Milk pH, acid base status, and growth in babies

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SUMMARY Metabolic acidosis is common in babies fed cows' milk-based formulae. Therefore the effects of adding alkaline salts (sodium and potassium citrate) to a demineralised whey formula were studied *in vitro* and in 26 low birthweight babies fed on the formula or formula plus citrate. The alkali altered the pH and titratable acidity to a value nearer human milk but it increased the buffering capacity to a value further away. This may affect the bacterial flora of the intestine. The babies fed on formula plus citrate did not make greater gains in weight, length, head circumference, skinfold thickness, or midarm muscle circumference, although they had a greater blood base excess. Some of these babies developed a mild metabolic alkalosis and 3 had hyponatraemia despite their increased sodium intakes. These babies also had lower levels of plasma transferrin but showed no differences in urea, albumin, cholesterol, and calcium levels. No baby fed on the demineralised whey formula without added citrate had a base deficit exceeding 5 mmol/l; late metabolic acidosis is less common in babies fed on this formula and the routine addition of alkali can have untoward metabolic effects.

Late metabolic acidosis is still a common finding in bottle-fed babies even when they are given low protein cows' milk formulae (Svenningsen and Lindquist, 1973; Moore *et al.*, 1977). We therefore studied the growth and biochemistry of low birthweight babies who were routinely given extra alkali in every feed to prevent this acidosis. As the alkali could also affect the intestinal flora by altering the pH and buffering capacity of the milk, the *in vitro* effects on the acid base properties of the formula were also investigated (Bullen and Willis, 1971; Harrison and Peat, 1972).

Methods

In vitro observations. Demineralised whey formula with a low curd whey ratio and electrolyte content was used (SMA Gold Cap). Samples of this formula were reconstituted with water according to manufacturer's instructions (12% w/v) and contained a Na, K, and Cl of 7, 14, and 12 mmol/l respectively. Both Na and K were then added to half the samples to raise their contents to that of ordinary SMA, which has been used safely in special care nurseries for several years. Thus Na 5 mmol/l was added as citrate salt, and K was added as 2 mmol/l as Cl salt, and 4 mmol/l as citrate salt. Citrate was chosen

as the major anion since it is the dominant base present in milk (Jenness and Sloan, 1970).

Samples of the formula and formula plus citrate together with pasteurised human and cows' milk were then examined as follows: three 20 ml samples of each milk were analysed in duplicate at room temperature for (a) pH (Radiometer microelectrode pH meter); (b) titratable acidity, i.e. volume of alkali required to raise pH from its starting level to pH 7.45 using 0.25 mmol/l NaHCO₃, a method similar to that of Harrison and Peat (1972); (c) buffering capacity, i.e. volume of added acid required to lower the pH when titrating milk from starting pH to pH 5.0 (equivalent to 100 mV) with 0.1 mmol/l hydrochloric acid, a method similar to that used by Bullen and Willis (1971).

Clinical trial. Formula and formula plus citrate were made up in the milk kitchen and then fed to two similar groups of low birthweight babies (Table 1). The quantity of electrolyte that was added (to 3 litres of formula) to make up the formula plus citrate milk to the concentration given above was prepared by the pharmacy in preweighed sachets. The babies were nursed in the special care baby unit at Sorrento Maternity Hospital and informed consent was obtained from their mothers. At the time the unit was performing nitrogen balances in low birthweight boys and so only girls were admitted to the study.

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Table 1 Details of babies

	Formula	Formula plus citrate
n	13	13
Gestation (weeks)	37±2*	37±2
Birthweight (kg)	2.0±0.15*	2.1±0.07
Preterm	5	5
Term (small-for-gestational-age)	8	8
Asian	3	5

*Mean±SD.

The feeding regimen was the usual one for the unit, i.e. preterm babies reached 200 ml/kg per day by day 9 while term light-for-dates babies reached this volume by day 6, the feeds were then adjusted according to this volume after twice-weekly weighings.

The babies were measured at birth and on days 7, 14, and 21 (weight, Salter spring balance; length, Harpenden neonatometer; head circumference, steel tape measure; skinfold thickness, expressed as mean from five sites—scapular, triceps, biceps, umbilical, and quadriceps, Harpenden skinfold calipers; and midarm muscle circumference (Gurney and Jelliffe, 1973). Samples of blood were collected by heel prick on days 7, 11, and 21, four hours after the 6 a.m. feed, for estimation of blood acid base status (Astrup apparatus) and plasma Na (flame photometer), Ca (Baginski *et al.*, 1973), P (Itaya and Ui, 1966), albumin, transferrin, and lipoprotein (radial immunoassay), cholesterol by application of the Leiberman Burchard reaction, and urea by application of the Berthelot reaction after enzymatic hydrolysis with urease.

Results

In vitro observations (Fig. 1). The pH, buffering capacity, and titratable acidity of the formula were between the values observed in cows' milk and breast milk. By adding the citrate the pH and titratable acidity were altered to values closer to breast milk but the buffering capacity was increased to a value further away from that of breast milk.

Clinical trial. The babies took their feeds well and those fed on formula plus citrate did not vomit or have more frequent stools than those on the unmodified formula. Increases in weights, lengths, and head circumferences (Table 2) were similar in both groups during the 21 days. There was no difference in skinfold thickness and midarm muscle circumference at 21 days.

There was no difference in plasma concentration

Fig. 1 Acid base properties of the milks.

- pH: human milk had the highest pH and cows' milk the lowest. Addition of citrate to formula raised its pH to near human milk.
- Titratable acidity: human milk required less sodium bicarbonate to raise its pH to 7.45 than cows' milk, i.e. human milk has a lower titratable acidity. Addition of citrate to formula lowered its titratable acidity to nearer that of human milk.
- Buffering capacity: human milk required less hydrochloric acid to lower its pH to 5 than did cows' milk, i.e. human milk had a lower buffering capacity. By adding citrate to the formula the buffering capacity was increased to nearer that of cows' milk.

of urea, calcium, phosphate, or cholesterol, but the babies fed on formula plus citrate had lower transferrin levels (Table 2). The babies on formula plus citrate had a significantly greater base excess on days 7 and 11 than the babies on unmodified formula, who had a similar base excess to that previously found in well low birthweight babies fed on a demineralised whey formula (Svenningsen and Lindquist, 1973). However, in individual babies there was no relationship between weight gain and the degree of metabolic acidosis (Fig. 2). Further examination of the results also showed no correlation between gain in weight, length, or head circumference and acidosis on days 7 and 21. In no baby was the acidosis considered severe enough to require treatment with sodium bicarbonate.

Although there was no difference in the mean plasma Na levels between the two groups, the babies on the added citrate had more variable Na levels (Fig. 3). Hyponatraemia (>150 mmol/l) occurred in one baby and hyponatraemia (<130 mmol/l) was found in 3.

Table 2 Biochemistry of babies receiving formula and 'formula plus citrate'

Measurements (means $\pm$ SD)	Infant formulae	
	Formula	Formula plus citrate
Growth increment (day 21—day 1)		
Weight (g)	300 $\pm$ 91	322 $\pm$ 109
Length (cm)	2.3 $\pm$ 0.3	2.2 $\pm$ 0.6
Head circumference (cm)	1.61 $\pm$ 0.55	1.69 $\pm$ 0.42
Plasma or blood concentration		
Urea (mmol/l)		
Day 11	2.9 $\pm$ 0.7	2.5 $\pm$ 0.8
21	3.1 $\pm$ 0.9	2.9 $\pm$ 0.8
Calcium (mmol/l)		
Day 7	2.45 $\pm$ 0.22	2.43 $\pm$ 0.23
11	2.43 $\pm$ 0.20	2.38 $\pm$ 0.15
21	2.43 $\pm$ 0.21	2.45 $\pm$ 0.20
Phosphate (mmol/l)		
Day 7	2.48 $\pm$ 0.35	2.48 $\pm$ 0.36
11	2.48 $\pm$ 0.36	2.48 $\pm$ 0.39
21	2.39 $\pm$ 0.30	2.32 $\pm$ 0.22
Transferrin (g/l)		
Day 11	1.96 $\pm$ 0.46	1.70 $\pm$ 0.46***
21	2.20 $\pm$ 0.41	1.72 $\pm$ 0.41***
Cholesterol (mmol/l)		
Day 11	4.20 $\pm$ 0.72 (12)	3.74 $\pm$ 1.23
21	3.74 $\pm$ 1.03	3.69 $\pm$ 1.01
pH		
Day 2	7.44 $\pm$ 0.05 (11)	7.43 $\pm$ 0.03 (10)
7	7.39 $\pm$ 0.04 (8)	7.43 $\pm$ 0.04
11	7.41 $\pm$ 0.05 (12)	7.44 $\pm$ 0.08 (12)
21	7.44 $\pm$ 0.04	7.48 $\pm$ 0.06
Carbon dioxide (kPa)		
Day 2	4.32 $\pm$ 1.13 (11)	5.40 $\pm$ 0.72** (10)
7	4.69 $\pm$ 0.75 (8)	4.58 $\pm$ 0.66
11	4.47 $\pm$ 1.00 (12)	4.60 $\pm$ 0.73 (12)
21	4.60 $\pm$ 0.98	4.47 $\pm$ 0.79
Base excess (mmol/l)		
Day 2	-0.1 $\pm$ 3.4 (11)	+2.7 $\pm$ 3.2 (10)
7	-3.0 $\pm$ 0.9 (8)	-0.2 $\pm$ 3.2*
11	-2.5 $\pm$ 2.1 (12)	-0.3 $\pm$ 2.6* (12)
21	+0.2 $\pm$ 2.4	+2.6 $\pm$ 3.5

*Significantly different from result in formula group using independent *t* test $P < 0.05$; ** $P < 0.02$; *** $P < 0.01$.

Numbers in parentheses show number of estimations performed. If no number is shown, estimations were performed on all 13 babies in the group. Conversion: SI to traditional units — urea: 1 mmol/l $\approx$ 6.02 mg/100 ml; calcium: 1 mmol/l $\approx$ 4 mg/100 ml; phosphate: 1 mmol/l $\approx$ 3.1 mg/100 ml; transferrin: 1 g/l $\approx$ 100 mg/100 ml; cholesterol: 1 mmol/l $\approx$ 38.7 mg/100 ml; carbon dioxide: 1 kPa $\approx$ 7.5 mmHg.

Fig. 2 Weight gain over 21 days and base excess on day 11 in babies on formula (■) and formula plus citrate (○). There was no correlation between weight gain and base excess ($r=0.2$, $P>0.1$). Note that no baby had a base excess of less than -5. There was also no correlation with gain in length and head circumference.

Fig. 3 Plasma sodium levels on days 7, 11, and 21 in babies fed on formula (■) and formula plus citrate (○). There was no difference in the mean sodium levels. Babies on added citrate had a wider range in sodium levels.

Discussion

Recently Moore *et al.* (1977) suggested that raising the pH of cows' milk formulae may prevent babies developing late metabolic acidosis. Our results show that adding alkali routinely to a formula does indeed reduce the degree of acidosis, but we could not find any clinical advantage. The babies grew no more quickly, some had a mild metabolic alkalosis, and 3 developed hyponatraemia.

It should be realised that the acid base effects of a milk on a baby are only partly dependent on its pH; milk is a buffered system with a low free hydrogen ion concentration and the total amount of preformed acid present can be obtained only by measurement of titratable acidity and not by the pH (analogous to the measurement of pH and titratable acidity in the urine). The titratable acidity still does not indicate the complete acid base effect of the milk since it does not take into account how the nutrients are metabolised. In most natural milks the measured titratable acid is mainly citric acid, which after absorption is rapidly metabolised to CO_2 and therefore does not contribute to the net load of metabolic acid. The major sources of acid are from endogenous production of organic acids, sulphuric acid from the sulphur amino-acids—methionine and cysteine, and from hydrogen ions released during bone deposition (Kildeberg and Winters, 1972); these sources can be determined only by balance studies on the babies and not by measurement of pH and titratable acid of the milk. The effect of diet on blood pH is shown in Fig. 4. The curd whey formula is less acidogenic than unmodified cows' milk because it has a lower titratable acid (although this is probably unimportant). However, it also has a reduced endogenous production of acid because it has (a) a lower protein content (Svenningsen and Lindquist, 1973), (b) possibly a greater content of

the better used sulphur amino-acid cysteine instead of methionine, and (c) a lower Ca and phosphate concentration. Theoretically if a milk or formula has a low protein content and particularly a low curd whey ratio—e.g. human milk or demineralised whey formulae—acidosis should be less common, even though its low protein and phosphate concentration may limit urinary acid excretion (Moore *et al.* 1977). This study and that of Rih *et al.* (1976) shows that this is so. None of the babies in our study had base deficits below 5—i.e. they did not develop late metabolic acidosis (Kildeberg, 1964). These formulae seem to lead to a satisfactory acid-base balance even in moderately low birthweight babies, and presumably this is why the group that received extra alkali showed no clinical advantage. If lower protein demineralised whey formulae are used, the routine use of dietary alkali is not necessary and should be reserved for the occasional baby with true late metabolic acidosis. Not only was the routine addition unnecessary, it also had 3 untoward effects. (a) Some babies had a mild metabolic alkalosis which can affect the ionised Ca (Radde *et al.*, 1975). (b) Three babies had hyponatraemia. The wide variation in plasma Na in the formula plus citrate group was unexpected, but Harrison and Peat (1975) also found a wider variation in their babies receiving extra sodium bicarbonate. Aperia *et al.* (1977) have shown that Na given as NaHCO_3 is not as well retained as NaCl, and it is possible that hyponatraemia developed as the babies attempted to excrete the increased base in their diet and correct their metabolic alkalosis (Seldin and Rector, 1972). It therefore appears that the attractive idea (Moore *et al.*, 1977) of preventing the problem of hyponatraemia in babies fed on low sodium milks (Roy *et al.*, 1976; Honour *et al.*, 1977) and at the same time preventing late metabolic acidosis by the routine addition of alkaline Na salts is not possible.

Fig. 4 Simplified diagram illustrating how blood pH is influenced by the diet (see Kildeberg *et al.*, 1969). Milk affects the net acid-base balance by influencing the input and output of acids and bases.

(c) It increased the buffering capacity. This would favour the growth of *Escherichia coli* rather than lactobacilli in the intestine (Bullen and Willis, 1971). However Harrison and Peat (1972) found an increase in lactobacilli in the flora when alkali was added to the feed.

Addition of citrate was not associated with a lower level of cholesterol, as found by Harrison and Peat (1975), but we did find a higher plasma transferrin concentration. We cannot explain these findings but they show that dietary manipulations for one purpose can have other unexpected metabolic effects which in themselves need study.

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