Symposium report

Protective properties of human milk and the effects of processing on them

A one-day symposium on the protective properties of human milk, organised jointly by the Neonatal Society and the National Institute for Research in Dairying, was held on 28 April 1978. Years ago the most important property of human milk was its freedom from bacterial contamination and hence the protection it afforded breast-fed infants from gastrointestinal infections. This is still so in many parts of the world. Isolated reports of specific protective substances in human milk appeared from time to time, most importantly Paul György's in 1953 on the importance of the 'bifidus factor', but it was not until the 1970s that it was appreciated how important and varied these substances were. Now that more and more human milk banks are being set up to meet the needs of the low-birthweight infant, contamination of human milk becomes a danger. To overcome this the milk is generally heated, and an important part of the symposium was concerned with the best method of processing human milk so that as far as possible harmful bacteria and viruses are destroyed, and the inherent protective properties are preserved.

Dr Mavis Gunther spoke first on the relationship of the composition of human milk and the baby's mechanisms of defence. Dr Gunther, perhaps before anyone else, saw the practical importance to the baby of the discoveries that were being made in laboratories, and while she covered the subject in a general way, she brought out a number of points not made by subsequent speakers. She emphasised that some bacteria in the gut are a good thing, and contrasted the greater abundance of the beneficial *Lactobacillus bifidus* in the gut of the breast-fed infant with the larger number of potentially harmful *Escherichia coli* in that of the infant fed on a preparation based on cows' milk. Bacteria get into the infant's gut, not only from its food, but also from the mouth of anyone who kisses it. So long as this person is its mother, and she is breast feeding it, this does not matter because she secretes antibodies imprinted with her immune experience in her milk, and the mother's germs may even be helpful by taking part in excluding other more harmful ones. In the discussion after Dr Gunther's paper it was

suggested that the situation was not quite so simple, and the conditions leading to a correct gut flora were not known.

Miss P. Evans (Department of Animal Husbandry, Langford, Bristol) spoke next on intestinal immunity in young animals. She referred particularly to her work on the stimulation of mammary antibodies in pigs. The immune system in the intestine in many mammalian species is characterised by a predominance of immunoglobulin A. In the absence of congenital infection, the immune system of the young animal at birth is relatively devoid of plasma cells which secrete immunoglobulins. The newborn therefore relies on antibody passively acquired from its mother through the colostrum and milk. Most of the antibody is synthesised in the mammary gland. When lactating sows were dosed orally with heat-killed *E. coli* there was no antibody response in the mammary gland, but when live bacteria were given there was an increase in antibody titre in the milk, which lasted for at least 4 weeks, and this was predominantly of the IgA class. There was also a secretory IgA response in the intestinal secretions of the sow, and it was suggested that antigenic stimulation in the gut is linked with and precedes stimulation of IgA antibody production in milk. Reference was made to a study by Ahlstedt *et al.* in Sweden in which women were given *E. coli* by mouth during pregnancy, and again live bacteria were much more powerful than dead in stimulating the local immune system.

The next two papers were concerned with the practical problems confronting those concerned with collecting human milk, storing it, processing it, and feeding it to low birthweight infants. Dr J. Hewitt (Public Health Laboratory Service) spoke about the bacteriological quality of human milk collected for milk banks. He started with a slide of a cow and a woman and pointed out the advantage the woman has, with her breasts high up away from the ground and from her excreta so that the likelihood of contamination of human milk is much less than of the milk from the cow. Hewitt described his collaborative work with the staff of the Department of Child Health, King's College Hospital, London, and the

Department of Microbiology, University of Surrey. The bank at King's was established by recruitment of mothers returning home after delivery in hospital. Surplus milk was stored in sterile plastic jars in the domestic refrigerator and called for 3 times a week. The instructions for the collection of expressed milk include—using disposable towels for washing the hands and breasts, avoiding collection from a recently suckled breast, and rejecting the first 10 ml of milk expressed. This milk was found to have a total bacterial count 10- to a 100-fold higher than the midstream milk. It is recommended that milk kept by donors at home for longer than 24 hours should be kept in the freezer compartment of the refrigerator. If the measures are followed the milk contains small to moderate numbers (10^2 to 10^4 per ml) of the normal skin bacteria, with a few Gram-negative organisms. When the milk arrives at the bank it is screened for bacterial content, and graded as suitable for use raw, for pasteurisation, or to be discarded. Hewitt was asked about the time-consuming plate-counts used in quality control, and whether simpler screening tests such as dye-reduction could be used: the answer was no. Another question concerned screening for viruses: Hewitt replied that screening of the donors was a first vital step, particularly to reduce enteric virus contamination. Cytomegalovirus was lost on pasteurisation. There was no evidence as yet for the transmission of a hepatitis B in breast milk.

Dr J. D. Baum (John Radcliffe Hospital, Oxford) described an alternative system. The milk during transport to the hospital reaches a temperature of $+5$ to $+15^\circ\text{C}$. On arriving at the hospital bank it is pooled, poured into individual bottles, pasteurised using the purpose-built automated Oxford pasteuriser (Vickers Medical Ltd), and stored frozen until used. A sample of the pooled milk after pasteurisation is examined bacteriologically and the presence or absence of named pathogens provide the index of the acceptability of the milk. Baum, like Hewitt, was much concerned with reduction of bacterial contamination and said that soaking the collecting vessels in hypochlorite (Milton) is effective in reducing the numbers of pathogens and nonpathogens.

There was much common ground between the systems at King's College Hospital and at Oxford. The major point of difference was that attempts were being made at King's to separate milk that could safely be used raw, whereas the Oxford system relies on a precise pasteurising process to eliminate pathogens, and at the same time to preserve the antimicrobial factors of milk.

Dr Pamela Davies (Department of Paediatrics, Hammersmith Hospital, London) concluded the morning session with a paper given jointly with Dr L.

Gothefors on feeding preterm babies with fresh and heat treated milk. She described studies on 27 preterm infants with median gestational age 32 weeks, many of whom were ill. Seven were fed fresh human milk, 11 sterilised human milk, and 9 a modified cows' milk preparation for several weeks after birth. The number of infants colonised with *E. coli*, *Klebsiella*, or *Enterobacter* did not differ in those fed fresh human milk or a modified dried cows' milk. The only obvious effects of human milk (fresh or pasteurised) were that the number of infants colonised with *Streptococcus faecalis* and clostridia were fewer, and bifidobacteria were somewhat more common, than in infants fed on cows' milk formula. The reduction in numbers of enterobacteria was considerably less than that expected in healthy mature breast-fed babies.

Dr Davies was asked whether the infants received their own mother's milk: she replied that most did so. Finally a member of the audience asked whether a colostrum bank was more desirable than a milk bank. The question was passed to Dr Gunther, who said that as anyone who had any experience of handling the thick and sticky colostrum would know this would be a practical impossibility.

In the afternoon session Dr H. Burton (NIRD) explained the basic principles of milk pasteurisation and the problem of achieving an acceptable bactericidal performance with the minimum of damage to the milk's nutritional quality. He recommended the in-bottle pasteurising process and suggested ways in which it might be modified to minimise damage to nutrients. He posed the question—What degree of bacteriological effectiveness do we need?—and in discussion it was suggested that any bacteriologically effective process might so impair the protective properties of the milk as to increase the risk of enteric infection in the recipient infant. Nevertheless, Dr L. A. Mabbitt expressed horror at the prospect that donor milk might be fed unheated without stringent control of its bacteriological quality. This would involve a large amount of routine bacteriological testing, but some such attempt to provide a grade A quality for milk for feeding raw to weak and premature babies might be practicable and it is clearly important to establish whether it would be beneficial.

Dr R. L. J. Lyster (NIRD) described a study of the kinetics of heat denaturation of α -lactalbumin, lactoferrin (LF), and IgA in human milk, and compared the rates of denaturation of these proteins at graded temperatures in the range 60 – 70°C . He commented on a wide variation between different workers' results for IgA and LF in heated milk. This he attributed partly to poor precision in the measurements, but mainly to variation in protein

concentration and pH between milk samples; he also urged the need for further work to resolve the discrepancies. In discussion it was pointed out that immunoassay values do not necessarily provide an accurate measure of the biological activity of the residual protein and that, with lactoferrin, the stability towards heat might be influenced by the degree of saturation with iron.

Dr J. E. Ford (NIRD) spoke on the nutritional significance of trace nutrient binders in breast milk. The vitamin B12 and folate are strongly attached to specific 'binder' proteins, which are present in large excess throughout lactation and strongly reinforce the endogenous binder-protein systems in the infants' intestines. This binding of the vitamins may promote their absorption, both directly, and indirectly by preventing their uptake by intestinal micro-organisms. Free cyanocobalamin and folic acid, unlike the bound forms in milk, were taken up avidly by several species of intestinal bacteria. Similarly with iron and zinc: both occur in breast milk in complex-bound forms that are efficiently absorbed from the baby's intestine. The likely influence of these various binders on the ecology of the gut microflora and on the nutrition of the milk-fed infant was discussed, and the possibly detrimental effect of their destruction by heat treatment of the milk.

Dr B. Reiter (NIRD) presented a paper on lactoperoxidase, which is present in milk and saliva and other external secretions and, with hydrogen peroxide and thiocyanate, comprises a potent antibacterial system of low specificity. *In vitro* all the main human serotypes of *E. coli* were susceptible, and in experiments with milk-fed calves the lactoperoxidase systems killed *E. coli* in the abomasum without affecting the numbers of lactobacilli. The system has been used experimentally to preserve bovine milk during storage under refrigeration and at ambient tem-

perature, by addition of a source of H_2O_2 , and preliminary indications suggest that it could be equally effective with human milk in reducing the bacterial load to acceptable levels, obviating the need for heat processing.

In the closing paper, Dr D. B. L. McClelland (Royal Infirmary, Edinburgh) assessed the influence of the leucocytes of milk as protective agents. Human colostrum contains large numbers of leucocytes, and their functions have attracted interest since the suggestion of Mohr that these cells could play a part in transmitting active immunity to the neonate. A problem in studying these cells is the shortage of data about their type and total numbers. McClelland found great variability in total cell numbers and a tendency for them to fall rapidly during the first week of lactation. The predominant cell is the macrophage. Lymphocytes rarely comprise more than 5% of the total cell population but, despite this, most studies of the biological role of milk cells have concentrated on the lymphocyte population. There is evidence that human maternal milk may transmit active immunity to tuberculo-protein, but this has not been shown to be related to the presence of viable cells. Macrophages in human milk phagocytose very actively, but have poor bactericidal activity *in vitro*. Although they are unlikely to kill bacteria within the neonatal digestive tract, they could possibly sequester organisms and so prevent colonisation of mucosal surfaces. Such a mechanism may explain the observation of Pitt that the viable leucocytes in milk protect against neonatal necrotising enterocolitis in rats.

The true importance of the milk leucocytes remains to be shown. Until more evidence is available, attempts to preserve viable leucocytes should not influence the processing and storage conditions used for human milk.

E. M. WIDDOWSON
University of Cambridge