PAPERS AND ORIGINALS

Organisation of bank of raw and pasteurised human milk for neonatal intensive care

S WILLIAMSON, J H HEWITT, E FINUCANE, H R GAMSU

British Medical Journal, 1978, **1**, 393-396

Summary and conclusions

In 1976 a human-milk bank was established at King's College Hospital to serve the neonatal intensive care unit. The bank is staffed by two part-time nurses, who interview prospective donors, organise collections, prepare samples for bacteriological screening, and process the milk. On average 25 litres a month may be collected from about 15 donors, of which at least two-thirds is free enough of bacteria to be fed raw (unheated) to sick and low-birth-weight infants. Most of the remainder may be used after holder pasteurisation.

The bank provides an adequate supply of milk of consistent nutritional quality and permits a more informed approach to the dietary management of infants of low birth weight.

Introduction

In the nutrition of full-term infants human milk is acknowledged to be superior to modified cows' milk. The ideal diet for infants of low birth weight is more controversial. Although the low sodium load, improved fat absorption, and defence against infection, including enterocolitis,[1-3] conferred by human milk are advantageous to these infants, its nutritional superiority is arguable.[4 5] Nevertheless, many believe that on balance it is the preferred food.[6]

Department of Child Health, King's College Hospital Medical School, London SE5 8RX

S WILLIAMSON, DCH, MRCP, senior registrar
E FINUCANE, HND(CHEM), senior technician
H R GAMSU, DCH, FRCPED, consultant paediatrician and senior lecturer

Public Health Laboratory, Dulwich Hospital, London SE22 8QF

J H HEWITT, MSC, MIBIOL, senior scientific officer

Many practical problems attend the use of human milk for these small infants. It is often impossible to obtain enough milk from the mother, who may be unwilling or unable to express milk, or if the baby has been transferred to an intensive care centre she may live too far away. Such babies are therefore dependent on milk from unrelated donors. Finding a sufficient and consistent supply creates a problem that is only partially overcome by donations from mothers in postnatal wards. With the trend to shorter hospital confinements fewer mothers can donate while still in hospital, and such contributions are therefore irregular. Until 1975 our intensive care unit depended on such a supply, which was enough for only a few of the infants who needed it. The milk was boiled after collection. In 1976, coincident with a campaign to encourage breast-feeding in our hospital, a larger milk bank was established, its main purpose being to secure an adequate supply of milk of consistent nutritional quality. In addition, most of the supply was to be suitable for use in the raw (unheated) state and most of the remainder used after pasteurisation. These aims had to be accomplished within the restraints of limited space and finance.

Operation of milk bank

RECRUITMENT OF DONORS AND COLLECTION OF MILK

Nursing and medical staff talk about the milk bank to all breast-feeding mothers in the postnatal wards. Those wishing to help are contacted a few weeks after returning home and given more detailed instructions. This gives them time to reflect on their willingness and ability to donate, those still eager to volunteer tending to be more reliable contributors. Some donors have been recruited from further afield by nurses, and some have responded to a poster campaign and appeals on local radio and television. Donors are usually accepted only if they live within three miles (five kilometres) of the hospital. No payment is made for the milk.

Most of the milk collected is expressed either manually or by using a hand breast reliever. Milk ejected from the opposite breast during feeding is trapped in a breast shield. Over 24 hours several hundred millilitres of milk may be so obtained. The milk is collected direct into sterile 250-ml screw-topped jars, or transferred to these jars from the shields or breast relievers, and stored in the household refrigerator for up to 24 hours or in the freezer compartment for up to three days. The jars are autoclaved in the hospital and delivered to the

home three times a week. Jars of donated milk are transferred back to the hospital in an insulated container.

The donors are given verbal and written instructions describing the technique of collection designed to ensure maximum cleanliness. This entails washing hands and breasts and drying with disposable towels. They are also advised not to collect from the breast that has just been suckled by the baby. After use, the breast relievers and shields are carefully washed with soap and water and disinfected in freshly prepared hypochlorite solution. Donors are asked to report any ill health in the family and whether they are taking drugs. If drugs are taken that may cross into the milk, or if there is any uncertainty that they might do so, the milk is not used. When mother or baby is unwell the milk is not collected. These instructions are reinforced by a home visit by a health visitor. If bacteriological screening suggests a poor collection technique a second visit is made, the instructions being repeated and the technique of collection scrutinised. Blood is taken from all new donors to exclude HBsAg carriers.

The milk bank is staffed by two part-time nurses. Their duties include interviewing prospective donors, organising collections, preparing samples for bacteriological screening, and processing the milk. On average 25 litres a month is collected from about 15 donors.

Nutritional and bacteriological considerations

Before the milk bank was established our practice of collecting milk from mothers in the postnatal wards and boiling the milk before feeding had given rise to concern. Such milk is predominantly colostrum, which has a higher content of protein and minerals than mature breast milk. Boiling and the resultant loss of water further increases its osmolality. In a group of light-for-dates infants fed this milk the osmolality of the urine on the sixth day was higher than that of a comparable group fed a commercial milk of low-solute concentration.[7]

Using standard analytical methods,[8] we compared the nutritional constituents in samples of 20 separate pools of milk taken after 1976 with 10 samples taken before 1975 (table I). As expected, the samples from the milk bank had the characteristics of mature milk, whereas the earlier samples represented colostrum and transitional milk. The colostric samples also showed a far greater variability in the concentrations of the constituents analysed, which is a consequence of pooling small volumes of milk from few donors. Assurance that the milk is now of a more constant composition permits a more informed approach to dietary management.

A problem common to all human-milk banks is that milk collected by a mother at home may be heavily contaminated with bacteria. Some form of heat treatment is the most common method used for reducing the content of viable bacteria but is done at some cost to the quality and quantity of many nutritional constituents.[8] We found that boiling, and to a less extent pasteurisation, profoundly affects the absorption of fat and results in poorer weight gain when compared with raw milk.[9] Heat treatment also has a deleterious effect on the anti-infective components of human milk.[10] Feeding heated breast milk to preterm infants results in a faecal flora different from that of breast-fed, mature infants.[11] Hence we now feed carefully selected and, if possible, unheated milk; otherwise we subject it to holder pasteurisation, which was chosen for its efficiency and ease of use on a small scale and its greater preservation of immunoglobulins, lysozyme, folic-acid-binding protein,[10] and fat absorption when compared with more severe treatment.

Bacteriological safeguards

The bacteriological standards required are determined by the known susceptibility of infants of low birth weight to infection. Healthy, full-term infants may ingest milk from their mothers with bacterial counts exceeding 100×10^6/l.[12] Even milk collected carefully may have counts of over 10^6/l.[13] The microflora of this milk is ideally that resident on the alveolar skin, though it may also include oral streptococci derived from the infant, and the count may be greatly increased if the milk remaining in the ducts of the nipple at the end of the feed has been colonised during the preceding feed.

Tables II and III give the total bacterial counts and predominant micro-organisms detected in the first 371 home-collected milk samples from 56 donors. The results indicate that unless careful methods of collection and storage are used the bacterial content may rise unacceptably but that with careful collection low counts (below 10×10^6 colony-forming units/l) are possible. Though *Staphylococcus aureus* was the predominant organism in three samples, in most the predominant ones were non-pathogenic staphylococci, micrococci, or diphtheroids. Poor storage of the milk at home in some cases was suggested by high counts of enterobacteria or non-fermentative, gram-negative rods—for example, acinetobacter, alcaligenes, and moraxella.

The bacteriological quality of the milk can be further improved if the mother discards the first 5-10 ml and collects the remaining "midstream" milk; by this means a tenfold to one-hundredfold decrease in bacteria can be achieved.[14] The proportion of milk satisfying the criteria for use in the raw state rose from 66% to 80% after mothers were advised to discard the first few millilitres.

SCREENING

All milk is screened on arrival with a modified dip-slide technique by pipetting samples on to the agar-surfaced slide. This is used to

TABLE II—*Total viable counts of bacteria in 371 milk samples on arrival at milk bank*

Log_{10} viable counts/l ($\times 10^{-3}$)	<2	2–	3–	4–	5–	≥6
No (%) of samples	111 (30)	108 (29)	82 (22)	33 (9)	13 (3·5)	24 (6·5)

TABLE III—*Predominant aerobic micro-organisms detected in 371 milk samples*

	No (%) of samples
Staphylococcus albus (DNAse-negative)	234 (63)
Oxidase-negative, non-fermentative Gram-negative rods (for example, *Acinetobacter*)	40 (11)
Lactose-fermenting enterobacteria (for example, *Escherichia coli*, *Klebsiella*)	26 (7)
Oxidase-positive, Gram-negative rods other than *Pseudomonas aeruginosa* (for example, *Alcaligenes*)	26 (7)
Streptococcus viridans	15 (4)
Unclassified Gram-negative rods	11 (3)
Diphtheroids	4 (1)
Pseudomonas aeruginosa	4 (1)
Staphylococcus aureus (DNAase positive)	3 (1)
Mixed growth	8 (2)

TABLE I—*Analyses of 20 pooled milk samples from milk bank compared with 10 pooled samples collected before establishment of bank*

	Nitrogen (mmol/l)	Sodium (mmol/l)	Calcium (mmol/l)	Total phosphorus (mmol/l)	Total fat (g/l)	Fat (% of free fatty acid)	Lactose (mmol/l)	Potassium (mmol/l)	Osmolality (mmol/l)	kcal/l* (ref [23])
Milk-bank samples										
Mean ± 1SD	136·1 ± 15·92	8·66 ± 1·96	6·92 ± 0·52	4·13 ± 0·23	34·79 ± 4·3	8·24 ± 2·24	184·85 ± 6·98	13·47 ± 0·93	292·8 ± 7·94	613·1 ± 33·8
Range	114·0-168·0	5·52-12·22	6·04-7·88	3·65-4·55	28·84-44·48	4·3-12·6	177·0-203·0	11·92-15·58	284·0-313·0	566-688
Pre-milk-bank samples										
Mean ± 1SD	173·0 ± 38·3	16·3 ± 9·15	6·78 ± 1·36	4·14 ± 0·83	29·2 ± 5·63					
Range	116·0-224·0	6·9-33·7	3·96-8·71	1·98-4·99	17·0-37·3					
Significance of difference between means	P<0·001	P<0·01	NS	NS	P<0·01					

*1000 kcal ≈ 4·2 MJ.
NS = Not significant.
Conversion: SI to traditional units—Nitrogen: 1 mmol/l ≈ 1·4 mg/100 ml. Sodium: 1 mmol/l = 1 mEq/l. Calcium: 1 mmol/l ≈ 4·0 mg/100 ml. Phosphorus: 1 mmol/l ≈ 3·1 mg/100 ml. Lactose: 1 mmol/l ≈ 34 mg/100 ml. Potassium: 1 mmol/l = 1 mEq/l. Osmolality: 1 mmol/kg = 1 mOsm/kg.

identify samples complying with our criteria for pasteurisation. Milk found to have a low bacterial count and whose donors consistently give more than 100 ml daily is screened for use in the raw state. Standard loopfuls (0·01 ml) are plated on blood agar and MacConkey agar, and a 1-ml sample of undiluted milk is added to 5 ml of MacConkey broth, which is used as an enrichment medium to detect enterobacteria.

Raw milk

The following three main criteria must be satisfied if the milk is to be used raw.

(*1*) *It should not contain enterobacteria*—We have not confined this criterion to milk containing lactose-fermenting enterobacteria since many infants and probably many adults do not harbour lactose-fermenting enterobacteria in their faecal flora. In addition, rare strains of enteropathogenic *Escherichia coli* may not ferment lactose.[15] Collection of milk is discontinued from any mother when one of the recognised enteropathogenic serotypes of *E coli* is found. This occurred only once in the first year of operation of the milk bank.

(*2*) *It should not contain more than* 10×10^5 *colony-forming units of Staph aureus/l*—Ingesting moderate numbers of *Staph aureus* presents no hazard to the normal suckling infant,[16] possibly because human milk contains heat-stable anti-staphylococcal components.[17] For some time, however, we declined to feed raw milk in which this organism could be detected by enrichment of a 1 ml sample. Some carefully collected milk contains up to 10×10^5 colony-forming units of this organism per litre, and we now believe that it is no longer justifiable to enforce our original strict limit. A screening test that detects 10×10^5 or more colony-forming units per litre is now in use.

(*3*) *The total count should not exceed* $2{\cdot}5 \times 10^6$ *colony-forming units/l, and normal skin flora should predominate.*

The milk is stored at −18°C until the results of the tests are known. Samples satisfying the above criteria are then thawed, pooled, mixed, and decanted into 220-ml plastic, screw-topped bottles. These are kept at −18°C until the day required, usually within three weeks of collection. Once thawed the milk is used within a few hours or poured into smaller bottles, which are kept at 4°C while awaiting use during that day. Raw milk is not administered from large-volume containers for continuous feeding because of the fear of bacterial replication at room temperature.

During the operation of the milk bank we have not encountered any unexpected gastrointestinal problems in neonates fed this milk despite the fact that raw milk may contain some organisms.

Milk that does not satisfy the above criteria and collections below 100 ml may be pasteurised.

Pasteurised milk

Three criteria have to be met before the milk is considered suitable to be pasteurised.

(*1*) *The aerobic count should not exceed* 100×10^6 *colony-forming units/l*—This conforms to the criterion used by the dairy industry before milk is accepted for pasteurisation. Extensive bacterial growth before pasteurisation leads to spoilage of the nutritional and immunological properties.

(*2*) *Staph aureus should not exceed* 10^6 *organisms/l*—Feeding heat-stable enterotoxins of *Staph aureus* is a risk of pasteurisation. In adults outbreaks of food poisoning have occurred with foods containing more than 100×10^6 *Staph aureus* organisms/l or kg.[18]

(*3*) *Counts of organisms not forming part of the normal flora of the skin of the breast should not exceed* $10^6/l$.

When the milk persistently has total counts exceeding 100×10^6/l despite checks on collection and storage collections are tactfully stopped.

Milk collected under the supervision of trained staff on the postnatal wards and frozen or pasteurised without delay is not usually screened.

TECHNIQUE OF PASTEURISATION

We use a thermostatically controlled water bath (Grant Instruments, Cambridge Ltd, model SS.30) fitted with a shaking trolley to ensure uniform heating. Bacteriologically acceptable samples are thawed and brought to room temperature, pooled, and poured into sterile 220-ml watertight plastic bottles (Axifeed, Non-Woven Fabrics Ltd, Windsor, Berks). These are completely immersed in a water-bath checked to be 63°C and held at this temperature for 40 minutes. This includes 10 minutes found to be necessary for the milk temperature to equilibrate with that of the water. The cycle is completed by emptying the bath and running cold tap water through it to cool the milk rapidly. The process takes less than 20 minutes of the nurses' time. Four litres of milk is processed at each cycle. The bath is emptied and wiped dry after use. All pasteurised milk not for immediate use is stored at −18°C to prevent multiplication of any residual organisms. In practice, the relative rarity of Lancefield group D streptococci, lactobacilli, or spore-bearing organisms in carefully collected human milk usually results in a sterile product. We used the alkaline phosphatase test[24] to establish the efficiency of pasteurisation.

Problems encountered

Jaundice—Breast-milk jaundice is a theoretical risk associated with the use of raw milk kept in the refrigerator before use.[19] The analysis of pooled milk (table I) stored at −18°C for two to three weeks showed a high free fatty-acid concentration. Bilirubin conjugation is inhibited in vitro by milk containing high concentrations of free fatty acids.[20] It therefore seems wise to store milk for as short a time as possible. We have identified some infants with prolonged, unexplained jaundice which after extensive search for other causes we have tentatively ascribed to the breast milk they were receiving.

Hyponatraemia—Certain infants of very low birth weight are prone to hyponatraemia consequent on renal sodium loss.[21] The serum sodium of those fed mature breast milk has to be regularly measured, and additional sodium may have to be given in selected cases. We have had to supplement the sodium intake to several very small infants for this reason.

Cost

The milk bank is accommodated satisfactorily in the existing milk kitchen,[22] now little used because of a change to prepacked modified cows'-milk feeds for the larger infants. The total capital outlay was £860 (deep-freeze and pasteuriser £520; breast pumps and breast shields £340), and yearly recurrent expenditure is about £3290. Yearly recurrent expenditure (rounded figures) comprises nurses' salaries (£1000); transport (£1000); bacteriological screening, including the time taken by a full-time, trained bacteriologist, the cost of media and glassware, but excluding the cost of equipment if use is made of an established bacteriology laboratory (£500); replacing glassware (£40); disposable bottles (£700); and hypochlorite tablets (£50). These recurrent costs do not include expenditure on sterilising jars, electricity, disposable paper towels, publicity, stationery, or secretarial help. We hope that transport costs will be reduced by asking ex-donors to collect locally and provide collection points for the hospital transport.

We acknowledge the help of Ruth Jerrett, Isabel Frith, and Mr B Hoarl, who are responsible for the smooth operation of the milk bank. We also thank the staff of the Public Health Laboratory, Dulwich Hospital, who have helped in the bacteriological screening programme, and Mrs Pamela Golding for preparing the typescript. The investigation relating to the possibility of establishing a milk bank was made possible by a Wates Research Fellowship to Dr S Williamson.

Requests for reprints should be addressed to: Dr S Williamson, c/o Department of Child Health, King's College Hospital Medical School, Denmark Hill, London SE5 8RX.

References

1 Widdowson, E M, *Lancet*, 1965, **2**, 1099.
2 Goldman, A S, and Smith, C W, *Journal of Pediatrics*, 1973, **82**, 1082.
3 Barlow, B, *et al*, *Journal of Pediatric Surgery*, 1977, **9**, 587.
4 Fomon, S J, Ziegler, E E, and Valquez, H D, *American Journal of Diseases of Children*, 1977, **131**, 463.
5 Heird, W C, *American Journal of Diseases of Children*, 1977, **131**, 468.
6 Human Milk in premature infant feeding: Summary of a workshop, *Pediatrics*, 1976, **57**, 741.
7 Gamsu, H R, *Postgraduate Medical Journal*, 1975, **51**, suppl No 3, p 31.
8 Sager, C A, *Archiv für Kinderheilkunde*, 1953, **37**, 1.
9 Williamson, S, *et al*, *Archives of Disease in Childhood*. In press.

[10] Ford, J E, *et al*, *Journal of Pediatrics*, 1976, **90**, 29.
[11] Wilkinson, B, *et al*, *Pediatric Research*, 1976, **10**, 361.
[12] Scherer, P, *Zeitschrift für Hygiene und Infektionskrankeiten*, 1951, **132**, 219.
[13] Wright, J, *Lancet*, 1947, **2**, 121.
[14] West, P A, *et al*. Paper in preparation.
[15] Gross, R J, personal communication.
[16] Wilson, G S, and Miles, A A, editors, in *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*, 6th edn, p 2620. London, Arnold, 1975.
[17] György, P, *American Journal of Clinical Nutrition*, 1971, **24**, 970.
[18] Gilbert, R J, *Postgraduate Medical Journal*, 1974, **50**, 603.
[19] Cole, A P, and Hargreaves, T, *Archives of Disease in Childhood*, 1972, **47**, 415.
[20] Foliot, A, *et al*, *Pediatric Research*, 1976, **10**, 594.
[21] Day, G M, *et al*, *Pediatric Research*, 1976, **10**, 522.
[22] Gamsu, H R, Cawdery, H M, and Taylor, C G, *Lancet*, 1969, **2**, 1049.
[23] Southgate, D A T, and Barret, I M, *British Journal of Nutrition*, 1966, **20**, 363.

(*Accepted 18 January 1978*)

Antibody to hepatitis B core antigen in chronic active hepatitis

P BORIES, P COURSAGET, A GOUDEAU, C DEGOTT, P MAUPAS, J P BENHAMOU

British Medical Journal, 1978, **1**, 396-397

Summary and conclusions

Antibody to hepatitis B core antigen (anti-HBc), which has been assumed to be a more sensitive indicator of hepatitis B virus replication than hepatitis B surface antigen (HBsAg), was detected in the sera of 26 of our 65 patients with HBsAg-negative chronic active hepatitis. Thus despite the absence of HBsAg the liver disease could be the consequence of chronic infection with hepatitis B virus in these patients. They differed, however, from a group of 35 patients with HBsAg-positive hepatitis in being older on average and having less active liver lesions. The two groups could represent either two stages of chronic infection with hepatitis B virus or two types of response to it.

Introduction

The presence in serum of hepatitis B surface antigen (HBsAg) is a recognised indicator of hepatitis B virus replication. HBsAg in the serum of patients with chronic active hepatitis is regarded as evidence that the liver disease results from infection with hepatitis B virus.[1] Serum antibody to hepatitis B core antigen (anti-HBc) is another indicator of virus replication, possibly more sensitive than HBsAg.[2] We carried out our investigation to see whether some patients with HBsAg-negative chronic active hepatitis have anti-HBc in their serum and thus have liver disease that could also be the consequence of infection with hepatitis B virus.

Unité de Recherches de Physiopathologie Hépatique (INSERM), Hôpital Beaujon, 92118 Clichy, France
P BORIES, MD
J P BENHAMOU, MD

Laboratoire de Virologie, Facultés de Médecine et de Pharmacie, 37000 Tours, France
P COURSAGET, MD
A GOUDEAU, MD
P MAUPAS, MD

Service d'Anatomie et de Cytologie Pathologiques, Hôpital Beaujon, 92118 Clichy, France
C DEGOTT, MD

Patients and methods

We investigated 100 patients, 50 men and 50 women (aged 16-80, mean 48 years), with histologically proved chronic active hepatitis, 36 of whom had spent 10 years or more in Africa or Asia. The hepatic lesions were classified as moderately (grade A) or extremely (grade B) active in 75 and 25 patients according to the histological criteria of De Groote *et al*.[3] Chronic active hepatitis was associated with cirrhosis in 64 and with hepatocellular carcinoma in three patients. The liver lesions were judged to be chronic on the basis of (*a*) histologically proved cirrhosis associated with chronic active hepatitis or (*b*) clinical signs, abnormal results of liver function tests, or histological lesions (or all three) persisting for a year or more after the histological recognition of the liver disease. We excluded patients who during the year before this study had resided in Africa or Asia, had received blood transfusions or intramuscular injections, or had had clinical manifestations or biochemical disorders compatible with acute viral hepatitis (that is, transient jaundice, episodes of fever, or appreciably increased serum transaminase activity).

Serum glutamic pyruvic transaminase (SGPT) activities ranged from 2 to 506 and averaged 94 international units (IU) (normal < 25 IU) at the time of the liver biopsy. HBsAg and antibody to it (anti-HBs), detected by radioimmunoassay (AusRIA II and AusAB, Abbott Laboratories, North Chicago, Illinois, USA), were present in the sera of 35 and 24 patients.

Anti-HBc was detected by counterelectrophoresis.[4] In all the patients in whom HBsAg was absent and anti-HBc was shown by counterelectrophoresis the anti-HBc titres were determined by complement fixation[5]; the results were expressed as values for the rate constant K_{37}.[6] In 10 of these patients anti-HBc titres were determined in paired samples taken 12-18 months apart.

The means were compared by the Student *t* test and the percentages by the χ^2_c test.[7]

Results

The patients were divided into three groups (table I) according to the presence or absence of HBsAg and anti-HBc in their serum. Group 1 included 35 HBsAg-positive patients, all having anti-HBc detected in their serum; in none of them was anti-HBs detected in the serum. Group 2 included 26 HBsAg-negative patients who had anti-HBc in their serum (see tables II and III for anti-HBc titres); in 16 anti-HBs was present. Group 3 included 39 HBsAg-negative, anti-HBc-negative patients, in 8 of whom anti-HBs was detected.

Statistically significant differences between the three groups are shown in table I. In group 2 the mean age was 15 years greater than in group 1, and there was a higher percentage of patients with moderately active liver lesions, a greater prevalence of cirrhosis associated with chronic active hepatitis, and a lower mean value for SGPT. The percentage of patients who had lived in Africa or Asia was higher in groups 1 and 2 than in group 3; the difference is statistically significant, however, only between groups 2 and 3. The three patients with hepato-