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Aspects of the Biochemistry, Physiology and Endocrinology of Lactation*

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Abstract

Recent research on the endocrine control of the initiation of lactation, on the hypothalamic control of prolactin secretion, and on the utilization of nutrients by the mammary gland for the synthesis of milk is reviewed. Particular emphasis is placed on the mechanism of action of prolactin, and the role played by prolactin receptors.

Introduction

The study of lactation and its control has received new impetus from the discovery of powerful drugs inhibiting prolactin secretion, which have application both to the inhibition of lactation, and to the treatment of infertility (see de Pozo and Lancranjan 1978). The clear identification of prolactin as a key hormone in lactogenesis in several species, and advancing knowledge on prolactin receptors and their role in the control of the mammary gland, has further stimulated research in this field. The mechanism of action of prolactin and the hypothalamic control of prolactin secretion are other areas of increasing research interest. While research into the synthesis of milk constituents is receiving less attention at present, the purpose of lactation in providing nutrition for the young mammal cannot be neglected, and is discussed in relation to the utilization of nutrients by the mammary gland.

Endocrine Control of the Initiation of Lactation

Three main lines of research have led to most of the information in this field: the *in vitro* culture of mammary tissue and examination of its hormone requirements for lactogenesis, the *in vivo* administration of exogenous hormones with or without removal of endocrine glands, and the measurement of endogenous concentrations of lactogenic hormones during pregnancy and lactation.

Studies using Organ Cultures of Mammary Tissue

The technique used *in vitro* is the culture of small pieces of mammary tissue containing ducts and alveoli from animals at varying stages of physiological development. The cultures can be maintained for 1–2 weeks, during which time hormone stimulation can be measured either histologically or biochemically. Tissues obtained from mice, rats, rabbits and women have been extensively studied with considerable general agreement as to the effects of hormones on development and lactogenesis (see review by Forsyth 1971 for background).

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Mammary ducts and alveoli are hormone responsive from early foetal development. The primary mammary sprout grows out on day 16 of foetal life in the mouse, and at 17 days mammary analagen of foetal rats respond to culture with insulin, aldosterone and prolactin by extensive development of a duct system and formation of intracellular organelles (Ceriani *et al.* 1970). Progesterone added to the three hormones gives rise to secretion containing casein-like proteins within 9 days of culture (Barnawell 1965). The characteristic changes seen in development of the mammary glands in the male rat foetus have been shown to be due to the secretion of the foetal testis (Raynaud 1971), thus further confirming the role of hormones in the control of growth and development of the mammary glands of the foetus.

Mammary alveolar tissue from adult female rats, mice and rabbits can be induced to grow, differentiate and secrete milk in organ culture, with a minimum hormone combination of an adrenal corticosteroid (corticosterone, cortisol, aldosterone), insulin and prolactin (see Forsyth 1971). The effects of oestrogen, progesterone and thyroid hormones are very dependent on the concentrations used, and oestrogen: progesterone ratio. Explants of human breast tissue cultured *in vitro* displayed growth of large ducts in the presence of 4×10^{-9}M oestrogen and of interlobular ducts in the presence of 4×10^{-6}M progesterone (Van Bogaert 1978). Triiodothyronine (1×10^{-9}M) was shown to increase lactogenic stimulation in explants of mammary gland from hypothyroid or euthyroid mice cultured with insulin, cortisol and prolactin.

Mammary tissue, obtained from rabbits on days 12–14 of pseudopregnancy which has proliferated *in vivo* under the influence of endogenous hormones, can be stimulated to lactate in culture by prolactin alone (Forsyth *et al.* 1972). Results of other studies have shown lactogenic stimulation of alveolar tissue, from rabbits, cultured in the presence of placental lactogens and human growth hormone—polypeptide hormones with close similarity to prolactin (Niall *et al.* 1971; Forsyth 1973).

To summarize the results obtained from studies using organ cultures of mammary tissue, insulin and an adrenal corticosteroid appear to be necessary for maximum growth and division of alveolar epithelial cells. In spite of this, these hormones are not lactogenic. Gonadal steroids stimulate selective duct and lobular growth in mammary explants, but are not lactogenic *in vitro*. Lactogenesis *in vitro* appears to be dependent upon the presence of prolactin, or the closely related hormones human placental lactogen and human growth hormone.

Studies in Surgically Modified or Intact Animals

Investigations of the effects of surgical ablation of endocrine glands on the initiation of lactation have resulted in a comprehensive understanding of the hormone requirements for mammary growth and lactation *in vivo* (see Cowie and Tindal 1971). In particular, replacement of hormones in hypophysectomized animals or hypophysectomized, ovariectomized and adrenalectomized animals have allowed the effects of individual hormones and combinations to be assessed (Lyons 1958). In the triply operated rat the minimum hormone requirements for mammary duct growth are oestrogen, adrenal steroids and growth hormone. For lobulo-alveolar growth, progesterone and prolactin are additionally required. Species differences in response to ovarian hormones have been observed, the sheep and cow in particular responding to oestrogen alone at physiological concentrations by lobulo-alveolar growth (Cowie and Tindal 1971).

Recent research on rodent, human and ruminant placental lactogens has raised the question of their physiological roles for the mammary gland. That their presence is not obligatory for mammary development and lactation is evident from the ability of the rabbit and bitch to lactate after pseudopregnancy, and the successful induction of lactation by exogenous steroid hormones in non-pregnant animals (see, for example, Fulkerson *et al.* 1976). In rats placental lactogen shows a peak in blood concentration at about the 12th day of pregnancy, with a fall until days 17–21 when a rise is followed by a sharp fall just before parturition (Shiu *et al.* 1973). Prolactin concentrations are low in the latter part of pregnancy rising just before parturition. It is thus likely that in the rat, placental lactogen has an effect during the middle of pregnancy, when major growth of the ducts and alveolar tissue of the mammary gland is occurring. In ruminants, the lactogenic activity in blood during pregnancy is largely attributable to placental lactogen, which rises to peak values in the region of 1 μg/ml or higher during the last third of pregnancy (Forsyth and Hart 1976). This may be responsible for a significant proportion of mammary growth during that period, as the hormone has activity in both lactogenic and growth hormone-like roles (Chan *et al.* 1976).

Initiation of Milk Secretion

The onset of milk secretion from the developed mammary gland occurs near the time of parturition, but the timing is difficult to determine with accuracy. Milk constituents begin to appear well before parturition in most species, but high rates of synthesis do not occur until after the birth of the young. For example, in the rabbit the milk-specific, medium-chain fatty acids appear in the gland during days 19–22 of pregnancy, but are not synthesized in quantity until day 27 and onwards, parturition occurring on day 31 (Strong and Dils 1972). Maximal rates of milk fat synthesis are reached at about 3 weeks after parturition (Cowie 1969).

In the rat, major biosynthetic changes occur much closer to parturition. Casein appears in appreciable concentrations in milk only 2 days before parturition whereas lactose begins to accumulate rapidly on the day of parturition itself (Kuhn 1977). Other species exhibit similar variation.

The hormonal trigger for the initiation of secretion appears to have two discrete components—one comprising a prolactin-induced stimulation, and the other removal of a progesterone-induced inhibition. The effects of prolactin on initiation and maintenance of secretion have best been demonstrated in the rabbit. In rabbits pseudopregnant for 12–14 days, the intravenous or intraductal injection of prolactin will initiate enzymic and morphological changes in the mammary tissue leading to milk secretion within 3 days (Fiddler *et al.* 1971). Conversely, administration of the prolactin-inhibiting drug bromocriptine will totally block milk secretion after parturition (Taylor and Peaker 1975).

Cowie *et al.* (1969) showed the effects of hypophysectomy and replacement with prolactin on lactation in the rabbit. After hypophysectomy lactation was effectively abolished, replacement with cortisol had no effect, but addition of prolactin returned lactation yield dramatically back to its original level.

In the cow, prolactin appears to be a key component for the onset of milk secretion, since inhibition of prolactin release with bromocriptine prior to parturition severely suppressed milk yield (Schams 1976). It is interesting to note that prolactin has less importance for maintenance of established lactation in the dairy cow, since use of bromocriptine during lactation has little or no effect on milk yield (Schams 1976).

Growth hormone has been implicated in the maintenance of milk yield in dairy cows, though whether this is a direct hormone effect on the mammary gland or an indirect effect through increasing the availability of nutrients is not clear (Hart *et al.* 1978). Measurements of serum concentrations of prolactin in several species have shown low fluctuating levels early in pregnancy, very low levels in mid–late pregnancy and a dramatic rise just prior to parturition (McNeilly and Friesen 1978). As discussed earlier, prolactin will induce lactogenesis in organ cultures of mammary tissue and in the mammary glands of pseudopregnant rabbits. The observed endogenous rise in prolactin at parturition is thus likely to be a major lactogenic stimulus in the normal animal.

The other aspect of the control of lactogenesis is the evidence for the withdrawal of progesterone as a trigger mechanism. It has been widely shown that removal of the corpus luteum, or ovaries, from pregnant animals initiates lactation, and that exogenous progesterone administration will inhibit this effect (see Kuhn 1977). Progesterone has been shown to act directly upon mammary alveolar tissue, since it will also inhibit lactogenesis in explants of mammary gland in culture (Turkington and Hill 1969). In the goat at parturition, the fall in circulating progesterone, the rise in prolactin, oestrogen and prostaglandin, and the increase in uptake of glucose by mammary tissue (indicating the onset of milk secretion), are effectively simultaneous (Davis *et al.* 1979).

Thus at parturition in the normal animal, both hormone mechanisms can be expected to play a part in the initiation of lactation. The rapid drop in progesterone, and rise in prolactin, in the blood just prior to parturition will therefore remove the inhibitory control by progesterone and simultaneously provide activation by prolactin.

Artificial Induction of Lactation in Domestic Animals

The induction of lactation in non-pregnant animals has been carried out both experimentally and as a mechanism for obtaining milk production from infertile cows. Recent work in Australia by Fulkerson and others on the artificial induction of lactation in the ewe has demonstrated the striking effect of glucocorticoid, as a lactogenic trigger, in animals previously treated with oestradiol and progesterone to develop the mammary glands. Dexamethasone (10 mg/day) initiated lactation within 4 days, without any apparent major increase in blood prolactin concentration. This occurred even when progesterone (20 mg/day) was also injected, which indicates the absence of suppression of lactogenesis by progesterone in these circumstances in the ewe (Fulkerson *et al.* 1976).

The mechanism by which exogenous glucocorticoids act in initiating lactation is not clear, and will probably require studies in hypophysectomized animals to ensure that it is not a pituitary-mediated response. It is unlikely that glucocorticoids represent a normal lactogenic trigger in the ewe, since the blood concentrations only rise after parturition, presumably due to a stress response (Chamley *et al.* 1973).

Non-pregnant cows have been induced to lactate by treatment for 7 days with oestradiol and progesterone. Lactation began at 13 days after commencement of hormone treatment, and followed an approximate doubling of plasma prolactin. The suppression of plasma prolactin by bromocriptine delayed the onset of lactation until an average of 11 days after bromocriptine was discontinued (Peel *et al.* 1978). This system therefore is closer to the endogenous hormone changes initiating lactation than the use of glucocorticoids in the ewe.

Prolactin Receptors in Lactogenesis

Since the discovery of prolactin binding to mammary tissue, with specific localization on the plasma membrane of the cell adjacent to the vascular supply (Birkinshaw and Falconer 1972), the characteristics of prolactin receptors have been thoroughly investigated (Shiu and Friesen 1974). The importance of prolactin receptors in mediating the actions of prolactin in mammary tissue was convincingly demonstrated by use of an antiserum to the receptors, which reduced the stimulation of casein synthesis by prolactin in organ culture of mammary explants (Shiu and Friesen 1976).

Receptor numbers vary with the physiological condition of the animal. In the rabbit, the total binding of prolactin in the mammary gland is low throughout pregnancy, rising slowly until day 29. A tenfold increase in prolactin binding occurs between day 29 of pregnancy and day 6 of lactation. This increase in prolactin receptors is synchronous with the onset of lactation, and occurs at the same time as the major rise in prolactin at parturition (Djiane *et al.* 1977). In the rat, prolactin binding by the mammary gland also increases sharply at parturition, approximately sixfold per unit wet weight between day 20 of pregnancy and day 5 of lactation (Hayden *et al.* 1979).

Oestradiol administration was shown to increase the number of prolactin receptors in mammary glands from virgin rats, whereas progesterone suppressed this increase. In hypophysectomized rats, prolactin injection dramatically increased the number of prolactin receptors. This and other studies indicate that prolactin induces its own receptors in the mammary gland (Hayden *et al.* 1979). Some of the increase in prolactin receptors occurring naturally at parturition and in early lactation is probably due to an increase in the number of secretory epithelial cells, since prolactin is demonstrably mitogenic for mammary epithelium (Bourne *et al.* 1974).

From this information it is clear that not only do fluctuating hormone concentrations affect mammary tissue directly, but the numbers of hormone receptors in mammary tissue also vary. The increase in prolactin receptors caused by prolactin appears to be a positive feedback system for accelerating lactogenesis, probably acting through an increase in receptors per cell, and an increase in cell number.

Mechanism of the Initiation of Lactation

While there is a considerable amount of data on the endocrinology of lactogenesis and also on the biochemistry of the mammary gland, the mechanism by which hormones initiate milk secretion is far from clear. Most studies on the initiation of lactogenesis have used one of the characteristic biochemical indicators of milk synthesis as a measure of hormone response. These have included synthesis of medium-chain fatty acids (Strong and Dils 1972; Falconer *et al.* 1978*a*, 1978*b*); casien (Rillema *et al.* 1977); casein mRNA (Devinoy *et al.* 1978; Matusik and Rosen 1978) and lactose (Jones and Cowie 1972). From the characteristic changes in the biochemical capacity of the mammary gland that occur at the onset of lactation, both in terms of synthesis of new compounds and quantity of synthesis, it is apparent that major gene activation takes place.

The only experiments to demonstrate this directly are those in which casein mRNA synthesis has been measured (Matusik and Rosen 1978). These workers demonstrated that within 1 h of addition of prolactin to organ cultures of rat mammary gland, casein mRNA increased above the amount observed in cultures containing insulin and

hydrocortisone alone. The effect was not dependent on the glucocorticoid, but maximal stimulation only occurred in its presence. The amount of casein mRNA increased steadily up to 48 h after prolactin addition. Thus the presence of a specific polypeptide hormone on its plasma membrane receptor had stimulated DNA transcription from a gene unique to milk synthesis. In this case some casein mRNA was present at the beginning of treatment—and casein is normally present in low concentrations in mammary gland during the latter part of pregnancy. However, the rate of transcription of the gene was greatly increased due to prolactin and the effect was further accelerated by hydrocortisone.

If prolactin exerts its biological effects while bound to receptors on the plasma membrane of the cell, it is necessary to postulate a 'second messenger' system whereby nuclear function can be modulated. Alternatively, if intact prolactin molecules or biologically active fragments are taken into the cells together with their receptors, and then the hormone or its fragments interact with the nucleus to stimulate lactogenesis, then the prolactin receptors are simply a system for internalizing prolactin in alveolar cells.

Although internalization of polypeptides does occur, and is a major degradative pathway (Goldstein *et al.* 1979), there is no evidence at present that prolactin is internalized in order to function or that hormone fragments are biologically active (Falconer, unpublished data). The postulated 'second messenger' for prolactin has therefore been assiduously sought.

Cyclic 3′,5′-adenosine monophosphate has no stimulatory effect, even at 10 mM in organ culture of mammary tissue with spermidine (0·5 mM) present as well (Rillema *et al.* 1977). Cyclic 3′,5′-guanosine monophosphate together with spermidine stimulates casein synthesis, but alone has no effect. A similar response has been measured in the presence of prostaglandin $F_{2\alpha}$ and polyamine. Increased tissue polyamines are widely associated with hormone responses, and the key enzyme for their synthesis, ornithine decarboxylase, rapidly increases in activity upon stimulation of many tissues (Thomson and Richards 1978). This effect is, however, highly unspecific, either for prolactin or for mammary gland. Polycations appear to interact with cell membranes, causing hormone-like effects, as do prostaglandins in a number of hormone-sensitive systems (Wolff and Cook 1977). Spermidine alone stimulates RNA synthesis in perfused lactating mammary gland (Mepham and Peters 1978), indicating an effect at nuclear level.

It is clear that these biologically active compounds have a potential role in the normal hormonal stimulation of a variety of tissues, but their implications for the initiation of lactation await further research.

Prolactin and Ion Transport

An alternative approach to the mechanism of action of prolactin has been through its role in vertebrates evolving at different times in the Earth's history. In fish and eels which migrate between salt and fresh water at different periods of their life cycles, prolactin plays a key osmoregulatory role in sodium retention during survival in fresh water. In one fish, the Amazonian discus fish, prolactin also stimulates a cutaneous secretion for nutrition of the young fish (Hildermann 1959). Amphibia use prolactin in both osmoregulatory and reproductive roles—the water-seeking behaviour of newts, for example, being stimulated by prolactin (for further examples see Bern 1975).

Prolactin in Killifish stimulates renal Na^+/K^+ activated adenosinetriphosphatase (Na^+/K^+ ATPase) and inhibits gill Na^+/K^+ ATPase (Pickford *et al.* 1970). In the marine birds and ducks, prolactin is one component of the control of salt excretion by the nasal salt gland (Peaker *et al.* 1970; Peaker and Linzell 1975). Prolactin also controls crop milk production in pigeons (Riddle *et al.* 1932). Thus, the evolutionary history of prolactin shows both osmoregulatory and reproductive functions, with the nutrition of the young through a prolactin-controlled epithelial secretion occurring in species of fish and birds as well as mammals.

Milk has an ionic composition more closely resembling intracellular fluid than extracellular fluid. This is brought about by the epithelial secretory cells having active monovalent-cation pumps which increase cell K^+ and decrease cell Na^+ only on the plasma membrane adjacent to the vascular supply (Johnson and Wooding 1978). By contrast, the apical surface of the cells adjacent to the milk appears to be a simple unselective permeability barrier to monovalent cations thereby allowing the ratio of intracellular K^+/Na^+ to largely regulate that of milk. Prolactin has effects on the ionic composition of milk, particularly in late lactation, part of which is due to tightening of cell junctions which prevents intercellular leakage of ions (Linzell *et al.* 1975). Prolactin has also been shown to rapidly activate the reciprocal transport of Na^+/K^+ in mammary tissue (Falconer and Rowe 1975) resulting in elevated tissue K^+ and depressed tissue Na^+ concentrations (Falconer and Rowe 1977). This effect is also seen when prolactin is added *in vitro* to explants of mammary tissue from pseudopregnant rabbits (Falconer *et al.* 1978*a*).

Examination of a possible 'second messenger' function through modulation of intracellular K^+ concentration has led to interesting results. It has been shown that prolactin-induced lactogenesis *in vitro* may be prevented by low concentrations of ouabain, a specific Na^+/K^+ pump inhibitor (Falconer *et al.* 1978*a*). Results of more recent investigations supported this possibility. In these studies, valinomycin depleted intracellular K^+ and inhibited lactogenesis *in vitro*. Small increases in extracellular concentration of K^+ opposed these effects of valinomycin (Falconer, unpublished data).

In this work initiation of synthesis of medium-chain fatty acids (Falconer *et al.* 1978*b*) and changes in protein and RNA synthesis as indices of prolactin stimulation and its inhibition were studied. Synthesis of medium-chain fatty acids, in particular, is a highly specific response to lactogenesis in the rabbit. It is due to the presence of the enzyme medium-chain hydrolase which is not otherwise present (Chivers *et al.* 1977). Thus, stimulation of synthesis of medium-chain fatty acids reflects gene activation by prolactin. Its inhibition could, however, occur at any step between initiation of DNA transcription and availability of substrates for fatty acid synthesis.

The role of monovalent cations in cell activation is receiving increasing attention at present, which hopefully will clarify their mechanisms of action (Koch and Leffert 1979).

The modulation of cytoplasmic Ca^{2+} concentration is implicated in a number of hormone activation processes in other tissues (Hales *et al.* 1977). The mammary alveolar cells in lactation secrete calcium complexed with casein, apparently through accumulation of Ca^{2+} in Golgi vesicles (Wooding 1977). Calcium does not freely traverse the glandular epithelium, and a regulated transport process would be expected in mammary cells (Neville and Peaker 1979). Whether transport of Ca^{2+} plays a part in lactogenesis remains to be clarified.

At present, therefore, it is not possible to state with certainty how prolactin controls lactogenesis.

Control of Prolactin Secretion

Prolactin secretion occurs in a pulsatile manner, associated particularly with parturition, suckling, or stress. Blood concentrations therefore vary markedly from hour to hour in a single animal or person. Prolactin secretion in response to suckling or teat stimulation has been particularly thoroughly researched, and the neuroendocrine pathways mapped (see Cowie and Tindal 1971). Sensory receptors in the teat connect through an ascending nervous pathway in the spinal cord which can be effectively blocked by local anaesthesia of the teat (Findlay 1968). The suckling-induced oxytocin release pathway has been mapped through the brain in detail (Cowie and Tindal 1971). Results of comparable studies on the pathway for prolactin release have demonstrated similarities with the mechanism for the release of oxytocin, but there is evidence for cerebral cortical involvement in prolactin release that is not present for oxytocin secretion (Cowie and Tindal 1971). The hypothalamic release of neurohumoral agents represents the termination of the neural pathways for prolactin secretion, and has received much recent research interest.

Earlier studies showed a tonic inhibition of prolactin secretion, in the intact animal, which could be released by severing the pituitary stalk or autotransplantation of the pituitary under the kidney capsule (Everett 1956). The inhibitory agent has been sought and identified as dopamine, which has been found in hypothalamic–pituitary portal blood in sufficient concentration to account for the inhibition of prolactin release (Gibbs and Neill 1978). Whether modulation of dopamine secretion is the mechanism whereby suckling releases prolactin is still unclear, and evidence exists that a positive stimulus of secretion may exist independently (see Krulich 1979). The serotoninergic system may activate prolactin secretion, since serotonin receptor blockade will prevent the release of prolactin by suckling in the lactating rat (Kordon *et al.* 1974). The inhibitory effects of dopamine appear to be blocked by endogenous opiates, which may relate to the release of prolactin during stress (Enjalbert *et al.* 1979).

The presence of a specific prolactin-releasing factor remains to be clarified, but thyroliberin (otherwise TRF, thyrotrophin-releasing factor) has potent prolactin-releasing capabilities. Thyroliberin administration will stimulate release of prolactin in monkeys with sectioned pituitary stalks, demonstrating that its effect does not require intermediate activation of other releasing factors, or that it is due to inhibition of dopamine secretion. Thyroliberin stimulation may be inhibited by L-dopa, showing that the two compounds have direct pituitary effects in opposing directions (Deifenbach *et al.* 1976). In circumstances when thyroliberin is administered together with sulpiride, a dopamine antagonist, the effect on prolactin secretion is synergistic, indicating that different mechanisms are involved (Portioli *et al.* 1976).

It is not clear whether thyroliberin is a natural controller of prolactin secretion. In the case of prolactin release after suckling it seems unlikely, since little change in TSH release occurs (Jeppson *et al.* 1976). In the same experiments thyroliberin was shown to induce release of TSH and prolactin after suckling in a normal manner.

Evidence is accumulating that there is a prolactin-releasing factor independent of thyroliberin or dopamine inhibition. Several groups have reported this activity in hypothalamic fractions, but it remains controversial (see Boyd *et al.* 1976). From

these studies it is apparent that prolactin secretion is controlled by several independent systems operating through hypothalamic neurosecretions. The dopamine system is inhibitory, but appears to be overridden by a serotoninergic system and by releasing factors. The physiological relationships between these mechanisms await clarification.

Nutrient Utilization by the Mammary Gland for Milk Production

Milk fat is synthesized from plasma glucose, from circulating free fatty acids, and from very low density lipoproteins and chylomicra. The proportion of each which is used will depend on species and on physiological state. In the rabbit, the predominant fatty acids of milk fat are of medium chain length and are synthesized *de novo* (Dils *et al.* 1977). In addition, some long-chain fatty acids are derived from hydrolysis of blood triglycerides as a result of the action of lipoprotein lipase—an enzyme activated by prolactin (Falconer and Fiddler 1970). Both ruminants and rabbits use acetate, β-hydroxybutyrate and acetoacetate extensively for milk fat synthesis, since these compounds are available from bacterial degradation of carbohydrate in the gut (Bickerstaffe *et al.* 1974; Jones and Parker 1978).

In some species milk fats reflect in composition mobilized body fats. An example is seal's milk, which contains long-chain polyunsaturated fatty acids (Jenness 1974) which are probably initially synthesized in phytoplankton and enter the seal following digestion of its fish diet.

Glucose does not appear to contribute to the synthesis of a major proportion of milk fatty acids in ruminants, but in monogastric animals such as the sow a substantial proportion of synthesized fatty acids is derived from glucose (Spincer *et al.* 1969).

In all species milk lactose is derived from plasma glucose. Even in ruminants in which plasma glucose is low, the extraction of blood glucose across the mammary gland can provide for the lactose synthesized (Davis and Bickerstaffe 1978). The rate-limiting enzyme in lactose synthesis is lactose synthase, which has as its components α-lactalbumin and UDP-galactose *N*-acetylglucosamine galactosyl transferase enzyme, the concentration of which increases rapidly at parturition. The specificity of the galactosyl transferase is altered by α-lactalbumin such that glucose is used for lactose synthesis rather than synthesis of *N*-acetylglucosamine. Synthesis of α-lactalbumin in the mammary gland commences at parturition, thus preventing synthesis of lactose prepartum (see Kuhn 1971). Lactose is a major osmole of milk and deprivation of the mammary gland of its precursor glucose leads to reduction in milk yield, since milk has an equal osmolarity to intracellular fluid (Annison 1971).

Milk proteins in mid-lactation are synthesized in the mammary gland from amino acids obtained from the blood. Essential amino acids are extracted by the udder of the lactating ewe more effectively than non-essential amino acids, the hydrophobic amino acids being taken up in excess of their requirements for milk protein synthesis (Davis *et al.* 1978).

The ewe's udder also obtained ornithine and citrulline from the blood, which are not found in milk proteins. The essential amino acids taken up in excess of the requirement for milk protein synthesis, and the basic non-protein amino acids, are metabolized in the mammary gland largely as substrates for production of energy and non-essential amino acids (Davis and Mepham 1976). In particular the basic

amino acids act as sources of arginine and proline in milk protein (Verbeke *et al.* 1968). The direct use of blood amino acids for synthesis of milk proteins accounts for nearly all the protein in milk in mid-lactation. However, colostrum is rich in immunoglobins and other plasma proteins, which are to a significant extent transferred from blood or tissue to milk through the secretory cells (Brandon *et al.* 1971).

The nutrient requirements of the mammary gland during lactation are therefore considerable, the largest proportion appearing again in the milk, but significant amounts are also totally oxidized to CO_2 within the gland. In the sow, which uses glucose as a major energy source as well as the precursor of milk lactose and a proportion of milk fat, 34% of the glucose entering the mammary gland is oxidized to CO_2 (Linzell *et al.* 1969). In ruminants, in the fed state, 25% of the glucose and 47% of the acetate entering the gland are oxidized for energy supply. The mammary gland of ruminants uses approximately 8 g of glucose, 3 g of amino acids and 6 g of fatty acids per 100 ml of milk secreted, and of this about one-fifth is oxidized for energy (Linzell 1974). In addition to the use of nutrients from the diet, the tissues of the lactating animal can be mobilized for milk production with energetic efficiencies in the whole animal of 80–85% (Flatt and Moe 1971).

Like other areas of biological science, the study of lactation leads to the posing of many questions which are unanswered at the present time. Three areas of lack of knowledge seem to be of particular interest. The first is the molecular mechanism by which hormones initiate genetic activation leading to milk secretion; the second is the identification of a positive secretory control for pituitary prolactin release under physiological conditions; and the third, the quantitive determination of the nutrient requirements for human lactation. While progress is being made in each of these fields the results are far from complete. When these areas have been clarified our understanding of the function of the mammary gland will be significantly advanced.

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