GH does not produce its effects by direct stimulation of the gland in ruminants. McDowell and Hart (92) were recently unable to stimulate milk yield in sheep by close arterial infusion of GH until the dose was high enough to raise circulating concentrations of the hormone when the effect was seen not locally but in both the infused and the uninfused control glands. Rather, GH appears to act by partitioning available energy away from body tissues and toward milk. In goats with one mammary gland transplanted to the neck, and therefore denervated, milk yield is normal, but there is no prolactin released in response to milking. However, release of GH occurs as a delayed response (59). This suggests that metabolic stimuli, in addition to or instead of tactile stimuli, may effect GH release at milking, in contrast to prolactin.

In vitro, bovine GH does not stimulate casein or fat synthesis or α-lactalbumin secretion by cow mammary gland (56) or ovine GH α-lactalbumin synthesis by sheep mammary gland (20). In contrast, effects of mouse GH on mouse mammary gland appear direct (87). We have, nevertheless, observed an effect of bovine GH in maintaining but not initiating fatty acid synthesis by bovine mammary gland explants (Table 5), so there may be some direct effects of the hormone on ruminant mammary tissue.

It has already been mentioned that milk yield can be enhanced in mice by increasing the number of young suckling (78). Milk yield in pigs is related to litter size (Table 6), but it is not known whether this is controlled entirely by suckling and milk removal or whether previous development in pregnancy plays any part (76). Even though in ruminants, such a high proportion of mammary growth occurs during pregnancy (2, 4, 51) and in goats (61) milk yield is related to number of kids born, milk yield can be further enhanced postpartum. In a study of 16 primiparous goats, the cor-

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fatty acid synthesis²</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
</tr>
<tr>
<td>Control (uncultured)</td>
<td>19.4</td>
</tr>
<tr>
<td>I + F</td>
<td>8.9</td>
</tr>
<tr>
<td>I + F + prolactin</td>
<td>48.1</td>
</tr>
<tr>
<td>I + F + GH</td>
<td>25.4</td>
</tr>
</tbody>
</table>

¹Explants were precultured for 2 d in insulin (1, 5 µg/ml) plus cortisol (F, 1 µg/ml), before transfer for a further 2 d to IF, IF plus bovine prolactin, 100 ng/ml, or IF plus bovine growth hormone (GH), 100 ng/ml.
²Values are means ± half difference between duplicates.
TABLE 6. Effect of litter size on milk yield in pigs.1

<table>
<thead>
<tr>
<th>Number of piglets in litter</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daily milk yield kg/litter</td>
<td>4.0</td>
<td>4.8</td>
<td>5.2</td>
<td>5.8</td>
<td>6.6</td>
<td>7.0</td>
<td>7.6</td>
<td>8.2</td>
<td>8.6</td>
</tr>
<tr>
<td>kg/piglet</td>
<td>1.0</td>
<td>1.0</td>
<td>.9</td>
<td>.9</td>
<td>.8</td>
<td>.8</td>
<td>.8</td>
<td>.7</td>
<td>.7</td>
</tr>
</tbody>
</table>

1 Data from Elsley (44).

relation between milk yield and PL concentrations between wk 16 and 20 of pregnancy was stronger at 50 d of lactation (r = .635) than at 203 d (r = .374), suggesting a declining influence of prepartum effects (52). Compensatory increases in milk yield, which became significant days or weeks after removing one mammary gland in goats (79) and ewes (30), may indicate that the lactating gland still has the capacity for growth. However, immediate increases in yield can be produced by experimental manipulation (66) or three times daily milking (65, 106), which suggests that the secretory capacity of cells can be increased. The mechanisms operating are not fully understood but may include removal of inhibitors and offer interesting possibilities for maximizing yield.

In a consideration of species differences in galactopoiesis, it is interesting to note that mammals vary widely in the frequency with which suckling occurs. This ranges from more than 50 times a day in rats, to once a day in rabbits, and to as infrequently as once a week in some marine mammals (139). In most species the cessation of suckling quite rapidly brings about involution as milk accumulates within the glands (84). There must be special mechanisms that prevent this in species with long suckling intervals.

**LACTATION IN MARSUPIALS**

Marsupials provide a further example of the diverse ways in which mammary development and function relate to patterns of hormone secretion.

The young are born very immature after a short gestation period similar in length to one estrous cycle. The neonate's requirement for milk increases slowly, in marked contrast to the immediate demands of the more mature young of eutherian mammals for a copious supply of milk directly after their birth. The key event in initiation of marsupial lactation is the attachment of the young to a teat after it has made its way from the birth canal to the marsupium. Lactation is not dependent on the occurrence of pregnancy, since nonpregnant females can lactate successfully if foster young are transferred to attach to a teat and suck, if the transfer is at the stage of the estrous cycle corresponding with the time of birth. As lactation progresses, milk composition changes from a dilute milk, high in carbohydrates, to a more concentrated milk in which protein and particularly fat concentrations increase. The four mammary glands of wallabies and kangaroos can be simultaneously at different stages of development with two quiescent, one supplying dilute milk to an attached pouch young and one secreting concentrated milk for an older joey outside the pouch (85, 144). Marsupial lactation consists then of three stages; 1) preparation of the gland during the estrous cycle, which requires the corpus luteum but is independent of pregnancy, 2) slow suckling-induced increase in milk production for the pouch young, which involves gland growth; and 3) maintenance of lactation by intermittent suckling by a juvenile. Hinds and Tyndale-Biscoe (67) have used a heterologous assay to measure prolactin in pregnant and lactating tammar wallabies (*Macropus eugeni*). Prolactin patterns during the estrous cycle and pregnancy were distinguished only by a peak of prolactin at the time of birth, which transfer experiments would indicate is not essential for initiating lactation. Prolactin remained low in the first 100 d of lactation but rose much higher, exceeding 100 ng/ml, in two animals that successfully maintained their pouch young. Prolactin declined when the young were artificially removed. It is known that hypo-
physsectomy terminates lactation in this species (64). The relatively low amounts of prolactin in early lactation may achieve their stimulatory effect via an increase in prolactin receptors per cell. Such an increase is observed, in the suckled mammary gland only, over the first 12 wk of lactation.

CONCLUSION

Some features relating to mammary physiology show remarkable similarity in all mammals. The structure of the secretory mammary tissue, the ultrastructure of epithelial cells and of the secretory process, and the milk ejection response to oxytocin appear to be essentially the same in all mammals, whether egg-laying monotremes, marsupials, or placental mammals. Studies in endocrinectomized animals defined the hormones involved in mammary growth and function, but until relatively recently, no assays were available to measure hormone concentrations. It is perhaps remarkable that so much could be learned using injection schedules that elevated hormone concentrations in ways quite different from normal secretion patterns and with exogenous protein hormones extracted from a quite different species than that used in the test. Now that radioimmunoassays and radioreceptor assays allow secretion patterns to be examined, we are discovering that species vary widely and show considerable complexity. There is still relatively little information about concentrations of GH in pregnancy and lactation. A surge of prolactin at parturition and a suckling-induced rise in prolactin secretion are generally observed, although uncertainties remain about their precise physiological significance and quantitative relevance. Prolactin secretion patterns in pregnancy vary widely and, except in rats and mice, we have little information on circadian variation and whether the onset of pregnancy affects it. In some species, the prolactin and GH stimulus is largely replaced by secretion of PL. It is of interest that specific binding of prolactin to the mammary gland appears much greater in species without PL (rabbits, pigs, ferrets, dogs) than in species in which the hormone can be demonstrated (rats, mice, ruminants, humans). Finally, ruminants appear to differ from other mammals in the maintenance of lactation. In most species, suppression of prolactin secretion rapidly inhibits milk production. However, ruminants appear much more dependent on the metabolic effect of GH in partitioning metabolic energy to the gland, although it cannot be assumed that prolactin has no role. Small amounts of prolactin remain in the circulation of bromo- criptine-treated animals and this may be sufficient, perhaps together with GH, to maintain functional integrity in the gland.

In early studies, the role of the young in mammary development was largely ignored. It is now clear that hormones from the conceptus play an important role in mammary development in pregnancy. After parturition, to meet their nutritional requirements, the suckling young provide the stimuli responsible for further development and maintenance of function of the lactating gland. Consideration of lactation as the final phase of reproduction, a phase that must be integrated with its other aspects, may help us to understand better lactational endocrine strategies. It is also clear that there are many findings that cannot be explained in terms of systemic changes in hormone and metabolite levels. Much control is at the mammary gland itself.

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