Transport of Milk Constituents by the Mammary Gland

D. B. SHENNAN AND M. PEAKER

Hannah Research Institute, Ayr, Scotland

I. Introduction

II. Experimental Methods Used to Study Mammary Gland Solute Transport

III. Routes of Secretion
   A. Description of routes
   B. Solute transport via the paracellular pathway

IV. Sodium, Potassium, and Chloride Transport
   A. Carrier mechanisms for Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\)
   B. Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) channels in the apical membrane
   C. Effects of prolactin on Na\(^{+}\), K\(^{+}\), and Cl\(^{-}\) transport

V. Relations Between Sodium, Potassium, and Chloride Transport and Water Movements

VI. Phosphate Transport

VII. Calcium Transport

VIII. Citrate Transport

IX. Iodide Transport

X. Stretch-Sensitive Ion Transport Mechanisms

XI. Choline and Carnitine Transport

XII. Glucose Transport
   A. Glucose transporters
   B. D-Glucose transport across the apical membrane
   C. Transport of D-glucose across the Golgi membrane
   D. Control of D-glucose transport

XIII. Amino Acid Transport
   A. Amino acid transporters
   B. Amino acid transport across the apical membrane of mammary secretory cells
   C. Control of amino acid transport
   D. Volume-sensitive amino acid transport
   E. Peptide transport
   F. Does γ-glutamyl transpeptidase play a role in mammary amino acid transport?

XIV. Fatty Acid Uptake

XV. Coordination and Control

Shennan, D. B., and M. Peaker. Transport of Milk Constituents by the Mammary Gland. *Physiol Rev* 80: 925–951, 2000.—This review deals with the cellular mechanisms that transport milk constituents or the precursors of milk constituents into, out of, and across the mammary secretory cell. The various milk constituents are secreted by different intracellular routes, and these are outlined, including the paracellular pathway between interstitial fluid and milk that is present in some physiological states and in some species throughout lactation. Also considered are the in vivo and in vitro methods used to study mammary transport and secretory mechanisms. The main part of the review addresses the mechanisms responsible for uptake across the basolateral cell membrane and, in some cases, for transport into the Golgi apparatus and for movement across the apical membrane of sodium, potassium, chloride, water, phosphate, calcium, citrate, iodide, choline, carnitine, glucose, amino acids and peptides, and fatty acids. Recent work on the control of these processes, by volume-sensitive mechanisms for example, is emphasized. The review points out where future work is needed to gain an overall view of milk secretion, for example, in marsupials where milk composition changes markedly during development of the young, and particularly on the intracellular coordination of the transport processes that result in the production of milk of relatively constant composition at a particular stage of lactation in both placental and marsupial mammals.
I. INTRODUCTION

The last Physiological Reviews article on the mechanism of milk secretion was written in 1971 (118) at a time when morphological information from electron microscopy was being combined with physiological and biochemical knowledge to provide the basis of our current understanding of milk secretion at the cellular level. Many of the hypotheses on the secretory mechanisms for the various milk constituents have survived more extensive work and amplification at the cellular and molecular levels, and it is not our intention to cover this older ground other than in outline. Rather, our intention is to extend the explanatory reductionist approach to examine the transport and secretory mechanisms for the major milk components or their precursors and their integration within the economy of the secretory cell.

The mammary gland is, in a number of respects, an unusual exocrine gland. Secretion, once initiated at about the time of parturition, is continuous but relatively slow in terms of fluid volume output per unit time. The secretion, milk in full lactation, is a complex mixture arising from different secretory pathways across, within, and sometimes between the mammary epithelial cells, comprising membrane-bound fat droplets, casein micelles, and an aqueous phase that usually contains lactose and sometimes complex carbohydrates, minerals in various ionic or bound forms, other proteins, and a bewildering array of soluble components, some of which may be biologically active in the young consuming the milk (78, 165). Another but related unusual feature is that secretion is stored within the lumen of the gland, either in the secretory alveoli or duct system, until it is removed at intervals (varying from several hours in many mammals, to once daily in rabbits, to once every 2 days in tree shrews, and to once a week in some pinnipeds) by the sucking young (46).

Studies in a number of species have shown that while there may be species-specific changes in milk composition with stage of lactation corresponding to growth phases of the young (a phenomenon particularly marked in marsupials), milk composition is markedly resistant to environmental, including nutritional, changes, whereas the quantity of milk secreted shows considerable plasticity (162).

The control of the rate of milk secretion will not be covered in detail, except for the effects of prolactin and cell stretch, since it has been reviewed extensively in recent years, particularly the autocrine control of milk secretion that acts to match supply by the mother to demand by the young within each individual mammary gland within the hormonal milieu of lactation that maintains the cells in a secretory state (163, 168, 169, 247–249).

The mammary gland is controlled by a number of hormones and local factors. When these are added to various mammary gland preparations, effects on transport may be observed. However, it is difficult to determine whether a hormone is having a primary, direct effect on a particular transport system or whether the effect is due to the overall response of the cell, to increase the degree of differentiation, for example, or simply to prolong its survival. Therefore, caution must be exercised in interpreting the individual effects of hormones on transport systems in such highly integrated differentiation, secretion, and transport systems.

II. EXPERIMENTAL METHODS USED TO STUDY MAMMARY GLAND SOLUTE TRANSPORT

The first physiological studies on the mechanism of milk secretion were done using conscious undisturbed animals. Although the range of measurements that can be made is limited, the major advantage is that artifacts are not introduced as part of the experimental procedure of removing tissue and trying to maintain it alive and undamaged. It is relatively easy to measure transepithelial solute gradients across the lactating mammary gland, and moreover, because of the slow rates of milk secretion, it is possible to make some meaningful electrophysiological measurements; the effect of changing the luminal contents on the transepithelial potential difference can be readily tested, for example (27).

Many studies on the mechanism of solute transport have been done using mammary tissue slices, otherwise termed fragments and explants. Explants are still a popular choice because they are easily and rapidly prepared; they do not require biochemical treatment, such as collagenase digestion, which could possibly alter the properties of the alveolar cells. Although explants are a mixed cell population, the vast majority, at least in those prepared from lactating tissue, are secretory cells. The alveolar nature of mammary tissue means that explants will give a good measure of transport across the basolateral membranes of the secretory cells particularly in experiments employing short time courses. However, the relatively large extracellular space of mammary tissue explants together with the presence of large unstirred fluid layers places limitations on the design of experiments. Isolated mammary epithelial cells and acini are an alternative to explants to study membrane transport. Like explants, they are relatively easy to prepare; however, the isolation process requires that the cells be exposed to digestive agents. The extracellular space is minimal compared with that associated with explants, but it is difficult to distinguish between transport occurring at the apical and basolateral membranes in both these preparations. There is also the possibility that dissociation of mammary tissue leads not only to a loss of polarity but also decreases cell viability.
The perfused lactating mammary gland has been successfully employed to characterize solute transport systems in both guinea pig and rat. This technique allows the transport properties of the blood-facing aspect of the mammary epithelium to be characterized under near-physiological conditions. One major assumption is that the endothelium does not constitute a major barrier to solute movement between blood and the interstitium. Recent work suggests that this is a reasonable assumption, since sucrose (an extracellular marker) has a larger dilution space than albumin (an intravascular marker) in the lactating bovine mammary gland (176). The major drawback is that the skills required to achieve a successful preparation take time to acquire. The perfused mammary gland, used in conjunction with the rapid, paired-tracer dilution technique (255), has been successfully employed to study amino acid transport.

Cultured mammary cells have also been used to study mammary transport processes. For example, cells cultured on collagen gels to form monolayers and subsequently mounted in Ussing chambers have allowed the polarity of the mammary epithelium to be examined. However, there is justifiable concern that cultured mammary epithelial cells are not fully active in terms of secretion, although an exception to this may be mammary epithelial cells cultured on a reconstituted basement membrane. Cells grown in this manner form polarized acinar structures that enclose a hollow, sealed lumen and secrete in a vectorial fashion (13, 42, 89). These acini, termed mammospheres, may prove particularly useful for characterizing transport across the basolateral membranes of secretory cells. Unfortunately, a technique that allows purified basolateral membrane vesicles to be prepared from mammary secretory cells has not yet been developed.

Membrane fractions isolated from milk have been used in attempts to study the transport properties of the apical (luminal) membrane. Wooding et al. (254) identified membrane-bound cytoplasmic droplets in goat’s milk containing fat and protein particles, mitochondria, and Golgi apparatus (but no nuclei); further studies showed that the droplets were capable of triacylglycerol biosynthesis (44). Because the cytoplasmic fragments originate from the apical aspect of mammary secretory cells, it is reasonable to assume that the surrounding membrane is indeed representative of the apical membrane. It has been shown that cytoplasmic droplets transport ions into an osmotically active space (213).

Because the milk fat globule becomes enveloped by membrane during its extrusion from the cell, the milk-fat-globule membrane has long been assumed to be representative of the apical membrane of the secretory cell. However, the finding that bovine milk-fat-globule membrane vesicles are impermeable to K⁺ casts doubt on the accepted origin or maintenance of original properties of these membranes (195, 196) (Fig. 1). There is the possibility that the milk-fat-globule membrane is derived from intracellular membranes rather than the apical membrane (228, 253). On the other hand, membrane vesicles prepared from bovine skim milk, which are distinct from milk-fat-globule membranes, are able to transport solutes into an intravesicular compartment (196). Skim milk membrane vesicles do appear to originate from the apical membrane (172).

In ending this section, it should be noted that the relatively complex morphology of the mammary gland precludes the use of several techniques to study solute transport. For example, the mammary epithelium is not flat and therefore, unlike frog skin and toad bladder, cannot be mounted in Ussing chambers.

III. ROUTES OF SECRETION

A. Description of Routes

There are five major, known routes of secretion across the mammary secretory epithelium from the blood side to milk, four transcellular and one paracellular (116, 118) (Fig. 2): 1) membrane route, 2) Golgi route, 3) milk fat route, 4) transcytosis, and 5) paracellular route.
1) In the membrane route, substances may traverse the apical cell membrane (and for those directly derived from blood, the basolateral membrane). Examples are water, urea, glucose, \( \text{Na}^+ \), \( \text{K}^+ \), and \( \text{Cl}^- \) (116).

2) In the Golgi route, secretory products are transported to or sequestered by the Golgi apparatus and secreted into the milk space by exocytosis. Examples are casein, whey proteins, lactose, citrate, and calcium (116, 118, 267).

3) In the milk fat route, milk fat globules are extruded from the apex of the secretory cell surrounded by membrane (milk-fat-globule membrane); some cytoplasm is sometimes included (126). Examples are milk fat, lipid-soluble hormones and drugs, some unknown growth factors (in milk-fat-globule membrane) (244), and the product of the \( \text{ob} \) gene, leptin (214).

4) In transcytosis, vesicular transport involves various organelles, possibly, in some cases also involving extrusion by route 2 (88, 154). Examples are immunoglobulins during colostrum formation (88), transferrin (154), and prolactin (154).

5) In the paracellular route, there is direct passage from interstitial fluid to milk.

In full lactation, routes 1, 2, 3, and, possibly, 4 predominate.

B. Solute Transport Via the Paracellular Pathway

Transcellular transport requires solutes to cross the constituent plasma membranes of the epithelium, whereas paracellular transfer involves movement be-
tween the cells via leaky tight junctions. In most species, solute transport across the lactating mammary epithelium appears to be mainly transcellular, i.e., the zonulae occludentes are tight junctions. However, there are situations where a significant flux occurs via a paracellular pathway. Movement of solutes across the paracellular shunt pathway occurs during pregnancy and, in some species, during late lactation. The early (160) and later work (150) on this aspect of mammary transport including physiological and morphological findings have been reviewed, and it is not our intention to provide a further detailed review here.

The transepithelial electrical resistance is a good measure of the tightness (or leakiness) of an epithelium. However, although it is possible to measure the transepithelial resistance of cultured mammary cell monolayers, it is more difficult to obtain reliable values of the electrical resistance across the lactating mammary gland in vivo. A common way of assessing paracellular permeability in the lactating mammary gland is to measure the solute content of milk; the concentrations of lactose and K\(^+\) in milk decrease (moving down their concentration gradients), whereas those of Na\(^+\) and Cl\(^-\) increase when the junctions become leaky. However, care must be exercised when relying solely on Na\(^+\) and K\(^+\) gradients because a change may simply reflect an alteration in the transport of these ions across transcellular pathways. A more preferable way of assessing tight junction permeability in vivo is to measure the rate of appearance of lactose in plasma or of an inert disaccharide introduced into plasma in milk (119, 216).

In general, the paracellular pathway is absent during lactation and present during pregnancy. However, in the rabbit during late lactation, when the rate of secretion is still relatively high, milk lactose and K\(^+\) concentrations decrease while those of Na\(^+\) and Cl\(^-\) increase. At this stage, the epithelium is permeable to lactose and sucrose and more permeable to Na\(^+\) and Cl\(^-\) (167). These changes were reversed by exogenous prolactin (121). Recent evidence suggests that prolactin may also be involved in some way in maintaining the integrity of the tight junctions (judged by the ability of the gland to maintain solute concentration gradients) in the rat mammary gland (71). There is also evidence that a paracellular pathway appears during other physiological states. Characteristic changes in milk composition were observed in many goats for 1–2 days up to 4 days before estrus (164), but the controlling mechanism remains unknown.

There is a major change in the permeability of the paracellular pathway at the onset of copious milk secretion around the time of parturition (119). There is also good evidence which suggests that glucocorticoids are involved in controlling the formation of tight junctions during mammary development and differentiation in pregnancy. The glucocorticoid dexamethasone increased the transepithelial electrical resistance and decreased the transepithelial movements of labeled mannitol and inulin across monolayers of a mouse mammary cell line (261). Glucocorticoid-induced formation of mouse mammary tight junctions involves an increase in the amount of the tight junction-associated protein ZO-1 and phosphorylation/dephosphorylation events since okadaic acid, a protein serine/threonine phosphatase inhibitor, attenuates the dexamethasone-induced increase in transepithelial resistance (212). However, mammary development is a complex series of events, and transforming growth factor (TGF)-\(\beta\) was found to antagonize the effect of dexamethasone (252). There is evidence from in vivo studies that glucocorticoids may control the permeability of tight junctions. In late-pregnant goats, an intraluminal injection of cortisol had marked effects on the composition of fluid within the lumen: K\(^+\) concentration increased and Na\(^+\) decreased, which is consistent with the closing of tight junctions (224). However, an effect of cortisol on transcellular ion transport cannot be ruled out at this stage. Hormonal events, other than or as well as a rise in circulating glucocorticoid concentration, appear to be involved in the final closure near parturition, with the hormonal events leading to parturition and the onset of copious milk secretion (lactogenesis stage II, Ref. 68) being closely linked. For example, in late pregnancy, the goat mammary gland synthesizes PGF\(_{2\alpha}\); near term, this production stops. Unilateral treatment with a PGF\(_{2\alpha}\) analog prevented the normal changes in milk composition and increase in the rate of secretion (127). The current view on the control of tight junctional permeability during lactogenesis has been well summarized by Nguyen and Neville (150).

We propose that progesterone inhibits tight junction closure during pregnancy, possibly through or along with local factors such as TGF-\(\beta\) and PGF\(_{2\alpha}\). As the level of progesterone drops during parturition or with ovariectomy, its inhibitory effect is removed, allowing the mammary epithelial tight junctions to close. However, glucocorticoid may be required for final differentiation of the mammary epithelium from the pregnant animal into the epithelium of the lactating animal with its highly impermeable tight junctions. Prolactin appears to play a role in the maintenance of the mammary epithelium in the lactating state and inhibition of programmed cell death.

The paracellular pathway reappears at the cessation of lactation. When milk removal was stopped in goats during late lactation, the key process was determined to be the local event of stretching the mammary epithelium by milk accumulating within the lumen of the gland, leading to a decrease in secretory and metabolic activity and an increase in the permeability of the tight junctions. Systemic control by hormones was excluded in this species as was pressure of the accumulated milk simply
bursting the taut epithelium (69, 161). Similarly, in the cow, milk stasis leads to an increase in the paracellular pathway that can be reversed by milking (217). These as well as other studies indicate an inverse link between secretory activity and permeability of the tight junctions. Whether maintenance of impermeable junctions is part of the secretory activity of the cell, like the synthesis of milk components, or whether disruption of the junctions is followed by partial depolarization of the cell which affects secretory activity, or whether there is signaling from the junctions to the synthetic and secretory pathways is not known (150).

The presence of a paracellular pathway may confound studies on milk composition and mammary epithelial permeability. Disruption of the epithelium is evident in mastitis but also when supraphysiological doses of oxytocin are given to contract the myoepithelium and obtain a milk sample. Care must be exercised when interpreting data on milk composition, particularly of the aqueous phase (160).

IV. SODIUM, POTASSIUM, AND CHLORIDE TRANSPORT

The mammary gland is able to generate and maintain large Na⁺, K⁺, and Cl⁻ gradients between milk and plasma. These ions make a substantial contribution to the osmolality of milk, especially in those species where the lactose content is relatively low. On the basis of measurements of the concentration gradients between interstitial or extracellular fluid, intracellular fluid, and milk, and the electrical potential gradients between these three compartments, Linzell and Peaker in 1971 (117, 118) proposed a scheme to account for the movement of Na⁺, K⁺, and Cl⁻ across the lactating mammary epithelium and the maintenance of milk composition (Fig. 3). In their hypothesis, the main features are that the Na⁺ concentration inside the cell is kept low, and the K⁺ concentration high, by the action of a Na⁺-K⁺ pump on the basolateral membrane, whereas these ions are freely distributed between the interior of the cell and milk according to the electrical potential difference across the apical membrane. Milk is electrically positive with respect to the cell, so the concentrations of Na⁺ and K⁺ are lower in milk, but the ratio of these ions is similar in both compartments, i.e., 3 K⁺:1 Na⁺. The situation for Cl⁻ is, however, different from that of Na⁺ and K⁺. The concentration of Cl⁻ is higher inside mammary cells than that expected for an equilibrium distribution, suggesting that a mechanism exists in both the basolateral and apical membranes which “actively” pumps Cl⁻ into the cells. Many experimental observations, at the level of individual transport systems, supporting the original hypothesis of Linzell and Peaker have since been made. The relations between lactose concentration, apical membrane potential difference, and ionic concentrations have also been considered (160, 159).

A. Carrier Mechanisms for Na⁺, K⁺, and Cl⁻

Three carrier mechanism were postulated in the scheme of Linzell and Peaker: 1) a Na⁺-K⁺ pump on the basolateral membrane, 2) an inwardly directed Cl⁻ pump on the apical membrane, and 3) a similar Cl⁻ transporter in the basolateral membrane of the secretory cell (117, 118). Evidence for the presence of a Na⁺-K⁺ pump in the blood-facing aspect of the mammary epithelium is strong, and several candidates for Cl⁻ pumps have been identified.

Many studies have shown that lactating mammary tissue from a variety of species displays ouabain-sensitive Na⁺-K⁺-ATPase activity (242) and ouabain-sensitive Na⁺.
and K\(^+\) transport (3, 61, 62, 117, 194). In addition, there is good histochemical evidence that Na\(^+\)-K\(^+\)-ATPase is located on the basal and lateral membranes, but not the apical membranes, of mammary secretory cells (96, 105). These findings taken together support the scheme of Linzell and Peaker that lactating mammary tissue expresses a functional Na\(^+\)-K\(^+\) pump on the blood-facing pole of the epithelium. Experiments using cultured mammary epithelial cells have provided evidence that the Na\(^+\)-K\(^+\) pump is located on the basolateral, but not the apical, membranes (24–26). However, there is evidence for Na\(^+\)-K\(^+\)-ATPase activity on the milk-fat-globule membrane, albeit at a relatively low specific activity (55).

Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport could be a candidate for the Cl\(^-\) pump in the model of Linzell and Peaker. Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport has been identified in lactating rat mammary tissue (194, 203). Thus K\(^+\) (Rb\(^+\)) transport (both influx and efflux) by mammary tissue explants is Na\(^+\) dependent, Cl\(^-\) independent, and inhibited by the loop diuretic furosemide. In addition, Cl\(^-\) efflux from rat mammary tissue explants is sensitive to furosemide (203). On the basis that cotransport activity was detected in explants, it is predicted that the Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport system is situated in the blood-facing aspect of the epithelium. Supporting evidence of Na\(^+\)-K\(^+\)-Cl\(^-\) expression in mammary tissue is the finding that bovine milk-fat-globule membrane binds bumetanide (an analog of furosemide) in a fashion that is dependent on Na\(^+\), K\(^+\), and Cl\(^-\) (166).

The triple cotransporter is able to drive the uphill transport of Cl\(^-\) (2). The Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport system is electroneutral and is generally assumed to operate with a stoichiometry of 1 Na\(^+\):1 K\(^+\):2 Cl\(^-\) (43, 79) The free-energy (\(\Delta G\)) available to a cotransporter operating with such a stoichiometry is given by

\[
\Delta G = RT \ln \left( \frac{[Na]^i[K]^i[Cl]^-_o}{[Na]^o[K]^o[Cl]^-_i} \right)
\]

where subscripts i and o refer to intracellular and extracellular, respectively. Applying the Na\(^+\), K\(^+\), and Cl\(^-\) concentrations of guinea pig mammary tissue and plasma (117) to Equation 1 gives a value +0.79RT for \(G\), suggesting that the triple cotransport system operating with a coupling ratio of 1 Na\(^+\):1 K\(^+\):2 Cl\(^-\) would require an input of free energy to drive inward transport. Alternatively, a Na\(^+\)-K\(^+\)-Cl\(^-\) cotransporter with a stoichiometry of 2 Na\(^+\):1 K\(^+\):3 Cl\(^-\), as found in the squid axon (190), would have sufficient freeenergy (\(G = -1.03RT\)) associated with the ion gradients to accomplish inward transport. However, it is clear that further experiments are required to determine both the stoichiometry and locus of the mammary Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport mechanism.

Cl\(^-\)/HCO\(_3\^-\) exchange could also be a candidate for a Cl\(^-\) pump in lactating mammary tissue (120). For example, a Cl\(^-\)/HCO\(_3\^-\) antiport drives the uphill accumulation of Cl\(^-\) into smooth muscle cells (2, 50). A DIDS-sensitive anion exchanger that accepts Cl\(^-\), SO\(_4\)^2\(^-\), I\(^-\), and NO\(_3\)^- as substrates has been reported in lactating rat mammary tissue explants (194, 203). However, it remains to be shown whether the rat mammary tissue anion-exchanger transports HCO\(_3\^-\) and, if so, whether it is able to act as a Cl\(^-\) pump.

**B. Na\(^+\), K\(^+\), and Cl\(^-\) Channels in the Apical Membrane**

The 1971 model of Linzell and Peaker predicts that the apical membrane of mammary secretory cells possesses Na\(^+\), K\(^+\), and Cl\(^-\) channels. Several lines of experimental evidence support this prediction. First, Blatchford and Peaker (27) have shown that the transepithelial potential difference across the lactating goat mammary gland can be altered by varying the Na\(^+\), K\(^+\), and/or Cl\(^-\) concentration in the lumen of the gland in a fashion that is consistent with the presence of channels for each of these ions in the apical aspect. However, it was found that the apical membrane is unable to discriminate between Na\(^+\) and K\(^+\), suggesting that the apical membrane may express a nonspecific cation channel rather than separate channels for Na\(^+\) and K\(^+\). In this connection, nonspecific cation channels have been identified in cultured mouse mammary epithelial cells (72). Second, cytoplasmic fragments isolated from goat’s milk that are surrounded by apical membrane, transport K\(^+\) (Rb\(^+\)) via barium- and quinoline-sensitive channels (213). Third, skim milk membranes, isolated from bovine milk, which appear to be derived from the apical aspect of secretory cells, express K\(^+\) and Cl\(^-\) channels (196). The channels identified in the membrane preparations need to be characterized using electrophysiological techniques.

**C. Effects of Prolactin on Na\(^+\), K\(^+\), and Cl\(^-\) Transport**

Prolactin was found to decrease the Na\(^+\) and increase the K\(^+\) content of mammary tissue taken from rabbits treated with bromocriptine, a drug that prevents prolactin release; ouabain inhibited the action (62, 63). In addition, the unidirectional uptake of K\(^+\) (Rb\(^+\)) by rabbit mammary explants was stimulated by prolactin via a pathway sensitive to ouabain (61). These observations are consistent with an effect of prolactin-induced differentiation of the cells on the number of Na\(^+\)-K\(^+\) pumps present either induced directly or through an increase in Na\(^+\) permeability. In this connection it is interesting to note that prolactin upregulates Na\(^+\)-K\(^+\)-Cl\(^-\) cotransport in rat mammary tissue (202). Work using cultured cells supports the claim that prolactin-induced differentiation in-
volves changes in mammary Na\(^{+}\) and K\(^{+}\) transport; prolactin increases the mucosal-to-serosal transfer of Na\(^{+}\) across monolayers of cultured mouse mammary epithelial cells via a ouabain-sensitive pathway (24–26).

V. RELATIONS BETWEEN SODIUM, POTASSIUM, AND CHLORIDE TRANSPORT AND WATER MOVEMENTS

It is recognized that the synthesis and secretion of lactose is responsible for the majority of water in milk. However, this cannot be the whole story, since water still appears in the milk of those species (i.e., pinnipeds) which contain little or even no lactose or any other sugar (151). In a number of secretory epithelia, it is accepted that the intracellular accumulation of Cl\(^{-}\), via Na\(^{+}\)-K\(^{+}\)-Cl\(^{-}\) cotransport across the basolateral membrane, is the driving force for the secretion of ions and water across the apical membrane (43, 153). The same process could occur in the mammary secretory cell, since the necessary gradients exist. Therefore, a situation could arise whereby the secretion of water by the mammary gland could be driven partly by the secretion of lactose and partly by the secretion of ions. Variations in the concentrations of lactose and ions within and between species could be related to the relative flux between these two mechanisms. This hypothesis, together with the involvement of paracellular ion flow in some species, may explain how secretion of ions and secretion of water by the mammary gland is achieved in all mammals.

VI. PHOSPHATE TRANSPORT

The suckling requires a considerable amount of phosphate for normal growth and development. There are at least three chemical forms of phosphate in milk: free inorganic orthophosphate (P\(_{i}\)) in solution as HPO\(_{4}\)\(^{2-}\) and H\(_{2}\)PO\(_{4}\); colloidal phosphate, the majority of which is calcium phosphate associated with casein micelles; and casein phosphate (86). For example, of the total phosphate (20.5 mM) present in goat’s milk, 6.7 mM is P\(_{i}\), 8.9 mM is colloidal, and 4.9 mM is casein bound (146). Despite the complex nature of phosphate in milk, it can be predicted that milk phosphate is derived from plasma P\(_{i}\) that has to be transported across the basolateral membranes of the secretory cells. In accordance with this prediction, the mammary gland has the capacity to extract large quantities of P\(_{i}\) from blood. For example, it is estimated that the entire pool of plasma P\(_{i}\) is replaced every 10 min in lactating rats (30).

The mechanism of P\(_{i}\) transport by the lactating mammary gland has only recently received attention. P\(_{i}\) uptake by rat mammary tissue explants and the perfused rat mammary gland occurs predominantly via a Na\(^{+}\)-dependent process (210). The Na\(^{+}\)-dependent moiety of P\(_{i}\) uptake in the latter preparation operates with a Michaelis constant (K\(_{m}\)) of 40 \(\mu\)M with respect to the extracellular P\(_{i}\) concentration. Furthermore, P\(_{i}\) efflux from mammary tissue can be stimulated by reversing the trans-membrane Na\(^{+}\) gradient. These findings are consistent with the presence of a Na\(^{+}\)-P\(_{i}\) cotransport system in the basolateral aspect of the mammary epithelium. Na\(^{+}\)-P\(_{i}\) cotransport mechanisms have been identified in a number of epithelia including the intestine, kidney, and placenta (21, 23, 33), several of which have been cloned and sequenced (125).

There are, however, several differences between the properties of the Na\(^{+}\)-dependent P\(_{i}\) transporter in mammary tissue and those described in other epithelia. First, P\(_{i}\) uptake by mammary tissue is only weakly inhibited by structural analogs; arsenate and phosphonoformic acid (PFA) were found to be weak or ineffective inhibitors of P\(_{i}\) transport. In contrast, both of these compounds have been shown to be high-affinity blockers of Na\(^{+}\)-dependent P\(_{i}\) transport in other tissues (103). Second, the kinetic parameters, with respect to Na\(^{+}\) activation, are markedly different; the stimulation of P\(_{i}\) uptake by external Na\(^{+}\) did not exhibit sigmoidal kinetics as found, for example, in the kidney (125).

Under certain experimental conditions P\(_{i}\) transport in lactating rat mammary tissue occurs via an anion-exchange system (210). External P\(_{i}\) is able to trans-accelerate P\(_{i}\) efflux via a mechanism sensitive to DIDS. It appears that the P\(_{i}\) self-exchange system is distinct from the anion exchanger that accepts Cl\(^{-}\) and SO\(_{4}\)\(^{2-}\) as substrates (194), since P\(_{i}\) efflux is not trans-stimulated by Cl\(^{-}\) and SO\(_{4}\)\(^{2-}\) and P\(_{i}\) does not trans-accelerate SO\(_{4}\)\(^{2-}\) efflux (210). Moreover, the finding that the P\(_{i}\) self-exchanger is not Na\(^{+}\) dependent suggests that the exchange mechanism is distinct from the Na\(^{+}\)-P\(_{i}\) cotransport system. However, the physiological significance of the P\(_{i}\) exchanger remains to be established.

The finding that P\(_{i}\) in milk is higher than that of plasma could, at first sight, be taken as evidence that the apical membrane expresses a P\(_{i}\) transport mechanism. However, the major pathway for P\(_{i}\) secretion into milk is believed to be via the Golgi vesicle route. This hypothesis is based on two important findings. First, Neville and Peaker (146) found that the apical aspect of the lactating goat mammary epithelium is almost impermeable to P\(_{i}\). Second, the time course of P\(_{i}\) secretion into milk, following an intravascular injection of radiolabeled P\(_{i}\), was similar to that of casein phosphate and colloidal phosphate. This finding raises questions about the nature of P\(_{i}\) transport across the Golgi membrane. There is, of course, the possibility that Golgi secretory vesicles do not need to transport P\(_{i}\) per se from the cytosol because sufficient P\(_{i}\) is generated in the Golgi lumen by the hydrolysis of UDP during lactose synthesis (Fig. 4). However, this mode of P\(_{i}\) generation will not occur in the Golgi lumen of pinnipeds.
VII. CALCIUM TRANSPORT

Milk is a calcium-rich fluid (94). For example, the total calcium concentration in rabbit’s milk can approach a concentration of 100 mM. Accordingly, during lactation, the mammary gland extracts large quantities of calcium from plasma to meet the requirements of the growing neonate. Milk calcium, like phosphate, exists in several chemical forms: free ionized calcium, casein-bound calcium, and calcium complexed to inorganic anions such as phosphate and citrate. In most milks, calcium associated with casein micelles comprises the majority of the total calcium present in milk (144). Neville and Peaker (146) found that the mammary gland does not transport calcium in the milk-to-blood direction, suggesting that calcium cannot cross the apical membrane or tight junctions. Thus it can be inferred that calcium transport into milk is transcellular. Furthermore, because the time course of appearance of labeled milk calcium is similar to that of casein and lactose, it has been suggested that calcium is secreted via the Golgi vesicle route (146). It follows that casein-bound calcium and colloidal calcium, along with free ionized calcium, are all derived from plasma Ca$^{2+}$, indicating that Ca$^{2+}$ must be able to cross both the basolateral and Golgi membranes before secretion.

The first question that arises is, What mechanism transports calcium across the basolateral membrane? Surprisingly, there is a paucity of information about the system(s) available for calcium transport across the blood-facing membrane except for the finding that it is a temperature-sensitive process that increases during the transition from pregnancy to lactation (147). Whatever the nature of the system(s), it must operate with a high capacity given the large amount of calcium secreted into milk. Calcium channels would of course fulfill such a role. However, until the mechanism of calcium uptake by mammary secretory cells is thoroughly studied, the presence of calcium channels in the basolateral membrane can only remain a suggestion.

Mammary epithelial cells maintain intracellular Ca$^{2+}$ at a low concentration (56, 75). This observation prompts the next question, How do mammary epithelial cells maintain a low cytosolic Ca$^{2+}$ concentration while facilitating a large transcellular calcium flux? A low calcium concentration could be maintained if Ca$^{2+}$ were pumped into intracellular organelles at a rate that matched the entry of calcium across the basolateral membranes of the secretory cells. In this connection, there is evidence suggesting that Golgi membranes express an ATP-driven Ca$^{2+}$ pump (149, 241, 245). Golgi-derived vesicles are able to accumulate Ca$^{2+}$ by a saturable process ($K_m = 0.8 \mu M$) that requires the hydrolysis of ATP (Fig. 4). A Ca$^{2+}$ pump situated in the basolateral membranes of mammary secretory cells could also act to keep intracellular Ca$^{2+}$ at a low concentration. However, no evidence for such a transport system has been presented. In addition, no evidence for a basolateral Na$^+/Ca^{2+}$ exchanger, another transport system which could regulate intracellular Ca$^{2+}$, has been found (143).

Mammary epithelial cells maintain intracellular Ca$^{2+}$ at a low concentration (56, 75). This observation prompts the next question, How do mammary epithelial cells maintain a low cytosolic Ca$^{2+}$ concentration while facilitating a large transcellular calcium flux? A low calcium concentration could be maintained if Ca$^{2+}$ were pumped into intracellular organelles at a rate that matched the entry of calcium across the basolateral membranes of the secretory cells. In this connection, there is evidence suggesting that Golgi membranes express an ATP-driven Ca$^{2+}$ pump (149, 241, 245). Golgi-derived vesicles are able to accumulate Ca$^{2+}$ by a saturable process ($K_m = 0.8 \mu M$) that requires the hydrolysis of ATP (Fig. 4). A Ca$^{2+}$ pump situated in the basolateral membranes of mammary secretory cells could also act to keep intracellular Ca$^{2+}$ at a low concentration. However, no evidence for such a transport system has been presented. In addition, no evidence for a basolateral Na$^+/Ca^{2+}$ exchanger, another transport system which could regulate intracellular Ca$^{2+}$, has been found (143).

Calcium uptake by mammary tissue may be a hormonally regulated process. Ca$^{2+}$ accumulation by cultured mouse mammary explants, presumably via the basolateral membranes, is stimulated by 1,25-(OH)$_2$D$_3$, the hormonal form of vitamin D, in a dose-dependent fashion (137), and Ca$^{2+}$ transfer into goat’s milk is increased by parathyroid hormone-related protein (15). However, in both of these examples, the target mechanism is unknown.
VIII. CITRATE TRANSPORT

Citrate exists in the aqueous phase of milk in a variety of chemical forms such as calcium citrate\(^{-}\), citrate\(^{2-}\), and citrate\(^{3-}\). The relative proportion of each chemical form depends on factors such as H\(^{+}\), Ca\(^{2+}\), and Mg\(^{2+}\) and thus citrate can be considered as an important milk buffer. The lactating goat mammary epithelium is impermeable to citrate in both directions, suggesting that citrate is synthesized within the secretory cells and released into milk after exocytosis of Golgi vesicles (116). In this connection, a Golgi-enriched fraction isolated from the bovine mammary gland accumulated citrate in a fashion sensitive to temperature, suggesting that a citrate transport system may be present in the Golgi apparatus membrane (207). The putative citrate transport mechanism in the Golgi membranes remains to be fully characterized.

IX. IODIDE TRANSPORT

The concentration of iodide in milk can be 20- to 30-fold higher than that found in maternal plasma (32), matching the requirements of the suckling for the synthesis of thyroid hormones. Iodide transport was investigated relatively early because of the contamination of land and milk supplies by radioactive iodine isotopes from nuclear explosions or accidents. In addition, it was thought that an understanding of the mammary iodide transport mechanism could help limit the transfer of radiolabeled iodide into the food chain via milk after environmental contamination.

The iodide transport system in the thyroid gland has recently been cloned (47). It is a Na\(^{+}\)-dependent mechanism sensitive to anions such as perchlorate and thiocyanate (155). A body of evidence suggests that mammary epithelial cells express a similar iodide transport mechanism. The lactating mammary gland has the ability to accumulate iodide into milk against a high concentration gradient by a system that is inhibited by perchlorate and thiocyanate (31, 32, 53, 114). Iodide uptake by mammary tissue explants prepared from midpregnant mice is a Na\(^{+}\)-dependent process stimulated by prolactin (184, 185). Moreover, mRNA encoding for the Na\(^{+}\)-dependent iodide transporter has been identified in the lactating human mammary gland (215).

Although the Na\(^{+}\)-dependent mechanism is probably the predominant pathway for iodide uptake by mammary cells, there is evidence that iodide also enters via a DIDS-sensitive anion-exchange pathway; sulfate efflux from rat mammary tissue can be stimulated by extracellular iodide (194).

X. STRETCH-SENSITIVE ION TRANSPORT MECHANISMS

Milk accumulation in the alveolar lumen leads to distension of the secretory alveoli, and cellular deformation may play an important role in determining secretory activity during the cessation of lactation. An increase in intramammary pressure at the end of lactation has been shown to reduce the rate of milk secretion in the goat (161), suggesting that, in addition to local, autocrine chemical control of milk secretion by the milk protein FIL, mammary distension could also play a part in controlling the rate of milk secretion in response to demand by the young (48, 163, 168, 169, 247–249).

Cell distension has marked effects on mammary gland ion transport. Enomoto et al. (58) found that mechanical stimulation of cultured mouse mammary cells, induced by touching with a blunt microelectrode, resulted in a short depolarization (1–8 s) followed by a prolonged (10–40 s) hyperpolarization. At present, the locus of the stretch-sensitive channels (apical and/or basolateral) on the mammary epithelial cells is not known. The mechanism underlying the depolarization remains to be determined, but it has been established that the hyperpolarization can be attributed to the activation of quininesensitive, Ca\(^{2+}\)-dependent K\(^{+}\) channels (58, 59, 72).

Accordingly, mechanical stimulation of cultured mouse mammary cells leads to an increase in the intracellular Ca\(^{2+}\) concentration. However, the increase in the Ca\(^{2+}\) concentration is not confined to the touched cell but also spreads to surrounding cells, even to those that have no direct physical contact with the stimulated cell, suggesting that a paracrine factor may be involved (60, 73). There is evidence that ATP is released from cultured mammary cells upon mechanical stimulation (73). In this connection, extracellular ATP, acting via P\(_{2}\)-purinergic receptors, has been shown to increase intracellular Ca\(^{2+}\) in human breast tumor cells (70). Cell swelling, induced by hypotonic shock, increases the intracellular Ca\(^{2+}\) concentration in acini isolated from lactating mouse mammary gland (221). The effect consists of a rapid and large transient increase in intracellular Ca\(^{2+}\) followed by a sustained plateau phase. The increase in Ca\(^{2+}\) is due to increased Ca\(^{2+}\) influx from the incubation medium rather than release from intracellular stores. The nature and precise locus of the Ca\(^{2+}\) transport pathway is unknown, but it is insensitive to nifedipine (221). Moreover, it is not yet known if the primary stimulus is membrane stretch or a change in the cellular hydration state.

Based on these still sparse observations, we hypothesize that stretch induces ATP release into the milk or interstitial space, where it interacts with neighboring cells leading to an increase in calcium entry into these cells.
XI. CHOLINE AND CARNITINE TRANSPORT

The organic cation choline is required by the suckling for normal growth and development including the synthesis of ACh, phosphatidylcholine, and sphingomyelin (258). The concentrations of choline (and its metabolites) in rat's milk and plasma are 1.5 mM and 12 µM, respectively, suggesting that lactation may put a considerable strain on the maternal choline stores (259). The concentration of free choline in rat's milk, 182 µM, is consistent with active choline transport. Lactating rat mammary epithelial cells possess a high-affinity \((K_m = 35 \mu M)\), Na\(^+\)-dependent choline carrier that can be inhibited by hemicholinium-3 (41). Rat mammary epithelial cells can maintain high concentrations of choline (41), suggesting that the Na\(^+\)-dependent carrier is situated in the basolateral membrane and that the transepithelial choline gradient is generated at the basal membrane of the mammary cell.

L-Carnitine (4-N-trimethylammonia-3-hydroxybutanoate) is essential for the transport of long-chain fatty acids between cell compartments. For example, the formation of long-chain acylcarnitines from their respective coenzyme A esters enables the acyl moieties to cross the mitochondrial membrane where they are subjected to \(\beta\)-oxidation (256). L-Carnitine-dependent oxidation of long-chain fatty acids is particularly important for brain development in the neonate. The transport of L-carnitine into milk is physiologically significant because the newborn has a limited capacity for L-carnitine biosynthesis. In rats, the lactating female depletes its reserves of L-carnitine through secretion into milk; the rate of L-carnitine secretion is high during the first 3–4 days of lactation but thereafter falls as the young develop the capacity to synthesize L-carnitine (186). In the early stages of lactation, the L-carnitine concentration in rat milk is almost an order of magnitude greater than that of plasma (320 vs. 35 µM), suggesting that mammary epithelial cells possess a carrier for L-carnitine. In accordance with this prediction, a concentrative, Na\(^+\)-dependent L-carnitine carrier has been identified in mammary explants isolated from rats during the early phase of lactation (201, 257). The carrier operates with a \(K_m\) of 132 µM and maximum velocity \(V_{\text{max}}\) of 100 pmol \(\cdot\ h^{-1} \cdot\ mg\ \text{intracellular water}^{-1}\). If, as expected, the carrier is situated on the basolateral membrane of the alveolar cells it will not be saturated with plasma L-carnitine under physiological conditions. However, the \(K_m\) value obtained with explants is probably an overestimate because of the presence of unstirred fluid layers. The rat mammary L-carnitine carrier is not stereospecific, since D-carnitine is an effective \(cis\)-inhibitor. In addition, acetylearnitine, but not choline or taurine, inhibits L-carnitine uptake by rat mammary explants. These characteristics suggest that the mammary tissue L-carnitine carrier is similar to that of renal brush-border membrane vesicles, neuroblastoma cells, and JAR cells (140, 174, 182). The activity of the Na\(^+\)-dependent L-carnitine transport system was found to decrease as lactation progressed (201).

L-Carnitine efflux from lactating rat mammary tissue is a slow process that is Na\(^+\) independent and cannot be stimulated by external L-carnitine. However, cell-swelling, induced by a hyposmotic shock, increases the fractional release of radiolabeled L-carnitine from rat mammary tissue (201). The possibility that volume-activated L-carnitine release is mediated via the volume-sensitive amino acid transport pathway should be investigated.

XII. GLUCOSE TRANSPORT

The concentration of lactose in the milk of most species is relatively high, and lactose is one of the major milk osmotyes; for example, the concentrations in human and bovine milk are, respectively, 204 and 140 mM (158). Lactose is synthesized within the lumen of the Golgi apparatus by an enzyme complex collectively known as lactose synthase. Exocytosis of the Golgi secretory vesicles then occurs at the luminal side of the cell. Golgi and apical membranes of mammary secretory cells are impermeable to lactose; therefore, because these membranes are freely permeable to water, the volume of milk secreted is closely related to the rate of lactose synthesis.

The lactating mammary gland has a high demand for D-glucose because it is the obligate precursor for lactose synthesis and, depending on species, is also used to synthesize lipids and amino acids (110, 115). Glucose transport across both the basolateral and Golgi membranes of the secretory cells is therefore an important process in milk secretion.

A. Glucose Transporters

1. GLUT1 expression

Lactating mammary tissue transports D-glucose via a facilitative transport system similar, if not identical, to GLUT1. Amato and Loizzi (4) found that guinea pig mammary tissue slices are capable of transporting 2-deoxyglucose, a substrate of GLUT1 type transporters (74), via a system inhibited by cytochalasin B. Rat mammary acini also transport 2-deoxyglucose and 3-O-methyl-D-glucose via a temperature-sensitive and saturable mechanism (\(K_m\) for 2-deoxyglucose = 16 mM) that is inhibited by cytochalasin B and phloretin (225). Carrier-mediated D-glucose uptake with characteristics of GLUT1 has also been described in mouse mammary acini (177); thus 3-O-methyl-D-glucose uptake by mouse mammary cells is via a saturable (\(K_m = 14 \mu M\)), cytochalasin B-sensitive pathway.

In accordance with the flux studies quoted in the previous paragraph, it has been shown that rat mammary...
tissue expresses mRNA that hybridizes with a cDNA probe for GLUT1 as well as a protein that reacts with an antibody raised against the COOH terminus region of the human erythrocyte glucose GLUT1 transporter (35, 124). Immunofluorescence and immuno-electron studies have shown that GLUT1 expression is on the basolateral aspect of the lactating rat mammary epithelium (40, 222), consistent with the finding that 2-deoxyglucose transport occurs at the blood-facing aspect of the intact mammary gland (226). There are also several reports of GLUT1 expression, both at the mRNA and protein level, in lactating bovine mammary tissue (262–264).

Although the available evidence suggests that GLUT1 mediates the majority of D-glucose uptake by lactating mammary tissue, this conclusion would be strengthened if sensitivity to phloretin and cytochalasin could be demonstrated in the perfused lactating mammary gland and if the sequence of the lactating mammary tissue GLUT1 mRNA were known. It is notable, however, that guinea pig mammary tissue apparently has more than one type of saturable transport system for D-glucose (4). Therefore, it appears that GLUT1 may not be the sole mechanism for glucose transport across the basolateral membrane of mammary secretory cells.

2. GLUT4 expression

No evidence has been found for the insulin-sensitive D-glucose transporter GLUT4 in lactating rat mammary epithelial cells (35, 40, 124). However, GLUT4 mRNA is expressed in mammary tissue taken from virgin and pregnant rats, but expression decreases as gestation progresses, suggesting that GLUT4 is expressed in mammary adipocytes rather than mammary epithelial cells (35, 40). No detectable expression of GLUT4 mRNA is evident in lactating bovine mammary tissue (263).

3. GLUT2, GLUT3, and GLUT5 expression

Lactating rat mammary epithelial cells do not appear to express GLUT2 or GLUT5 (35, 40). Similarly, lactating bovine mammary tissue does not express GLUT2 mRNA. Although low levels of GLUT3 and GLUT5 mRNA are found in the bovine mammary gland, the cellular location (i.e., epithelial or stromal) of these transcripts in the gland is not known (263).

4. SGLT1 expression

There is evidence for the presence of a Na+-dependent glucose transporter in lactating rat mammary tissue (198). 1) The efflux of 3-O-methyl-D-glucose from lactating rat mammary tissue explants is stimulated by reversing the transmembrane Na+ gradient. This finding is consistent with a Na+-dependent D-glucose transporter operating in the reverse mode or the inhibition of D-glucose (re)uptake via a Na+-dependent mechanism. 2) mRNA extracted from the rat mammary gland contains a 4-kb transcript that hybridizes with the cDNA for the rabbit intestinal SGLT1 (211). SGLT1 mRNA has also been found in lactating bovine mammary tissue (265).

The precise locus and functional significance of the mammary SGLT1-like transporter remains to be determined. If it is situated in the basolateral aspect of the mammary epithelium, then it may operate in parallel with GLUT1 to provide mammary acinar cells with D-glucose. However, an intracellular or apical location cannot be ruled out at this stage.

B. D-Glucose Transport Across the Apical Membrane

D-Glucose, along with a number of other monosaccharides, is present in milk of most species at a much lower concentration than that found in plasma (65). The available experimental evidence is consistent with the presence of a D-glucose transport system on the apical membrane of mammary secretory cells. Indeed, it has been suggested that milk D-glucose concentrations can be taken as a measure of the intracellular concentration (65, 142). D-Glucose and galactose introduced into the lactating goat mammary gland via the teat canal are rapidly lost from the milk space. The finding that fructose does not cross the mammary epithelium, even after 16 h, suggests that D-glucose and galactose cross the mammary epithelium via a transcellular route and not via a paracellular pathway (64). This conclusion is supported by the finding that radiolabeled 3-O-methyl-D-glucose introduced into the milk space of the goat mammary gland via the teat enters venous blood at a faster rate than radiolabeled...
sorbitol (65). The nature and significance of d-glucose transport across the apical membrane remains to be determined. If d-glucose is able to cross the apical membrane of rat mammary epithelial cells, then the available evidence suggests that the pathway is one other than GLUT1 because GLUT1 protein could not be detected on the apical membrane (40).

In contrast to most species (i.e., eutherian mammals), the Tammar wallaby can, in late lactation, maintain higher concentrations of glucose and galactose in milk than those in plasma (133, 134). This finding raises interesting questions about the nature of d-glucose and galactose transport across the apical membrane of the mammary epithelium in this species and other marsupials. For example, why aren’t these monosaccharides rapidly lost from the milk space as would be predicted if the apical membrane possessed a facilitative transport mechanism?

C. Transport of d-Glucose Across the Golgi Membrane

d-Glucose must cross the Golgi membrane to reach the site of lactose synthesis. A Golgi vesicle fraction prepared from lactating rat mammary tissue was found to transport d-glucose; it was postulated that a pore was involved (246). However, the precise nature of d-glucose transport across the Golgi membrane remains to be established, although one report indicates that Golgi membranes possess a protein similar to GLUT1. Therefore, it is possible that GLUT1 transports d-glucose to the site of lactose synthesis; alternatively, Golgi-derived GLUT1 proteins may be destined for the plasma membrane (124).

D. Control of d-Glucose Transport

Given the important role of d-glucose in the process of milk secretion, it is of fundamental importance that the factors that control the transport of d-glucose by mammary epithelial cells are elucidated. However, it is surprising to find that our knowledge of this important area is relatively patchy. Nevertheless, there are a number of findings which suggest that d-glucose uptake by mammary epithelial cells is influenced by the stage of mammary development and lactation (and, therefore, by inference, prolactational hormones like prolactin, and growth hormone) as well as nutritional status.

1. Ontogeny and hormonal control

Lactose synthesis and glucose uptake by the mammary gland increase abruptly at the time of parturition and decline rapidly as the gland stops secretion before involution (49, 69). Accordingly, the transport of 3-O-methyl-d-glucose by isolated mouse mammary cells increased during progression from the pregnant to the midlactating state (177). A sharp decline in uptake was observed in the immediate postlactational period. A change in the \( V_{\text{max}} \) but not the \( K_m \) of 3-O-methyl-d-glucose uptake suggests that the number of transporters is affected, rather than the affinity of the carrier, by the stage of lactation. The expression of GLUT1 mRNA and protein fall within 24 h of litter removal in the rat (40). These observations suggest that the differences in d-glucose transport by mammary tissue during development, lactation, and involution reflect the expression and function of GLUT1 under the prevailing hormonal milieu of these physiological states.

Milk secretion in the rat is controlled by both prolactin and growth hormone. Fawcett et al. (66) investigated the effect of these hormones on the expression of GLUT1 protein in mammary secretory cell membranes isolated from lactating rats receiving various treatments for 48 h. Administration of bromocriptine reduced GLUT1 in mammary membranes, as would be expected. In contrast, GLUT1 levels in membranes from animals that had been treated with an anti-rat growth hormone serum were not significantly altered. However, when the two treatments were combined, GLUT1 expression was reduced to a level below that found with bromocriptine treatment alone. The results suggest that prolactin and growth hormone act synergistically to maintain GLUT1 transporter expression in rat mammary gland plasma membranes. A role for prolactin in the regulation of GLUT1 expression in cultured mouse mammary epithelial cells has also been reported (87). Prolactin stimulates 2-deoxyglucose uptake by mammary gland explants isolated from midpregnant mice (170). Although the target mechanism was not identified, it can be predicted, on the basis of experiments using rats, that the prolactin effect is on GLUT1.

Zhoa et al. (264) found that administration of bovine growth hormone releasing factor, but not bovine growth hormone itself, increased by 21% the amount of GLUT1 mRNA in the lactating bovine mammary gland. However, the physiological significance is unclear, since the increase in message was not accompanied by a rise in GLUT1 protein in mammary cell membranes.

The uptake of 2-deoxyglucose by the lactating rat mammary gland in vivo was found in one study to be enhanced by insulin (226). This observation is difficult to reconcile with the failure of insulin to produce acute stimulation of 3-O-methyl-d-glucose transport by isolated lactating mouse mammary epithelial cells (177). If insulin does have a direct effect on d-glucose transport by lactating mammary epithelial cells, then the target must be a transporter other than GLUT1 (see sect. xvi). It is possible that insulin stimulates d-glucose uptake by the rat mammary gland as a result of altering the magnitude of the transmembrane d-glucose gradient rather than by affecting the transport proteins directly (34).
2. Effects of starvation

Overnight starvation of lactating rats reduces 2-deoxyglucose and 3-O-methyl-D-glucose uptake by the mammary gland by >90% (226); the arteriovenous difference in D-glucose concentration across the mammary gland is decreased to a similar extent (156). However, refeeding starved animals rapidly restores the uptake of glucose analogs and increases the arteriovenous difference. It is apparent that the effect of overnight fasting is a consequence of a reduction of D-glucose transport via GLUT1. Thus starvation decreases cytochalasin B-sensitive 3-O-methyl-D-glucose uptake by isolated lactating mouse mammary epithelial cells. This reduction in transport occurs as a result of a decrease in the \( V_{max} \) of uptake and is accompanied by a decrease in the number of cytochalasin B binding sites on mammary cell plasma membranes (175). However, it has been reported that starvation does not alter the total GLUT1 protein content of lactating rat mammary tissue, suggesting that the reduction in D-glucose transport induced by starvation is brought about by the translocation of GLUT1 carriers from the plasma membrane to an intracellular site (40).

XIII. AMINO ACID TRANSPORT

The lactating mammary gland has a large demand for amino acids to meet the requirements of milk protein synthesis. For example, a dairy cow secreting 35 l milk/day (by no means exceptional) with a protein content of 3.3% needs over 1 kg/day of amino acids to sustain milk protein synthesis. The first studies on amino acid transport by the lactating mammary gland focused on arteriovenous differences in amino acid concentrations and combining these with measurement of mammary blood flow to establish whether amino acids were the blood source of milk proteins and then whether their quantitative uptake was sufficient to account for milk output of protein (129). Many studies in goats, cows, sheep, pigs, guinea pigs, and rats have shown that the arteriovenous differences across the gland are substantial, especially for some of the essential amino acids (82, 119, 129, 136, 240). This extraction of amino acids from blood is generated by amino acid transport across the basolateral membranes of the secretory cells. Despite the importance of amino acids to milk secretion and mammary metabolism, it was some time before the cellular mechanisms responsible for amino acid uptake by the mammary gland received the attention they deserved. Although our current understanding of mammary amino acid transport systems and their regulation lags behind what is known in other tissues, it is now becoming clear that the lactating mammary gland possesses amino acid transport systems analogous to those described in other organs such as the intestine, kidney, and placenta (209). However, it appears that some of the mammary transport systems may have unique kinetic properties and mechanisms of control.

A. Amino Acid Transporters

1. \( \text{Na}^+ \)-dependent transport of neutral amino acids

There is good evidence for system A-like transport of neutral amino acids in lactating mouse, rat, and bovine mammary tissue (Table 1). System A is a \( \text{Na}^+ \)-dependent transport mechanism that prefers short-chain neutral amino acids as substrates and has the distinguishing feature of being able to tolerate \( N \)-methylated amino acids (14). Mouse and rat mammary tissue explants transport aminoisobutyric acid (AIB) via a pathway that is both \( \text{Na}^+ \) dependent and sensitive to methylaminoisobutyric acid (MeAIB), a characteristic of system A (145, 204). However, the substrate specificity of system A in rodent mammary tissue for naturally occurring amino acids remains to be established. The lactating bovine mammary gland also appears to express system A; mammary explants transport MeAIB in a \( \text{Na}^+ \)-dependent fashion (18).

The transport of amino acids via system A may, in part, be responsible for the accumulation of certain neutral amino acids within mammary cells with respect to plasma (209). In contrast to mouse, rat, and bovine mammary tissue, it appears that the guinea pig mammary gland does not possess system A activity. This conclusion is based on the lack of MeAIB-sensitive amino acid transport by the perfused guinea pig mammary gland (131).

Bovine mammary tissue transports AIB via a system that is \( \text{Na}^+ \) dependent but not inhibited by MeAIB (18). The simplest interpretation of this experimental observation is that bovine mammary tissue possesses system ASC-like activity. Similarly, it has been concluded that lactating guinea pig mammary tissue expresses system ASC-like activity (131). The presence of system ASC in mouse mammary tissue is a matter of controversy. On the one hand, Verma and Kansal (231) have shown that methionine may utilize system ASC; on the other hand, Neville et al. (145) found no evidence for AIB transport via system ASC in mouse mammary tissue. The difference in results is difficult to reconcile but may reflect metabolism of methionine in the former study. In this connection, no evidence for system ASC in rat mammary tissue has been found (204).

L-Glutamine transport by the lactating rat mammary gland is partly dependent on the presence of \( \text{Na}^+ \) (39). The \( \text{Na}^+ \)-dependent component, a low-affinity pathway that has been localized to the basolateral surface of the epithelium, is not mediated by system A, since L-glutamine transport is insensitive to MeAIB. The \( \text{Na}^+ \)-dependent moiety of L-glutamine transport remains to be identified, but a mechanism akin to system N (104) must be a strong candidate.
indicating mammary cells do not rely on intracellular amino acid efflux. In addition, the mammary system is different from EAAC1 is the effect of GLAST is inhibited by neither AAD nor DHK (98, 109). Another piece of evidence which suggests that the mammary system is different is that the carrier differentiates between the optical isomers of glutamate but not those of aspartate.

Another XAG-like feature of the transport system in the rat is that the carrier differentiates between the optical isomers of glutamate but not those of aspartate. XAG-like transport is the predominant, if not only, mechanism for anionic amino acid transport in rat mammary tissue; no evidence for L-glutamate transport by system X̄, a Na+-independent transporter which catalyzes the exchange of L-glutamate and L-cystine (11), was found (139). Studies using the perfused lactating rat mammary gland suggest that the system X̄AG-like activity is situated, as predicted, in the basolateral membrane of the mammary epithelium (138, 139). Although the kinetics suggest that there is only a single saturable system, a detailed study of anionic amino acid transport at the molecular level may reveal that there is more than one system (although their kinetics will be similar). However, if it transpires that the Na+-dependent moiety of L-glutamate transport in rat mammary tissue is mediated by a single mechanism, then the mammary system has a pharmacological profile unlike that of the high-affinity, Na+-dependent systems that have been cloned (EAAC1, GLAST, and GLT1) and characterized (97, 171, 219). The evidence for such a difference is based on the effects of dihydrokainate (DHK) and α-aminoacidopate (AAD); anionic amino acid transport by mammary tissue is inhibited by DHK but not AAD (139); EAAC1 is inhibited by AAD but not DHK; GLT1 is sensitive to both AAD and DHK; and GLAST is inhibited by neither AAD nor DHK (98, 109). Another piece of evidence which suggests that the mammary system is different from EAAC1 is the effect of extracellular amino acids on the efflux of substrates via the Na+-dependent carrier. L-Cysteine is capable of trans-accelerating L-glutamate efflux via EAAT3 (260), a human homolog of EAAC1, whereas it has little or no effect on amino acid efflux from rat mammary tissue (139). The mammary gland L-glutamate transport mechanism does display Na+-dependent exchange activity; anionic amino acids such as L-glutamate, L-aspartate, L-aspartate, and L-cysteine sulfinate are transported by the carrier operating as an exchanger (139) (Table 2). This mode of operation may allow the uptake of L-glutamate to regulate the

### Table 1. Kinetic properties of mammary tissue amino acid transport systems

<table>
<thead>
<tr>
<th>Amino Acid Transport System</th>
<th>Species</th>
<th>Experimental Preparation</th>
<th>( K_m )</th>
<th>( V_{\text{max}} )</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺-dependent L-glutamate uptake (system X̄AG)</td>
<td>Rat</td>
<td>Explants</td>
<td>112.5 ( \mu \text{M} )</td>
<td>71.3 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>138</td>
</tr>
<tr>
<td>Na⁺-dependent L-glutamate uptake (system X̄AG)</td>
<td>Rat</td>
<td>Perfused gland</td>
<td>18.1 ( \mu \text{M} )</td>
<td>40.3 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g tissue}^{-1} )</td>
<td>138</td>
</tr>
<tr>
<td>Na⁺-dependent D-aspartate uptake (system X̄AG)</td>
<td>Rat</td>
<td>Explants</td>
<td>32.4 ( \mu \text{M} )</td>
<td>24.5 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>139</td>
</tr>
<tr>
<td>Na⁺ + Cl⁻-dependent taurine uptake (system ( \beta ))</td>
<td>Rat</td>
<td>Explants</td>
<td>37.5 ( \mu \text{M} )</td>
<td>2.5 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g ICW}^{-1} )</td>
<td>205</td>
</tr>
<tr>
<td>Na⁺-dependent taurine uptake (possibly system ( \beta ))</td>
<td>Gerbil</td>
<td>Explants</td>
<td>70 ( \mu \text{M} )</td>
<td>7.9 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g ICW}^{-1} )</td>
<td>197</td>
</tr>
<tr>
<td>Na⁺-dependent AIB uptake (system A)</td>
<td>Mouse</td>
<td>Explants</td>
<td>2.0 ( \text{mM} )</td>
<td>197 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>145</td>
</tr>
<tr>
<td>Na⁺-independent AIB uptake (system L)</td>
<td>Mouse</td>
<td>Explants</td>
<td>2.7 ( \text{mM} )</td>
<td>150 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>145</td>
</tr>
<tr>
<td>Na⁺-dependent methionine uptake (system A)</td>
<td>Mouse</td>
<td>Explants</td>
<td>0.47 ( \mu \text{M} )</td>
<td>18.8 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>231</td>
</tr>
<tr>
<td>Na⁺-independent, MeAIB-sensitive methionine uptake (system L)</td>
<td>Mouse</td>
<td>Explants</td>
<td>0.46 ( \mu \text{M} )</td>
<td>30.0 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>231</td>
</tr>
<tr>
<td>Na⁺-dependent methionine uptake (system ASC)</td>
<td>Mouse</td>
<td>Explants</td>
<td>0.46 ( \mu \text{M} )</td>
<td>12.4 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>231</td>
</tr>
<tr>
<td>Na⁺-dependent tyrosine uptake (system unknown)</td>
<td>Mouse</td>
<td>Explants</td>
<td>1.67 ( \text{mM} )</td>
<td>75.1 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>100</td>
</tr>
<tr>
<td>Na⁺-independent, BCH-insensitive tyrosine uptake (system T)</td>
<td>Mouse</td>
<td>Explants</td>
<td>15.75 ( \text{mM} )</td>
<td>157.5 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>100</td>
</tr>
<tr>
<td>Na⁺-independent, BCH-sensitive tyrosine uptake (system L)</td>
<td>Mouse</td>
<td>Explants</td>
<td>0.23 ( \text{mM} )</td>
<td>31.0 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g cells}^{-1} )</td>
<td>100</td>
</tr>
<tr>
<td>AIB uptake (in presence of prolactin)</td>
<td>Mouse</td>
<td>Cultured explants</td>
<td>0.75 ( \text{mM} )</td>
<td>430 ( \text{nmol} \cdot \text{min}^{-1} \cdot \text{g ICW}^{-1} )</td>
<td>183</td>
</tr>
</tbody>
</table>

\( K_m \), Michaelis constant; \( V_{\text{max}} \), maximum velocity; AIB, aminoisobutyric acid; MeAIB, methylaminoisobutyric acid; BCH, 2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid.

2. Na⁺-dependent transport of anionic amino acids

In some species, L-glutamate is the most abundant amino acid in milk protein. In addition, the mammary gland of some species, notably humans, is able to generate a large transepithelial gradient of free glutamate in favor of milk (5). In vivo, the mammary gland extracts large quantities of glutamate from arterial blood (115), indicating mammary cells do not rely on intracellular L-glutamate synthesis. In lactating mammary tissue, there is convincing evidence that L-glutamate and L-aspartate are transported by a system that is analogous to system X̄AG (138, 139); the mechanism is of high affinity, is Na⁺ dependent, and interacts only with anionic amino acids. Another X̄AG-like feature of the transport system in the rat is that the carrier differentiates between the optical isomers of glutamate but not those of aspartate.
The table below shows the effect of external amino acids on $\alpha$-aspartate efflux from lactating rat mammary tissue.

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>n</th>
<th>Control</th>
<th>+ Test</th>
<th>Difference</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Glutamate</td>
<td>7</td>
<td>75 ± 10</td>
<td>362 ± 32</td>
<td>287 ± 38</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>$\alpha$-Aspartate</td>
<td>6</td>
<td>82 ± 12</td>
<td>311 ± 32</td>
<td>229 ± 43</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L-Aspartate</td>
<td>4</td>
<td>86 ± 8</td>
<td>415 ± 32</td>
<td>329 ± 36</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>CSA</td>
<td>4</td>
<td>58 ± 18</td>
<td>369 ± 55</td>
<td>311 ± 70</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>L-Cysteine</td>
<td>5</td>
<td>163 ± 9</td>
<td>161 ± 14</td>
<td>58 ± 9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>3</td>
<td>80 ± 12</td>
<td>88 ± 23</td>
<td>8 ± 11</td>
<td>NS</td>
</tr>
<tr>
<td>$\alpha$-Glutamate</td>
<td>5</td>
<td>87 ± 20</td>
<td>105 ± 19</td>
<td>18 ± 9</td>
<td>NS</td>
</tr>
<tr>
<td>DHK</td>
<td>4</td>
<td>54 ± 11</td>
<td>100 ± 14</td>
<td>46 ± 3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AAD</td>
<td>3</td>
<td>73 ± 16</td>
<td>87 ± 22</td>
<td>14 ± 9</td>
<td>NS</td>
</tr>
</tbody>
</table>

$\alpha$-Aspartate efflux was measured into a medium containing (in mM) 135 NaCl, 5 KCl, 2 CaCl$_2$, 1 MgSO$_4$, 10 glucose, and 20 Tris-MOPS, pH 7.4, and then to a similar medium supplemented with a “test” amino acid at 500 $\mu$M. CSA, $\alpha$-cysteinesulfinate; DHK, dihydrokainate; AAD, aminoadipate. Only those amino acids that inhibited L-Glu and $\alpha$-Asp uptake, with the exception of DHK, were effective at stimulating $\alpha$-Asp efflux, suggesting that high-affinity Na$^+$-dependent carrier is able to act as an exchanger as well as a cotransport system. The finding that DHK did not markedly stimulate $\alpha$-Asp efflux indicates that DHK is a nontransported inhibitor. [Data from Millar et al. (139).]

The intracellular concentration of L-aspartate (and vice versa); however, it is not yet known if the exchange of intracellular amino acids is required for amino acid uptake by the high-affinity anionic amino acid carrier.

3. Na$^+$-dependent transport of $\beta$-amino acids

Taurine (ethanesulfonic acid), a nonprotein $\beta$-amino acid, is present in the milk of a large number of species (181); in some species, notably pinnipeds, exceptionally high concentrations are found, 13 mM in the Antarctic fur seal for example (192). It has been suggested that the high taurine levels in the milk of certain marine mammals are related to their high milk fat content (152), since taurine is required for bile salt synthesis. There is a strong possibility that milk taurine may play an important role in growth and development of the suckling (90), particularly in those species whose neonates are not capable of synthesizing taurine. However, taurine is also present in the milk of species whose young are able to synthesize taurine, suggesting that the transfer of the $\beta$-amino acid into milk has other roles.

Lactating rat mammary tissue accumulates taurine to higher concentrations than plasma, generates a transepithelial concentration gradient with milk concentrations higher than plasma, and transports radiolabeled taurine from blood to milk (205, 218, 220). Collectively, these results suggest that the rat mammary gland possesses an active transport mechanism for taurine. The predominant pathway for taurine uptake by rat mammary tissue explants is a high-affinity ($K_m = 43 \mu$M), Na$^+$- and Cl$^-$-dependent transport mechanism selective for $\beta$-amino acids including $\beta$-alanine and hypotaurine (205). Another pathway for taurine uptake in rat mammary tissue may exist, since a small portion of the Na$^+$-dependent component is not Cl$^-$ dependent (205). The mammary taurine transport system has similar characteristics to the Na$^+$- and Cl$^-$-dependent taurine transporters that have been described (and cloned) in other epithelia (122, 178, 229). However, the molecular identity of the rat mammary taurine transport mechanism(s) remains to be determined. Maintenance of a high intracellular concentration of taurine by the Na$^+$-dependent system may be important in relation to mammary cell volume regulation; there is good evidence that taurine (and other amino acids) may play a role following cell swelling (see section on Na$^+$-dependent transport of neutral amino acids).

In view of the finding that gerbil milk contains a high concentration of taurine (~5 mM) (181), the uptake of taurine by lactating mammary tissue from the gerbil has also been investigated (197). Taurine uptake by gerbil mammary tissue explants, like that of the rat, is mediated by a high-affinity ($K_m = 70 \mu$M), Na$^+$-dependent transport system that is selective for $\beta$-amino acids. However, the Na$^+$-dependent moiety of taurine uptake is not Cl$^-$ dependent, and anions such as NO$_3^-$ and SCN$^-$ can substitute for Cl$^-$ (197). In this connection, a Cl$^-$-independent, Na$^+$-dependent taurine transport system has been found in cultured human kidney cells (95); however, this mechanish has a low affinity ($K_m = 1$ mM) for taurine. Similarly, a Na$^+$-dependent taurine transport system that is not coupled to Cl$^-$ has been identified in rabbit proximal kidney tubule (29).

4. Na$^+$-independent transport of neutral amino acids

System L, a well-studied system for neutral amino acids that is sensitive to 2-aminobicyclo-[2,2,1]-heptane-2-carboxylic acid (BCH), has been identified in lactating mouse, guinea pig, bovine, and rat mammary tissue (18, 131, 145, 206, 231) (Table 1). This conclusion is based on the finding that mammary tissue from these species exhibits BCH-sensitive, Na$^+$-independent transport of neutral amino acids. System L in mammary tissue appears, on the basis of cis-inhibition studies, to have a wide substrate specificity and a low stereospecificity (145, 206). This mechanism may be the single most important pathway for the uptake of the essential neutral amino acids by the lactating gland. Studies using the perfused lactating guinea pig and rat mammary gland preparations have shown that system L is situated in the blood-facing aspect of the mammary epithelium (39, 131). There is evidence to suggest that in bovine mammary tissue system L acts as an exchanger: intracellular cycloleucine is able to trans-stimulate the uptake of cycloleucine by bovine mammary explants. Similarly, L-methionine uptake by mouse mammary explants via system L is trans-accelerated by intracellular L-methionine (99). However, AIB efflux from rat mammary tissue is not trans-stimulated by substrates of
system L (206). Likewise, L-phenylalanine egress from rat mammary tissue, in the absence of extracellular Na\(^+\), is not significantly affected by supplementing the incubation medium with system L substrates (D. B. Shennan, unpublished data). If it is assumed that system L in rat mammary tissue is identical to system L in bovine and mouse mammary glands, then the finding that amino acid uptake, but not efflux, is trans-stimulated suggests that system L in mammary tissue operates with asymmetrical kinetics. This form of asymmetry between the internal and external face of the carrier favors amino acid retention by the mammary epithelium particularly after a postprandial increase in plasma amino acids. In this connection, a sulfate carrier with asymmetrical kinetics for trans-stimulation between its internal and external face has been described in human placental brush-border membrane vesicles (36). However, there is a distinct possibility that system L in rat mammary tissue may not be able to act as an exchange mechanism, suggesting that there may be tissue-specific variants of the transport mechanism. This is already apparent with respect to substrate specificity given that system L in some tissues (e.g., the human placenta) does not transport AIB (57).

System T, a Na\(^+\)-independent mechanism originally identified in human erythrocytes (189), has been described in mouse mammary tissue (100). Thus the portion of L-tyrosine uptake by mouse mammary tissue explants that is not dependent on extracellular Na\(^+\) and is not inhibited by BCH has been ascribed to system T. This mechanism prefers aromatic amino acids (L-tyrosine, L-tryptophan, and L-phenylalanine) as substrates.

5. Cationic amino acid transport

The transport of cationic amino acids by mammary tissue is of particular interest because it has been shown in several species that L-arginine is taken up by mammary tissue in excess of its requirement for milk protein synthesis (45). It has been suggested that L-arginine is used as a precursor for amino acids whose uptake is less than that required for milk protein synthesis as well as for urea (115, 130). In addition, L-arginine is needed by mammary epithelial cells for the synthesis of nitric oxide (112). The transport of cationic amino acids by lactating bovine and rat mammary tissue does not depend on Na\(^+\) (17, 208). L-Lysine and L-arginine (and ornithine) share a pathway for transport in bovine mammary tissue that is not cis-inhibited by MeAIB and L-histidine (but possibly by c-Leu) (17). However, the results of these experiments must be interpreted with caution, since the effects of the amino acids were not tested on the initial rate of L-lysine uptake. Nevertheless, Baumrucker (17) concluded that the cationic amino acid transport in bovine mammary tissue is mediated via system y\(^+\). L-Lysine and L-arginine also share a pathway for transport in lactating rat mammary tissue, but it is evident, on the basis of cis-inhibition studies, that neutral amino acids also interact with the L-lysine carrier (208). Thus a wide range of neutral amino acids are able to reduce L-lysine uptake by mammary tissue explants. The finding that L-lysine is trans-stimulated by L-leucine and L-glutamine (even in the absence of extracellular Na\(^+\)) suggests that neutral amino acids are actually transported by the cationic amino acid carrier rather than simply acting as inhibitors. It is interesting to note that Neville et al. (145) found that L-arginine inhibited AIB uptake by lactating mouse mammary tissue. The cationic amino acid carrier that interacts with neutral amino acids has also been found using the perfused rat mammary gland, suggesting that it is located in the basolateral membrane of the secretory cells (38). The available evidence suggests that the cationic amino acid transport system that interacts with neutral amino acids in lactating rat mammary tissue is not system γ\(^+\). However, the uptake of cationic amino acids by rat mammary tissue may be mediated by more than one pathway as the kinetics of L-lysine transport have not been thoroughly examined. The molecular identity of the rat mammary tissue cationic amino acid transport mechanism remains to be identified, but there is the possibility that the system may be related to one of the amino acid transport systems (b\(^0\), γ\(^-\)) that accepts both cationic and neutral amino acids as substrates (22, 52). On a cautionary note, it must be borne in mind that metabolism of L-lysine (particularly in the experiments employing explants) may have confounded the interpretation of the results.

B. Amino Acid Transport Across the Apical Membrane of Mammary Secretory Cells

To date, the amino acid transport systems that have been identified in mammary tissue probably reside in the blood-facing aspect of the mammary epithelium. However, the fact remains that the milk of some species contains significant quantities of free amino acids. A striking example is the milk of pinnipeds, where the total concentration of free amino acids is in the range 12–21 mM (192). Invariably, the most abundant free amino acids in milk are taurine and L-glutamate (181, 192). Although paired measurements of free amino acids in milk and plasma from single animals are lacking, it would appear that some amino acids are concentrated in milk. For example, L-glutamate is concentrated in human milk (5). There is the possibility that amino acids can cross the apical membrane via specific carriers, but no studies have been reported.

C. Control of Amino Acid Transport

Amino acid uptake by lactating mammary tissue is regulated by a variety of factors including prolactin, milk
stasis, and starvation. Most of the studies have involved measurement of arteriovenous differences, sometimes combined with blood flow determinations, and it was not possible to identify which transport systems were affected. Nevertheless, it is possible in a few cases to be almost certain what pathways would have been involved; for example, if a manipulation affected the extraction of anionic amino acids, then it can safely be assumed that the effect was on the high-affinity, Na$^+$-dependent, anionic amino acid transporter given that this is the only pathway available for this class of amino acids.

Because prolactin is of major importance to milk secretion in rats, it is not surprising that full secretory activity stimulated by prolactin is associated with changes to amino acid transport. Treating rats with bromocriptine during peak lactation, to block prolactin release, reduced the arteriovenous concentration difference across the mammary gland for all amino acids with the exception of L-aspartate, L-glutamate, and L-valine. However, arteriovenous differences were restored (but not for L-threonine, L-isoleucine, and L-leucine) by exogenous prolactin (234). AIB uptake by mammary tissue explants from rats treated with bromocriptine was reduced; the reduction in AIB uptake is attributable to an amino acid transport systems A and L (206). Similarly, c-Leu transport by cultured rat mammary acini isolated from lactating rats during late lactation can be stimulated by prolactin, a finding consistent with an increase in system L (187). Amino acid transport by mammary tissue taken from midpregnant mice also responds to prolactin; AIB transport was increased but could be inhibited by cycloheximide and actinomycin D (183).

As would be expected from the arrest of secretion that occurs in the glands of rats that are sealed to prevent milk removal, the uptake (as interpreted from arteriovenous differences) of all amino acids was reduced (240). Similarly, starvation for 24 h decreased the arteriovenous difference in concentration of all the amino acids in a reversible manner (237). Consistent with the in vivo observations, it was subsequently shown that the uptake of radiolabeled AIB by mammary acini isolated from starved lactating rats is markedly reduced (223, 239). It has been suggested that the reduction in amino acid transport can be attributed to an increase in the circulating concentrations of ketone bodies (237). In contrast, in the mouse, Verma and Kansal (232) found that L-methionine uptake by mammary explants from animals that had been starved, for the very long period of 48 h, was markedly stimulated. It appeared that the increase in uptake can be attributed to systems A and ASC (but not system L) and reflects an increase in the $V_{\text{max}}$ of the transport mechanisms. These results are difficult to reconcile with those found using the rat but could be associated with the longer period of starvation.

D. Volume-Sensitive Amino Acid Transport

Most cells regulate their volume after swelling or shrinking. Perturbations induced by anisosmotic conditions and substrate accumulation activate membrane transport mechanisms (85, 106). Swelling activates the release of K$^+$, Cl$^-$, and organic osmolytes such as amino acids (especially taurine); this solute efflux together with osmotically obliged water returns cells to their normal volume. Shrinkage has the opposite effect, leading to the uptake of solutes such as Na$^+$, K$^+$, Cl$^-$, and amino acids and, consequently, water.

Mammary tissue has a relatively high intracellular free amino acid pool (93, 207, 209). In particular, the concentrations of nonessential amino acids are higher than in plasma or milk. It appears that mammary tissue may utilize amino acids to regulate volume after swelling because exposing rat mammary explants to hyposmotic conditions increased the efflux of radiolabeled taurine and glycine in a rapid and reversible manner (207). This efflux is seen in the perfused rat mammary gland, suggesting that the amino acids were exiting via the basolateral membrane of the secretory cell (39). However, the magnitude of the contribution of amino acids to mammary cell volume regulation is not known.

It is not yet known if the amino acids whose transport is affected by cell swelling utilize one or more pathways. The simplest explanation of the available evidence is that amino acids, such as taurine and glycine, exit mammary tissue via a single pathway. Volume-sensitive taurine efflux from mammary tissue may be channel mediated, since the process is relatively temperature insensitive and does not exhibit trans-stimulation (207). However, the exact identity of the mammary tissue pathway is unknown. Apparently, volume-activated taurine efflux from some, but not all, cell types is via volume-activated anion channels. For example, it is beyond doubt that taurine is able to traverse anion channels in Madin-Darby canine kidney, C6 glioma, and inner medullary collecting duct cells (9, 28, 92). The finding that volume-activated taurine efflux is also inhibited by anion transport blockers such as DIDS, 5-nitro-2(3-phenylpropylamino) benzoic acid (NPPB), and niflumic acid has also been used to support the claim that amino acids use volume-activated anion channels (81, 107, 108, 157, 191). However, care must be exercised in interpreting studies employing anion transport inhibitors because these compounds are notoriously nonspecific (37). Interestingly, volume-sensitive taurine efflux from lactating rat mammary is inhibited by DIDS, NPPB, and niflumic acid, albeit at relatively high concentrations. This could, at first sight, be taken as evidence that volume-sensitive taurine efflux from mammary tissue utilizes anion channels. However, Shennan and co-workers (200, 207) found that the effluxes of radiolabeled I$^-$ and D-aspartate, substrates of volume-activated anion channels, were not increased when mammary explants were subjected to a
from the perfused lactating rat mammary gland. Data are means ± SE (n = 3). The results suggest that the volume-activated system is selective for taurine and is situated in the basolateral aspect of the mammary epithelium. [From Calvert and Shennan (39).]

Figure 5. Effect of a hyposmotic shock (307–182 mosmol/kgH₂O) on the fractional release of radiolabeled taurine (▲) and D-aspartate (●) from the perfused lactating rat mammary gland. Data are means ± SE (n = 3). The results suggest that the volume-activated system is selective for taurine and is situated in the basolateral aspect of the mammary epithelium.

E. Peptide Transport

Because of the nature of the digestive system and the diverse sources of protein available to ruminant animals, particular diets can be devised that alter the availability of amino acids. Dairy animals have very large mammary glands for their body size (83) and, therefore, extract considerable quantities of amino acids from blood. Early studies in the goat and cow showed that “the mammary uptake of essential amino acids is sufficient to account for the output of their corresponding residues in the protein” (129). Later, there have been suggestions that the mammary uptake of some essential amino acids is insufficient to account for their output in milk protein (135). For example, in lactating goats, L-phenylalanine and L-histidine uptake were found to be insufficient for the output of these residues in milk. (8). On the basis that these amino acids cannot be synthesized within mammary cells, it has been suggested that amino acids derived from circulating peptides (and/or proteins) may make up the apparent shortfall. There is good evidence that amino acids derived from intravenously administered dipeptides can be incorporated into milk proteins in the goat (7, 8). The possible mechanisms for this incorporation are transport of intact peptides with intracellular hydrolysis and extracellular hydrolysis followed by uptake of the free amino acids. In this connection, Wang et al. (243) claim to have evidence, albeit indirect, for the transport of intact peptides by mouse mammary tissue explants; however, the mechanism(s) remains to be characterized.

In a recent study, an attempt was made to quantify the transport of intact peptides across the basolateral membrane of rat mammary secretory cells (199). The transport of dipeptides containing a D-isomer at the NH₂ terminus, a configuration which confers resistance to hydrolysis (132), by the perfused lactating rat mammary gland was examined. This experimental approach demonstrated dipeptide transport by a low-affinity pathway. However, the rate of peptide transport was low compared with the uptake of free amino acids (199). The results suggest that the pathway for dipeptide transport across the basolateral membrane of rat mammary secretory cells is different from the peptide transport systems that have been cloned from the intestine (PepT1) and kidney (PepT2) (67, 123).

The study by Shennan et al. (199) does not rule out a role for a peptide transport system such as PepT1 or PepT2 in the apical membrane of mammary secretory cells. A specific peptide transporter on the apical aspect of the epithelium could act as a scavenger system to transport the products of milk protein hydrolysis back into secretory cells. It is interesting to note that a favorable pH gradient exists across the apical membrane for proton-coupled peptide transport, and rabbit mammary gland has been reported to express mRNA encoding the high-affinity PepT2 transport system (54). However, the location of the transporter remains to be established.

It has been suggested that peptides may cross the basolateral membranes of epithelia via an anion transporter (132). Support for this hypothesis comes from the finding that a range of aromatic peptides and cephalosporin antibiotics are able to interact with the renal organic anion transporter (230). In this connection, benzylpenicillin, an antibiotic which contains an amide bond, is actively transported from blood to milk across the lactating caprine mammary gland via a pathway sensitive to probenecid, an inhibitor of the renal organic anion transport system (193). Similarly, probenecid reduces the transfer of benzylpenicillin into ovine milk (266). Evidence for an organic anion transporter in mammary tissue is strengthened by the finding that an analog of p-aminohippuric acid (N⁴-acetylated p-aminohippuric acid), a model substrate of the kidney organic anion transport mechanism, is concentrated in bovine and caprine...
milk (179). Active transport of 4-amoantipyrine, N-acetyl-
ated sulphanilamide, and nitrofurantoin across the bovine,
caprine, and rat mammary gland, respectively, has also been
described (10, 101, 102, 180, 227). There is the possibility that
an organic anion transport system, analogous to the one
expressed in the kidney, may provide a route for small
peptides across the basolateral membrane of mammary se-
cretry cells.

The rat mammary gland is able to hydrolyze peptides
extracellularly followed by uptake of the free amino acids
(199). Thus anionic peptides, presented to mammary tis-
ue explants or the perfused gland, are able to trans-
stimulate D-aspartate efflux. It is envisaged that anionic
amino acids, produced as a consequence of peptidases
that hydrolyze anionic dipeptides extracellularly, stimu-
late D-aspartate efflux via the high-affinity anionic amino
acid carrier, system X_{AG} (199) (Fig 6). It appears that the
rat mammary gland has a large capacity to hydrolyze
anionic peptides, since some dipeptides were almost as
effective as free L-glutamate in eliciting an increase in
D-aspartate efflux. It is possible that the uptake of free
amino acids following the extracellular hydrolysis of plas-
ma-derived peptides could make an important contribu-
tion to the supply of certain amino acids for milk protein
synthesis. The exact identity of the peptidases and the
quantitative importance of blood peptides, as opposed to
amino acids, remain to be established.

**F. Does γ-Glutamyl Transpeptidase Play a Role
in Mammary Amino Acid Transport?**

Meister (128) proposed that γ-glutamyl transpepti-
dase (γ-GT; EC 2.3.2.2.), an enzyme whose substrates
include glutathione (γ-glutamyl-cysteinylglycine) and cer-
tain amino acids, is involved in amino acid transport. It
was envisaged that γ-GT (in conjunction with other en-
zymes) utilized intracellular glutathione to effect the
transport of extracellular amino acids into the cell. How-
ever, the original model was revised on the finding that
the enzymatic site for glutathione was on the extracellular
face (80). The model predicts that following the hydro-
lysis of extracellular glutathione, the γ-glutamyl moiety
is transferred to an amino acid to form a γ-glutamyl-dipep-
tide that is subsequently taken up by cells. The initial
hypothesis that γ-GT was involved in amino acid trans-
port was based on the correlation between high rates of
amino acid transport and γ-GT activity. In this conncet-
ction, lactating bovine and rat mammary tissue express
γ-GT activity with a specific activity second only to that
found in the kidney (19). In the rat, γ-GT activity increases
with the onset of lactation and is regulated by prolactin. A
role for γ-GT in rat mammary tissue amino acid transport
was inferred from the finding that the arteriovenous con-
centration gradients for those amino acids that are good
substrates of γ-GT were reduced after the administration
of inhibitors (serine borate or anthglutin) of γ-GT (233,235).
However, interpretation of these results relies
heavily on the assumption that the inhibitors are specific
for γ-GT. The hypothesis that γ-GT has a direct role in
amino acid transfer is weakened in light of the finding that
the turnover of glutathione required to sustain amino acid
transport is much higher than the predicted capacity of
mammary tissue (236). An alternative explanation for the
effect of γ-GT on amino acid metabolism is that γ-GT
produces a signal that is capable of regulating amino acid
uptake by rat mammary tissue. The main candidates are
γ-glutamyl-amino acids or 5-oxoproline; the latter com-
ound is formed intracellularly during the metabolism of
γ-glutamyl-amino acids (236, 238). Treating rats with bro-
mocriptine to reduce prolactin secretion inhibited γ-GT
and decreased the arteriovenous concentration difference
of a number of amino acids. However, amino acid extrac-
tion by the rat mammary gland was restored to control
levels by a product of γ-GT, namely, γ-glutamyl-glut-
tamine. Similarly, 5-oxoproline was shown to reverse the
bromocriptine-induced decrease in amino acid uptake by
the rat mammary gland.

Kansal and Kansal (99) have cast doubt on a func-
tional relation between the activity of γ-GT and amino
acid uptake by the lactating mammary gland. They found
that inhibition of γ-GT had no effect on the uptake of
L-methionine and L-alanine by lactating mouse mammary
tissue explants.

Bovine mammary tissue γ-GT is able to hydrolyze
extracellular glutathione; the liberated amino acids were
subsequently transported into the intracellular compart-
ment (20). This observation coupled with the finding that
there is an arteriovenous glutathione concentration dif-
ference across the bovine mammary gland suggested that glutathione, derived from red blood cells, may be an important source of cysteine for milk protein synthesis (173). Similarly, it has been concluded that the goat mammary gland also utilizes erythrocyte-derived glutathione as a source of amino acids (6).

XIV. FATTY ACID UPTAKE

The amount of fat in milk, the majority of which is triacylglycerol, ranges from 0.2% in rhinos to >50% in pinnipeds (151). Lactating mammary tissue is able to synthesize fatty acids intracellularly from a supply of substrates extracted from plasma. In addition, fatty acids are extracted from lipids in the blood. The relative importance of intracellular synthesis compared with plasma-derived fatty acids depends on species, stage of lactation, and diet (16, 115, 141). The plasma sources of fatty acids include triacylglycerols, cholesterol esters, and nonesterified fatty acids; quantitatively, triacylglycerols carried in chylomicrons and very-low-density lipoproteins are the most important in fed animals. Nonesterified fatty acids are, however, important for mammary lipid uptake in the fasting state (148). Nonesterified fatty acids are produced from triacylglycerols in mammary tissue by the enzyme lipoprotein lipase which is situated in the capillary endothelium (and possibly the basolateral surface of the secretory cells). The mechanism of fatty acid uptake by mammary epithelial cells has not been identified. However, it has been suggested that fatty acid binding proteins, located in the plasma membrane, may play a role in fatty acid uptake (reviewed by Barber et al., Ref. 12). Expression of a protein termed FAT (fatty acid translocator) in fibroblasts induces the uptake/binding of long-chain fatty acids (91). FAT, which is predicted to have two substrates extracted from plasma. In addition, fatty acids are extracted from lipids in the blood. The relative importance of intracellular synthesis compared with plasma-derived fatty acids depends on species, stage of lactation, and diet (16, 115, 141). The plasma sources of fatty acids include triacylglycerols, cholesterol esters, and nonesterified fatty acids; quantitatively, triacylglycerols carried in chylomicrons and very-low-density lipoproteins are the most important in fed animals. Nonesterified fatty acids are, however, important for mammary lipid uptake in the fasting state (148). Nonesterified fatty acids are produced from triacylglycerols in mammary tissue by the enzyme lipoprotein lipase which is situated in the capillary endothelium (and possibly the basolateral surface of the secretory cells). The mechanism of fatty acid uptake by mammary epithelial cells has not been identified. However, it has been suggested that fatty acid binding proteins, located in the plasma membrane, may play a role in fatty acid uptake (reviewed by Barber et al., Ref. 12). Expression of a protein termed FAT (fatty acid translocator) in fibroblasts induces the uptake/binding of long-chain fatty acids (91). FAT, which is predicted to have two membrane-spanning domains, may be either a fatty acid transporter or a regulator of fatty acid transport (1). Interestingly, FAT has 85% homology with CD36, a protein expressed in the lactating mammary epithelium (77).

XV. COORDINATION AND CONTROL

It is clear from this review of the transport mechanisms operating across the basolateral membrane of the secretory cell that the degree of coordination between synthetic, secretory, and transport processes to produce milk of virtually constant but complex composition must be very high. Even for protein synthesis, a key question is how the numerous transport processes for amino acids act in concert to deliver the fixed-proportion mixture required.

While efforts will continue to identify which transporters of those available within the genome are expressed by the mammary gland and what systemic and local factors control that expression and activity after transcription, a major topic for future research must be how all the intracellular events are integrated, whether by quantitatively coordinated gene expression in response to hormonal or local signals or by cross-talk and feedback between the various pathways. In this respect, it is interesting to note that the majority of work on intracellular signaling within the mammary gland has been concerned with mammary development, because of the obvious relation to breast cancer, rather than with the control of secretion (250, 251).

It is time for investigations of the coordinated regulation to extend to those changes in the composition of the secretion that occur with physiological state, the formation of colostrum in late pregnancy, and the shift to milk production at around the time of parturition, for example. There are also several major changes in milk composition during lactation in marsupials, matching the requirements of the young at different stages in development (76, 84) that should receive increased attention.

Address for reprint requests and other correspondence: D. B. Shenman, Hannah Research Institute, Ayr, Scotland KA6 5HL (E-mail: shenmand@hri.sari.ac.uk and/or peakerm@hri.sari.ac.uk).

REFERENCES


61. FLINT DJ AND GARDNER M. Evidence that growth hormone stimulates milk synthesis by direct action on the mammary gland and that prolactin exerts effects on milk secretion by maintenance of mammary DNA content and tight junction structure. Endocrinology 135: 1119–1214, 1994.


171. PINES G, DANBOLT NC, BJORAS M, ZHANG Y, BENDAHAN A, EIDE L, KOEPSELL H, STORM-MATHISEN J, SEEBERG E, AND EIDE L. Functional charac-


