 0095–5108/87 0.00 + .20

Special Properties of Human Milk

Cutberto Garza, MD, PhD, Richard J. Schanler, MD,†*
Nancy F. Butte, PhD,‡ and Kathleen J. Motil, MD, PhD§

Current research findings support the conclusion that human milk constituents are not interchangeable with those of other nutrient sources. The intense clinical interest in breastfeeding has prompted a remarkable increase in investigations of human milk and a careful review of health policies that promote breastfeeding, support its practice, and foster investigations of lactation physiology.[76] Because of data generated by current investigations, clinical nutritionists recommend human milk as the ideal food for full-term infants. Much of their enthusiasm is based on the concept that human milk constituents play unique dual roles: the classically recognized role associated with most nutrients, that is, the provision of enzymatic cofactors or substrates for energy or structural components, and a complex functional role in which constituents complement the developing abilities of maturing infants.[26, 34]

Each of the major classes of organic nutrients is represented in human milk. Proteins provide amino acids for growth, but they occur as polypeptides that aid digestion,[61] host defense,[34] and other functions.[51] Fats provide energy, but some also have antiviral properties.[105] Carbohydrates provide energy but also may enhance mineral absorption,[103] modulate the growth of bacteria,[38] and prevent the attachment of selected bacteria to epithelial cells along the respiratory and gastrointestinal tracts, which are exposed to human milk at each nursing.[41] Recent findings suggest that human milk may actively stimulate the production of selected immune factors by the infant.[85] This

* Professor, Department of Pediatrics, Baylor College of Medicine, Houston, Texas
† Assistant Professor, Department of Pediatrics, Baylor College of Medicine; Staff, Texas Children's Hospital, Houston, Texas
‡ Research Assistant Professor, Department of Pediatrics, Baylor College of Medicine, Houston, Texas
§ Assistant Professor, Department of Pediatrics, Baylor College of Medicine, Houston, Texas

From the USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital, Houston, Texas

discussion will focus on these characteristics of human milk components.

PROTEIN: NUTRITIONAL CONSIDERATIONS

Protein Needs During Early Infancy

The National Research Council (NRC) has set protein allowances for infants 6 months of age or younger at 2.2 gm protein per kg body weight.[12] This estimate was based on "the amount of protein provided by the quantity of milk required to ensure a satisfactory rate of growth." Allowances were estimated to fall gradually from 2.4 g per kg per day during the first months of life to approximately 1.5 gm per kg per day by the sixth month. A 75 per cent adjustment is made in the allowance for older children to account for the decreased efficiency of protein utilization from a mixed diet. Observed mean intakes for infants 1 to 4 months of age are summarized in Table 1. The mean protein intake dropped from 1.6 gm per kg per day at month 1 to 0.9 gm per kg per day at month 4. The mean intake plus two standard deviations for months 1 and 4 were 2.2 and 1.3 gm per kg per day, respectively.

The values for the mean plus two standard deviations agree well with protein allowances recommended by the NRC, but the mean values are substantially below factorially derived estimates of protein requirements.[8] The factorial method to determine protein requirements sums up the amounts of nitrogen needed for maintenance and growth.[20] This sum should represent the mean minimal amount of protein required for normal growth and development. These estimates also are found in Table 1. The mean requirements derived by the factorial method are 17 per cent, 33 per cent, 29 per cent, and 30 per cent above the intakes of protein N measured for months 1, 2, 3, and 4 respectively. If we assume that both protein and nonprotein nitrogen in human milk are nutritionally available to the infant, mean intakes match factorially derived estimates of requirement well. The close

Table 1. *Protein and Total N Intakes Compared with Protein and Nitrogen Requirements Derived by the Factorial Method*

AGE (MO)	PROTEIN INTAKE (GM/KG/DAY)	TOTAL NITROGEN INTAKE (MG/KG/DAY)	NITROGEN REQUIREMENT (MG/KG/DAY)	PROTEIN REQUIREMENT (GM/KG/DAY)
1	1.6 ± 0.3	345 ± 48	310	1.97
2	1.1 ± 0.2	250 ± 31	270	1.68
3	1.0 ± 0.2	215 ± 22	220	1.40
4	0.9 ± 0.2	200 ± 23	200	1.27

Adapted from data from references 8 and 9.

agreement between observed mean intakes and estimates of requirement suggest, however, that only a small margin may exist to account for potential disparities between individual intakes and a normal person's protein requirement.[75, 95] Furthermore, no surfeit nitrogen is available to offset the effects of infections and other stresses that transiently increase nitrogen needs.[87]

Nonetheless, hypoproteinemia is seldom, if ever, encountered in exclusively breastfed infants whose mothers manage their lactation successfully. Although failure to thrive has been documented in breastfed infants, abnormal milk composition has rarely been implicated without evidence of concomitant mammary gland involution. Low milk volumes due to mismanagement usually have been the most likely cause of inadequate energy and protein intakes.[53] What then are the properties of human milk nitrogen that contribute to its apparent efficient utilization? The answer to this question will be based on the acceptance of two premises: (1) that protein requirements can be estimated by the factorial approach, and (2) that exclusive breastfeeding for the first 4 months of life provides adequate nutrition. We shall return to the second premise in a later section.

Protein: General Nutritional Aspects

The nutritional value of a protein food source may be assessed by examining the general composition of its nitrogen contents, amino acid pattern, and apparent digestibility. The protein content of mature human milk is approximately 0.8 per cent to 0.9 per cent.[9] Protein concentration, however, changes as lactation progresses.[9] Usually by the end of the second postpartum week the transition from colostrum to mature milk is complete and the protein concentration is approximately 1.3 g per cent.[9] This value falls to approximately 0.9 per cent by the end of the second month. Values continue to decrease after this time, although after the third month mean values do not fall much below approximately 0.8 per cent. The concentration of nonprotein nitrogen also decreases,[9] but to a lesser extent (approximately 15 per cent over the first 4 months of lactation) than that of protein. Protein concentrations also may be expressed per 100 kcal: human milk protein is approximately 1.5 gm per 100 kcal at the end of the first month and 1.3 gm per 100 kcal at the end of the fourth month.

Nonprotein Nitrogen

Nonprotein nitrogen (NPN) accounts for 18 per cent to 30 per cent of total milk N and consists mostly of urea, free amino acids, the amino sugars N-acetyl glucosamine and N-acetyl neuraminic acid, creatine, nucleic acids, the amino alcohols, choline and ethanolamine, and smaller amounts of several other substances.[11] Fifty-two per cent of the NPN originates from N-acetyl glucosamine, approximately 30 per cent from urea, and less than 10 per cent from N-acetyl neuraminic acid. All other sources individually contribute less than 5 per cent of the total NPN.[11]

Protein Composition

Human milk proteins are not homogeneous and may be grouped into two principal categories (casein and whey), which differ in solubility characteristics. There is significant heterogeneity within individual proteins in each category, which is primarily due to genetic polymorphism, post-translational phosphorylation and glycosylation, and in the case of the immunoglobulins, maternal antigenic exposure. The significance of the heterogeneity is difficult to assess but is likely to be more important from a functional than from a nutritional consideration.

The casein group of human milk proteins constitutes approximately 20 per cent to 40 per cent of the total protein and consists of at least two subtypes of alpha caseins and various forms of beta, gamma, and kappa caseins.[40, 48] The major component of the casein family in human milk is a protein similar in size, composition, and electrophoretic mobility to bovine beta-casein. The major difference between the two is that the human protein occurs in multiphosphorylated forms (0–5 phosphates per molecule). The bovine protein contains a specific number of phosphate groups per molecule.[35] The nutritional and functional significance of this difference is not understood. Debate continues whether human milk contains alpha or kappa caseins. The major whey proteins that constitute 60 per cent to 80 per cent of total human milk protein are alpha-lactalbumin, lactoferrin, and secretory IgA (sIgA).[40] The concentration of lactalbumin is 0.22 to 0.46 gm per dl.[3] Changes in the concentration of lactalbumin as lactation progresses have not been characterized as well as those of lactoferrin and sIgA. Lactoferrin concentrations fall from approximately 0.5 gm per dl at week 1 of lactation to 0.1 per cent at week 12,[32] and remain relatively stable for the next 2 years of lactation.[31] Secretory IgA concentrations follow a similar pattern,[32] that is a fall from approximately 0.2 gm per dl at week 1 to approximately 0.08 gm per dl at week 12. Despite these decreases in concentration, the intakes of the immunoproteins are substantial[10] whenever milk provides a significant proportion of the total diet. The immunoprotein concentrations generally rise or remain constant after the onset of gradual weaning.[33] The interindividual variability in their concentration is substantial, approximately 20 to 30 per cent or more.[32] A study conducted in the Gambia of the variation in the concentration of the protective factors in human milk identified parity as the major determinant of the variation.[73] Mothers of parity 1 and 2 produced the highest concentrations of immunoproteins.

Amino Acid Composition

The significance of the general amino acid pattern of human milk has been the topic of many investigations.[26] Generally, differences between the amino acid content of human milk- and cow milk-based formulas appear to be of greater significance to the premature infant than to the infant born at term. The nutritional significance of recent

reformulations of cow milk-based formulas with contents augmented with bovine whey proteins is difficult to evaluate because of persistent differences between the amino acid composition of human milk and that of the reformulations and the denatured character of the cow milk proteins.

To understand amino acid utilization by the exclusively breastfed infant, we have followed their postprandial amino acid responses at 1, 2, and 3 months of age.

Plasma amino acid levels were determined before nursing and 1 and 2 hours after the initiation of feeding. We expected to find that postprandial changes in plasma amino acid concentrations were related to the ratio of the index amino acid's dietary level to its uptake for the synthesis of proteins or energy. Decreases in postprandial plasma concentrations below preprandial levels would suggest an inadequate intake.[60] The postprandial plasma concentrations of several essential and nonessential amino acids fell below preprandial concentrations. The most marked reduction occurred in plasma glycine concentration, which suggests that glycine intake may have been marginal. Indeed, a preliminary comparison of the total amount of glycine ingested by exclusively breastfed infants over the first 3 months of postnatal life with the net amount of glycine accumulated for growth[23, 106] during this period indicated that approximately 40 per cent of the net glycine that accumulates must be synthesized *de novo*. All other amino acids were ingested in amounts that either equaled or exceeded the quantities that accumulate during these 3 months.

If the infant uses the nonprotein component of human milk to meet his or her need for nitrogen, there may be significant reliance on the hydrolysis of human milk urea by colonic bacteria.[79] Ammonia liberated by this hydrolysis could be used for glutamine synthesis. To identify the possible physiologic bases for the low intakes of glycine, it is of interest to note that of all negative feedback inhibitors of glutamine synthetase, the major enzyme for the utilization of NH_3, only two are not end products of glutamine metabolism: alanine and glycine.[54] The low levels of ingested glycine would promote the activity of glutamine synthetase. The introduction of liberated ammonia into glutamine would make a potential waste product readily available to amino acid metabolism. A waste product in maternal metabolism also would be converted into a component of lean tissue by the infant. This partial explanation for the efficient utilization of human milk nitrogen requires experimental verification.

Digestibility of Human Milk

The digestibility of specific human milk proteins has been assessed only indirectly. Apparent absorption of human milk nitrogen has been estimated in term and preterm infants fed unpasteurized and pasteurized human milk. Apparent digestibility in very low birthweight infants (VLBW) is between 80 and 90 per cent[84]; in full-term infants the values are also between 80 and 90 per cent.[89] Infants fed either cow's milk-based formula or human milk excreted similar

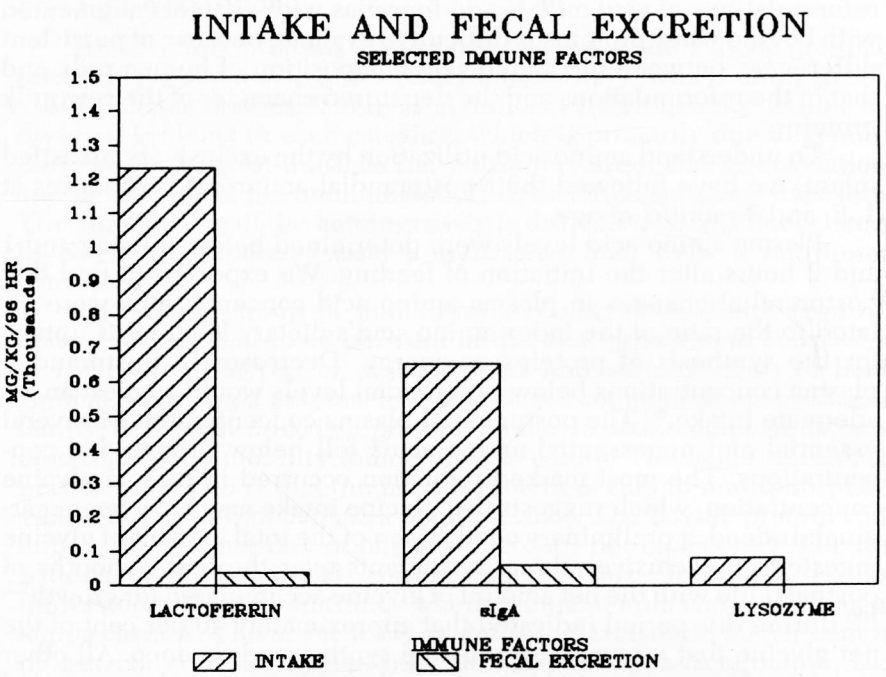

Figure 1. Intakes and fecal excretion (see key under figure) of lactoferrin, sIgA, and lysozyme are compared. Subjects were very low birthweight infants fed fortified human milk.

amounts of N in their feces despite unequal intakes of N. Human milk-fed infants consumed less total N than did those infants fed cow's milk.[89] The term "apparent," as it applies to digestibility and absorption, should be noted. Methods that have been used to assess the digestibility of human milk do not allow the differential quantitation of fecal N losses of endogenous and dietary origins. Therefore, true digestibility of milk protein is likely to be considerably higher than these values indicate.

These estimates are of particular interest because of recent suggestions that major whey proteins are largely unavailable to the infant.[39] In vitro assessments of the digestibility of lactoferrin[80] and sIgA[68] and preliminary assessments[66, 90] of the fecal output of these components in neonates have led to the suggestions. More recent studies of VLBW[85] and full-term infants[16] do not support these views. As summarized in Figure 1, fecal lactoferrin and sIgA accounted for less than 5 and 10 per cent, respectively, of the amounts of each of the proteins fed to VLBW infants.[85] These observations of apparent digestibility of specific whey proteins coupled with clinical studies assessing the general digestibility of human milk nitrogen indicate that more than 90 per cent of human milk nitrogen is probably absorbed in most infants.

PROTEINS: FUNCTIONAL CONSIDERATIONS

Casein

The most notable property of casein proteins is their ability to form stable aggregates, casein micelles, which include calcium and phosphorus. This property increases the Ca and P content of human milk beyond the amounts permitted by the solubility of either mineral. Most investigations of the functional properties of human milk proteins have focused on whey rather than casein proteins.

Whey Protien: sIgA

The whey proteins that comprise the immunologic system in human milk have been studied most often. In collaboration with Goldman and Goldblum of the University of Texas Medical Branch, Galveston, we have focused on sIgA, lactoferrin, and lysozyme. Secretory IgA is a dimer that includes the J chain found generally in polymeric immunoglobulins and the secretory piece that is specific to sIgA and IgM. The secretory piece may help to protect this form of IgA against proteolytic digestion.[42]

Specific sIgA against a wide array of enteric and respiratory bacterial and viral pathogens is found in human milk. Various investigators have demonstrated that the specificity of human milk sIgA depend on the mother's antigenic exposure.[22,29] The mechanism responsible for the appearance of these antibodies is understood only partially. Sensitized plasma cells are transported from the gastrointestinal,[29] and bronchotracheal-associated[22] lymphatic tissues to multiple mucosal surfaces, including breast alveoli during lactation. The participation of the maternal urinary tract in this general response also has been suggested.[43] During lactation, the "homing" of these cells to the breast appears to be activated by lactogenic hormones.[104] This mechanism provides similar specific immunities to most mucosal surfaces.

Secretory IgA has the ability to attach itself to mucosal epithelium and prevent the attachment, and possibly invasion, of specific infectious agents.[65] Animal models have demonstrated binding to the glycocalyx of epithelial cells in microvilli of the small intestine. Studies have shown that human milk sIgA may bind to an infant's buccal mucosa, providing a potential physical mechanism for sIgA to act as a protective agent in the infant's hypopharynx[17] (pars laryngeapharyngis); sIgA also has the ability to prevent the adhesion of *Escherichia coli* to epithelial cells of the urinary tract.[96] These findings are of potential importance if the infant can absorb milk sIgA.

Environmental Specificity of Human Milk

The ability of the mother to secrete antibodies directed against specific antigens that she and her infant encounter in the environment gives human milk an environmental specificity with significant protective potential. The dynamics and properties of this response, how-

ever, are not well understood. Evidence suggests that the sIgA response in human milk after maternal mucosal exposure is not uniform among antigens. In the rabbit model, for example, a response to respiratory syncytial virus has been well documented; comparable exposure to bovine serum albumin does not induce a similar response.[69] Whether requirements necessary for a positive sIgA response in milk reside exclusively on the antigen or in their immunologic processing is unknown. This processing includes the route of immunization and possibly undefined characteristics of cells that initially take up the antigen. For example, epithelial cells overlaying lymphoid follicles in the gastrointestinal tract appear to be specialized to sample the intestinal contents and possibly participate actively in the processing of potential antigens for eventual sensitization of the host.[5]

The complexities of this sIgA response also are evident from the work of Hanson and his collaborators.[13, 41, 97, 98] Oral immunization of lactating women with live poliovirus vaccine after natural exposure to the virus resulted in reduction rather than enhancement of specific sIgA antibodies in milk.[97] Simultaneous cholera vaccination further decreased the response to the polio vaccine.[41] Oral administration of a cow pea antigen to Guatemalan lactating women also resulted in a decreased titer in those with significantly high titers before the index administration.[13] In related experiments, subcutaneous immunization against cholera resulted in a significant rise in sIgA titer in 70 per cent of Pakistani women tested, but in none of the Swedish women used as a comparison group.[98] The Swedish group did not respond to a booster dose administered 14 days after their initial immunization. These results suggest a significant difference in the capacity of parenterally administered cholera vaccine to stimulate sIgA antibody formation in naturally sensitized and nonsensitized women.

Nonspecific Protective Factors

In contrast to the highly specific protective proteins in human milk, there are a number of nonspecific factors that may play protective roles in vivo (e.g., lactoferrin). Because lactoferrin binds free iron in human milk avidly, presumably lactoferrin also limits iron availability to potentially pathogenic enteric flora by competing with bacterial enterochelin for iron.[36] Lysozyme, another nonspecific protective factor, catalyzes the hydrolysis of beta-1,4-glycosidic bonds in bacterial cell walls. The pattern of lysozyme secretion differs from that of lactoferrin.[31, 32] As discussed previously, lactoferrin levels decrease rapidly in early lactation and remain fairly stable after the first 4 months. Lysozyme levels also decrease initially to approximately 25 μg per ml at 2 to 4 months but rise steadily thereafter to 250 μg per ml at 6 months and remain close to 200 μg per ml for as long as 2 years of lactation.

Several nonspecific protective proteins act synergistically with each other and with sIgA to enhance specific functional effects. The anti-*E. coli* effects of lactoferrin and sIgA acting in concert are greater than those of either component acting alone.[92] These two components also act synergistically to enhance the antibacterial effects of perox-

idase.[63] Lactoferrin appears to modulate inflammation by influencing macrophage antibody responses.[19] Secretory IgA also interacts with cellular elements of the immune system. It appears to enhance specifically the natural antibacterial activity of gut-associated lymphocytes and induces antibacterial activity in splenic leukocytes in animal models; no comparable effects are noted on cells from the thymus or popliteal nodes.[99] The function of these and other protective factors in human milk in the development of normal gut permeability is not well understood, although a portion of the nonspecific protective properties may be mediated by an enhancement of the gut's role as a barrier between the infant and the environment.[100]

Potentially, an even more dramatic demonstration of the multiple functions of human milk proteins may be that of bile salt-stimulated lipase. Lipase activity in human milk complements the normally low level of pancreatic lipase activity of infants. Bile salt-stimulated lipase activity is stable at a *p*H of 3.5 at 37°C for 1 hour. This stability should provide significant activity at the level of the small intestine to digest the amounts of fat normally consumed. Although lipase is inactivated by trypsin and chymotrypsin at a *p*H of 6.5, inactivation is prevented by bile salts. The physiologic significance of this enzyme to fat digestion has been reviewed.[4] More recently, investigators have reported that human milk has a cholate-dependent ability to kill a common intestinal parasite (*Giardia lamblia*) known to cause diarrhea and to be associated with failure to thrive in young infants.[27] Bile salt-stimulated lipase has been proposed as the active agent. Field studies, however, assessing the antigiardial activity of human milk have been unable to confirm whether the lower giardial infection rates observed in breastfed infants are due to the protective properties of human milk or to a decreased rate of exposure to the parasite.[47] The possibility of a similar activity against other intestinal parasites has been investigated only partially. If this or other antiparasitic activities are confirmed by future work, a molecule will have been identified that complements the digestive abilities of the infant and protects him or her from common parasites associated with significant morbidity.

Active or Passive Protection

Two general mechanisms have been proposed to explain the manner in which specific components in human milk may protect the infant from infection. One is the interaction between specific constituents in milk with epithelial surfaces or with specific substances in the gastrointestinal lumen during the digestion and absorption of human milk. Evidence for this mechanism is strong and rests on experiments that support the survival of the protective factors in human milk in the infant's gastrointestinal tract.[66, 68, 80, 90] These experiments are basically of two types: in vitro demonstrations of the resistance of these proteins to proteolytic digestion[68, 80] and the demonstration of higher levels of these proteins in the feces of infants fed human milk.[66, 90] The other mechanism is the possible modulation of the infant's immune system by protective factors in the milk, which results in

selective production of immune factors by the infant.[4, 30, 72, 74, 86, 91] No conclusive data, however, have been published to demonstrate that either of the mechanisms explains the putative protective effects of human milk or that the mechanisms are mutually exclusive.

Our laboratory has conducted a series of experiments to evaluate these possibilities.[85] The amounts of lactoferrin, sIgA, lysozyme, and specific antibodies to a pool of *E. coli* O somatic antigens were measured in four consecutive 24-hour collections of urine and one concomitant 96-hour collection of feces from infants fed fortified human milk or cow-milk formula designed specifically for VLBW infants. The protocol, which has been described,[84] called for the addition of human milk protein and cream to milk produced by mothers of VLBW infants. The feces obtained from infants fed human milk had significantly greater amounts of lactoferrin, lysozyme, and sIgA than those of infants fed the cow milk-based formula. Specific sIgA antibodies to *E. coli* were detected in the feces of 90 per cent of the human milk-fed infants, but in none of the infants fed the cow milk-based formula. A significant correlation was found between the fecal and human milk concentrations of specific sIgA antibodies to *E. coli* O somatic antigens.

Had the analyses of these data been limited to those described in the preceding paragraph, one might have concluded that the expected survival of protective factors after passage through the infant's gastrointestinal tract had been noted once again. We found, however, no other significant relationships among the milk's concentrations of any other of the selected immune factors and their fecal excretion or between the infant's intakes and excretion. Significant relationships were nonetheless, noted among the immune factors in the feces. The increased quantity of selected immune factors in the feces of VLBW infants fed human milk may have resulted, therefore, from the passive ingestion and persistence of these factors throughout the gastrointestinal tract or from endogenous synthesis induced by unidentified factors in the milk.

Several reports[4, 30, 72, 74, 86, 91] support the hypothesis that human milk may induce the production of selected immune factors by the infant. Preliminary analyses have detected significantly greater amounts of sIgA and lactoferrin in the urine of the VLBW infants fed fortified human milk than in those fed the cow milk-based formula.[30] The urinary concentration and output of these materials were 100- to 200-fold greater in the group fed human milk. Although both complete proteins and fragments of each of the proteins are present in the urine, the fragments appear larger than peptides commonly found in urine. Unconfirmed reports also suggest that human milk-fed infants may passively acquire T cell responsiveness to specific antigens through an unidentified mechanism.[86]

Plasma concentrations of immune factors, however, do not fully corroborate the concept that human milk stimulates the production of immune factors. Previous investigations reporting the absorption of small amounts of sIgA from milk have been limited to the first few days of life.[101] Investigators comparing the concentrations of serum

antibodies to commensal *E. coli* O lipopolysaccharide antigens in later stages of infancy report higher levels in bottlefed infants.[93] It is not clear, however, if these higher levels are the result of greater antigen exposure, differences in gut permeability,[100] or differences in the maturation of the capacity to respond immunologically. Investigators have found higher levels of salivary sIgA in very young infants fed human milk than in those fed a synthetic formula,[91] whereas others have failed to confirm this observation.[37] Although the ontogeny of secretory immunity has been a topic of investigation for several years,[7] the mechanisms underlying the disparate responses between feeding groups have not been identified.

In vitro studies have detected an increase in the synthesis of IgA by cord blood lymphocytes when milk from early lactation is added to the culture media.[71] In more recent studies, this unidentified factor was credited with the selective stimulation of IgA synthesis by human tonsilar lymphocytes.[74]

The potential role of human milk as an immunization vehicle is also of interest. The functional significance of cytomegalovirus (CMV) excretion in milk has been of particular concern.[107] In a study that describes the nature of the debate, CMV was detected in 39 per cent of the milk, vaginal secretions, urine, or saliva of a lactating population. Sixty-nine per cent of the infants of these women became infected and infected infants shed CMV chronically, but none demonstrated chronic clinical illness. Two premature infants developed a transient pneumonitis. Does this type of viral transmission coupled with maternal immunologic protection provided transplacentally and via the breast result in long- and short-term protection of the infant? The risk is not clear. The case of the seronegative premature infant consuming CMV-infected human milk from a donor other than the infant's mother is of most concern.

FAT: NUTRITIONAL AND FUNCTIONAL PROPERTIES

Fat provides 50 per cent of the energy in human milk; the total fat content of human milk is approximately 3.5 per cent, which remains fairly stable throughout the first few months of established lactation.[9] Human milk fat produced by women who consume at least 30 to 40 per cent of dietary energy as fat is derived from blood triglycerides. The mother's diet determines the composition of the fat in her milk,[46] (her milk fat composition may reflect a high-fat, energy-adequate diet or if her diet fails to meet her energy needs, the composition will reflect that of her adipose tissue stores. If a high-carbohydrate, energy-replete diet is consumed, the proportion of triglycerides of medium-chain length fatty acids is increased and fatty acids of 18-carbon chain length are decreased almost proportionally. Experiments are underway in our laboratory to determine if the fatty acid composition of milk can be altered by manipulating the mother's diet to improve the digestibility of human milk fat by VLBW infants.[88] Unlike the coeffi-

cients of fat absorption of approximately 95 per cent in full-term infants,[89] those of VLBW infants commonly are 80 per cent or less.[84]

The triglycerides in human milk have a unique positional distribution of fatty acids compared with the equal distribution of the fatty acids in dietary and depot fat triglycerides.[6] Fully saturated triglycerides usually contain stearate in position 1, palmitate in position 2, and fatty acids of 14- and 18-carbon chain length in position 3. Triglycerides that contain monoenoic fats also have palmitate in position 2. This fatty acid distribution complements pancreatic lipase's preferential hydrolysis of fatty acids in positions 1 and 3.[61] The 2-monoglyceride of palmitic acid is better absorbed than is free palmitic acid.[59] Evaluation of the physiologic significance of triglyceride positional specificity and the action of pancreatic lipase in human milk is difficult because of the absence of any positional preference of bile salt-stimulated lipase to hydrolyze fats in the 1 or 2 position.[49] Nonetheless, human milk fat appears to be highly digestible, possibly as the result of many factors, that include the combination of fatty acid composition, triglyceride positional specificity, and complementary enzymatic activities that originate in the milk and in the infant's gastrointestinal tract.

The lipid fraction of human milk also provides essential fatty acids for structural (cellular membranes) and other functional (prostaglandins) purposes and fat-soluble vitamins. Additionally, milk lipids appear to contribute to the infant's immunologic protection.[105] Although studies have reported close correlations between lipase activity in milk and lipid-mediated antiviral activity, the research has demonstrated that milk lipase activity is not directly responsible; the free fatty acids and monoglycerides generated by milk lipases account for activity directed against viruses with envelopes. Palmitoleic, oleic, and lauric acids were found to be the most effective and also have been reported to have antibacterial and antifungal activities. The fat globule membrane in the sow is able to bind *E. coli*, which suggests yet another potential protective property of the lipid fraction.[2]

The influence of fat and cholesterol intake in early infancy on lipid metabolism in later life remains unresolved.[44] Evidence that the consumption of human milk either protects the infant or places him or her at greater risk to disorders of lipid metabolism in adulthood is inconclusive. The complexity of the problem has been reviewed recently.[45]

CARBOHYDRATE: NUTRITIONAL AND FUNCTIONAL PROPERTIES

Lactose is the principal carbohydrate in human milk and provides approximately 50 per cent of the energy content. The utilization of lactose as an energy substrate depends on its hydrolysis by lactase, beta-1,4 galactosidase, a disaccharidase maximally active during infancy. Lactose is found almost exclusively in milk. The physiologic

advantage of lactose as a primary energy source in milk has not been identified. The increased susceptibility of lactase (relative to the other intestinal disaccharidases) during infections and other stresses is particularly puzzling. Transient lactose intolerance is a common complication of viral and bacterial gastroenteritis. The common need to replace lactose with sucrose in the diet of bottlefed infants is ample evidence of the increased vulnerability imposed by lactase.[56] Lactose intolerance under similar clinical conditions does not appear to be as common in human milk-fed infants.[50] Evidence that lactose promotes the absorption of calcium has been presented,[103] but it is not fully accepted.[83]

Lactose is proposed often as a determinant of the infant's gastrointestinal flora. Because lactose is digested slowly, significant amounts may reach the large intestine where they possible modulate the resident flora. Recent work evaluating breath H_2, an accepted marker of carbohydrate fermentation in the colon, demonstrates that significant amounts of lactose reach the large intestine of breastfed children.[55]

Human milk also contains trace amounts of glucose and small but significant amounts of oligosaccharides and glycoproteins. These components of the carbohydrate fraction are thought to participate in the modulation of colonic flora by promoting the growth of the *Lactobacillus bifidus* and thus limit the growth of other bacteria. The resulting commensal flora may act in a protective manner by occupying the limited number of potential binding sites, thus the sites become unavailable to potential pathogens.[67] Of more recent interest, however, is the presence of oligosaccharides, which are structurally similar to the oligosaccharides found on the surface of retropharyngeal epithelial cells. These human milk oligosaccharides may bind potential pathogens by mimicking receptors normally found on epithelial cells.[82]

The significance of these factors to bacterial colonization of the colon appears clear. The colonization pattern of breastfed and bottlefed infants is similar at day 7, although by day 30, stable and distinct patterns may be identified in each group.[78] By this age, bifidobacteria are the predominant colonic flora of breastfed infants and outnumber enterobacteria by greater than two magnitudes. Enterococci are reduced and Clostridia and Bacteroides are rarely isolated. In the bottlefed infant, the flora observed at day 7 have changed little by day 30; at day 7 the flora are dominated by enterococci and bifidobacteria with enterobacteria a close second. Clostridia is also common, but Bacteroides are rarely detected. At day 30, bifidobacteria are dominant but outnumber enterococci by only a small margin; Clostridia become even more prevalent, but Bacteroides remain rare.

Dietary carbohydrates are unlikely to be the sole determinants of these flora. Two theories have been proposed to explain the colonization patterns[78]: one maintains that the distinct floras are a consequence of physiochemical properties of human milk. Proponents suggest that although amounts of carbohydrate that reach and are

fermented in the colon are similar in breastfed and bottlefed infants, the lower buffering capacity of human milk (a consequence of its lower mineral and protein contents) results in a more acidic environment, which favors the bifidobacteria.

The second theory underscores the presence of lactoferrin, lysozyme, sIgA, and the other components of human milk with functional properties. Interactions of these components with the floras acquired by the infant from his or her environment and the mother during birth determine the final flora. It is likely that the determinants are both the physiochemical properties of milk and the unique array of functional components.

IN VIVO SIGNIFICANCE OF UNIQUE NUTRITIONAL AND FUNCTIONAL PROPERTIES OF HUMAN MILK

Energy intake and utilization have been key factors in understanding the significance of feeding human milk. Energy utilization is the common denominator that underlies all metabolic processes and is a key indicator of functional well-being. Until recently, little information was available regarding normal volumes and composition of milk produced under optimal nutritional and environmental conditions. The lack of data has prevented the resolution of apparent discrepancies between the projected volumes of milk required to meet an infant's energy and protein requirements and the lower volumes of milk consumed by apparently healthy infants studied in field evaluations. Both the NRC[12] and the World Health Organization[20] have estimated that the average healthy 3-month-old infant needed approximately 850 gm of milk per day and that the 5- to 6-month old infant needed over 1100 gm of milk per day to meet energy requirements. Commonly observed rates of milk production, however, ranged from 600 to 800 gm per day.[9, 18, 70, 102] Despite this discrepancy, human milk has supported adequate infant growth throughout the first 4 to 6 months of life, and possibly longer.[1] Resolution of this discrepancy has been difficult because many longitudinal and cross-sectional studies have not addressed disparities in breastfeeding skills among subjects and the possible inclusion of a disproportionate number of high-volume milk producers. Recent studies have begun to document more carefully the subjects' lactation management skills and the causes for subject attrition from longitudinal studies.

In our longitudinal studies of 45 infants, the attrition rate was less than 10 per cent; subjects who left the study did so for reasons unrelated to their breastfeeding.[9] A support staff trained to help mothers manage lactation successfully was available to the subjects throughout the study. Over the 4 months of investigation, milk intake of the infants plateaued at 733 gm per day. Nutrient intakes were based on the analysis of individual milk samples collected through a 24-hour period. On a body weight basis, energy intakes fell from 110 kcal per gm at month 1 to 71 kcal per kg at month 4. By the fourth month, therefore,

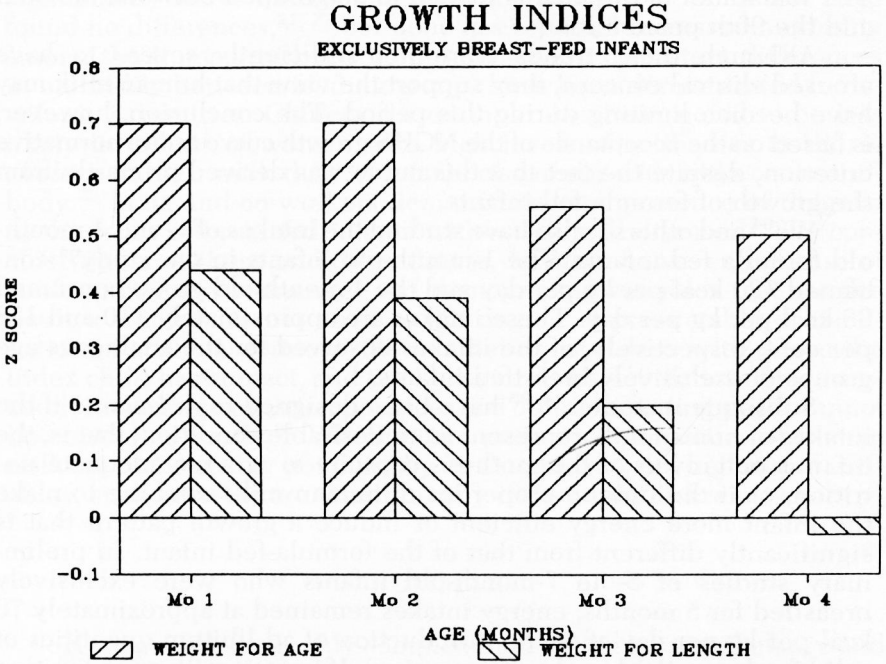

Figure 2. Weight for length and weight expressed as Z scores of exclusively breastfed 1- to 4-month-old infants.

these infants were consuming less than 65 per cent of the energy recommended for their age by the NRC.

Assessing the functional consequences of these apparently low intakes is difficult. Growth, as the criterion for adequate intake, appears to be an obvious choice. Weight-for-age, length-for-age, and weight-for-length percentiles of the study subjects were above 50[9] and remained above this level for the 4 months of observation. There was, however, a statistically significant negative trend for the weight-age-percentile per month. Length for age was stable from month 1 to month 4. Weight for length declined at a rate of 3.9 percentiles per month. The weight-for-length Z scores are illustrated for months 1 through 4 in Figure 2.[25] This growth index is particularly informative because it accounts for both the expected mean weight for length and the distribution of this growth parameter at the ages specified. A Z score of 1 indicates that the group was one standard deviation above the mean and a Z score of -1, one standard deviation below the mean. A Z score of 0 is coincident with the mean. In terms of individual children, 7 per cent of the group fell below the 10th weight-for-age percentile at birth; thereafter none of the infants were below the 10th percentile ranking. On the upper end, 19 per cent of the infants were above the 90th percentile at birth, and 14 per cent were at this level at 4 months.

The remainder of the infants tended to be grouped between the 50th and the 90th percentiles.

Although these trends were not sufficiently severe to have aroused clinical concern, they support the view that human milk may have become limiting during this period. The conclusion, however, is based on the acceptance of the NCHS growth curve as the normative criterion, despite the fact that this curve was derived primarily from the growth of formula fed infants.

We[64] and others[24] also have studied the intakes of 1- and 4-month-old formula fed infants. The 1-month-old infants in our study[64] consumed 120 kcal per kg per day and the 4-month-old group consumed 98 kcal per kg per day. These intakes are approximately 110 and 138 per cent, respectively, of the intakes observed for the analogous age groups of exclusively breastfed infants.

Subsequent studies[94, 95] have been designed to determine if the intakes of human milk represent an undesirable condition, that is, the infant's outgrowth of the mother's capacity to provide adequate nutrition, or if the unique properties of human milk combine to make the infant more energy efficient or induce a growth pattern that is significantly different from that of the formula-fed infant. In preliminary studies of 5- to 7-month-old infants who were exclusively breastfed for 5 months, energy intakes remained at approximately 70 kcal per kg per day after the introduction of ad libitum quantities of solid foods (available only after nursing). If human milk were limiting by the fourth month, an increase in energy consumption and possibly an acceleration in the growth rate would be likely after the ad libitum introduction of solid food. Neither was observed in these preliminary studies. The intake of exclusively breastfed infants at month 5 was 66 ± 7 kcal per kg. Energy intakes from human milk and solid foods at months 6 and 7 (solid foods were first offered at month 6) were 73 ± 11 kcal per kg per day and 71 ± 13, respectively. Weight-for-age percentiles also decreased slightly in this study, but the identification of normative growth criteria represents a problem for reasons previously discussed.

Do formula-fed infants require "more" food to attain approximately the same endpoint as their human milk-fed counterparts or do differences in intake between formula- and breastfed infants represent a more or less active "gatekeeping" role by mothers of formula-fed infants? These different intakes may represent physiologically sound responses to different nutrient sources. One mechanism that may contribute to a more efficient utilization of energy and protein is a significant reduction in clinical and subclinical infections. The nutritional cost of such stresses has been documented in developing countries[58] and controlled laboratory studies of adults. Nonetheless, the potential role of protective factors in reducing infections in infants has been extrapolated to a significant degree from in vitro studies. A definitive demonstration of protective functions in free-living populations is much more complex. Most studies comparing morbidity among children fed human milk or synthetic formula have reported

significantly fewer illnesses in breastfed infants.[14, 15, 52] A few have found no differences,[21, 77] but none has reported increased morbidity among the human milk-fed groups. The most consistent differences in morbidity between feeding groups have been demonstrated in developing countries. Cholera, for example, has been noted to be less likely in infants of women with high levels of antibodies to *Vibrio cholera* in their milk than in infants of women with little or no antibody.[28] Mata and co-workers demonstrated the presence of Shigella in the feces of breastfed infants without clinical disease.[57] The conclusiveness of these and other data is debated, however, because of confounding environmental and demographic variables that are difficult to control. Issues such as the degree of preventable contamination of artificial formula, the number of caretakers with whom the index child has contact, and the behavioral characteristics of the caretaker, including sanitation practices and other mothering skills, cannot be controlled easily.[81] Whether the protective properties of human milk are made real or potential by environmental and behavioral variables, such benefits are possible only if the infant is breastfed.

SUMMARY

Human milk is a highly complex fluid with a nutrient balance and an array of functional properties that may promote a level of metabolic efficiency that is not attainable when a cow milk-based formula is fed. This is not a novel idea. Mitchell in 1933[62] proposed that the level of efficiency of energy use is determined by the nutrient "balance" in the diet. Nonetheless, difficulties remain in the attempt to reconcile the low levels of intake with established estimates of energy needs.[8] If the amount of energy that appears necessary for growth and maintenance of a 4-month-old infant is compared with that from his or her intake of an exclusive human milk diet, the infant should have little or no energy left for activity. Do metabolic economies contribute to more efficient uses of energy for growth and maintenance in breastfed infants? Are there differences in body composition? Does more efficient use of energy occur as a result of a decrease in clinical and subclinical infections? Is efficient energy utilization accomplished by significant curtailment in activity? If an excess level of energy is consumed by bottlefed infants, what are the positive or negative short-term or long-term consequences? These questions are the focus of research in numerous laboratories. Field and clinical studies of breastfed infants and in vitro studies of human milk offer unique opportunities to understand basic mechanisms of human adaptations to nutrient intake and environmental challenges.

ACKNOWLEDGMENTS

This work is a publication of the USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine and Texas Children's Hospital,

Houston, Texas. This project has been funded in part with federal funds from the U.S. Department of Agriculture, Agricultural Research Service under Cooperative Agreement number 58-7MNI-6-100. The contents of this publication do not necessarily reflect the views or policies of the U.S. Department of Agriculture, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government. We thank E. Roseland Klein for editorial assistance.

REFERENCES

1. American Academy of Pediatrics and American College of Obstetricians and Gynecologists. Guidelines for Perinatal Care. Washington, DC, AAP and ACOG. 172, 1983
2. Atroshi F, Alaviuhkola T, Schildt R et al: Fat globule membrane of sow milk as a target for adhesion of K-88-positive *Escherichia coli*. Comp Immun Microbiol Infect Dis 6:235–245, 1983
3. Bezkorovainy A: Human milk and colostrum proteins: A review. J Dairy Sci 60:1023–1037, 1977
4. Blau H, Passwell JH, Levanon M et al: Studies on human milk macrophages: Effect of activation of phygocytosis, and secretion of prostaglandin E2 and lysozyme. Pediatr Res 17:241–245, 1983
5. Bockman DE, Cooper MD: Pinocytosis by epithelium associated with lymphoid follicles in the bursa of Fabricius, appendix, and Peyer's patches. An electron microscopic study. Am J Anat 136:455–478, 1973
6. Breckenbridge WC, Marai L, Kuksis A: Triglyceride structure of human milk fat. Can J Biochem 47:761–769, 1969
7. Burgio GR, Lanzauecchia A, Plebani A et al: Ontogeny of secretory immunity: Levels of secretory IgA and natural antibodies in saliva. Pediatr Res 14:1111–1114, 1980
8. Butte NF, Garza C: Energy and protein intakes of exclusively breastfed infants during the first four months of life. In Gracey M, Falkner F (eds.): Nutritional Needs and Assessment of Normal Growth. New York, Raven Press, 1985, pp. 63–83
9. Butte NF, Garza C, Smith EO et al: Human milk intake and growth performance of exclusively breast-fed infants. J Pediatr 104:187–195, 1984
10. Butte NF, Goldblum RM, Fehl LM et al: Daily ingestion of immunologic components in human milk during the first four months of life. Acta Paediatr Scand 73:296–301, 1984
11. Carolson SE: Human milk nonprotein nitrogen: Occurrence and possible functions. Adv Pediatr 32:43–70, 1985
12. Committee on Dietary Allowances: Washington, DC, National Academy of Sciences, 1980, pp 46–47
13. Cruz JR, Hanson LA: Specific immune response in human milk to oral immunization with food proteins. Ann NY Acad Sci 409:808–809, 1983
14. Cunningham AS: Morbidity in breastfed and artificially fed infants II. J Pediatr 95:685–689, 1979
15. Dagan R, Pridan H: Relationship of breastfeeding versus bottlefeeding with emergency room visit and hospitalization for infectious disease. Eur J Pediatr 139:192–194, 1982
16. Davidson LA, Lonnerdal B: Effect of development on the fecal excretion of lactoferrin and secretory IgA in breast-fed infants. Am J Clin Nutr 43:699, 1986
17. Davis CP, Houston CW, Fader RC et al: Immunoglobulin A and secretory immunoglobulin A antibodies to purified type 1 *Klebsiella pneumoniae* pili in human colostrum. Infect Immunol 38:496–501, 1982
18. Dewey KG, Lonnerdal B: Milk and nutrient intake of breast-fed infants from 1 to 6 months: relation to growth and fatness. J Pediatr Gastroenterol Nutr 2:479–506, 1983
19. Duncan RL Jr, McArthur WP: Lactoferrin-mediated modulation of mononuclear cell activities. Cell Immunol 63:308–320, 1981

20. FAO/WHO: Energy and Protein requirements. WHO technical report series No. 522, Geneva, 1973
21. Fergusson DM, Hormwood LJ et al: Infant health and breastfeeding during the first 16 weeks of life. Aust Pediatr J 14:254–258, 1978
22. Fishuat M, Murphy D, Neifert M et al: Bronchomammary axis in the immune response to respiratory syncytial virus. J Pediatr 99:186–191, 1981
23. Fomon SJ: Body composition of the male reference infant during the first year of life. Pediatrics 40:863–870, 1967
24. Fomon SJ, Thomas LN, Filer LJ et al: Food consumption and growth of normal infants fed milk-based formulas. Acta Paediatr Scand (Suppl) 223:1–36, 1971
25. Garza C, Stuff J, Butte NF et al: Complementation and weaning phases of lactation. In Hamosh, M, goldman AS (ed): Maternal Factors in Lactation. New York, Plenum Press, 1986, pp 155–164
26. Gaull GE, Jensen RG, Rassin DK et al: Human milk as food. In Milunsky A, Friedman EA, Gluck L (eds): Advances in Perinatal Medicine, Vol 2. New York, Plenum Publishing, 1982 pp 47–120
27. Gillin FD, Reiner DS, Gault MJ: Cholate-dependent killing of *Giardia lamblia* by human milk. Infect Immunol 47:619–622, 1985
28. Glass RI, Svennerholm AM, Stoll BJ et al: Protection against cholera in breast-fed children by antibodies in breast milk. N Engl J Med 308:1389–1392, 1983
29. Goldblum RM, Ahlstedt S, Carlsson B et al: Antibody-forming cells in human colostrum after oral immunization. Nature (London) 257:797–799, 1975
30. Goldblum RM, Schanler RS, Garza C et al: Enhance urinary lactoferrin excretion in premature infants fed human milk. Pediatr Res 19:342A, 1985
31. Goldman AS, Garza C, Goldblum RM: Immunologic components in human milk during the second year of lactation. Acta Pediatr Scand 72:461–462, 1983
32. Goldman AS, Garza C, Nichols BL et al: Immunologic factors in human milk during the first year of lactation. J Pediatr 100:563–567, 1982
33. Goldman AS, Garza C, Nichols BL et al: Immunologic components in human milk during gradual weaning. Acta Paediatr Scand 72:133–134, 1983
34. Goldman AS, Smith CW: Host resistance factors in human milk. J Pediatr 82:1082–1090, 1973
35. Greenberg R, Groves ML: Human beta casein. J Dairy Res 46:235–239, 1979
36. Griffiths E and Humphreys J: Bacteriostatic effect of human milk and bovine colostrum on *Escherichia coli*: Importance of bicarbonate. Infect Immunol 15:396–401, 1977
37. Gross SJ, Buckley RH: IgA in saliva of breast-fed and bottle-fed infants. Lancet 2:543, 1980
38. Gyorgy P: The uniqueness of human milk: Biochemical aspects. Am J Clin Nutr 24:970–995, 1971
39. Hambraeus L, Fransson GB, Lonnerdal B: Nutritional availability of breast milk protein. Lancet 2:167–168, 1984
40. Hambreaus L, Lonnerdal B, Forsum E et al: Nitrogen and protein components of human milk. Acta Pediatr Scand 67:561–567, 1978
41. Hanson LA, Alhstedt S, Andersson G et al: Mucosal immunity. Ann NY Acad Sci 409:1–21, 1983
42. Hanson LA, Alhstedt S, Carlsson B et al: New knowledge in human milk immunoglobulin. Acta Pediatr Scand 67:577–582, 1978
43. Hanson LA, Ahlstedt S, Carlsson B et al: Secretory IgA antibodies to enterobacterial virulence antigens: their induction and possible relevance. Adv Exp Med Biol 107:165–176, 1978
44. Holman RL: Atherosclerosis—a pediatric problem. Am J Clin Nutr 9:565–569, 1961
45. Innis SM: The role of diet during development of the regulation of adult cholesterol homeostasis. Can J Physiol Pharmacol 63:557–564, 1985
46. Insull W, Hirsch J, James T et al: The fatty acids of human milk. II. Alterations produced by manipulation of caloric balance and exchange of dietary fats. J Clin Invest 165:118–122, 1959
47. Islam A, Stoll BJ, Ljungstrom I et al: *Giardia lamblia* infections in cohort of Bangladeshi mothers and infants followed for one year. J Pediatr 103:996–1000, 1983

48. Jenness R: Comparative aspects of milk proteins. J Dairy Res 46:197–210, 1979
49. Jensen RG, Clark RM, deJong FA et al: The lipolytic triad: Human lingual, breast milk and pancreatic lipase: Physiologic implications of their characteristics in digestion of dietary fats. J Pediatr Gastroenterol Nutr 1:243–255, 1982
50. Khin-Maung-U, Nyunt-Nyunt-Wai, Myo-Khin et al: Effect on clinical outcome of breastfeeding during acute diarrhea. Br Med J 290:587–589, 1985
51. Klagsbrun M: Human milk stimulates DNA synthesis and cellular proliferation in cultured fibroblasts. Proc Nat Acad Sci 75:5057–5061, 1978
52. Larsen SA, Homer DR: Relation of breast versus bottlefeeding to hospitalization for gastroenteritis in a middle class US population. J Pediatr 92:417–418, 1978
53. Lawrence RA: Breastfeeding: A Guide for the Medical Professional. St. Louis, CV Mosby, 1980
54. Lehninger AL: Biochemistry: The Molecular Basis of Cell Structure and Function. New York, Worth Publishers 1970, pp 556–557
55. Lifschitz CH, Bautista A, Gopalakrishna GS et al: Absorption and tolerance of lactose in infants recovering from severe diarrhea. J Pediatr Gastroenterol Nutr 4:942–948, 1985
56. Lifshitz F: Perspectives of carbohydrate intolerance in infants with diarrhea. Lifshitz F (ed): In Carbohydrate Intolerance in Infancy. New York, Marcel Dekker, 1982, pp 3–19
57. Mata LJ, Urrutia JJ, Garcia B et al: Shigella infection in breast-fed Guatemalan Indian neonates. Am J Dis Child 117:142–146, 1969
58. Mata LJ, Urrutia JJ, Gordon JE: Diarrheal disease in a cohort in Guatemalan children observed from birth to age two years. Trop Georg Med 19:247–257, 1967
59. Mattson FH, Volpenheim RA: Rearrangement of glyceride fatty acids during digestion and absorption. J Biol Chem 237:53–55, 1962
60. McLaughlin JM, Campbell JA: Methodology of protein evaluation. In Munro H (ed): Mammalian Protein Metabolism. New York, Academic Press 1969, pp 391–422
61. Mehta NR, Jones JB, Hamosh M: Lipases in preterm human milk: Ontogeny and physiologic significance. J Pediatr Gastroenterol Nutr 1:317–326, 1982
62. Mitchell HH: Balanced diets, net energy values, and specific dynamic effects. Science 80:558–561, 1933
63. Moldoveanu Z, Tenovuo J, Mestecky J et al: Human milk peroxidase is derived from milk leukocytes. Biochem Biophys Acta. 718:103–108, 1982
64. Montandon CM, Wills CA, Garza A et al: Formula intake of one- and four-month-old infants. J Pediatr Gastroenterol Nutr 5:434–438, 1986
65. Nagura H, Nakane PK, Brown WR: Breast milk IgA binds to jejunal epithelium in suckling rats. J Immunol 120:1333–1339, 1978
66. Ogra SS, Weintraub D, Ogra PL: Immunologic aspects of human colostrum and milk. III. Fate and absorption of cellular and soluble components in the gastrointestinal tract of the newborn. J Immunol 119:245–248, 1977
67. Op den Camp JHM, Oosterhof A et al: Interaction of bifidobacterial lipoteichoic acid with human intestinal epithelial cells. Infect Immunol 47:332–334, 1985
68. Parkin DM, McClelland DBL, Samson RR et al: The effect of acid pepsin and trypsin on human colostral IgA agglutinins. Eur J Clin Invest 3:66–71, 1973
69. Peri BA, Theodore CM, Leonsky GA et al: Antibody content of rabbit milk and serum following inhalation or ingestion of respiratory syncytial virus and bovine serum albumin. Clin Exp Immunol 48:91–101, 1982
70. Piciciano MF, Calkins EJ, Garrick JR et al: Milk and mineral intake of breast-fed infants. Acta Paediatr Scand 70:189–194, 1981
71. Pittard W, Bill K: Differentiation of cord blood lymphocytes into IgA producing cells in response to a breast milk stimulatory factor. Clin Immunol Immunopathol 13:430–434, 1979
72. Pittard WB, Bill K: Immunoregulation by breast milk cells. Cell Immunol 42:437–441, 1979
73. Prentice A, Prentice AM, Cole TJ et al: Determinants of variations in breast milk protective factor concentrations of rural Gambian mothers. Arch Dis Child 58:18–22, 1983

74. Puskas M, Antoni F, Peterfy F et al: Pokeweed mitogen, Bordetella pertussis, and breast milk cell factor induce preferentially the synthesis of different immunoglobulin classes. Boll Ist Sieroter Milan 62:262–267, 1983
75. Rand WM: Statistical considerations in the estimation of protein requirements. In Torun B, Young VR, Rand WM (eds): Protein-Energy Requirements of Developing Countries: Evaluation of New Data. Tokyo, United Nations University, 1981, pp 3–7
76. Report of the Surgeon General's Workshop. Breastfeeding and human lactation. US Dept. Health and Human Services, Publication No. HRS-D-MC-84-2. Washington, DC, US Government Printing Office, 1984
77. Research Subcommittee South East England Faculty Royal College of General Practitioners. The influence of breastfeeding on the incidence of infectious illness during the first year of life. Practioner 209:356–362, 1972
78. Roberts AK, Van Biervliet JP, Harzer G: Factors of human milk influencing the bacterial flora of infant feces. In Schaub J (ed): Composition and Physiological Properties of Human Milk. New York, Elsevier, 1985, pp 259–270
79. Rose WC, Dekker EE: Urea as a source of nitrogen for the biosynthesis of amino acids. J Biol Chem 223:107–121, 1956
80. Samson RR, Mirtle C, McClelland DBL: The effects of digestive enzymes on the binding and bacteriostatic properties of lactoferrin and vitamin B_{12} binder in human milk. Acta Paediatr Scand 59:517–523, 1980
81. Sauls HS: Potential effects of demographic and other variables in studies comparing morbidity of breastfed and bottlefed infants. Pediatrics 64:523–527, 1979
82. Savanborg EC, Andersson B, Hagberg L et al: Receptor analogues and anti-pili antibodies as inhibitors of bacterial attachment in vivo and in vitro. Ann NY Acad Sci 409:580–582, 1983
83. Schaafsma G, deWaard H: Nutritional interrelationships among Ca, P, and lactose. Am J Clin Nutr 31:5–6, 1978
84. Schanler RJ, Garza C, Nichols BL: Fortified mothers' milk for very low birthweight infants: Results of growth and nutrient balance studies. J Pediatr 107:437–445, 1985
85. Schanler R, Goldman R, Garza C, et al: Enhanced fecal excretion of selected immune factors in very low birthweight infants fed fortified human milk. Pediatr Res 1986 (In Press)
86. Schlessinger JJ, Covelli HD: Evidence for transmission of lymphocyte responses to tuberculin by breastfeeding. Lancet 2:529–532, 1977
87. Scrimshaw NS: Protein deficiency and infective disease. In Munro HN, Allison JB (eds): Mammalian Protein Metabolism, Vol II. New York, Academic Press, 1964, pp 569–592
88. Silber G, Hopkinson J, Hachey D, et al: Modification of fatty acid composition in human milk. Am J Clin Nutr 43:688, 1986
89. Southgate DAT, Barrett IM: The intake and excretion of calorific constituents of milk by babies. Br J Nutr 20:363–372, 1966
90. Spik G, Brunet G, Mazurdier-Dehaine C et al: Characterization and properties of the human and bovine lactotransferrins extracted from the faeces of newborn infants. Acta Paediatr Scand 71:979–985, 1982
91. Stephens S: Development of secretory immunity in breast-fed and bottle-fed infants. Arch Dis Child 61:263–269, 1986
92. Stephens S, Dolby JM, Montreuil J et al: Differences in inhibition of the growth of commensal and enteropathogenic strains of *Escherichia coli* by lactotransferrin and secretory immunoglobulin A isolated from human milk. Immunology 41:597–603, 1980
93. Stephens S, Kennedy CR, Lakhani PK et al: In-vivo immune responses of breast- and bottle-fed infants to tetanus toxoid antigen and to normal gut flora. Acta Pediatr Scand 73:426–432, 1984
94. Stuff JE, Garza C, Boutte C, et al: Caloric intakes of older breast-fed infants: human milk and solid food. Am J Clin Nutr 43:418, 1986
95. Stuff J, Garza C, Fraley JK et al: Sources of variation in milk and caloric intakes in breast-fed infants: Implications for lactation study design and interpretation. Am J Clin Nutr 43:361–366, 1986

96. Svanborg EC, Svennerholm AM: Secretory immunoglobulin A and G antibodies prevent adhesion of *Escherichia coli* to human urinary tract epithelial cells. Infect Immunol 22:790–797, 1978
97. Svennerholm AM, Hanson LA, Holmgren et al: Milk antibodies to live and killed polio vaccines in Pakistani and Swedish women. J Infect Dis 143:707–711, 1981
98. Svennerholm AM, Hanson LA, Holmgren J et al: Different secretory IgA antibody response to cholera vaccination in Swedish and Pakistani women. Infect Immunol 30:427–430, 1980
99. Tagiliabue A, Nencioni L, Villa et al: Antibody-dependent cell-mediated antibacterial activity of intestinal lymphocytes with secretory IgA. Nature 306:184–186, 1983
100. Udall JN, Colony P, Fritze L et al: Development of the gastrointestinal mucosal barrier. II. The effect of natural versus artificial feeding on intestinal permeability to macromolecules. Pediatr Res 15:245–249, 1981
101. Vukavic T: Intestinal absorption of IgA in the newborn. J Pediatr Gastroenterol Nutr 2:248–251, 1983
102. Wallgren A: Breast milk consumption of healthy, full-term infants. Acta Paediatr Scand. 32:778–790, 1945
103. Wasserman RH: Lactose-stimulated absorption of calcium: A theory. Nature. 201:997–999, 1964
104. Weisz-Carrington P, Roux ME et al: Hormonal induction of the secretory immune system in the mammary gland. Proc Nat Acad Sci USA 75:2928–2932, 1978
105. Welsh JK, Skurrie IJ, May JT: Use of Semliki forest virus to identify lipid-mediated antiviral activity and anti-alphavirus immunoglobulin A in human milk. Infect Immun 19:395–401, 1978
106. Widdowson EM, Southgate DAT, Hey EN: Body composition of the fetus and infant. In Jisser HKA (Ed): Nutrition and Metabolism of the Fetus and Infant, Boston, Martinus Nijhoff Publishers 1979, pp 169–178
107. Yow M, Stagno S, Pass RF et al: Cytomegalovirus infection of breast milk and transmission in infancy. Pediatrics 72:295–299, 1983

Children's Nutrition Research Center
Medical Towers Building, Suite 601
6608 Fannin
Houston, Texas 77030