Milk Secretion
An Overview

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MILK SECRETION: AN OVERVIEW

Margaret C. Neville

INTRODUCTION

The defining characteristic of the class Mammalia is the ability to produce milk, an externally secreted fluid designed specifically to nourish the young. The provision of milk frees the mother from the necessity of providing a specialized environment for rearing of the young. It allows birth to occur at a relatively early stage of development and provides a time of intense maternal interaction with the newborn during early behavioral development. In addition the nutritional reserves of the mother may be able to sustain the suckling through a period of famine. Milk composition is species specific. In humans it is becoming increasingly clear that breast milk is the most appropriate source of nutrition up to the age of 6 months. Many components of human milk including, but not-limited to the protein lactoferrin, growth factors, long chain polyunsaturated fatty acids, bile salt stimulated lipase, and anti-infectious oligosaccharides and glycoconjugates are not duplicated in the cow's milk upon which formula manufacture is based. This article is primarily directed to a description of milk secretion in women, but material from studies in animals is drawn on as necessary to define the mechanisms involved.

MILK AS A FLUID

Milk is a complex fluid composed of several phases that can be separated by centrifugation (Neville, 1995a) into a cream layer, an aqueous phase, and a two-phase pellet. The upper phase consists of milk cells and membranous debris, the lower of casein micelles. Casein can also be precipitated by micelle-destroying treatments such as the enzyme rennin or low pH leaving an aqueous phase that is often termed whey. If the whey is made from skim milk it is a true solution that contains all the milk sugar as well as the major milk proteins lactoferrin and secretory immunoglobulin A (sIgA), the monovalent ions sodium, potassium and chloride, citrate, calcium, free phosphate and most of the water-soluble minor components of milk. Depending on the species, varying proportions of the lactoferrin, lysozyme, citrate and calcium may be found associated with the casein pellet . The casein fraction is a small proportion of human milk, about 0.2% by weight. However, casein makes up 4% of cow's milk and as much as 12% of rodent milks. The casein fraction from cow’s milk, usually obtained by rennin precipitation, is used in cheese-making while the whey finds a multiplicity of uses, most notably as the base for infant formula.

FUNCTIONAL ANATOMY OF THE HUMAN BREAST

The parenchyma of the breast consists of approximately 10 to 15 ducts extending from the nipple and coursing through the mammary fat pad to terminate in grape-like clusters of alveoli (Fig. 1). Each duct serves a specific lobule. The lobules are separated and supported by thick connective tissue septa and, in the non-pregnant, non-lactating breast, by large amounts of adipose tissue. Blood vessels, nerves and lymphatics run in the septa which merge imperceptibly with the fascia at the anterior thoracic wall. The nipple, which serves as the termination point for the lactiferous ducts, is surrounded by an area of pigmented skin, the areola, containing sebaceous glands and sweat glands. The areola serves as the termination point for the fourth intercostal nerve, which carries sensory information about suckling to the spinal cord and brain. This is extremely important in the regulation of oxytocin secretion from the

posterior pituitary and prolactin from the anterior pituitary.

During pregnancy the alveolar complexes increase in number and complexity and the cells lining the alveoli and small ducts mature, acquiring the capability to secrete milk. However, milk secretion is kept in check by high concentrations of circulating sex steroids, primarily progesterone. At parturition a series of programmed changes transforms the cells into the fully secretory state. The modern term for this transformation is *secretory activation* although the term *lactogenesis* is still common in the literature. Thereafter milk is secreted more or less continuously into the alveolar lumens and stored there until the let-down reflex brings about contraction of the myoepithelial cells, forcing milk through the ducts to the sinuses beneath the areola where it becomes available to the suckling infant.

OVERVIEW OF THE MAMMARY ALVEOLUS

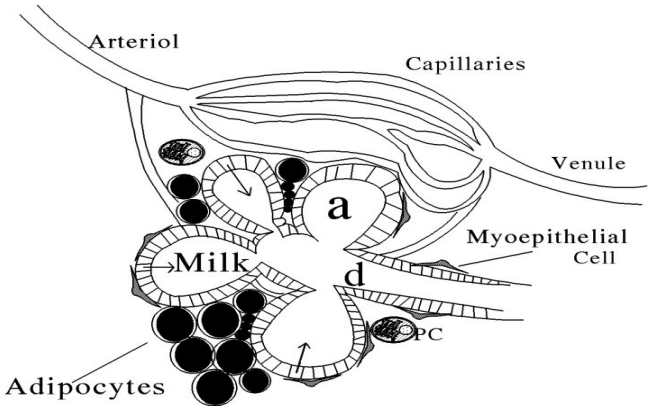

Figure 2. Model alveolus (a) with subtending duct (d) showing blood supply, adipocyte stroma, myoepithelial cells, and plasma cells (PC).

Although the location and external form of the mammary glands differ from one species to another, the mechanisms of milk production are remarkably similar. Milk is produced and stored in alveolar units like that diagramed in Figure 2. Removal of milk from the alveoli is accomplished by contraction of the myoepithelial cells surrounding the alveoli (a) and ducts (d). This process is called milk ejection. Milk exits through ductules into ducts draining several clusters of alveoli. In the human the small ducts coalesce into 8 to 15 main ducts that drain sectors of the gland. The main ducts open directly on the nipple. While earlier pictures show a sinus below the areola, more recent ultrasound studies of milk secretion do not show this structure (Ramsay et al, 2004). In many species including both ruminants and rodents the ducts empty into a single primary duct or a cistern which in turn is drained by a single teat canal. In dairy animals the cistern provides additional milk storage.

In comparison to related dermal glands such as the salivary and sweat glands, the rate of milk secretion is slow, about 1.5 ml of milk per gram of tissue per day (Peaker, 1977). Histologically, the cells lining the smaller ducts resemble the alveolar cells, even reacting with anticasein antibodies (Smith & Vonderhaar, 1981). The larger ducts play a passive role in milk secretion, merely transferring the milk from the alveolar stores to the sub-areolar sinuses where it becomes available to the suckling infant. Because the composition of the aqueous phase of milk changes very little during a feed or milking (Neville et al.,1984), it is unlikely that reabsorptive processes, like those important in the formation of saliva or sweat, play a significant role in determining milk composition.

Although the mammary epithelial cells are ultimately responsible for converting most precursors into milk constituents and transporting them to the mammary lumen, as illustrated in Figure 2 other cell types are also

intimately involved in milk production. We have already mentioned the myoepithelial cells responsible for milk ejection from the breast. The mammary ducts and alveoli are embedded in a stroma that contains fibroblasts, adipocytes, plasma cells and blood vessels. Blood flow is greatly expanded during lactation to make available the large amounts of substrate required for milk synthesis. Interactions with stromal cells are intimately involved in mammary development and milk secretion. Stromal fibroblasts and\or adipocytes are known to be the source of growth factors such as hepatic growth factor/scatter factor and IGF-1 and are probably responsible for production of the enzyme lipoprotein lipase, important in milk lipid synthesis. During lactation B lymphocytes "home" to the mammary gland where they become plasma cells and settle in the interstitial space producing the immunoglobulins that ultimately find their way into milk (Hayward, 1983). The mammary epithelium should, therefore, be viewed as an integrator of activities in many cells and tissues that contribute in a coordinated fashion to the synthesis of milk.

Five distinct processes are utilized by the mammary epithelium in the secretion of milk (Figure 3). These pathways operate in parallel to transform precursors derived from the blood or interstitial fluid into milk constituents. Although the biochemical processes involved are fundamentally the same in all mammals, differences in their relative rates and, in some cases, in the nature of the products synthesized result in milks whose composition differs widely from species to species. Some of the milk secretion pathways, e.g., exocytosis of protein-containing vesicles and transcytosis of immunoglobulins, are similar to processes in many exocrine organs. In contrast, the mechanism for fat secretion is unique to the mammary gland.

CELLULAR MECHANISMS FOR MILK SYNTHESIS AND SECRETION

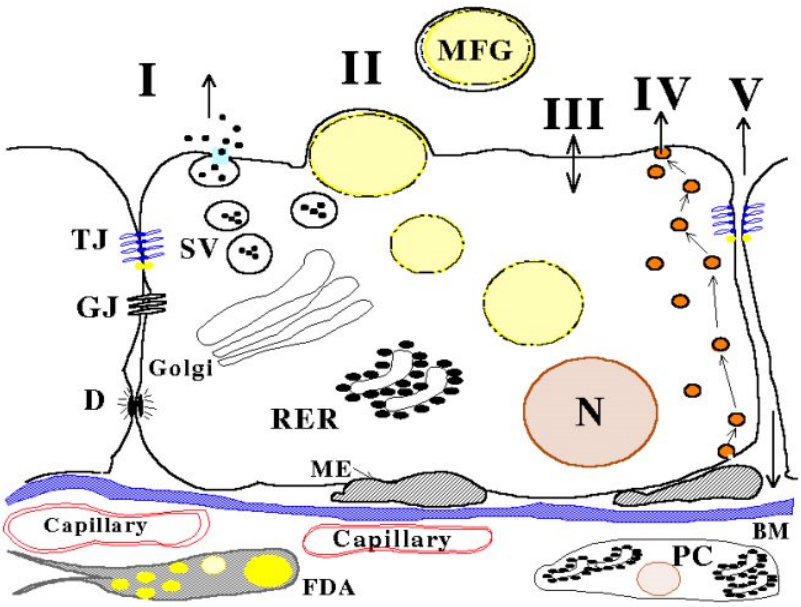

Figure 3. Alveolar Cell from lactating mammary gland. N, nucleus; TJ, tight junction; GJ, gap junction; D, desmosome; SV, secretory vesicle; FDA, fat-depleted adipocyte; PC, Plasma Cell; BM, basement membrane; ME, cross section through process of myoepithelial cell; RER, rough endoplasmic reticulum. See text for explanation of secretory pathways I (exocytosis), II (lipid), III (apical transport), IV (transcytosis) and V (paracellular pathway).

Four secretory processes are synchronized in the mammary epithelial cell of the lactating mammary gland: *exocytosis*, *lipid synthesis* and secretion, *transmembrane secretion* of ions and water and *transcytosis* of extra-alveolar proteins such as immunoglobulins, hormones and albumin from the interstitial space. A fifth pathway, the *paracellular pathway*, allows the direct transfer of materials between the milk space and the interstitial space. This pathway is open in the pregnant gland and allows the transfer of molecules at least as large as intact immunoglobulins. It is closed in the fully lactating gland providing a tight barrier between the

milk and interstitial spaces. This barrier opens again in the presence of mastitis and during involution (Neville, 1995b).

- *Exocytosis*

Most of the components of the aqueous phase of milk are secreted by the exocytotic pathway (Pathway I, Figure 3). Proteins synthesized on ribosomes are transferred to the lumen of the rough endoplasmic reticulum where their signal sequences are cleaved and the protein molecules folded. Vesicles transfer the proteins to the Golgi stack where they are further processed by the addition of carbohydrate, phosphate or other groups and packaged into secretory vesicles.

In addition to processing of milk proteins *per se*, the Golgi vesicles in the lactating mammary cell synthesize lactose from precursor UDP-galactose and glucose that enter from the cytoplasm. Because the Golgi membrane is impermeable to lactose, the sugar is osmotically active and water is drawn into the terminal Golgi vesicles. The swollen appearance of the *trans*-Golgi and the secretory vesicles which arise from it are specific characteristics of the lactating mammary cell. Casein micelle formation begins in the *cis*-Golgi with condensation of casein molecules; addition of calcium, possibly in the secretory vesicle, leads to maturation of the casein micelles into particles sufficiently dense to be seen in the electron microscope. Secretory vesicles are thought to be the source of most of the constituents of the aqueous phase of milk including citrate, nucleotides, calcium, phosphate and probably monovalent ions and glucose. However, the apical membrane of the mammary alveolar cell has transporters for monovalent ions, calcium, and glucose and the concentration of these substances may be adjusted by direct membrane transport (see below).

Secretory vesicles move to the plasma membrane where they fuse and release their contents into the milk space by exocytosis. The exocytotic pathway involved in milk secretion appears to be largely constitutive. This means that, once secretion begins after parturition, exocytosis is continuous and secretory products are not stored within the epithelial cell. However, Burgoyne and his colleagues (Turner et al.,1992) have observed that a portion of the mammary secretion could be stimulated by increased cell calcium in an *in vitro* system suggesting the presence of a regulated secretion pathway in the lactating mammary epithelial cell.

- *Lipid Synthesis and Secretion.*

Triglycerides, synthesized in the smooth endoplasmic reticulum of the mammary alveolar cell from precursor fatty acids and glycerol, coalesce into large droplets that are drawn to the apex of the cell (Pathway II, Figure 3). The lipid droplets bulge against and gradually become enveloped in apical plasma membrane, finally separating from the cell as the milk fat globule. The occasional inclusion of a crescent of cytoplasm within the membrane-bound globule enables any substance contained in the cytoplasm to enter milk. The membrane surrounding the milk fat globule has two functions: it is the primary dietary source of phospholipids and cholesterol for the breast-fed infant and it prevents the fat globules from coalescing into large fat droplets that might prove difficult to secrete. Butter results when a suspension of separated milk fat droplets is beaten or churned to remove the membranes allowing the fat droplets to condense.

- *Transport across the Apical Membrane.*

In contrast to the other pathways for milk secretion the pathways for the direct transport of substances across the apical membrane of the mammary alveolar cell are poorly understood (Pathway 3, Figure 3). Linzell and Peaker (Linzell & Peaker, (1971) devised a clever technique to determine what molecules could utilize this pathway, infusing isotopes of small molecules up the teat of a goat and calculating how much of the substance

left the milk and entered the blood. They found that sodium, potassium, chloride and certain monosaccharides as well as water directly permeated this membrane (Linzell & Peaker, 1971) but calcium, phosphate and citrate did not (Neville & Peaker, 1981). Studies of bicarbonate secretion led these investigators to postulate the presence of chloride-bicarbonate exchange at the apical membrane (Linzell & Peaker, 1975). Stable isotope studies in women confirmed the presence of a glucose pathway across the apical membrane in the human mammary gland as well (Neville et al., 1990). Recent studies by Van Houten and colleagues show that calcium is actively transported across the apical membrane as well Van Houten et al, 2007). What is clear is that apical pathways are limited to a modest number of small molecules. Although often overlooked, many drugs enter milk by direct transfer across both basolateral and apical membranes of the mammary alveolar cell. For this reason most therapeutic drugs are transferred efficiently into milk (Fleishaker & McNamara, 1988)

- *Transcytosis of Interstitial Molecules.*

Intact proteins can cross the mammary epithelium from the interstitial fluid either by *transcytosis* or through the *paracellular* pathway. During lactation only the transcytotic pathway is available (Pathway IV, Figure 3). Immunoglobulins are the best studied of the molecules that enter milk via transcytosis. In most-non-ruminants IgA is synthesized by plasma cells in the interstitial spaces of the mammary gland or elsewhere in the body (Hayward, 1983). The protein binds to receptors, the polymeric immunoglobulin receptor, on the basal surface of the mammary alveolar cell; the entire IgA-receptor complex is endocytosed and transferred across the cell. At the apical membrane the extracellular portion of the receptor is cleaved and secreted together with the IgA. The cleaved receptor portion is known as secretory component and the secreted product is thus secretory IgA or sIgA. The many proteins, hormones and growth factors that find their way into milk from the plasma are also thought to be secreted by similar, but much less well-studied, mechanisms.

- *The Paracellular Pathway*

The paracellular pathway (Pathway V, Figure 3) allows passage of substances between epithelial cells, rather than through them. During full lactation the passage of even small molecular weight substances between alveolar cells is impeded by a gasket-like structure called the tight junctions (*Zonula occludens*) that joins the epithelial cells tightly, one to another. Although immune cells apparently can diapedese between epithelial cells to reach the milk (Lin et al.,1995; Seelig, Jr. & Beer, 1981), the junctions seal tightly behind them leaving no permanent gap. During pregnancy and with mastitis the tight junctions become leaky and allow components of the interstitial space to pass unimpeded into the milk. At the same time milk components can enter the plasma. This leakiness is useful during these periods since secretion products are allowed to leave the gland, and inflammatory cells and protective molecules can enter the milk space. When the junctions are open the mammary secretion has high sodium and chloride concentrations, a fact that is sometimes useful in diagnosing breastfeeding problems (Morton, 1994).

Secretory Activation: THE TRANSITION FROM PREGNANCY TO LACTATION

Formally secretory activation (formerly lactogenesis II) is defined as the onset of milk secretion. Hartmann (1973) and Linzell and colleagues (Fleet et al, 1975), based on work in ruminants, divided lactogenesis into two stages. Secretory differentiation (formerly Lactogenesis Stage 1) occurs during pregnancy when the gland becomes sufficiently differentiated to secrete small quantities of specific milk components such as casein and lactose. Secretory activation (formerly Lactogenesis Stage 2) is defined as the onset of copious milk secretion associated with parturition. It is brought about by a decline in progesterone around the time of parturition in the presence of maintained prolactin concentrations. A differentiated mammary epithelium is necessary for

stage 2 lactogenesis to occur. In humans the epithelium reaches this stage of differentiation about mid-pregnancy. The terminology for these stages has recently changed: the interested reader is referred to Pang and Hartmann (2007) for an excellent discussion of secretory activation in women.

In the early post-partum period the secretion product of the mammary gland is called *colostrum*. This fluid contains high concentrations of immunoglobulins and the protective protein, lactoferrin. In species such as ruminants that lack transplacental transport of immunoglobulins, feeding of colostrum is necessary to provide passive immune protection to the young until their immune systems become mature. In other species, such as humans, where transplacental transport of immunoglobulins provides humoral immunity in the early post-partum period, the presence of secretory IgA, lactoferrin and high concentrations of oligosaccharides is important in protection of mucosal surfaces from infection, particularly under conditions where optimal sanitation cannot be maintained.

Lactogenesis represents a profound and rapid series of changes in the activity of differentiated mammary epithelial cells from a quiescent state to a fully active secretory state. As can be illustrated by examining the composition of human milk during the first week post-partum, these changes occur as an orderly progression of events that starts with closure of the tight junctions between the epithelial cells, followed by a transient increase in the secretion of the protective proteins sIgA and lactoferrin. After about 36 hours a rapid increase in the synthesis of all the components of mature milk begins that is complete by about day 5 postpartum.

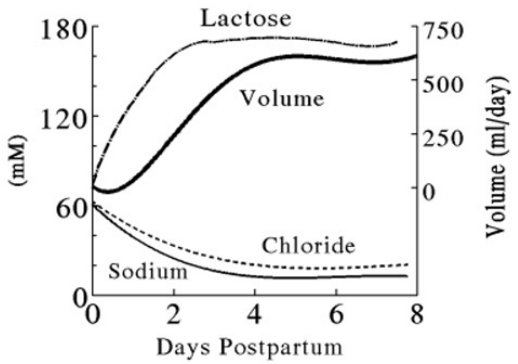

Figure 4. Changes in human milk composition and volume in the early postpartum period. The concentration scale for lactose sodium and chloride, whose concentrations begin to change immediately post-partum, is on the left. Volume increases from 50 ml/day on day 1 to 600 ml/day on day 5 with the greatest increase taking place between days two and four.

The first change to occur is a fall in the sodium and chloride concentrations in the milk and an increase in the lactose concentration (Figure 4). These modifications commence immediately after birth and are largely complete by 72 hours postpartum (Neville et al., 1991). They precede the onset of the increase in milk volume by at least 36 hours and can be explained by closure of the tight junctions. With blockage of the paracellular pathway lactose, made by the epithelial cells, can no longer pass into the plasma. Sodium and chloride can no longer pass from the interstitial space into the lumina of the mammary alveoli. Thus this phase is marked by a rapid fall in the sodium and chloride concentrations of milk and a rapid increase in the lactose concentration.

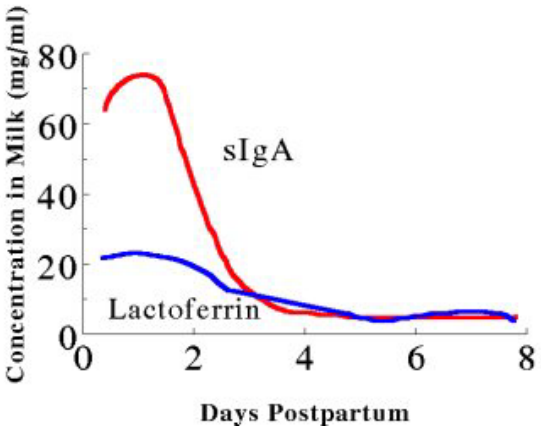

Figure 5. Changes in the concentrations of IgA and lactoferrin in human milk in the post-partum period.

The next change is an increase in the rates of secretion of sIgA and lactoferrin (Figure 5). The concentrations of these two important protective proteins remain high for the first 48 hours after birth, together comprising as much as 10% by weight of the milk. Colostrum also contains high concentrations of cells including lymphocytes, macrophages, neutrophils and sloughed secretory epithelium (Ho et al., 1979). The concentrations of lactoferrin, sIgA and cellular elements fall rapidly after day 2, a consequence of both dilution as milk volume secretion increases and a decrease in their absolute rate of secretion. Although both lactoferrin and IgA are found at high concentrations in colostrum, their secretion is likely under separate control since lactoferrin is secreted by the exocytotic pathway (I in Figure 3) while sIgA is secreted by the transcytotic pathway (IV in Figure 3). Further the secretion rate of lactoferrin peaks about one day after the peak of sIgA secretion.

Finally, starting about 36 hours postpartum there is a 10-fold increase in milk volume from about 50 ml/day to 500 ml/day (Figure 4). This volume increase may be perceived by the parturient woman as the "coming in" of the milk and is brought about by a massive increase in the rates of synthesis and/or secretion of almost all the components of mature milk (Neville et al.1991), most notably lactose, casein (Patton et al., 1986), alpha-lactalbumin, lipid, calcium, potassium, etc. Three milk components, citrate, glucose and free phosphate, are quite interesting because their concentrations increase in proportion to the increase in milk volume In the case of glucose the change in concentration has been shown to be due to an increase in glucose transport from the interstitial space into the mammary alveolar cell (Neville et al.,1990) whereas the increase in phosphate possibly results from dephosphorylation of the UDP generated during lactose synthesis. The increase in citrate may be related to fat synthesis; however, it does not occur in all species since rodents do not have citrate in their milks.

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