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THE SECOND JOHN SOOTHILL LECTURE. BREASTFEEDING, INFECTIONS AND IMMUNOLOGY

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HISTORICAL NOTES ON BREASTFEEDING

Today none of us doubts the advantages of breastfeeding nutritionally, psychologically, as well as immunologically. It is obvious that, if at all possible, an infant should be exclusively breastfed directly from birth on.

It is remarkable that there are so many sources in history advocating that especially colostrum is unsuitable for the infant. The Indian document *Susruta Samhita* from the fourth to the second centuries B.C. prescribes that the newborn's mouth is to be cleaned with ghee, which is cleared butter, and salt. Then it should be given three times daily honey, ghee with certain roots and leaf juices till lactation starts after a few days (1). Similar customs are still common in parts of the Indian subcontinent (1, 2). Soranus of Ephesos, living around AD 100 recommended boiled honey, possibly with goat's milk, but preferred not to give the newborn anything for the first two days. The mother's milk was unsuitable for the first 20 days in his view and he attacked a colleague who advocated immediate breastfeeding (1). Soranus was quoted through the centuries and it seems that it took a long time before physicians and others contemplated the idea that one reason for the very high infant mortality of that time could be that breastfeeding in some areas was scarce. A Liverpool surgeon, W. Moss wrote in 1781 that problems of neonatal diarrhoea decreased if the babies were put to the breast (3). The high infant

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mortality in Sweden in the late 18th century made the authorities start a school for midwives where also the advantages of breastfeeding were taught. Data from the next several decades showed that in some parts of Sweden where breastfeeding was rare, infant mortality due to diarrhoea was much higher during the summer months than in areas where breastfeeding was the rule (4). This may be the first relatively good evidence for the capacity of human milk to protect against infections.

MODES OF FEEDING IN PAKISTAN, A DEVELOPING COUNTRY

With the aim of describing the prevalence and forms of breastfeeding today in a developing community with many infections a field study was carried through (5). All children born during two years among 1000 families in each of three poor areas in and outside of Lahore, Pakistan were followed. These children from a village, a mud hut area and an urban slum were compared with those of an upper middle class group in Lahore; altogether there were 1476 children.

First it was realized that exclusive breastfeeding was quite uncommon (6). It was seen only initially in the village in about 18% and in the mud hut area in some 10% (Fig. 1a). Partial breastfeeding was the major mode of feeding among the three poor groups, whereas artificial feeding was most common in the upper middle class group (Fig. 1b). It was noted that exclusive breastfeeding became less prevalent during the hot summer months. At the same time intake of other fluids increased, due to the mothers' belief that the children require extra water during that time, something that does not find support in studies of ours and others (7). It was remarkable that commercial formulas were rarely used in the village and just in a few percent in the urban slum and mud hut area (Fig. 2a). Only in the upper middle class group were commercial formulas common, but still they usually amounted to less than 50%.

Determining instead the prevalence of the use of the bottle gave a very different view (6). Just about every child not fed exclusively with human milk was given their food by a bottle (Fig. 2b). Via the bottle they were most often fed buffalo milk, in many instances diluted with water, which often may have been contaminated.

It is obvious that commercial formulas per se do not provide a major route of infection in these poor population groups. However, the extensive use of the bottle mostly with other foods, brings a risk in areas where the water is not always potable, the educational level low and the hygiene poor.

In this situation we have tried to promote breastfeeding in a group of 300 poor mothers in the village and the urban slum (8). A special team from the project visited each pregnant mother and used various educational materials to illustrate the advantages of exclusive breastfeeding. These activities turned out to be very efficient according to a preliminary analysis. The rate of breastfeeding increased so that there was almost no control group of non breastfed left. Thus at 1 month of lactation exclusive breastfeeding had increased by about 40% both in the village and the city slum compared to the field study. Partial breastfeeding also increased by some 40%.

Another effect was that breastfeeding was initiated much earlier in this group, than in those of the field study where e.g. only about 50% of the newborns had received any human milk by 48 hours of age (2,6). The promotion of breastfeeding increased the percentage of neonates started at 24 hours of age from 15% to about 75% in the city slum and from 5% to around 90%

in the village. These observations suggest that it may be possible to enhance considerably breastfeeding in poor populations with a high morbidity and mortality in infections and little exclusive breastfeeding.

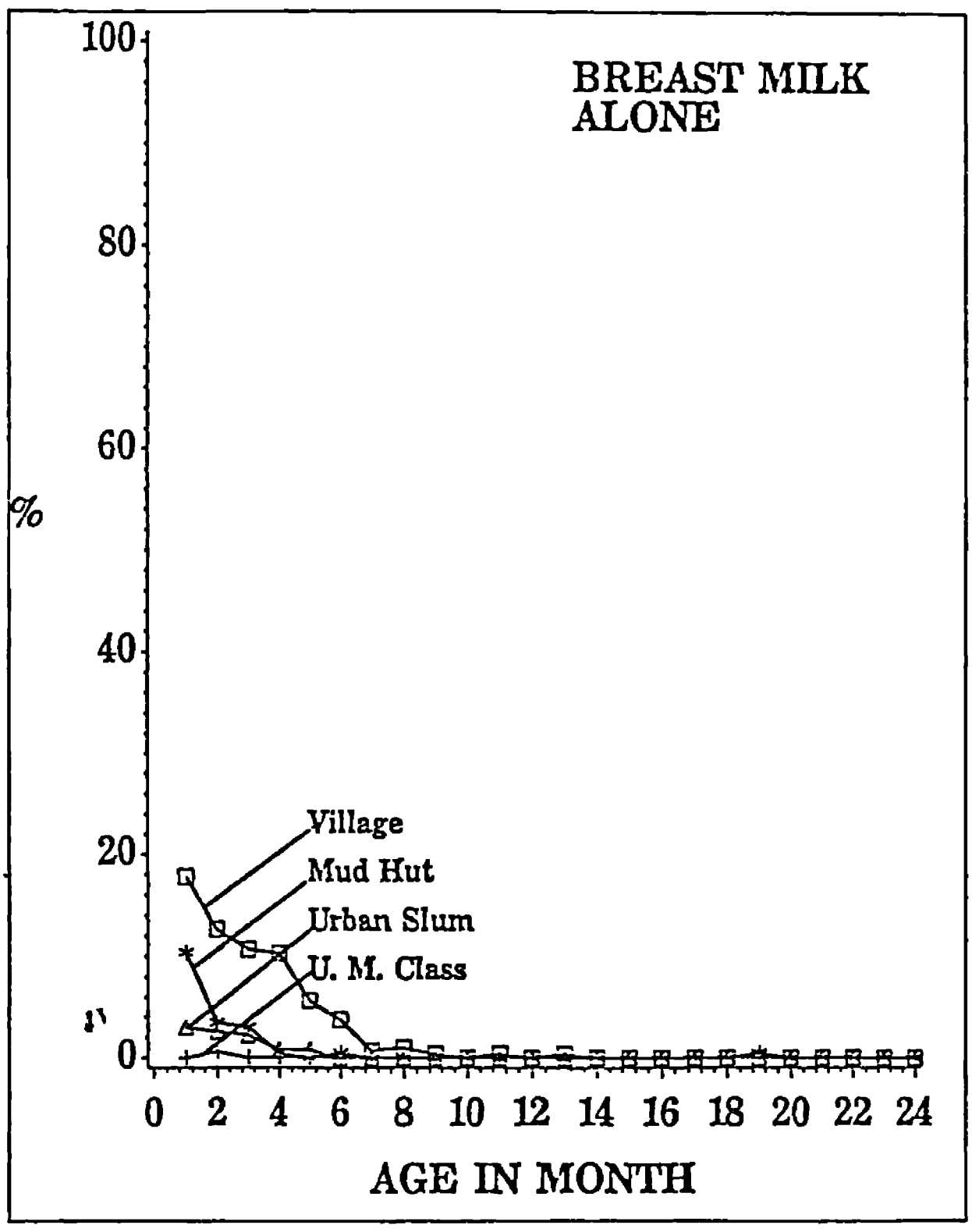

Fig. 1a. Exclusive breastfeeding in relation to age in months in the four population groups studied.

BREASTFEEDING AND THE PROTECTION AGAINST INFECTIONS

Numerous studies have analyzed the capacity of human milk to prevent, or at least ameliorate, the cause of infections. Critical reviews have shown that the methodological problems with such studies are numerous and that it is difficult to control for all the confounding factors (9-12). Still they found good evidence for breastfeeding protecting against morbidity and mortality caused by diarrhoeal diseases.

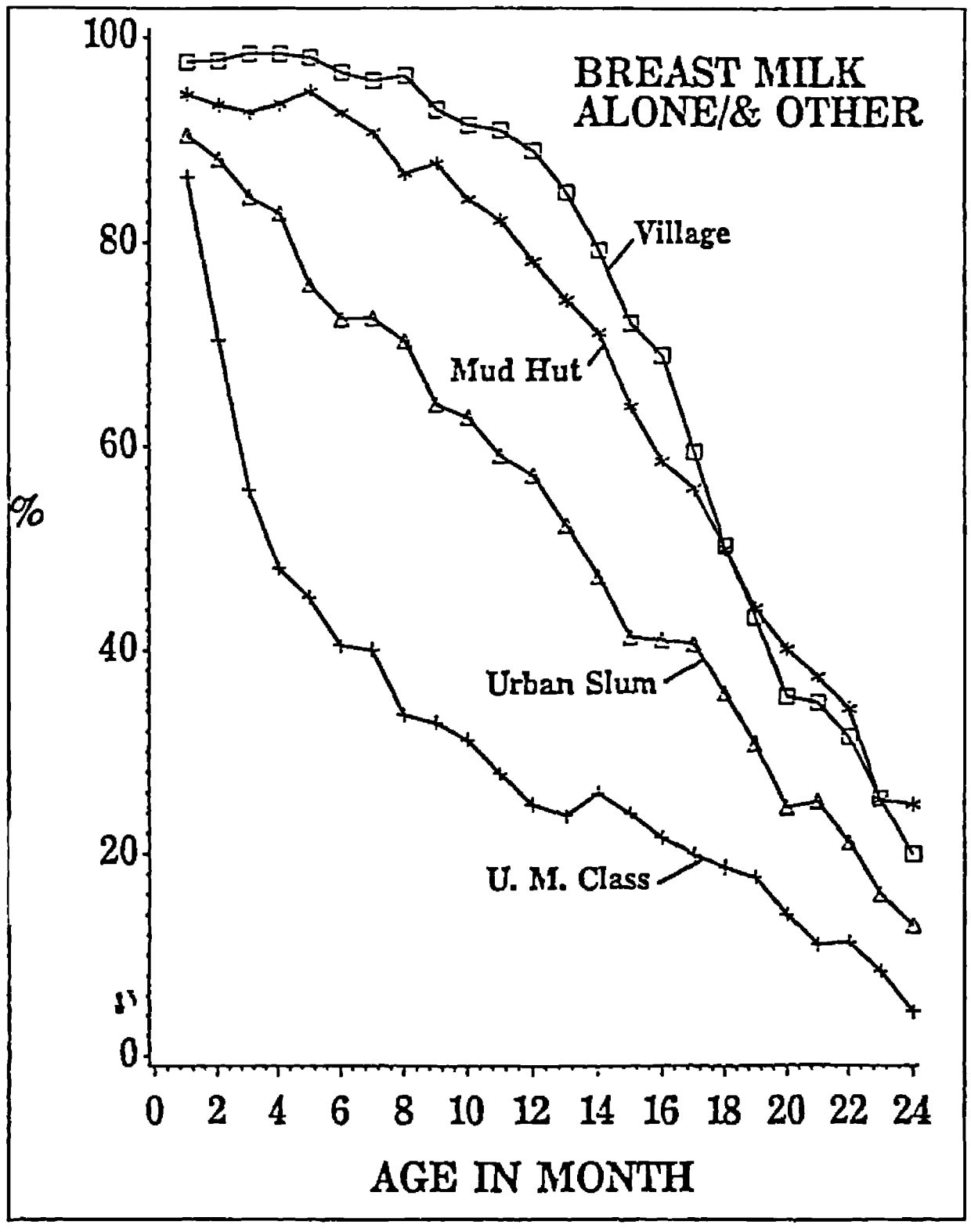

Fig. 1b. Breastfeeding, partial as well as exclusive in relation to age in the four population groups.

Recently a study of the prevalence of gastroenteritis was performed in relation to the mode of feeding in the four Pakistani population groups referred to above (5,6). Protection against gastroenteritis was seen for the first 24 months of life among the breastfed infants (13). This was observed in spite of the fact that the majority was only partially breastfed as previously demonstrated (6). Protection was evident even during the hot summer with increased risk of diarrhoea when breastfeeding decreased in connection with the feeding of additional fluids as mentioned above (6). Determining the protective efficacy, i.e. the reduction in incidence of diarrhoea in percent, showed striking effects of breastfeeding. Thus the efficacy among the youngest infants was 60-80% in the three poor groups, the village, mud hut area and urban slum. The protection decreased with age, but remained at 10-25% even at 24 months of age (Table 1). Protection was also seen in the upper middle class group up towards 40% in the youngest infants and down to 10% at 24 months.

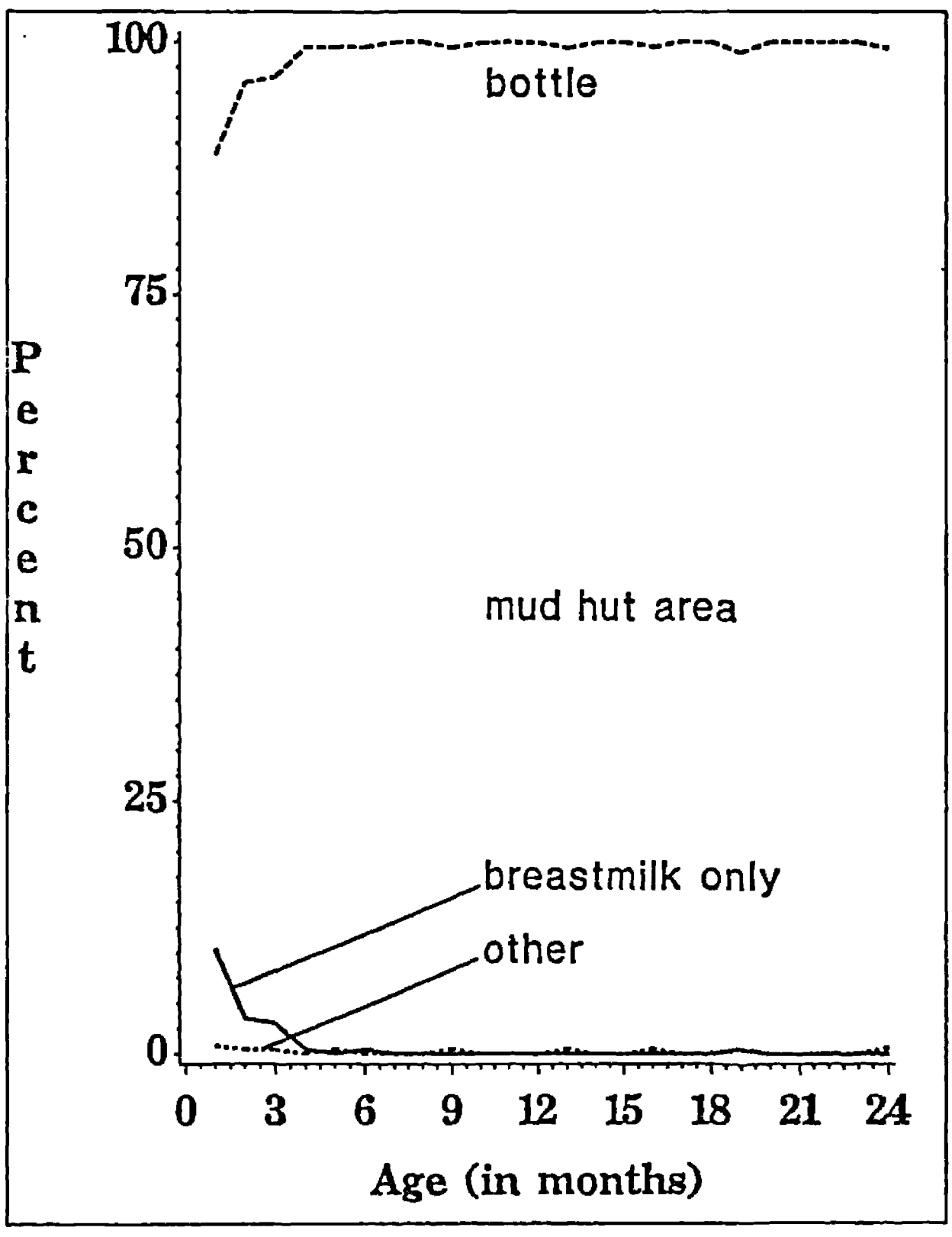

Fig. 2a. The extent of the use of commercial formula in the mud hut area.

It was suggested from an early investigation from Sweden that breastfeeding may decrease the risk of neonatal sepsis (14). We have recently analyzed this possibility in a high risk population in Pakistan (15). Excluding a number of potentially confounding factors such as sex, socioeconomic situation, hygienic and other conditions at delivery etc, the only factor that differed between the 42 cases of neonatal sepsis and the 270 controls was the mode by feeding. Exclusive breastfeeding hardly existed and partial breastfeeding was predominant. Many more controls than cases were partially breastfed and the odds ratio for protection by breastfeeding against neonatal sepsis was 18. The non breastfed were mainly given buffalo milk after the initial honey, ghee and herb extract traditionally given in this region.

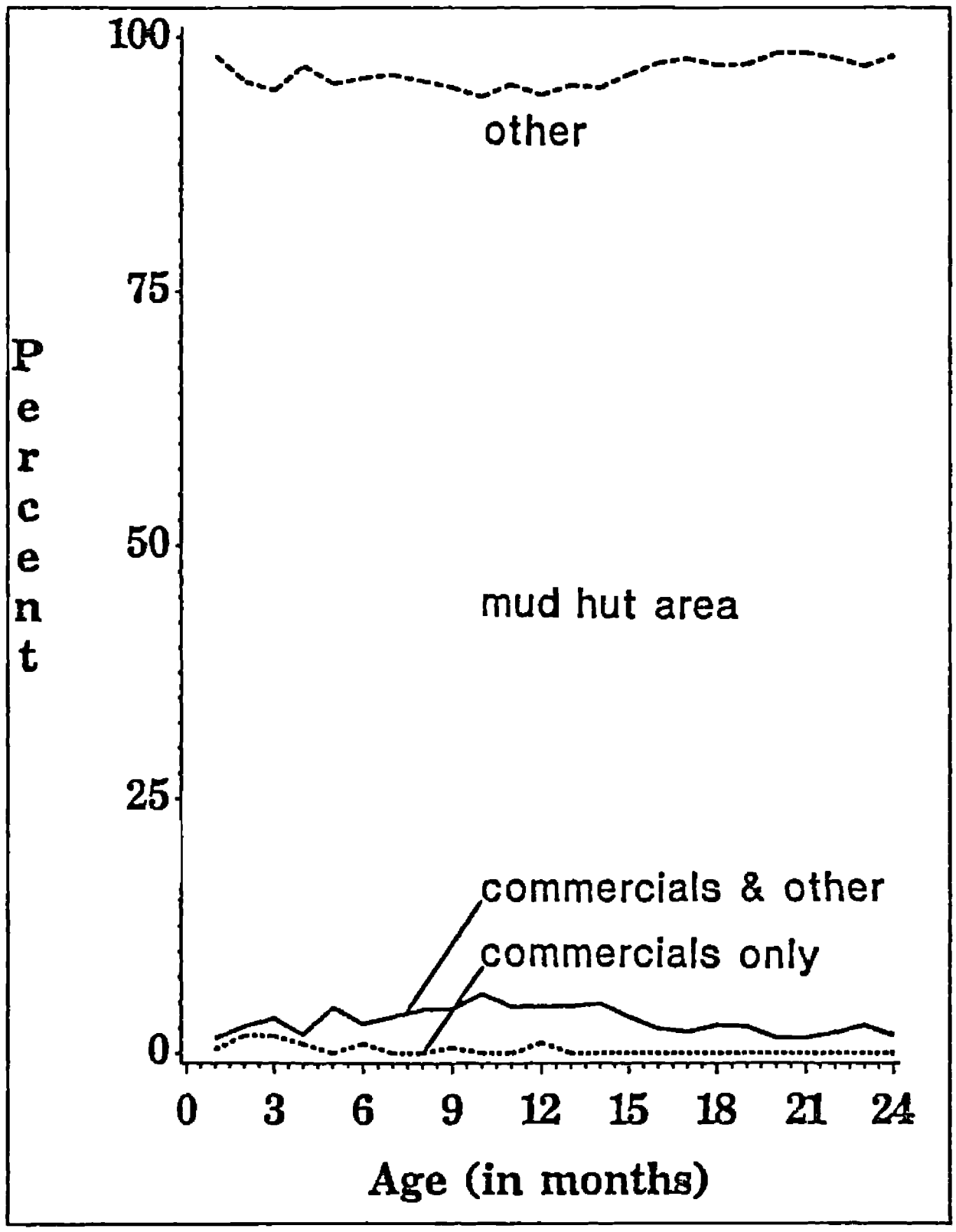

Fig. 2b. The extent of the use of bottle feeding in the mud hut area.

In a recent review it was stressed that by now there is good evidence that breastfeeding protects against gastrointestinal and lower respiratory tract infections, otitis media, bacteremia, meningitis (16), and necrotizing enterocolitis (17).

Table 1

Efficacy of the protection of breastfeeding against diarrhoeal disease in poor population groups compared to a upper middle class control, Lahore, Pakistan.

	Efficacy of protection* (%) at various ages (months)		
	1	12	24
Village and mud hut area**	62	24	22
Urban slum	70	10	18
Upper middle class control	32	24	10

* Efficacy (E) of protection is the ratio between the incidence rate of diarrhoea in the breastfed infants (rB) and the non-breastfed infants (rC);

$$E=(1-\frac{rB}{rC})X10$$

The underlying observed values have been smoothed by a third degree polynomial function.

** These two areas are combined since the results are so similar.

HOST DEFENCE FACTORS IN HUMAN MILK

How can partial breastfeeding have the capacity to prevent neonatal sepsis so strikingly in children so heavily exposed to microbes? How can partial breastfeeding prevent up to 70-80% of diarrhoeal diseases in such populations? This is especially surprising considering the many pathogens which can cause diarrhoea in this region, together with the fact that human milk does not seem to be so efficient in protecting against such a common causative agent as rotavirus. Below a few aspects on potential protective factors are reviewed.

BREASTFEEDING AND EFFECTS ON THE INTESTINAL GRAM-NEGATIVE BACTERIAL FLORA

It was striking that the Pakistani infants were quickly colonized in the gut with aerobic gram-negative bacteria after birth (18). Many Pakistani infants delivered both normally and by caesarian section were colonized already the first day of life. In contrast, the Swedish infants were not always colonized with enterobacteria even on their fifth or sixth day of life. Whereas the Swedish infants usually yielded only one kind of enterobacteria in their stool cultures, mainly *Escherichia coli* or *Klebsiella* the Pakistani infants had a more variable flora; e.g. they were more frequently colonized with *Proteus*, *Klebsiella* and *Citrobacter*. Breastfeeding from the first day of life, which was rare, reduced colonization with such bacteria. It is obvious that the heavy microbial exposure of the Pakistani infants from the milieux and the initially given foods and fluids (2) play a major role in creating an intestinal flora which can be an important source of the pathogens causing neonatal sepsis and diarrhoea, which are predominant causes of the neonatal mortality in the South Asian region studied by us (19).

In addition to the influence of breastfeeding on intestinal colonization, other effects on the flora are also notable. Aerobic Gram-negative bacteria from the intestine of breastfed infants are more sensitive to bactericidal antibodies than are those from formula fed infants (20). This suggests a decrease of virulence of the bacteria. A similar conclusion might be drawn from recent observations on the effect of breastfeeding on *E.coli* 083 given orally to newborns during the first week of life (21). This strain colonizes infants very efficiently. The *E.coli* 083 from the breastfed infants were often carrying type 1 fimbriae, which do not seem to be a virulence factor. Rather they bind via mannose residues to the carbohydrate moiety of secretory IgA (22), of which milk is a rich source. The colonization of the type 1 fimbriated 083 was in fact favoured by breastfeeding (Table 2). As a consequence of the enhanced fimbriation the *E.coli* 083 from the breastfed infants also adhered better to colon epithelial cells (Table 2). This does not have to be a sign of virulence since secretory IgA antibodies prevent the adhesion and since such type 1 fimbriated bacteria are quickly fagocytized when exposed to granulocytes (23). This occurs because the type 1 fimbriae bind to mannose residues on the fagocytes which therefore can quickly engulf and kill the bacteria.

THE ANTI-INFLAMMATORY CAPACITY OF HUMAN MILK

Host defence mediated by human milk does not seem to depend on inflammatory mechanisms (24,25). In milk there is little complement and e.g. the coagulation system, fibrinolytic factors and kallikrein are virtually absent. Mature milk has a low concentration of complement- activating IgM and IgG antibodies, but much of secretory IgA which does not activate complement. The leukocytes found in milk are quite refractory to bacterial ligands such as N-formylmethionyl peptides and the chemoattractant anaphylatoxin.

Instead human milk contains growth factors which may augment mucosal barriers. Not only secretory IgA antibodies, but also receptor analogues such as oligosaccharides and glycoconjugates from the milk can prevent the adherence of microbes to mucosal membranes,

e.g. in the gut. This prevents inflammatory tissue reactions which would be initiated if the microorganisms manage to attach to mucosal epithelium and start an infection.

Table 2

Recovery of type 1 fimbriated *E. coli* 083 from colonized breastfed and formula fed infants.

	No of infant	No of samples	Recovery of type 1 fimbriated 083	Adherence to gut epithelium bacteria/cell mean (SEM)
Breastfed	8	45	46%	18 (1.4)
			$p < 0.05$	$p < 0.05$
Formula fed	9	40	24%	11.6 (2.7)

* HT 29 colon epithelium cell line.

Secretory IgA and lysozyme decrease the response of neutrophils to certain chemoattractants. Lactoferrin inhibits the complement system. The milk also contains a number of factors which act as anti-oxidants, such as catalase, lactoferrin, glutathion peroxidase etc as reviewed recently by Goldman et al (25).

It may be that the human milk prevents the microbial flora, especially in the gut, not only from initiating infections, but also from causing inflammatory responses. Bacterial components, especially endotoxin or LPS, can induce production and release of cytokines such as Interleukin-1 (IL-1), IL-6 and TNF from macrophages and intestinal epithelium. We have been interested in the question whether or not the colonization with gram-negative bacteria in the gut of newborns and their release of free LPS induces cytokine production in the gut mucosa. Could it even be that such LPS-induced release of e.g. TNF can explain catabolic effects in the neonate, even part of the early weight loss?

To study this in vitro we exposed human cell lines of macrophages (U937) and of colon epithelium (HT29) to LPS in the presence and absence of various protein fractions of human milk and determined the release of IL-6 with a bioassay. Our preliminary data show that human, but not bovine lactoferrin, could decrease the release of IL-6. This may agree with recent work demonstrating that lactoferrin, both can bind to LPS and in a complex with LPS via a LPS receptor can also bind to macrophages. In addition lactoferrin can bind directly to macrophages (26). It is thus possible that one role of human milk lactoferrin may be to depress the infant's potentially harmful cytokine response to LPS.

THE MAIN ANTIBODIES OF HUMAN MILK: SECRETORY IGA

The predominant antibody in human milk, the secretory IgA is common to all parts of the mucosal defence system in the body. Actually, secretory IgA was first discovered in human milk (27). The entero-mammaric link results in a close connection between the mucosal immune system in the gut and the mammary gland. The homing of lymphoid cells to the mammary gland after antigenic exposure in the Peyer's patches explains the presence in milk of IgA antibodies to numerous microbial and food antigens (28). Secretory IgA responses are usually rather short-lasting. Still milk samples contain IgA antibodies against so many different antigens that it seems unlikely that the mother can recently have met them all. The fact that we find quite high avidities of milk IgA antibodies against e.g. diphteria toxoid and *E.coli* O antigens (29) may be due to that milk antibodies are made by memory cells which produce a rather mature antibody response with high avidity antibodies in the mammary gland. Actually the avidity of milk antibodies to *Vibrio cholerae* LPS in Pakistani women did not even increase after parenteral vaccination, suggesting an already mature response (30). Presumably the memory cells are homing to the glands via effects of the lactogenic hormones as originally shown in mice (31).

The only human milk component that has been proven to be protective per se is secretory IgA. Secretory IgA antibodies in milk have been related to protection against *V.cholerae*, ETEC and *Campylobacter*-induced diarrhoea in breastfed infants (32,33,34).

In previous studies with Dr Cruz at INCAP, Guatemala we have not found evidence for impaired milk IgA antibody levels or responses in protein undernourished mother compared to priviledged controls (35,36,37). More recently we found, however, that milk antibody avidities were lower in Pakistani than Swedish mothers (29). These Pakistani mothers were not overtly undernourished, but it cannot be excluded that nutritional deficiencies still may be the most likely explanation for the difference. Actually Dr Cruz will report a study at this meeting which indicates a possible difference in milk antibody avidity comparing undernourished mothers supplemented with a high or a low calory intake. This difference at the end of 20 weeks of supplementation might indicate an effect, although minimal, of undernutrition on the quality of the antibodies as to their binding capacity, or avidity.

However, there might be other explanations since we also found lower antibody avidities in colostrum from Costa Rican than Swedish mothers. However, in the mature milk they were not different. There is no evidence of nutritional differences between these mothers.

VACCINATION AND HUMAN MILK

In previous studies we have noticed that parenteral vaccination of mothers e.g. with *V.cholerae* or poliovirus vaccine quite consistently increase milk secretory IgA antibody titres as well as serum titres (38-40). In contrast, live peroral vaccines against poliovirus and typhoid bacteria, or a food protein, very often decreased preexisting milk antibody titres (37,39,40), although the serum antibodies increased (38,39,40). The explanation is still not known.

Pakistani mothers have high neutralizing capacity against poliovirus in colostrum (41). If oral live poliovirus vaccine is given on the day of birth as presently adviced by WHO it is likely that this vaccine dose of virus may be neutralized if the mother starts breastfeeding at once. On the

other hand colostrum is presently not given in many traditional societies as discussed above, even in newborns who are to be breastfed. Advocating immediate start of breastfeeding as we should, there may be an increased risk of interference with the first dose of oral poliovirus vaccine.

In a preliminary study we noticed that breastfeeding seemed to enhance vaccine responses in the infant (42). Actually the study was performed to investigate whether or not there would be a difference in the vaccine response in infants given a low (1.1 g/100ml) or conventional (1.5 g/100 ml) protein formula. There was no difference so the formula groups were combined and compared with the breastfed control group. The breastfed infants produced more secretory IgA antibodies than the formula fed in the saliva against the parenteral tetanus, diphtheria and peroral poliovirus vaccine. The fecal IgM antibody responses to the tetanus and poliovirus vaccines were also higher among the breastfed than the formula fed infants. This was seen in the responses shortly after the vaccination as could be expected for the short lasting local mucosal antibody production. The serum IgG antibodies to diphtheria toxoid and the serum neutralizing activity against poliovirus were significantly higher in the breastfed than the formula fed groups at 21-40 months of age.

The explanation how breastfeeding could enhance vaccine responses in the infant is not obvious. Growth factors in the milk could for instance be important. Another possibility occurs from our studies of anti-idiotypic antibodies to poliovirus. We find such antibodies in human serum, including cord serum and commercial immunglobulin preparations (43). We have proposed that such antibodies passing via placenta can initiate antibody production to poliovirus in the fetus. This would explain how we can find secretory IgA and IgM antibodies to poliovirus in newborns in Sweden, where vaccination with inactivated poliovirus vaccine has eliminated both wild and vaccine virus strains. Such antibodies are also found in neonates of mothers who lack them due to hypogamma-globulinemia or IgA deficiency (44). In the hypogammaglobulinemia mothers the stimulating anti-idiotypic antibodies should come from the immunoglobulin prophylaxis given to protect them.

We also find anti-idiotypic antibodies to poliovirus in human milk (43). With a similar reasoning as above we would like to suggest that the anti-idiotypic antibodies present in human milk might enhance the vaccine responses of the breastfed infant by priming its immune system. Actually, there is support for such a concept from studies in mouse models (45,46).

INFANT MORTALITY, BIRTH RATES AND BREASTFEEDING

It is quite clear that breastfeeding can decrease morbidity and mortality among infants. A change in nursery routines with increased breastfeeding in rural Costa Rica decreased mortality in diarrhoea, pneumonia, bacteremia and meningitis fourfold (47). It is obvious that by promoting breastfeeding the lives of many children can be saved and the morbidity of the survivors can be decreased.

However, according to the disturbing note by Maurice King in The Lancet last year we may by saving lives in many poor areas only "add to the man-years of misery" because of the drastic population increase which ultimately results in ecologic destruction (48). Due to these ecological effects the increasing population can no longer support itself. One conclusion of Dr King is that

we perhaps should not try to decrease infant mortality indiscriminately. This is a serious and important debate about one of the major problems of our time. However, we think there is a significant factor left out in this debate: breastfeeding does not only decrease morbidity and mortality but it is also anti-conceptional. Breastfeeding may prevent more births than all family planning programs together in the world according to UNICEF. There are good data to show the importance of breastfeeding to decrease fertility or fecundity in developing countries (49). Demographers, as well as Dr King, doubt that there is a connection between the decrease of infant mortality and birth rates. Such a lack of relation seems strange when breastfeeding so clearly can decrease infant mortality as well as birth rates. Furthermore, with the increased spacing between births that results from breastfeeding, there is also a decreased mortality (49,50). Also, it has been shown that with smaller family size the risk of death in infections may decrease, presumably due to less crowding. These factors taken together makes it obvious that promotion of breastfeeding is an urgent and important task.

CONCLUSIONS

Exclusive breastfeeding sems to be much less common than partial breastfeeding with a delayed onset in many poor societies. Promotion of breastfeeding was quite successful in such a community in Pakistan. Breastfeeding clearly protects against gastroenteritis, neonatal sepsis, lower respiratory tract infections, otitis media and necrotizing enterocolitis. Promotion of breastfeeding could save many lives, but would also prevent many births due to the anti-conceptional effect of breastfeeding.

The host defence factors of human milk are not all well defined. The predominant antibody, the secretory IgA is important for defense and the major whey protein lactoferrin may be anti-inflammatory by preventing LPS induced cytokine release in the infant. Many other milk components are anti-inflammatory.

Protein undernutrition does not have striking effects on the amount of milk antibodies produced, but some data suggest that the binding capacity, or avidity of the antibodies may be affected, although only slightly.

Breastfeeding influences the intestinal flora, both by decreasing colonization with potential pathogens and possibly by decreasing microbial virulence.

Breastfeeding also seems to enhance the vaccine responses of infants.

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REFERENCES

1. Fildes VA. Breast, Bottles and Babies, a History of Infant Feeding. Edinburgh University Press, Edinburgh, 1986.
2. Hanson LÅ, Adlerberth I, Carlsson B, Jalil F, Karlberg J, Lindblad BS, Mellander L, Khan SR, Hasan R, Sheikh AK, Söderström T. Breastfeeding in reality. In: Hamosh M, Goldman AS, Eds. Human Lactation 2. Maternal and Environmental Factors. Raven Press, New York, 1986:1-12.
3. Moss W. An essay on the management and nursing of children in the earlier periods of infancy. London, 1781.
4. Brändström A. The loveless mothers. Acta Universitalis Umaniensis, Umeå. Studies in the Humanities 62, 1984. (In Swedish with English summary).
5. Jalil F, Lindblad BS, Hanson LÅ, Khan SR, Carlsson B, Zaman S, Karlberg J. Early child health in Lahore, Pakistan. I. Study design. In manuscript.
6. Ashraf RN, Jalil F, Khan SR, Zaman S, Karlberg J, Lindblad BS, Hanson LÅ. Early child health in Lahore, Pakistan. IX. Feeding patterns. In manuscript.
7. Ashraf RN, Jalil F, Aperia A, Lindblad BS. Additional water is not needed for healthy breastfed babies in a hot climate. In manuscript.
8. Ashraf RN, Jalil F, Zaman S, Karlberg J, Lindblad BS, Hanson LÅ. The effect of promotion of breastfeeding in a Pakistani village and city slums. In manuscript.
9. Feachem RG, Koblinsky MA. Interventions for the control of diarrhoeal diseases among young children: promotion of breast-feeding. *Bull WHO* 1984; 62:271-291.
10. Jalil F, Adlerberth I, Ashraf N, Carlsson B, Hanson LÅ, Karlberg J, Khalil K, Lindblad BS, Nazir R, Zaman S, Khan SR. Methodological problems in assessment of long-term health outcomes in breastfed versus bottle-fed infants. In: Atkinson SA, Hanson LÅ, Chandra RK, Eds. Human Lactation 4. Breastfeeding, Nutrition, Infection and Infant Growth in Developed and Emerging Countries. ARTS Biomed Publ, St John's 1990; 381-394.
11. Bauchner H. Breastfeeding and infections: methodological issues and approaches. In: Atkinson, SA, Hanson LÅ, Chandra RK, Eds. Human Lactation 4. Breastfeeding, Nutrition, Infection and Infant Growth in Developed and Emerging Countries. ARTS Biomed Publ, St John's 1990; 395-404.
12. Victora CG. Case-control studies of the influence of breastfeeding on child morbidity and mortality: methodological issues. In: Atkinson SA, Hanson LÅ, Chandra RK, Eds. Human Lactation 4. Breastfeeding, Nutrition, Infection and Infant Growth in Developed and Emerging Countries. ARTS Biomed Publ, St John's 1990; 405-418.
13. Jalil F, Mahmud A, Ashraf RN, Zaman S, Karlberg J, Hanson LÅ, Lindblad BS, Epidemiology of breastfeeding and diarrhoea in a developing country. In manuscript.
14. Winberg J, Wessner G. Does breast milk protect against septic-aemia in the newborn? *Lancet* 1971; i:1091-1094.
15. Ashraf RN, Jalil F, Zaman S, Karlberg J, Khan SR, Lindblad BS, Hanson LÅ. Breastfeeding and protection against neonatal sepsis in a high risk population. *Arch Dis Childh* 1991; 66:488-490.

16. Cunningham AS, Jelliffe DB, Jelliffe EFP. Breastfeeding and health in the 1980s. A global epidemiologic review. *J Pediatr* 1991; 118:659-666.
17. Lucal A, Cole TJ. Breast milk and neonatal necrotizing entero-colitis. *Lancet* 1990; 336:1519-1523.
18. Adlerberth I, Carlsson B, de Man P, Jalil F, Khan SR, Larsson P, Mellander L, Svanborg Edén C, Wold AE, Hanson LÅ. Intestinal colonization with *Enterobacteriaceae* in Pakistani and Swedish hospital delivered infants. *Acta Paediatr Scand* 1991; 80:602-610.
19. Khan SR, Karlberg J, Jalil F. Early child health in Lahore, Pakistan II. Mortality. In manuscript.
20. Gothefors L, Olling S, Winberg J. Breastfeeding and biological properties of fecal *E.coli* strains. *Acta Paediatr Scand* 1975; 64:807-811.
21. Lodinóva-Zadnikóva, R, Slavikóva M, Tlaskalóva-Hógenóva H, Adlerberth I, Hanson LÅ, Wold A, Carlsson B, Svanborg C, Mellander L. The antibody response in breastfed and formula-fed infants after artificial colonization of the intestine with *Escherichia coli* O83. *Pediatr Res* 1991; 29:396-399.
22. Wold A, Mestecky J, Tomana M, Kobata A, Ohbayashi H, Endo T, Svanborg Edén C. Secretory IgA carries oligosaccharide receptors for *Escherichia coli* type 1 fimbrial lectin. *Infect Immun* 1990; 58:3073-3077.
23. Söderström T, Öhman L. The effect of monoclonal antibodies against *Escherichia coli* type 1 pili and capsular polysaccharides on the interaction between bacteria and human granulocytes. *Scand J Immunol* 1985; 20:299-305.
24. Goldman AS, Thorpe LW, Goldblum RM, Hanson LÅ. Anti-inflammatory properties of human milk. *Acta Paediatr Scand* 1986; 75:689-694.
25. Goldman AS, Goldblum RM, Hanson LÅ. Anti-inflammatory systems in human milk. In: Bendich A, Phillips M, Tengerdy RB, Eds. Anti-oxidant Nutrients and Immune Functions. Plenum Press, New York, 1989:69-76.
26. Miyasawa K, Mantel C, Lu L, Morrison DC, Broxmeyer HE. Lactoferrin and lipopolysaccharide interactions. Effect on lacto-ferrin binding to monocyte/macrophage - differentiated HL-60 cells. *J Immunol* 1991; 146:723-729.
27. Hanson LÅ, Adlerberth I, Carlsson B, Coffman K, Dahlgren U, Nilsson K, Jalil F, Roberton DM. Mucosal immunity - from past to present. *Monogr Allergy* 1988; 24:1-8.
28. Hanson LÅ, Brandtzaeg P. Mucosal defense system. In: Stiehm ER, Ed. Immunological Diseases in Infants and Children. Saunders 3rd edition, Philadelphia, 1988:116-155.
29. Roberton DM, Carlsson B, Coffman K, Hahn-Zoric M, Jalil F, Jones F, Hanson LÅ. Avidity of IgA antibody to *Escherichia coli* polysaccharide and diphtheria toxin in breast milk from Swedish and Pakistani mothers. *Scand J Immunol* 1988; 28:783-789.
30. Dahlgren U, Carlsson B, Jalil F, MacDonald R, Mascart-Lemone F, Nilsson K, Roberton DM, Sennhauser F, Wold A, Hanson LÅ. Induction of the mucosal immune response. *Curr Top Microbial Immunol* 1989; 146:155-160.
31. Weisz-Carrington P, Roux ME, Lamm ME. Plasma cells and epithelial immunoglobulins in the mouse mammary gland during pregnancy and lactation. *J Immunol* 1977; 199:1306-1310.

32. Glass RE, Svennerholm AM, Stoll BJ, Khan MR, Hossein KMB, Huq MI, Holmgren J. Protection against cholera in breast-fed children by antibodies in breast-milk. *N Engl J Med* 1983; 308:1389-1392.
33. Cruz JR, Gil L, Cano P, Caceres P, Pareja G. Breast-milk anti-*Escherichia coli* heat-labile toxin IgA antibodies protect against toxin-induced infantile diarrhoea. *Acta Paediatr Scand* 1988; 77:658-662.
34. Ruiz-Palacios GM, Calva JJ, Pickering LK. Protection of breastfed infants against *Campylobacter* diarrhoea by antibodies in human milk. *J Pediatr* 1990; 116:707-713.
35. Cruz JR, Carlsson B, Hofvander Y, Holme D, Hanson LÅ. Studies of human milk II. Concentration of antibodies against *Salmonella* and *Shigella* in milk of women from different populations and the daily intake by their breast-fed infants. *Acta Paediatr Scand* 1985; 74:338-341.
36. Cruz JR, Carlsson B, Garcia B, Gebre-Medhin M, Gothefors L, Urrutia JJ, Hanson LÅ. Studies of human milk III. Secretory IgA quantities and antibody levels against *Escherichia coli* in colostrum and milk samples from underpriviledged mothers. *Pediatr Res* 1982; 16:272-276.
37. Cruz JR, Hanson LÅ, Specific milk immune response of rural and urban Guatemalan mothers. *J Pediatr Nutr Gastroenterol* 1987; 5:450-454.
38. Svennerholm AM, Hanson LÅ, Holmgren J, Lindblad BS, Nilsson B, Quereshi F. Different secretory IgA antibody response to cholera vaccination in Swedish and Pakistani women. *Infect Immun* 1980; 30:427-430.
39. Svennerholm AM, Hanson LA, Holmgren J, Lindblad BS, Khan SR, Nilsson A, Svennerholm B. Milk antibodies to live and killed polio vaccines in Pakistani and Swedish women. *J Infect Dis* 1981; 143:707-711.
40. Hahn-Zoric M, Carlsson B, Jalil F, Mellander L, Germanier R, Hanson LÅ. The influence on the secretory IgA antibody levels in lactating women of oral typhoid and parenteral cholera vaccines given alone or in combination. *Scand J Infect Dis* 1989; 21:421-426.
41. Zaman S, Carlsson B, Morikawa A, Jeansson S, Narayanan I, Thiringer K, Jalil F, Hanson LÅ. Secretory antibodies and neutra-lizing activity in the milk of mothers with different exposures to poliovirus antigen. In manuscript.
42. Hahn-Zoric M, Fulconis F, Minoli I, Moro G, Carlsson B, Böttiger M, Räihä N, Hanson LÅ. Antibody responses to parenteral and oral vaccines are impaired by conventional and low protein formulas as compared to breastfeeding. *Acta Paediatr Scand* 1990; 79:1137-1142.
43. Hahn-Zoric M, Carlsson B, Jeansson S, Ekre O, Osterhaus AD, Roberton DM, Hanson LÅ. Anti-idiotypic antibodies to poliovirus in commercial immunoglobulin, human serum and human milk. In manuscript.
44. Hahn-Zoric M, Carlsson B, Björkander J, Osterhaus ADME, Mellander L, Hanson LÅ. Presence of non-maternal antibodies in newborn of mothers with antibody deficiencies. In manuscript.
45. Stein KE, Söderström T. Neonatal administration of idiotype or anti-idiotype primes for protection against *E.coli* K13 infection in mice. *J Exp Med* 1984; 160:1001-1011.

46. Okamoto Y, Tsutsumi H, Kumar N, Ogra P. Effect of breast-feeding on the development of anti-idiotype antibody response to F glycoprotein of respiratory syncylial virus in infant mice after postpartum maternal immunization. *J Immunol* 1989; 142:2507-2512.
47. Mata L, Allen MA, Jimenez P, Garcia ME, Vargas V, Rodrigues ME, Valerin C. Promotion of breastfeeding, health and growth among hospital-born neonates and among infants of a rural area of Costa Rica. In: Chen LC, Scrimshaw NS, Eds. Diarrhoea and Malnutrition: Interactions, Mechanisms and Interventions. Plenum Press, New York, 1983:177-202.
48. King M. Health is a sustainable state. *Lancet* 1990; 336:664-667.
49. Thorpe S, Short RV, Potts M. Breastfeeding, birth spacing and their effect on child survival. *Nature* 1988; 335:679-682.
50. Hanson LÅ, Bergström S. The link between infant mortality and birth rates - the importance of breastfeeding as a common factor. *Acta Paediat Scand* 1990; 79:481-489.