RESEARCH PAPERS

Human Milk and Colostrum Proteins: A Review

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ABSTRACT

Human milk contains a number of factors that are protein in nature, which apparently enhance the resistance of the breast-fed infant against disease. Among such factors are lactoferrin, immunoglobulin A, the Lactobacillus bifidus growth-promoting glycoproteins, and lysozyme. Lactoferrin is believed to exert its bacteriostatic action mainly in concert with specific antibacterial immunoglobulins. The immunoglobulins may, in turn, act in concert with the large numbers of white blood cells in human milk and colostrum. α-Lactalbumin, a major component of human milk, is important both from a nutritional point of view, and as a component of the enzyme system biosynthesizing lactose. It is structurally homologous to lysozyme of both human milk and hen's egg white. The most abundant component of the casein fraction of human milk is β-casein, which exists in the form of genetically-determined polymorphs. The polymorphs apparently differ in their phosphate content only. The amino acid sequence of the N-terminal region of human milk β-casein bears a strong structural homology to that of bovine milk β-casein. Human α-casein is also in the form of genetically-determined polymorphs; however, it has not been purified and characterized. Human κ-casein is a glycoprotein which releases a glycomacropeptide when acted upon by rennin. Though it has not been characterized to any great extent, there is evidence that there may be several forms of κ-casein in human milk. Also the nature of the glycomacropeptide varies depending on whether purified κ-casein or whole casein fractions are subjected to the action of rennin. The amino acid sequence of a glycomacropeptide produced from κ-casein-enriched fraction of human milk casein was structurally homologous to the glycomacropeptides from other species.

INTRODUCTION

Since the introduction of artificial feeding into the field of pediatrics, there has been controversy regarding merits of breast feeding as opposed to bottle feeding. At the turn of the century, there was no question of which method was more desirable. However, as man's knowledge of sterilization grew and a vigorous educational program of expectant mothers was instituted, differences in morbidity and mortality rates between the two groups of children became less apparent. It became eventually necessary to evaluate the possible advantages or disadvantages of breast feeding under rigorous (insofar as is possible in such circumstances) conditions with sufficiently large samples to permit a sound statistical work-up of the data. Of several such studies over the years, the older ones invariably showed that human breast milk was superior to cow milk in minimizing both the morbidity and mortality of infants. Thus, in Great Britain (101) with 3266 infants, mortality rates in the first 7 mo of life were 10.2 and 57.3 per 1000 in breast- and bottle-fed babies. Morbidity rates were 223.4 and 573.7 per 1000. The bottle-fed infants were also much more likely to die if they became ill than were breast-fed infants. The difference in death rates was especially great for respiratory diseases (8.2 and 31.6 per 1000). In addition, there were no deaths among breast-fed children from gastroenteritis and middle ear infections whereas bottle-fed children showed rates of 7 and 8.1 per 1000.

In a Chicago study (44) involving 20,061 infants, the pattern was similar. Of the 9,749

Received January 19, 1977.
breast-fed infants, 15 died from various causes whereas of 8,605 partially breast-fed infants, 75 died, and finally, of 1,707 bottle-fed infants, 129 succumbed to various diseases. Respiratory infections were the most prevalent causes of death.

Studies in later years have shown little, if any, difference between the development of breast-fed and bottle-fed infants in the developed countries (1, 118). These findings referred to both the growth and development of the infants, as well as their apparent resistance to disease. To a large part, this was due to the nutritional adequacy of artificial milk formulae, which have been manufactured to approximate the nutrient content of human milk (27, 31, 66). The idea has emerged that breast feeding offered no advantages over bottle feeding as long as the formulae were nutritionally adequate and hygienic measures were appropriate (105).

Breast feeding in the less affluent and informed populations of the world is far superior to bottle feeding in both morbidity and mortality of the neonates (17, 34). In fact, "...if one consciously sets out to kill infants of the poor, he might begin by persuading or forcing their mothers to artificially feed them" (77).

Many investigators believe, however, that the poor and the ignorant are not the only segment of the world's population that can benefit from breast feeding. Thus, the artificial formulae, though similar to breast milk in calories, vitamins, minerals, etc. contained therein, are not similar qualitatively with respect to fats, proteins, and other substances. It is possible that substitution of bovine milk fat and protein for human materials may increase the infant's future risks of developing degenerative diseases (70). In addition, it is believed that the premature infant can benefit from breast milk to an even greater extent than can the full-term infant (124). The most compelling argument in favor of breast feeding is, however, the contention that breast milk contains factors that protect the infant against both systemic and gastrointestinal infections. The most dramatic testimonials in this regard are provided by the results of nursery epidemics where otherwise normal infants quickly succumb to a variety of infectious agents unless they are fed raw human milk (28, 34). The protection accorded the newborn by breast milk is also critical for premature infants where a relatively common fatal syndrome called necrotizing enterocolitis can be prevented by breast feeding (5, 79, 114). It also has been proposed that the incidence of cot deaths (sudden death syndrome) in infants is lower in breast-fed infants than it is in bottle-fed infants (80). The resistance of breast-fed infants against infection is summarized in several review articles (34, 35, 46, 50, 75), which ascribe the beneficial effects of human milk to factors that are therein but are absent from bovine milk. These factors are, for the most part, protein in nature, the most important of which are the immunoglobulins, lactoferrin, and lysozyme.

Aside from their function in protecting the infant against infectious disease, proteins of human milk are concerned with providing the infant with the proper balance of essential amino acids, with acting as carriers for a number of vitamins and minerals, and with acting in an enzymatic capacity to biosynthesize lactose and possibly other oligosaccharides. The proteins most concerned with these nutritional activities are the caseins, α-lactalbumin, and the folate and vitamin B<sub>12</sub>-binding proteins.

Lastly, many human milk proteins can serve as model systems for the study of the biochemical properties of proteins in general. We have, for instance, the rather well-defined IgA antibody system that is specific for the E. Coli microorganism. It may, therefore, serve as a convenient system for the study of antigen-antibody reactions involving naturally-occurring antigens. Lactoferrin is an excellent model for the investigation of metal-protein interactions, and the lactose synthetase system involving proteins A and B is an unique enzymatic system that well may have a wide distribution in nature.

This review article is concerned with properties of major protein components of human milk, whereby it is hoped that it will serve as a stimulus for a more extensive investigation of this unique biological fluid.

**Protein Content of Human Milk**

Human milk, compared to the milks of other species, is relatively poor in protein content, and the amount of casein that it contains is
TABLE 1. Protein content of mature human milk (in g/100 ml).

<table>
<thead>
<tr>
<th>Average value</th>
<th>Range</th>
<th>Casein</th>
<th>Whey</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.25 ± .28</td>
<td>.79 - 2.04</td>
<td>...</td>
<td>...</td>
<td>95</td>
</tr>
<tr>
<td>1.1</td>
<td>...</td>
<td>.44</td>
<td>.66</td>
<td>47</td>
</tr>
<tr>
<td>1.1</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>118</td>
</tr>
<tr>
<td>1.0</td>
<td>...</td>
<td>.4</td>
<td>.6</td>
<td>57</td>
</tr>
<tr>
<td>.88 ± .14</td>
<td>.70 - 1.084</td>
<td>.35</td>
<td>.53</td>
<td>69</td>
</tr>
<tr>
<td>1.1</td>
<td>.7 - 2.0</td>
<td>.37</td>
<td>...</td>
<td>72</td>
</tr>
<tr>
<td>1.04</td>
<td>...</td>
<td>.44</td>
<td>.60</td>
<td>86</td>
</tr>
</tbody>
</table>

lower than its content of whey protein. In addition, day-to-day and week-to-week variations in its protein content have been rather wide. Table 1 presents the total protein, casein, and whey protein contents of human milk by various authors.

Colostrum (milk obtained 1 to 5 days following parturition) and transitional milk (6 to 10 days following parturition) have higher amounts of protein: 2.3 g/100 ml for colostrum (range 1.5 to 6.8), and 1.6 g/100 ml for transitional milk (range 1.3 to 1.9) (72). The increased protein content in these early milk samples is largely due to increases in whey protein rather than casein.

Major proteins of human milk whey are α-lactalbumin, lactoferrin, serum albumin, and lysozyme. Concentrations of each are in Table 2. The major components of the casein fraction of human milk are the κ-, β-, and α-like caseins comprising approximately 20 to 27, 64, and 9% of the total protein in the casein fraction (83, 116).

TABLE 2. Concentration of the major whey protein components of human milk (in g/100 ml milk).

<table>
<thead>
<tr>
<th>Lactoferrin</th>
<th>α-Lactalbumin</th>
<th>Serum albumin</th>
<th>Lysozyme</th>
<th>Reference</th>
</tr>
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<tbody>
<tr>
<td>.155</td>
<td>.46</td>
<td>.049</td>
<td>.046</td>
<td>69</td>
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<tr>
<td>.10</td>
<td>...</td>
<td>...</td>
<td>.035</td>
<td>35</td>
</tr>
<tr>
<td>...</td>
<td>.36a</td>
<td>...</td>
<td>...</td>
<td>72</td>
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<tr>
<td>.10 (.4)b</td>
<td>...</td>
<td>...</td>
<td>...</td>
<td>51</td>
</tr>
<tr>
<td>...</td>
<td>.224</td>
<td>.072</td>
<td>...</td>
<td>82</td>
</tr>
<tr>
<td>.21 (.49)b</td>
<td>.28</td>
<td>.060</td>
<td>...</td>
<td>86</td>
</tr>
<tr>
<td>...</td>
<td>...</td>
<td>.016c</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

a Listed as “lactalbumin”.
b Value in parentheses refers to colostrum.
c Single donor.
transferrin (67), the amino acid side-chains that may be involved in chelating the iron are the tyrosyl groups (112, 113) and histidyl residues (63). When lactoferrin binds iron, it assumes a salmon pink color, hence, its older designation as “red protein”. Amino acid composition and physical constants of lactoferrins are summarized in Tables 3 and 4, respectively.

There has been much activity in studies of primary structure and structural homology of the transferrin group of proteins. Transferrin (71), ovotransferrin (conalbumin) (123), and lactoferrin (68) are composed of two structurally homologous and apparently independent regions, each containing one iron-binding site. The homology may have arisen during evolution through gene duplication. In spite of the structural homologies of the two halves in each type of iron-binding protein, the two halves apparently have undergone a sufficiently large number of point mutations during evolution to yield the expected number of unique cyanogen bromide fragments (based on methionine content (6, 76). There are amino acid sequences that are common to lactoferrin, transferrin, and ovotransferrin, indicating that they were derived from a common ancestral protein (11, 60).

### TABLE 3. Amino acid and carbohydrate composition of some human milk proteins (in moles/mole protein).

<table>
<thead>
<tr>
<th></th>
<th>Lactoferrin&lt;sup&gt;b&lt;/sup&gt; (76)</th>
<th>Lactalbumin&lt;sup&gt;c&lt;/sup&gt; (104)</th>
<th>Lysozyme&lt;sup&gt;d&lt;/sup&gt; (61)</th>
<th>Galacto-therming&lt;sup&gt;e&lt;/sup&gt; (103)</th>
<th>β-Casein&lt;sup&gt;f&lt;/sup&gt; (42)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asp</td>
<td>71</td>
<td>14</td>
<td>18&lt;sup&gt;e&lt;/sup&gt;</td>
<td>7</td>
<td>11</td>
</tr>
<tr>
<td>Thr</td>
<td>30</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>9</td>
</tr>
<tr>
<td>Ser</td>
<td>54</td>
<td>7</td>
<td>6</td>
<td>2</td>
<td>9</td>
</tr>
<tr>
<td>Glu</td>
<td>62</td>
<td>13</td>
<td>9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>19</td>
<td>39</td>
</tr>
<tr>
<td>Pro</td>
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<td>8</td>
<td>0</td>
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<tr>
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<td>2</td>
<td>2</td>
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<td>54</td>
<td>12</td>
<td>8</td>
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<tr>
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<td>5</td>
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<tr>
<td>His</td>
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<tr>
<td>Arg</td>
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<td>14</td>
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<td>3</td>
</tr>
<tr>
<td>Trp</td>
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<td>None</td>
<td>None</td>
<td>None</td>
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<td>None</td>
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<td>None</td>
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<tr>
<td>Mannose</td>
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<td>None</td>
<td>None</td>
<td>None</td>
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<tr>
<td>Fucose</td>
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<td>None</td>
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<tr>
<td>NAcGlc</td>
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<td>None</td>
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<tr>
<td>NANA</td>
<td>3</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>N-terminus</td>
<td>None&lt;sup&gt;d&lt;/sup&gt;</td>
<td>Lys</td>
<td>Lys</td>
<td>...</td>
<td>Arg</td>
</tr>
<tr>
<td>C-terminus</td>
<td>...</td>
<td>Leu</td>
<td>Val</td>
<td>...</td>
<td>...</td>
</tr>
</tbody>
</table>

<sup>a</sup>No distinction is made between Asp and Asn and between Glu and Gln.

<sup>b</sup>Molecular weight of 77,000 assumed.

<sup>c</sup>Molecular weight 14,302. Slightly different values were obtained by Findlay and Brew (30).

<sup>d</sup>Other authors have found N-terminal glycine (9, 68).

<sup>e</sup>10 Asn, 8 Asp. All other amino acid residues calculated on basis of 18 Asp.

<sup>f</sup>6 Gln, 4 Glu.

<sup>g</sup>Molecular weight assumed was 14,000.

<sup>h</sup>Molecular weight assumed was 24,000.
Lactoferrin is a glycoprotein and contains some 5 to 6% carbohydrate consisting of galactose, mannose, fucose, N-acetylglucosamine, and sialic acid (76). The carbohydrate apparently is arranged in the form of two oligosaccharide chains that are attached to the polypeptide backbone through asparaginylglycan linkages (109).

It has been proposed that lactoferrin is involved in the host resistance mechanism of the breast-fed infant. The lay press has described its action as: "Lactoferrin is a protein which kills harmful bacteria by engulfing them—swallowing them whole, you might say." (29). Lactoferrin, of course, does no such thing; however, it does inhibit the growth of a number of microorganisms in vitro (91). To show this effect, lactoferrin must not be saturated with iron. If iron is added to saturate the protein, the bacteriostatic action is lost. This effect of lactoferrin, as well as other transferrin-like proteins, has been ascribed to these proteins making iron unavailable for bacterial growth.

Though lactoferrin is bacteriostatic in a synthetic medium, its bacteriostatic effects are enhanced several-fold if it is permitted to act in concert with antibodies to a microorganism. Thus, the bacteriostatic effect of whole human milk toward E. Coli may be reproduced by the appropriate mixture of iron-poor lactoferrin and specific E. Coli antibodies from human milk. Lactoferrin alone had a smaller inhibitory effect, whereas the antibody alone had almost none (20). Findings have been similar with Clostridium welchii, Pasteurella septica, Pasteurella pestis, Klebsiella pneumoniae, and others (18, 19). The mechanism of this effect is unclear; however, it is believed that in the absence of the specific antibody, the microorganism will secrete an iron chelater (in case of E. Coli 0111, the chelator is called enterochelin), which successfully can compete for iron with lactoferrin and make it available for growth of the microorganism. A specific antibody serves to inhibit secretion of this type of a chelator. Even in the presence of lactoferrin and specific antibody, there will be no bacteriostasis if citrate is in the medium. Apparently citrate can mediate iron exchange between lactoferrin and the microorganism. However, bicarbonate will nullify effects of citrate and bring about bacteriostasis (40).

It is believed milk proteins in the breast-fed
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infant are digested slowly in the stomach and pass largely unchanged into the intestine (19). It is probable that lactoferrin, in concert with the various specific antibodies in human milk and in the presence of the rather large amounts of bicarbonate in the small intestine, will protect the infant against enteric, and perhaps even systemic infections.

α-Lactalbumin

According to some authors, α-lactalbumin is the most abundant whey protein of human milk (see Table 2). It was first isolated and described by Johansson, who found it was similar to bovine α-lactalbumin (59). Its amino acid content is similar if not identical to that of bovine α-lactalbumin (94, 104, see Table 3). It is of major importance to the infant from a nutritional view (32).

The complete amino acid sequence of human α-lactalbumin has been determined (30), as it has for the α-lactalbumins of a number of other species (12, 13, 14, e.g.'s). All of these α-lactalbumins have segments of identical amino acid sequences, and all cross-react immunologically if the immunoadsorption method is used. The degree of cross-reactivity is smaller with the Ouchterlony system (precipitating antibodies) (99).

Human α-lactalbumin consists of 123 amino acids and a single polypeptide chain. Seventy-two percent of its amino acid residues are in positions identical to those in bovine α-lactalbumin (30).

There is a substantial number of regions in human α-lactalbumin that share the same amino acid sequences with several regions of human and hen's egg lysozyme. For this reason, it has been proposed that the α-lactalbumins and lysozymes trace their origins to a common ancestral gene (52). Thus, human α-lactalbumin and human leukemic lysozyme (as well as hen's egg lysozyme) have identical amino acids in 39% of the positions. Since many of these “conserved” amino acids are believed to be crucial in maintenance of both secondary and tertiary structures of these proteins, it is assumed that the lysozymes and α-lactalbumins have nearly identical three-dimensional shapes.

The two types of proteins do not, however, cross-react immunologically. Lysozyme from human milk will be discussed in greater detail infra.

In addition to serving as a source of essential amino acids for the infant, α-lactalbumin is a crucial component of an enzyme system that biosynthesizes lactose (15). This enzyme system consists of “protein A”, or UDP-galactosyl transferase, and “protein B” which is α-lactalbumin. In the absence of protein B, protein A catalyzes the formation of β-galactosyl-1,4-N-acetyl glucosamine (N-acetyllactosamine) from UDP-galactose and N-acetylgalactosamine whereas in the presence of protein B, the galactose acceptor is glucose, and the product is lactose.

A functional similarity between α-lactalbumin and lysozyme, is that the former catalyzes the biosynthesis of β-1,4-glycosidic bonds whereas the latter catalyzes the hydrolysis of the same bonds in bacterial cell walls. The mechanism of lactose formation has been reviewed (36).

Galactosyl transferase is in both the breast tissue and in milk. It is believed that the function of the tissue enzyme is to biosynthesize oligosaccharides and glycoproteins whereas the milk enzyme is concerned largely with lactose biosynthesis. Presumably, the amount of α-lactalbumin in the milk controls the rate of lactose biosynthesis since lactose contents were highest in those milks that also had the highest concentrations of α-lactalbumin. Of six species, human milk had the highest lactose (7.0 g/100 ml) and α-lactalbumin concentrations (.48 g/100 ml) (65). Galactosyl transferase is in human milk in the form of three molecular species, molecular weights 38,000, 43,000, and 50,000. The species of lower-molecular weight are derived from the higher ones by partial proteolysis. This is apparently of importance physiologically, as the 38,000-dalton species has less affinity for N-acetyl glucosamine than does the 50,000-dalton molecule (98). It also has been proposed that thiol groups may be involved in the UDP-galactosyl transferase activity (62).

α-Lactalbumin has no function in protecting the breast-fed infant against disease; however, it has the indispensable roles of providing a balanced complement of essential amino acids to the infant and of participating in the biosynthesis of lactose.

Immunoglobulins

Some years ago, it was demonstrated that human milk and colostrum contained proteins...
that cross-reacted with antisera to IgG, IgA, and IgM (49). The concentration of immunoglobulins is highest in colostrum, which falls drastically with the first few days of lactation. Thus, the IgA, IgG, and IgM contents of colostrum (1st day of lactation) were 1735, 43, and 159 mg/100 ml and only 100, 4, and 10 mg/100 ml on the 4th day of lactation (4). These values tend to drop even lower with increasing time of lactation, where individual variation in immunoglobulin is considerable (93). The most abundant immunoglobulin of human milk is IgA, which is unlike the situation in the cow, whose colostrum immunoglobulin is almost entirely of the IgG class. However, unlike in the bovine newborn, the IgA of human colostrum does not penetrate the intestinal wall of the infant, the gut forming an effective "barrier to penetration of macromolecules" (33). Apparently the function of the high concentrations of immunoglobulins in human colostrum and milk is to provide the infant with protection against enteric infection and to serve as an "intestinal paint" to prevent the passage of various foreign proteins and bacteria from the intestinal tract into the circulation (55). Table 5 lists some of the specific antibodies discovered in human milk.

Of greatest importance among these are the E. Coli type O antibodies, which protect the infant against the most common type of enteric infection. These antibodies are primarily of the IgA type. The specific antibodies may act in concert with lactoferrin, as was indicated supra (20, 40), or they may act in conjunction with the white blood cells that are normally in human milk. The white cell count of human milk is between 1000 and 4000 per mm³ during the first 2 wk following delivery, and slightly lower thereafter (50). This includes lymphocytes (that secrete IgA), macrophages, and neutrophils.

Though IgG and IgM of human milk are apparently identical to their respective counterparts in blood serum, the IgA possesses a different structure and is termed secretory IgA. This protein (m. w. 420,000) consists of an IgA dimer (m. w. 330,000) in association with a secretory piece (or component) and a J-chain. The secretory piece is a protein with a molecular weight of near 78,000, and the J-chain has a molecular weight of near 15,000. The secretory component is apparently specific for IgA, though it may also associate with IgM under laboratory conditions (122). It is attached to the IgA dimer via disulfide linkages(s) whereas its association with IgM, is of physical nature only. The secretory piece occurs in the human milk in the free form as well as in association with IgA (10, 117, 122). Its role may be that of protecting the IgA against proteolytic digestion. The J-chain normally is found with all polymeric immunoglobulins, including serum IgM, serum IgA dimers, and secretory IgA. Its function is apparently to join the immunoglobulin molecules together.

TABLE 5. Partial list of antitoxins and specific antibodies found in human milk and colostrum.

<table>
<thead>
<tr>
<th>Microorganism or toxin</th>
<th>References</th>
</tr>
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<tbody>
<tr>
<td>Shigella dysenteriae</td>
<td>25, 75</td>
</tr>
<tr>
<td>Shigella flexneri 1</td>
<td>75</td>
</tr>
<tr>
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<td>Salmonella</td>
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</tr>
</tbody>
</table>

Enzymes

There are a number of enzymes in human milk, though they have not been studied as extensively as those in bovine milk (107). The most abundant enzyme of human milk (if a-lactalbumin is excluded) is lysozyme. It is believed that its function is to act as an antibacterial agent since it catalyzes the hydrolysis of the β-1, 4-glycosidic bonds in the cell walls of many microorganisms, or it may act in concert with immunoglobulins or white blood cells (56). However, suspicion is growing that the main function of lysozyme in the human organism is concerned with proteoglycan and glycoprotein metabolism; lysozyme, because of its basic character, chemically combines with the acidic proteoglycans and/or glycoproteins.
and may influence their turnover. Moreover, it is speculated that an as-yet unidentified glycoprotein, and not bacterial cell walls, is the true substrate of mammalian lysozymes (64).

The primary structure of human milk lysozyme (61) is a basic single-chain protein, molecular weight 14,400, consisting of 130 amino acid residues, and with internal homology of about 74% with respect to hen’s egg lysozyme. Human milk and leukemic urine lysozymes have identical primary structures (22). Structural homologies between lysozymes and the α-lactalbumins as well as the function of α-lactalbumin and UDP-galactosyl transferase in the biosynthesis of lactose have been discussed supra.

Among other enzymes in the human milk and colostrum are the amylases which are in the form of several isoenzymes (37), oxytocinase (16), lactoperoxidase (38), lipoprotein lipases (58), and xanthine oxidase (125).

**Miscellaneous Proteins of Human Milk Whey**

There are a number of proteins in human milk whey, whose function is not established well or which have not been thoroughly characterized. Human serum albumin was first isolated from human milk by Johansson (59). It is apparently in large amounts in colostrum; than it declines during the first 9 to 10 wk of lactation, and increases again with further lactation (56). It is immunologically and chemically identical to albumin of blood serum.

An interesting protein that precipitates when the milk is at room temperature but dissolves at refrigerator temperatures has been isolated by Schade and Reinhart and termed galacto-thermin (103). This protein had a molecular weight of near 14,000 by the ultracentrifuge method and a high proline content. A similar protein was isolated by Bezkorovainy and coworkers from heat-treated human milk (8). However, the molecular weight was near 30,000 by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate and mercaptoethanol. This protein had a single phosphate residue per molecule of protein, suggesting some relationship to casein. Similar proteins have been isolated from bovine and dog milks (106, 111).

Human milk contains a number of factors, some of them nondialyzable, that stimulate the growth of *L. bifidus var. Penn.* (45). Among the nondialyzable, or slowly dialyzable substances with *L. bifidus* growth-promoting activities, are oligosaccharides (e.g., 48) and glycoproteins. Such glycoproteins have been isolated from human colostrum by Hirano and coworkers (53, 54) and by Bezkorovainy and coworkers (88, 89, 90) as well as from human milk whey by Bezkorovainy and coworkers (7). There were at least five such glycoproteins in human colostrum, ranging in molecular weights from 26,000 to 35,000, and in carbohydrate content of 54 to 75% (88, 90). The carbohydrate components were galactose, sialic acid, fucose, N-acetylglucosamine, and N-acetylgalactosamine. Human milk contained a glycoprotein with a molecular weight of near 30,000 and a carbohydrate content of 70%, and a mixture of glycopeptides with an average molecular weight of near 4000 and a carbohydrate content of 76% (7).

Factors promoting the growth of the *Lactobacillus* species are in human milk but not in bovine milk. This difference is apparently responsible for the fact that the dominant species of the intestinal flora of the breast-fed infant is the *Lactobacillus*, which, by secreting lactic acid, maintains the pH of the intestinal content lower than in the formulated infant. The relatively low pH apparently inhibits the establishment of pathogenic microorganisms in the intestinal tract of the breast-fed infant (35, 46, 89).

In addition to the glycoproteins promoting microbial growth, human milk and colostrum contain orosomucoid, a serum protein without any such activity (7) and an influenza virus hemagglutination inhibitor (108).

Human milk and colostrum contain a variety of other proteins, some of which may have nutritional significance. There are the folate binding (121) and vitamin B₁₂-binding proteins (21), the fat globule membrane protein (81), β-microglobulin (24), and corticosteroid-binding proteins (92, 102). One of the latter is the cortisol-binding globulin of plasma (CBG).

Bovine milk whey contains a heat-stable fraction comprising some 20% of total whey protein. It is termed the proteose-peptone fraction, and it consists of at least three unique components (41). Human milk and colostrum do not contain a comparable fraction. When human milk and colostrum were subjected to
the procedure whereby the proteose-peptone fraction was produced from bovine milk, a heat-stable fraction was obtained that consisted largely of α-lactalbumin, the temperature-sensitive protein, and the Lactobacillus bifidus growth-promoting glycoproteins (8).

The Casein Fraction of Human Milk

When the casein fraction of human milk is subjected to gel electrophoresis in the presence of urea, three fractions are fairly comparable to those of bovine casein. These correspond to the α-, the β- and κ-caseins in order of decreasing mobility towards the anode at pH 8.6 (73, 120) (Fig. 1). Both the α- and β-caseins show polymorphism whereas little is known about κ-casein. Human casein may be prepared by the classical acid precipitation method and fractionated either by ion-exchange chromatography or by gel filtration (83, 87). Colostral and transitional milk caseins give electrophoretic patterns differing from those of mature milk. The former apparently contain more α-casein, and their β-casein fraction does not resolve into the typical six-band polymorphic pattern. There are also slight differences in the overall amino acid compositions (84).

The major component of the casein fraction of human milk is β-casein, representing some 50% of the total casein fraction (116). Its molecular weight is near 25,000 (43, 115), and, like bovine β-casein, it polymerizes at room temperature and de-polymerizes at 4 C (115). Electrophoretically, human β-casein separates into six bands (termed A to F) in the presence of urea. Each of the six components has been purified by DEAE-cellulose chromatography, and they differ only in their phosphate content (42, 85). When dephosphorylated by a phosphatase preparation, the electrophoretic migration of all components became the same. The six β-casein components contain from zero to five phosphate groups and apparently do not differ in amino acid composition (42).

When the β-caseins of individual milk samples are examined electrophoretically, it becomes clear that individual donors differ in intensity of the individual β-casein bands, i.e., there are quantitative variations among the six β-casein bands. These differences have been ascribed to genetic control mechanisms (120) where three main phenotypes have been recognized: the β-A, β-AD, and β-D. In the β-A variant, the A-band is high; in the β-AD variant, bands A and D are of equal intensity; and in the β-D variant, band D is of higher intensity than band A. It was proposed that the β-casein pattern is controlled by a biallelic gene, with β-A and β-D frequencies of .678 and .322. Such gene frequencies differ among populations as in Table 6.

In addition to the above three patterns, other rare β-casein variants have been detected (119). This has suggested that the biallelic mechanism proposed is an over-simplification of the situation at hand and that in addition to the amount of phosphorylation, the β-casein variants may differ in amino acid sequences. The resolution of these questions must await determination of the complete amino acid sequence of each β-casein band. Such a study is well under way (39). β-Caseins with zero to five
TABLE 6. Gene frequencies for human caseins in various populations.

<table>
<thead>
<tr>
<th>Population</th>
<th>β-A</th>
<th>β-D</th>
<th>α-A</th>
<th>α-B</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Turin, Italy</td>
<td>.677</td>
<td>.323</td>
<td>.766</td>
<td>.234</td>
<td>120</td>
</tr>
<tr>
<td>Sardinia, Italy</td>
<td>.750</td>
<td>.250</td>
<td>.855</td>
<td>.145</td>
<td>96</td>
</tr>
<tr>
<td>Kikuyu, Africa</td>
<td>.311</td>
<td>.662</td>
<td>.822</td>
<td>.178</td>
<td>97</td>
</tr>
</tbody>
</table>

phosphate residues have identical amino acid sequences in the N-terminal region (28 residues), and all phosphate groups are located in a cluster attached to serine and threonine residues at positions 3, 6, 8, 9, and 10. There was also a high degree of similarity between the sequences of the human and bovine milk β-caseins.

α-Casein is a minor component of human milk and has not been isolated and characterized. An α₄-casein was described by Malpress and Seid-Akhavan (74); however, it was apparently what we today recognize as β-casein (42). Electrophoretically, the α-casein fraction also exhibits a polymorphic character whereby three distinct bands have been labeled A, B, and C in the order of decreasing electrophoretic migration (120). In the phenotype α-A, the A-band is present but B is absent; in the α-B phenotype, band B is present but A is absent; and in the α-AB phenotype, both bands A and B are present. All three phenotypes also show band C. The frequencies of these phenotypes were 86, 10, and 4% for α-A, α-AB, and α-B, and their gene frequencies are in Table 6.

The most enigmatic component of the human casein fraction is probably κ-casein. Though many attempts have been made to purify this material, the task remains. However, much information has accumulated on this material, and it is hoped that we will soon be provided with an accurate description of its properties. κ-Casein-containing fractions have been isolated by DEAE-cellulose chromatography (2), by CM-Sephadex chromatography (74), and by gel filtration (116). Most of these techniques involve inclusion of large amounts of urea into the elution buffers. All preparations described were able to stabilize human β-casein against precipitation by calcium, were themselves calcium-insensitive, and released carbohydrate-containing polypeptides following treatment with rennin. κ-Casein accounts for 27% of total casein protein and has a monomer molecular weight of near 19,000 (116). Its carbohydrate content was given as 4.4 to 8.8% (74) consisting of hexose (2.6 to 4.3%), hexosamine (1.0 to 2.9%), fucose (0 to 1.0%), and sialic acid (.36 to .70%). Human milk may contain more than one species of κ-casein (2).

When rennin acts on κ-casein of bovine milk, it removes a so-called glycomacropeptide therefrom which then loses its ability to stabilize α₄-casein, and the latter then precipitates with calcium. Glycomacropeptide also can be produced from human milk by the action of rennin on either the purified κ-casein component or the whole casein fraction. The two types of glycomacropeptide preparations have not proven to be identical, the one from whole casein containing much more carbohydrate (55%) identified as glucosamine, galactosamine, galactose, fucose, and sialic acid (74). This carbohydrate composition, as well as the monosaccharide ratios therein are strongly reminiscent of the carbohydrate portion of the Lactobacillus bifidus growth-promoting glycoprotein isolated from human milk and colostrum by Bezkorovainy and co-workers (7, 88). Judging from these admittedly incomplete data, it is entirely possible that the glycoproteins of human milk whey with Lactobacillus bifidus growth-promoting activity are either normal components of the human casein system and are abstracted therefrom by endogenous proteases or are whey proteins with an unusually great propensity to associate with casein. On the other hand, the glycomacropeptide isolated earlier by Alais and Jolles from whole human casein (3) contained only 16% carbohydrate.

A partial amino acid sequence of the human glycomacropeptide has been published by Chobert et al. (26). The substrate for the rennin digestion was a κ-casein-rich fraction prepared by DEAE-cellulose chromatography in the pres-
ence of urea and mercaptoethanol. The glycomacropeptide contained some 59 amino acid residues though no information was given on its carbohydrate moiety. There was an extensive sequence homology with respect to glycomacropeptides from other mammalian species as well as a conservation of the acidic amino acids (78).

CONCLUSIONS

There is little doubt that human milk serves a role in infant physiology much greater than being a mere supply of calories, essential amino acids, and other nutrients. The antibacterial properties of human milk are documented: lactoferrin acts in concert with the immunoglobulins to inhibit the growth of pathogenic species of \textit{E. coli} and other microorganisms; the \textit{Lactobacillus bifidus} factors stimulate the growth of Lactobacilli with the result that the pH of the infant’s intestinal tract is lower and does not serve as a good host for pathogens; lysozyme may act to destroy bacterial cell walls; and the large number of white blood cells results in biosynthesis of IgA and phagocytosis of pathogens. However, what remains to be done is an inquiry into how the human milk protects the breast-fed infant against systemic infection, as has been documented by a number of investigators (44, 101). It is known that, unlike the situation in the newborn calf, the human infant does not absorb significant amounts of immunoglobulins from his mother’s milk into his circulation; hence, the mechanism for such a resistance is probably unique.

Of the major proteins in whey of human milk, all seem well characterized. The primary structures of lactalbumin and lysozyme have been established, and the sequencing of lactoferrin seems to be well underway. The availability of the amino acid sequence of lactoferrin should shed considerable light on the mode of evolution of the non-heme iron-binding proteins of mammalian milks and serums, as well as the mode of iron attachment thereto. The latter information may have considerable bearing on the elucidation of the etiology of certain types of anemias and iron-storage diseases. No polymorphism has been discovered among whey proteins of human milk.

The casein fraction of human milk has not received the attention it deserves. Surprisingly, human milk contains a greater quantity of whey protein than it does of casein, and, in contradistinction to bovine casein, the most abundant component of the human milk casein fraction is \( \beta \)-casein. Its amino acid sequence should be elucidated in the near future, at which time it will become known whether the genetically-controlled polymorphism of \( \beta \)-casein does depend solely upon the amount of phosphorylation of the \( \beta \)-casein.

Much work remains toward characterization of \( \alpha \)-casein and \( \kappa \)-casein components of the casein fraction of human milk. The suggestion that two or more unique \( \kappa \)-caseins are in human milk merits further investigation as does the carbohydrate content and the oligosaccharide sequence of \( \kappa \)-casein.

An intriguing question without much attention is the evolutionary origins of proteins in human milk. In regard to lactoferrin and \( \alpha \)-lactalbumin, this question tentatively has been answered; lactoferrin shares a common ancestor with the other non-heme iron-binding proteins, transferrin and ovotransferrin, whereas \( \alpha \)-lactalbumin and lysozyme probably have arisen from a common ancestral gene. There is, however, no hint from where the caseins came, and the availability of their amino acid sequences should contribute toward the search for other human proteins that are their structural homologues.

In summary, human milk protein chemistry and physiology promise to be a fruitful field of research that in the future may yield information of fundamental biological importance.

ACKNOWLEDGMENTS

The author gratefully acknowledges the collaboration of W. F. Line, R. J. Miller, J. H. Nichols, and D. A. Sly, and of D. Grohlich in many of these investigations; the financial support of the National Institute of Child Health and Human Development (Grant No. HD-05657), the National Science Foundation (Grant No. GB-36008), and the Faculty Research Committee, Rush-Presbyterian-St. Luke’s Medical Center; and the Pediatrics Department, Evanston Hospital, Evanston, IL for their generous gifts of human milk and colostrum.
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