The nutritive and metabolic advantages of homologous milk

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In discussing whether or not homologous milk is an advantage to the young (i.e., whether feeding the young of a species milk of the same species confers an advantage over a substitute derived from milk of another species), we are still largely reduced to the old teleological arguments: the milk of a species is appropriate for the growth of the young of that species, therefore, growth and development on homologous milk are the optima and the standards by which alternatives are to be judged.

To some extent the development of scientific interest in human milk and breast-feeding must be credited to early attempts at making substitutes for maternal milk. This is true, also, for other species, such as those held in zoos, and thoroughbred racehorses. The primary function of mammalian milks is to feed the young of the species, but the recognition that milk contains numerous important, non-nutritive substances, including immune, growth and regulatory factors, has led to a tremendous increase in investigations, especially of human milk, its biological functions, and the lactation process, in the last 10–15 years (Peaker *et al.* 1984; Hamosh & Goldman, 1986; Howell *et al.* 1986; Goldman *et al.* 1987).

The interest in functions of human milk, other than its nutritional properties, has led to it being probably the best documented of all milks. With the exception of the major domestic dairy animals (cow, goat, sheep, buffalo, camel), the information base of milk composition is very poor. Composition information is available for about 200 of the more than 4000 mammalian species; in only about fifty-five of these have more than ten milk samples been analysed, often with poor documentation of sampling, so that nothing can be said about variability in composition (Oftedal, 1984; Gittleman & Oftedal, 1987; Oftedal & Jenness, 1988). The effects of milk on the young have been studied in even fewer species, so that most of the discussion relates to the effect of human milk on the human infant.

Colostrum

In almost all species for which sufficient information is available, duration of lactation has been found to be one of the most important determinants of variation in milk composition within a species. The stages of lactation are commonly identified as: (1) the initial or colostral secretion produced in the first few hours to days post partum; (2) mid-lactation milk, secreted at peak lactation, the duration of which varies widely among species (e.g. from 3 d post partum in the harp seal (*Phoca groenlandica*) up to 140 d in the Tammar Wallaby (*Dama pademelon*) (Oftedal, 1984). In humans, mid-lactation or 'mature' milk is usually regarded as 1–3 months post partum but can be sustained for over 1 year; (3) late-lactation milk produced in the often prolonged period of declining volumes as the young are weaned onto other foods (Mepham, 1987).

Of the relatively few species for which variation with duration has been documented, differences between colostrum and mid-lactation milk are usually the most marked, but both absolute values and direction of changes vary from species to species. For example, in the New Zealand white rabbit, there is a marked fall in fat in milk and a tripling of lactose content in the first 2–3 d after parturition, but little change in protein levels (Mepham, 1987). In contrast, in human milk, protein levels fall as much as fivefold,

Table 1. *Composition of human colostrum and mature milk (/l)*

	Colostrum	Mature milk
Energy: MJ	2·4	2·9
kcal	580	700
Lactose (mmol)	80	230
Glucose (mmol)	0·2	2
Protein (g)	90	8
Urea (mg)	100	300
Sodium (mmol)	60	10
Potassium (mmol)	18	16
β-Carotene (μg)	1120	230
Zinc (mg)	8	1
Copper (mg)	0·6	0·3

lactose increases by about 50%, but to a final concentration three times that in rabbit milk, and fat content changes little (McClelland *et al.* 1978; Casey & Hambidge, 1983; Bitman *et al.* 1986; Casey *et al.* 1986).

The changes in human milk are the best documented, and Table 1 gives the relation of various substances in colostrum to the level in mature human milk. Some of this change (e.g. fall in sodium and protein, rise in lactose and glucose) may be explained by the physiology of the onset of lactation (Neville *et al.* 1983). A proportion of the very high level of proteins in colostrum may be due to the presence of plasma proteins, including serum albumin, but it arises mainly from the very high levels of immunoglobulins (McClelland *et al.* 1978).

In the human, and other species for which there is information, the volume of material secreted by the breast in the early post-partum period is low in comparison with full production. The human mother produces about 100–400 ml colostrum in the first 48 h (Casey *et al.* 1986), compared with a full lactation of about 850 ml/24 h. In contrast, the dairy cow will provide about 3·5 litres colostrum at the first milking after parturition, compared with a full daily lactation of about 30 kg (Ternouth, 1986).

However, it should be noted that the very high levels of some substances in the immediate post-partum secretions and the fall with time do not appear to be entirely volume-related phenomena: the total daily excretion of many constituents reaches a maximum before volume production does (Casey *et al.* 1985); in an analysis of information from thirteen women, it was found that the decline in zinc concentration during the first week post partum did not correlate well with the rise in volume in individuals (Casey *et al.* 1989).

Thus colostrum may supply the infant with quite large amounts of protective substances (immunoglobulins, lactoferrin), essential trace elements (Zn, Cu) and other nutrients (β-carotene, protein). In feeding human infants, there are many customs and traditions in different parts of the world concerning the use of colostrum, from strong encouragement to outright taboo. Colostrum and precolostrum (the breast secretions obtained during pregnancy) have been used to combat diarrhoea due to *Escherichia coli* in infants (Larguia *et al.* 1977), and its use prophylactically has been advocated to protect sick and preterm infants from infection (Lewis-Jones & Reynolds, 1983). However, I am not aware of any studies showing an added advantage of colostrum to the infant who continues to be breast-fed.

Table 2. *Immunoglobulins in milks (mg/l)*

		IgG	IgA	IgM
Sheep:	Colostrum	60 000	2000	4100
	Milk	300	60	30
Cow:	Colostrum	79 000	4400	4900
	Milk	400	50	40
Goat:	Colostrum	58 000	1700	3800
	Milk	250	60	30
Rabbit:	Colostrum	2400	4500	100
Human:	Colostrum	500	90 000	1770
	Milk	280	1000	250

Immunoglobulins

Most species appear to provide some immune protection to their young, via the milk, which contains immune proteins (immunoglobulins, lactoferrin, complement), macrophages and other leucocytes, bacteriocidal enzymes (e.g. lysozyme) and specialized growth factors (e.g. Bifidus factor); a number of other components, such as some polysaccharides and fatty acids, may exercise a protective as well as a nutritional function (McClelland *et al.* 1978; Goldman *et al.* 1986, 1987; Howell *et al.* 1986; Porras *et al.* 1986). Species differ in the range and importance of milk-provided immune protection, with primate milks having the widest repertoire. Nonetheless, all milks contain immunoglobulins, with highest concentrations in colostrum but present throughout lactation (Lascelles, 1977). Table 2 shows the levels of different immunoglobulins in colostrum and mature milk from a number of species.

In species such as the sheep and cow, there is no immune transfer *in utero*, and colostrum is used to confer passive immunological competence on the young. These species have a colostrum in which IgG predominates. The gut of the neonate is permeable to immunoglobulins, which are taken up into the lymphatic system, for as much as 2–3 d after birth, by which time serum IgG levels have generally reached adult concentrations. Colostrum is essential for the health and survival of these types of species; diarrhoea leading to death, in association with low circulating immunoglobulin levels, may occur in up to 25% of newborn calves in some herds in the USA, emphasizing the need for an external source of passive immunity (Ternouth, 1986). Calves may continue to be protected after the colostral stage when milk immunoglobulins can act to create a local intestinal immunity; continuing access to 'mother's' milk is being advocated to improve survival in tropical herds (Hovell, personal communication).

This continuing protective effect of breast milk has been well documented in human infants. A number of studies in both Western and Third World countries provide strong evidence that breast-fed infants have a lower morbidity from gastrointestinal and upper respiratory tract infections than infants fed on other milks (Kramer, 1987).

Unlike cows and sheep, humans and other primates provide some passive immunity to their offspring by placental transfer of IgG before birth, and the predominant milk immunoglobulin is IgA. It is generally thought that the protective role of IgA occurs directly in the gut, forming an 'immunopaint' to prevent exposure of the mucosa, and hence of the immature immune system, to environmental pathogens, and possibly also to other antigenic proteins. Even in these infants, however, there is some uptake of intact immunoglobulins from the colostrum. Ogra *et al.* (1977) fed a bolus of colostrum to

healthy formula-fed infants and found IgA was detectable in the serum up to 24 h after birth. By 72 h, more than 70% of the total IgA ingested was recovered in the faeces.

Several other recent studies have reported the fate of ingested immunoglobulins and lactoferrin by breast-fed infants (Jatsyk *et al.* 1985; Schanler *et al.* 1986; Davidson & Lönnerdal, 1987; Prentice *et al.* 1987). (High levels of lactoferrin are found only in primate milks, where it appears to have a protective and anti-inflammatory role (Goldman *et al.* 1986), in addition to its nutritional function of enhancing iron and trace element absorption.) In general, results show a high recovery of ingested IgA in the stools of breast-fed infants in the first week, as much as 85% in one study (Davidson & Lönnerdal, 1987), falling to about 10% by 12 weeks after birth. Faecal excretion of ingested lactoferrin is much lower, about 6% at 1 week, falling to <1% by 12 weeks. In contrast, IgA is usually undetectable in the faeces of formula-fed infants during the first week, and even by 12 weeks total excretion is less than half that in the breast-fed infant. Faecal IgA in formula-fed infants appears to be of endogenous origin (Goldblum *et al.* 1987; Prentice *et al.* 1987).

Thus it is apparent that quite a high percentage of the relatively high intake of IgA from colostrum (30% of total protein intake on day 3) passes intact through the gastrointestinal tract of the neonate. Even by 3 months of age a significant proportion may still escape digestion, although by this time the total IgA intake is much reduced, to about one-tenth of the lower protein intake, but still appears to be sufficient to confer considerable protection on the infant.

Non-protein nitrogen

Milks from different species vary not only in the amount and types of proteins they contain, but also in the proportion of total N present as other non-protein nitrogenous substances (NPN) (Rassin *et al.* 1978; Oftedal, 1984; Mepham, 1987). Human milk is the best characterized; it contains about 26 mmol NPN/l, 20–30% of the total N, which is distributed as shown in Table 3 (Atkinson, 1985). With the exception of a few species such as the pig, mink (*Mustela* spp.) and horse which have 15–20% NPN, other milks contain less than 10% of the total N as NPN.

The functions of the relatively high concentrations of urea (up to 50% of NPN in human milk) and free amino acids in milks are not known. Rassin *et al.* (1978) found that in several species, particularly carnivores, the free amino acid content was greater than that of urea. Of the fifteen species which they studied, the β-amino acid taurine was found to be the first or second most abundant in eleven, ranging from 72% of total free amino acids in cat's milk to 2% in cow's milk.

Apart from its role in the conjugation and subsequent excretion of secondary bile acids, taurine appears to play an important role in the structural and functional integrity of cell membranes, especially those of the retina and central nervous system (Sturman & Hayes, 1980; Wright *et al.* 1986). This protective role may be in part asserted by

Table 3. *Distribution of non-protein nitrogen in human milk*

Total N	80 mmol/l
Non-protein N	26 mmol/l
Urea	50%
Creatinine	14%
α-Amino	26%
Amino sugars	9%
Ammonia	<1%

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functioning as an antioxidant. Taurine also plays a major role in osmoregulation, maintaining cell volume (Trachtman *et al.* 1988). In the cat, although some taurine is made endogenously, there is a definite dietary requirement. Kittens have a low rate of endogenous synthesis and can readily be made deficient by low dietary supply. Taurine deficiency has severe consequences, causing retinal degeneration, abnormal brain morphology and signs of cerebellar dysfunction (Wright *et al.* 1986).

Given the apparent importance of taurine for central nervous system development and function, considerable concern developed over its role in human nutrition, with the recognition that, whereas human milk was abundant in the amino acid (250 μmol/l), cow's milk, and especially cow's-milk-based preterm formulas, contained very low levels (<40 μmol/l) (Erbersdobler *et al.* 1984). Taurine depletion has been reported in patients on long-term intravenous nutrition; supplementation with taurine reversed the relatively minor disturbances of low plasma taurine and abnormal electroretinograms (Geggel *et al.* 1985). Studies with healthy full- and preterm infants are less conclusive. Supplementation of formulas caused an increase in plasma levels and urinary excretion of taurine, and a switch from glycine to taurine in bile acid conjugation. Plasma levels may reflect dietary intake, but do not reflect tissue levels which may be as much as 400 times higher. Renal clearance of taurine can be greatly depressed in face of a low dietary intake, providing a highly efficient conservation mechanism. Supplementation did not affect the rate of bile acid synthesis or the size of the bile acid pool, and there were no effects on fat absorption, growth, serum cholesterol or indices of protein metabolism (Gaull, 1983; Hayes, 1985; Chesney, 1988). It is probable that a healthy infant with an adequate intake of precursor amino acids (methionine and cysteine) can maintain adequate endogenous production, but a dietary supply may become essential under conditions of illness or extreme prematurity.

Compartmentation

For some substances, it is not only the amount in milk that is important for meeting the infant's needs, but equally significant is the physicochemical environment. Milk is usually divided into three main physical compartments: fat, casein precipitate or pellet, and the aqueous remainder or whey. The size and macromolecular character of these compartments will differ among species according to the macronutrient composition of the milk. Small solute molecules and ionic species, including minerals, will be distributed among these compartments according to the binding properties and physical chemistry of the macrocomponents (Lönnerdal, 1985*a*; Rumball & Baker, 1985). The binding properties of milk components and the relative amounts and digestibility of the macrocomponents in the gut of the infant, particularly hydrolysis of fat and proteolysis of caseins, can markedly affect the bioavailability of Zn, Fe, Ca and other minerals for absorption (Lönnerdal, 1985*b*).

The differences in the availability of Zn from human milk and cow's milk formulas have been well documented (Casey *et al.* 1981; Sandström *et al.* 1983). The use of stable isotopes has recently permitted direct measurement of Zn in infants; in preterm neonates, Ehrenkranz *et al.* (1986) found absorption of Zn added to their mother's milk was 53%, compared with 32% from a cow's-milk-based formula.

It was originally postulated that this difference in Zn bioavailability was due to the presence in human milk, but not cow's milk, of a low-molecular-weight Zn-binding ligand, possibly citrate (Lönnerdal *et al.* 1980). It is now appreciated that the situation is considerably more complex (Martin *et al.* 1984). In human milk, Zn is distributed evenly between the casein and whey fractions, with about 10% of the total in the fat fraction (Casey *et al.* 1987). It appears to be loosely bound and readily redistributed (Cousins &

Smith, 1980). In human milk the predominant casein is β-casein, which forms open micelles readily accessible to proteolytic enzymes. In cow's milk and formulas, about 80% of the Zn is associated with the casein pellet. Cow's milk caseins form tighter, less readily hydrolysed micelles; the higher phosphate content may also contribute to tighter mineral binding by the undigested curd, lowering the availability of minerals to the intestinal absorptive mucosa (Greenberg, 1986; Mepham, 1987).

If milk is expressed before being fed to an infant, care must be taken to maintain the physicochemical integrity. Expressed milk gradually loses carbon dioxide, with a consequent fall in pH and a redistribution of ionizable species such as Ca (Allen & Neville, 1983). The magnitude of this effect is unlikely to be of practical importance, but harsher treatment, pasteurization, caused a decline in Zn bioavailability sufficient to markedly affect Zn balance in preterm infants (Casey & Hambidge, 1985).

Mode of delivery

The apparent importance of the compartmentalization of milk constituents, and of maintaining the active physicochemical composition, highlights a major advantageous difference between species-specific and cross-species milk feeding: the mode of delivery of the milk.

Delivery of milk direct from maternal teat to infant mouth preserves a number of important functions which are lost when the milk is expressed, stored and treated. One important consequence of direct delivery is the opportunity it gives the infant to regulate his food supply, that is, the volume of milk ingested. Several recent studies have shown that the average rate of milk removal by the infant is an important control of the overall rate of milk production, after the first few weeks post partum (Dewey & Lönnerdal, 1986; Neville *et al.* 1988). At the more immediate level, there is quite a marked difference in the way a human infant takes a feed from the breast compared with the bottle. Lucas *et al.* (1981) found that while both feeds lasted about the same length of time, the breast-fed infant had a lower cumulative milk intake. From the bottle there was a steady increase in milk intake with time, with a slight slowing near the end of the feed. From the breast, there was a rapid increase in milk removal over the first 2–5 min, followed by a period of suckling with little or no milk removal, a break to change breasts, followed by another 5 min burst of milk removal, then several minutes of non-nutritive suckling. Whether these different patterns of milk ingestion have any physiological significance is unknown.

Milk is a 'live' substance, that is, it contains living cells and other bioactive substances which may be killed or rendered inactive by any sort of storage or processing. The bioactive factors include hormones, growth factors and enzymes (Howell *et al.* 1986; Koldovský & Thornburg, 1987).

As well as sloughed off mammary epithelia and cell debris, there are present in milk various types of leucocytes, including, particularly, neutrophils and lymphocytes. Cell numbers tend to be highest in colostrum and fall with duration of lactation, but leucocytes are found in milk at all times (Huang *et al.* 1984; Paape & Keller, 1985). Cells in human milk have various functional abilities that may enhance the host defence mechanisms of the neonate, but their exact role in either local intestinal or systemic immunity remains to be elucidated (Buescher & Pickering, 1986; Mandyla & Xanthou, 1986).

Hamosh *et al.* (1985) classified the enzymes in human milk into three groups. Some, such as lactose synthase (*EC* 2.4.1.22), Krebs cycle enzymes and phosphoglucomutase (*EC* 5.4.2.2), probably enter milk from epithelial cell wastage. They appear to have no

purpose in milk, simply reflecting the physiology of the functioning mammary gland. The second group of enzymes are those with a role in neonatal development, and these have specific, often protective, functions in milk and complement the physiology of the neonate. This group includes proteases and antiproteases, the latter protecting other milk proteins and enzymes from destruction by leucocyte and lysosomal proteases (Lindberg *et al.* 1982). Sulphydryl oxidase is another enzyme in milk which appears to have to function both in the milk and in the gastrointestinal system of the neonate, possibly to maintain structural and functional integrity of milk proteins, including immunoglobulins. It may also assist in the uptake of macromolecules by altering the physical state of the intestinal mucous diffusion barrier (Isaacs *et al.* 1984).

The third group includes two compensatory digestive enzymes present in human milk, α-amylase (*EC* 3.2.1.1) and bile-salt-stimulated lipase (BSSL). Full-term neonates have only about 0·5% of the amylase activity of the adult, and provision of α-amylase in milk assists the breakdown of oligosaccharides, about 15% of milk carbohydrates (Heitlinger *et al.* 1983). Similarly, fat digestion requires adequate lipase (*EC* 3.1.1.3) activity and concentration of bile salts. In the neonate, levels of both pancreatic lipase and bile salts are low. About 30% of dietary fat can be hydrolysed in the stomach by lingual and gastric lipases, but the breast-fed infant has an additional digestive enzyme in the BSSL ingested in the milk. This lipase, which has an optimum pH of 7–9, hydrolyses a wide variety of triglycerides to free fatty acids and glycerol in the small intestine. Bile salts are obligatory for its action, which hydrolyses up to 40% of milk fat. BSSL is produced in the mammary gland and is present in the milk by about 26 weeks gestation (Hamosh, 1988*a*). So far, it has been found only in the milks of humans, gorillas and carnivores (Freed *et al.* 1986).

Breast is best?

One can show very many differences in the composition of milks, and consequently in biochemical measures in the recipient young. Such differences are not necessarily of any importance in themselves; it is their possible longer-term effects on development and functioning that must determine the appropriateness or otherwise of a feeding regimen. In general, mammals, including the newborn, are fairly robust organisms and can utilize a wide range of food and nutrient intakes to fulfil their needs. (This does not, of course, apply to the sick or preterm neonate.) It is relatively easy now to choose or formulate a 'milk' that will provide the macronutrient composition for growth and gross body composition similar to that of homologous milk; but with humans (and, it must be acknowledged, expensive animals such as thoroughbred racehorses) more sophisticated outcomes are becoming of prime interest and importance, so that far more attention is being paid to the relative advantages of the finer details of milk composition.

In humans, outcomes of different types of infant feeding may be compared at three levels: short-term, relating to the immediate response to an individual feed; intermediate-term, the effects during infancy while the feeding regimen lasts; long-term, the follow-up throughout life after the end of the milk-feeding period.

Short-term differences, for example hormonal responses to a meal or the level of nutrients or metabolites in the plasma, are well documented for infants on different types of feeds, and some are covered by other speakers in this symposium. Differences in absorption of nutrients have been documented over the years, including poorer Ca and lower N and fat uptakes from early cow's milk formulas (Southgate *et al.* 1969), and more recently the problems discussed with Zn. These differences have led to overt, clinical problems in the formula-fed infant, and have been corrected as they have been discovered by appropriate changes in formulation.

Differences in intermediate outcomes are well documented at the population level.

The growth rate and pattern in breast-fed infants are different from those of current widely used growth standards, which were largely made from growth of children receiving older types of cow's-milk-based formulas. Typically, breast-fed babies grow more rapidly than the standards during the first 2–4 months, and then growth slows to a greater extent, so that after 4 months breast-fed infants cross down centiles (Waterlow *et al.* 1980; Whitehead & Paul, 1981; Salmenperä *et al.* 1985). The effect of changing feeding patterns in a society is nicely illustrated in a study from Australia by Hitchcock *et al.* (1981). They found that over the first year, infants born in 1980 had the same rate of weight gain as those in 1933, both groups being predominantly breast-fed, but the cohort born in 1964, a largely formula-fed group, were heavier at all ages after 4 weeks post partum.

The most important intermediate-term benefit of breast-feeding is the protection it bestows on the infant against infection. The evidence from developed countries, where exposure to pathogens is minimal, is perhaps equivocal (Bauchner *et al.* 1986; Leventhal *et al.* 1986). In poorer communities it is often difficult to distinguish between the protective properties of breast milk *per se*, and the protection afforded by breast-feeding from exposure to contaminated and inadequate feeds and contaminated water. However, epidemiological studies have shown that breast-feeding strongly protects against the development and severity of diarrhoea (Kramer, 1987). Several well controlled population-based studies have produced evidence that breast-feeding has a significant effect in lowering infant mortality in developing countries (Plank & Milanesi, 1973; Goldberg *et al.* 1984; Habicht *et al.* 1986).

These advantages are obviously very important during infancy, and the protective effect probably continues into early childhood whilst some breast milk continues to be given. However, the real test of the advantages of a homologous milk must lie in its long-term effects on the organism, in the impact on health and mortality patterns in later life. Are there any differences in fitness for function in humans—the important question is does type of infant feeding affect brain development: in the long term, do people who were breast-fed as infants have a better intellectual capacity or respond better to their environment; and, the ultimate Darwinian test, do offspring fed on milk of their own species have the advantage when it comes to continuation of the species?

In a large follow-up study of all the children born in Dunedin in 1972–3, Birkbeck *et al.* (1985) found that by 7 years of age there were no differences in weight or fatness of children according to type of infant feeding. Children breast-fed as infants were taller at 7 years, but had been larger at birth and had taller mothers. A number of other studies have attempted to investigate the influence of infant feeding on fatness and serum cholesterol levels, indices of risk of cardiovascular and other degenerative diseases, later in life. Study designs have included both prospective and retrospective, with follow-up periods of 1–32 years. Results have been generally inconclusive (Hamosh & Hamosh, 1987; Hamosh, 1988*b*).

To properly answer questions on the impact of early nutrition on later health and development would require an immensely complex study design, taking into account factors such as maternal and prenatal nutrition, a proper measure of total intake of breast milk, duration of breast-feeding, introduction of other foods, diet after weaning, genetic variability, and other factors known to influence the outcome of interest. Until such a study becomes possible, we are left with the conviction that homologous milk ought to be better for the neonate, from both the teleological and the aesthetic standpoint.

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