Curd and whey proteins in the nutrition of low birthweight babies

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SUMMARY Some animals thrive more satisfactorily on a milk that contains whey and curd protein. For this reason human milk protein (which contains about 40% whey) may have some advantages over cows' milk protein (which contains about 15% whey) and so infants feeding formulae based on demineralised whey in which the protein has been modified to achieve a curd:whey ratio similar to that in human milk may also thrive more satisfactorily. As the exact situation in the human newborn is unclear, the effects of feeding a formula containing unmodified cows' milk protein (mainly curd) and one containing the same amount of modified cows' milk protein (curd and whey) were studied in 57 low birthweight babies during the first 3 months of life. During the early weeks of life the curd and whey group grew bigger, absorbed more nitrogen, and excreted proportionately less urea. These results suggest that a curd and whey formula has advantages in the protein nutrition of low birthweight babies, especially the preterm ones. We feel it would be unwise to reduce the protein content of a formula based on cows' milk below 15 g/l unless it was modified to achieve a larger proportion of whey protein and hence, among other qualities, more cysteine. Although some of the qualities of human milk protein can be mimicked by the use of demineralised whey formulae, others cannot.

Some animals thrive more satisfactorily on a milk that contains whey and curd protein, but the significance of this for the human baby is unclear. It is relevant to re-examine this point because of recent trends in infant feeding practice.

Whey protein (lactalbumin) is more effective in promoting the growth of rats, dogs, mice, and chicks than curd protein (casein) (Mueller and Cox, 1947); it is also more effective in regenerating the plasma albumin of protein-depleted dogs (Chow *et al.*, 1948), and in rats it has a higher 'biological value'—that is, retention of absorbed nitrogen is greater (Tomarelli and Bernhart, 1962). The significance of these observations for the human baby is not known. Empirically cows' milk protein (containing <15% of whey protein compared with >40% in human milk protein) has for many years been used with satisfaction for infant feeding; growth velocities of

babies receiving a predominantly curd protein formula were similar to those receiving a curd and whey formula (Natelson *et al.*, 1955; Barness *et al.*, 1963), and nitrogen retention on cows' milk was similar to that of human milk (Waterlow *et al.*, 1960).

The Medical Research Council and Agricultural Research Council (1974) drew attention to two recent changes in the understanding of protein metabolism. Firstly, although the effective amino-acid patterns of human and cows' milk protein may be similar, the nutritive value of milk proteins does not depend only on the component amino-acids—e.g. the absorption of intact peptides by the intestine indicates that proteins of similar amino-acid pattern might, nevertheless, be handled quite differently. Secondly, a developmental deficiency in the newborn of the liver enzyme cystathionase may prevent adequate conversion of the high methionine content of curd protein into cysteine, so limiting nitrogen retention and making cysteine—an essential amino-acid for the newborn (Gaull *et al.*, 1972).

Empirically two trends in infant feeding make it relevant to reinvestigate the matter. Firstly, the reduction in protein concentration of infant feeding formulae to 1.5–1.8 g per 100 ml—i.e. well below that in cows' milk—makes consideration of protein

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quality in these formulae more important than when most babies received formulae containing about 3.5 g cows' milk protein per 100 ml. The Department of Health and Social Security (1974) stated that the lower protein formulae should supply the 'essential amino-acids in amounts at least equal to those in an equal volume of breast milk'. More importantly, however, formulae based on demineralised whey are now available—such as Gold Cap SMA-S26, Osterfeed, and Cow and Gate Premium. One advantage of these formulae is their lower mineral content but, in addition, the curd:whey protein ratio has been altered to a value similar to that of human milk. This has a teleological appeal but it is unclear whether it has any other advantages.

For these reasons we studied the nutritional effects of curd and whey protein by comparing babies fed on two formulae: one based on demineralised whey with a curd:whey ratio similar to that in breast milk (group 1) and one containing unmodified cows' milk protein—i.e. mainly curd protein (group 2).

Patients and methods

Formulae. Fig. 1 gives details of the formulae used. The curd and whey formula was based on Gold Cap SMA-S26, and the curd formula was SMA. They were manufactured in special batches for this study by the manufacturer, John Wyeth, and the fat blend was identical. The main difference was the quality of the protein—predominantly curd protein in SMA given to group 2; curd and whey protein in the S26 given to group 1. However, as a result of the demineralisation process, the sodium and potassium concentrations in S26 were lower than those in SMA (Table 1). At reconstitution of the feeds, therefore, extra sodium (5 mmol/l as citrate) and extra potassium (4 mmol/l as citrate, 2 mmol/l as chloride)

were added to the S26 to bring the concentrations of sodium, potassium, and chloride up to those in SMA. Citrate and chloride were chosen as the other anions because they are the main milk anions (Jenness and Sloan, 1970) and also because this combination lessened the differences in the electrolyte concentrations and acid base characteristics of the two formulae. There were still differences however; although the buffering capacity was similar, the pH was higher while the titratable acidity was lower than in the curd formula; we were unable to devise any method of eliminating this difference without introducing others. There was one other (unwanted) difference between the two formulae. The curd and whey formula contained less calcium and phosphate, thus partly reflecting its lower casein content (Jenness and Sloan, 1970).

Table 1 shows the composition of the reconstituted formulae and also the composition of human milk and unmodified cows' milk. In the curd and whey formula only 41% of the nitrogen was casein, so it contained more cysteine than the curd formula in which 82% of the nitrogen was casein.

Patients. Table 2 gives details of the patients. Beginning in January 1974 all low birthweight babies weighing 1.75–2.26 kg admitted to the special care baby unit of the Sorrento Maternity Hospital, entered the study if their mothers were unable to provide breast milk and had given their informed consent. Babies with severe respiratory distress requiring more than 40% ambient oxygen and not able to tolerate tube feeding were not studied.

Feeding regimen. The first feeds were given within 4 hours of birth to babies nursed initially at the optimum environmental temperature (Hey, 1971), subsequently aiming to maintain rectal temperature at 36.5–37.5°C. Indwelling polyvinyl intragastric

Fig. 1 The constituents of the demineralised whey formulae and the curd formula.

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Table 1 Constituents of curd and curd whey formula, mature human breast milk and unmodified cows milk (per litre whole milk)

Constituent	Milk			
	Curd formula	Curd whey formula	Human*	Cows*
Protein (N $\times$ 6.37) g	15.0	15.0	12.0	33.0
% casein (of total N)	82	41	31.7	80.0
% whey (of total N)	13	44	48.2	16.3
nonprotein nitrogen	5	15	20.0	5.8
of energy	9	9	7	20
Amino-acids (mmol/l)				
Cysteine mmol	0.8	2.0	2.4	2.4
mg per 100 kcal	16	38	43	43
Methionine mmol	2.49	2.49	1.54	5.83
mg per 100 kcal	60	60	34	130
Fat g	36		38	37
% of energy		48	51	51
Lactose g	7		7	4.8
% of energy	43		42	29
Energy KJ	2814		2814	2772
Minerals mmol†				
Sodium	12.0 (7.0)		6.5	25.0
Potassium	20.0 (14.0)		14.0	35.5
Calcium	14.0	10.5		
Magnesium	2.5	2.1		
Chloride	14.0 (12.0)		12.1	29.0
Phosphate	14.2	10.6	4.8	31.0
Acid base‡				
pH	6.75	7.15 (6.90)	7.22	6.70
Buffering capacity	1.0	1.0 (0.9)	0.64	2.4
Titrate acidity	1.85	0.60 (0.93)	0.22	3.30

*Derived from Macie and Kelly (1961).

†Figures in parentheses are values before adding electrolyte.

‡Derived experimentally (Berger *et al.*, 1978).

§§§Buffering capacity: ml 0.1 mmol/l hydrochloric acid to lower pH to 5.5; titrate acidity: ml 0.25 mmol/l sodium bicarbonate to raise pH to 7.45.

Table 2 Details of babies

Babies	Feeding formula	
	Curd	Curd and whey
Number		
Starting trial	37	33
Withdrawn in first 21 days	8*	5†
Number		
Completing 21 days	29	28
Gestation (completed weeks)‡		
Mean $\pm$ SD	36.2 $\pm$ 2.1	36.3 $\pm$ 2.0
Range	(33-40)	(33-40)
Preterm	16	17
Term	13	11
Male	18	15
Asian	6	10
Number completing 90 days§§	12	12

*Lactation established 1, severe RDS 1, IV treatment for hypoglycaemia 1, parenteral antibiotics 2, convulsion 3.

†Lactation established 2, early discharge possible 1, parenteral antibiotics 2.

‡Determined from reliable maternal dates or method described by Dubowitz *et al.*, 1970.

§§§Left trial after 21 days: 21 did not keep to trial formulae for various reasons, 12 did not attend for follow-up.

tubes were used if necessary. Appropriate weight-for-dates babies (weight 10-90th centile for gestation, Gairdner and Pearson, 1971) received 40 ml/kg during the first 24 hours of life, the volume increasing by 20 ml/kg each day, reaching 200 ml by the 9th day. Small-for-dates babies (weight < 10th centile) received 60 ml/kg on the 1st day, increasing by 30 ml/kg each day, reaching 200 ml by day 6. Thereafter, the volume remained at 200 ml/kg per day until day 21, after which the mothers were advised to give approximately 200 ml/kg per day according to appetite. For volume calculations, birthweight was used until this had been regained and then the volume was recalculated after each twice-weekly weighing. Plasma bilirubin concentrations exceeded 170 μ mol/l (9.9 mg/100 ml) in 11 of group 2 and in 14 of group 1, so extra sterile water (30% of the above volume) was given to each baby until the bilirubin level had fallen to 170 μ mol/l for 24 hours. As plasma bilirubin concentrations exceeded 250 μ mol/l (14.6 mg/100 ml) in 3 babies in group 2 and 6 in group 1, these babies received phototherapy until these levels were below 220 μ mol/l (12.9 mg/100 ml).

Method of assessment and analysis.

Anthropometry

This was performed at birth and at intervals thereafter by H.M.B. (Weight: Salter spring balance; length: Harpenden neonatometer; head and midarm circumference: steel tape measure; skinfold thickness: Harpenden skin calipers).

Plasma biochemistry

Samples of blood were collected by heel prick on days 2, 7, 11, and 21, four hours after the 6 a.m. feed. On days 60 and 90 parents were asked to give no milk for 8 hours before arrival but to give dextrose water as necessary.

The samples of blood were spun down within one hour and the plasma separated and stored at -20°C until analysis as follows: albumin (Northam and Widdowson, 1967), urea by application of the Berthelot reaction after enzymatic hydrolysis with urease, calcium (Baginski *et al.*, 1973), and phosphate (Itaya and Ui, 1966).

Nitrogen balance and partition

24-hour samples of urine were collected from 17 baby boys on days 3, 10, and 19. Collection was made directly into a polyethylene container kept on ice and containing one thymol crystal. The urine was deep frozen until it was analysed for uric acid (Caraway, 1955), amino nitrogen (Goodwin, 1970), total nitrogen (Shahinian and Reinhold, 1971), urea and creatinine by standard autoanalyser methods.

Nitrogen balances were performed (Fomon, 1974) on 7 baby boys in each dietary group for 3- or 5-day periods ending on day 21 (group 1 compared with group 2 mean and SD: birthweight 2.06 ± 0.16 compared with 2.04 ± 0.16 kg; gestation 36 ± 2.1 compared with 36 ± 2.5 weeks). The total nitrogen in the formulae, urine, and faeces was determined by the method of Shahinian and Reinhold (1971).

Results

Clinical. Three of the babies in group 2 had convulsions in the first 2 days, and were withdrawn from the trial (Table 2). Neither was hypocalcaemic or hypoglycaemic but they both had low Apgar scores. Many babies did not complete 3 months, mainly because they were also receiving solid foods (usually wheat cereal). Both infant feeding formulae were well accepted with no undue vomiting, abdominal distension, or diarrhoea.

Anthropometry (Table 3). At birth the two groups of babies were similar in size apart from a slightly greater head circumference in group 1. By 21 days these babies had also become heavier and longer. This significant difference in weight gain was present in the preterm babies but not in the term light-for-dates babies (Fig. 2).

By day 90 the difference in growth was no longer significant in the small number of babies that remained in the trial. There were no differences at any age in mean skinfold thickness (using biceps, triceps, subscapular, umbilical, and quadriceps measurements) and mid upper arm muscle circumference (Gurney and Jelliffe, 1973), so the results are not given.

Plasma biochemistry (Table 4). Group 1 babies were less acidotic on days 7, 11, and 21 (these differences

Table 3 Anthropometry from birth to day 90 in the babies on curd, and curd and whey formulae.

Measurement (mean $\pm$ SD)	Birth	Day 21	Day 90
Number of babies			
Curd	29	29	12
Curd and whey	28	28	12
Weight (g)			
Curd	2051 $\pm$ 156	2304 $\pm$ 204	4486 $\pm$ 342
Curd and whey	2091 $\pm$ 107	2430 $\pm$ 140	4728 $\pm$ 417
P*		<0.01	
Length (cm)			
Curd	45.2 $\pm$ 1.4	47.1 $\pm$ 1.4	56.0 $\pm$ 1.5
Curd and whey	45.7 $\pm$ 1.0	47.9 $\pm$ 1.1	56.4 $\pm$ 1.3
P		<0.05	
Head circumference (cm)			
Curd	31.1 $\pm$ 0.9	32.9 $\pm$ 1.1	38.3 $\pm$ 1.1
Curd and whey	31.6 $\pm$ 0.6	33.5 $\pm$ 0.9	39.1 $\pm$ 1.5
P		<0.02	<0.05

*P shows significance of difference between the means of the two groups using independent *t* test; values >0.10 not shown.

Fig. 2 Weight gain (mean $\pm$ SEM) in preterm and term light-for-dates babies fed on curd (●) and curd and whey (○) formula. A greater increase in weight was found in the preterm but not the term light-for-dates babies fed on the curd and whey formula.

Table 4 Blood acid base status

	Day 2	Day 7	Day 11	Day 21
pH				
Curd	7.42 $\pm$ 0.03	7.41 $\pm$ 0.04	7.40 $\pm$ 0.07	7.45 $\pm$ 0.04
Curd and whey	7.43 $\pm$ 0.03	7.44 $\pm$ 0.04	7.44 $\pm$ 0.03	7.47 $\pm$ 0.05
P		<0.02	<0.01	$\approx$ 0.08
Carbon dioxide (kPa)				
Curd	4.66 $\pm$ 0.93	3.99 $\pm$ 0.93	3.99 $\pm$ 0.80	4.12 $\pm$ 0.67
Curd and whey	5.32 $\pm$ 0.80	4.66 $\pm$ 0.67	4.52 $\pm$ 0.80	4.39 $\pm$ 0.80
P		<0.02	<0.01	<0.05
Base excess (mmol)				
Curd	1.2 $\pm$ 2.7	-3.1 $\pm$ 2.8	-3.9 $\pm$ 3.7	-0.6 $\pm$ 2.8
Curd and whey	2.1 $\pm$ 2.3	0.6 $\pm$ 3.0	0.4 $\pm$ 3.0	2.2 $\pm$ 2.9
P		<0.001	<0.001	<0.001

Conversion: SI to traditional units—carbon dioxide 1 kPa $\approx$ 7.5 mmHg.

P significance of difference between means using independent *t* value $P > 0.10$ not shown. Numbers in parentheses are numbers of babies studied when fewer than 27 on any day.

had disappeared by days 60 and 90, so these results are not given). There was no correlation between base deficit on day 11 and weight gain during the first 3 weeks of life ($r = 0.34$). There was no difference in plasma concentration of albumin, urea, calcium, and phosphorus throughout the 90-day study period, so the results are not given.

Nitrogen balance and partition (Table 5; Fig. 2). Group 1 babies absorbed a significantly greater proportion of the ingested nitrogen; mean nitrogen retention was higher but not significantly so. These babies excreted less urea nitrogen in the urine (day 10) and had higher urinary urea nitrogen:total nitrogen ratios (Fig. 3).

Table 5 Nitrogen balance and urine urea excretion (mean $\pm$ SD) of babies receiving curd, and curd and whey formulae (mmol/kg⁻¹ 24h⁻¹)

Nitrogen	Formulae		P*
	Curd (n = 7)	Curd and whey (n = 7)	
Intake (I)	35.3 $\pm$ 1.1	35.5 $\pm$ 1.6	
Faeces (F)	3.1 $\pm$ 1.0	2.3 $\pm$ 0.7	
Urine (U)	10.3 $\pm$ 3.2	9.9 $\pm$ 2.3	<0.03
Absorbed (I-F)	31.9 $\pm$ 1.4	33.1 $\pm$ 1.5	
Absorption as % of intake	90 $\pm$ 3	93 $\pm$ 1	
Retention (I-F-U)	21.5 $\pm$ 3.4	23.2 $\pm$ 1.1	<0.03
Retention as % of intake	61 $\pm$ 9	65 $\pm$ 5	
Urinary urea nitrogen	(n = 9)	(n = 8)	
Day 3	2.07 $\pm$ 0.57	1.76 $\pm$ 0.43	
Day 10	2.69 $\pm$ 0.77	1.87 $\pm$ 0.31	
Days 19	3.70 $\pm$ 1.52	3.76 $\pm$ 1.28	<0.05

*P, significance of difference between means using Mann Whitney U test values >0.10 not shown.

Fig. 3 Urinary urea nitrogen: total nitrogen over 3 weeks. In the curd and whey group (○) the urea: total nitrogen ratio was lower on days 3 and 10 than in the curd group (●).

Discussion

During the early weeks the babies in group 1 grew bigger (particularly if preterm), absorbed more nitrogen, and lost less nitrogen as urinary urea.

Were these differences in growth and nitrogen metabolism due solely to the quality of the dietary protein? There were also differences in the acid base qualities of the formulae and indeed the babies in group 1 were significantly less acidotic.

Differences in the acid base status due to the protein quality were expected (Kildeberg and Winters, 1972) and, since addition of the citrate salts to the curd and whey formula equalised the potential base in the formulae, it is probable that the differences in the base deficits were caused by the dietary protein. A lower incidence of metabolic acidosis in babies fed a curd and whey formula was also found by Rih *et al.* (1976) in studies on protein quality. Whatever the cause, however, it is unlikely that these acid base findings explain the differences in growth; there was no correlation between base excess and weight gain; the degree of acidosis was generally mild, and only 4 babies had base deficits great enough to be diagnosed as late metabolic acidosis (Svenningsen and Lindquist, 1973); Heird *et al.* (1976) suggested that acidosis affects growth by limiting appetite but in our study babies received a prescribed volume of feed.

It seems that the differences in growth velocity and nitrogen metabolism were at least primarily related to the difference in the quality of the dietary protein.

We are uncertain which of the various qualities of whey protein accounts for these differences. Whey protein leads to quicker gastric emptying and a lower intestinal buffering capacity than curd protein (Platt, 1961; Bullen and Willis, 1971) and this may be connected with the higher nitrogen absorption in group 1, but the differences in growth and nitrogen balance may be related to the higher cysteine content of whey protein (Table 1). The curd and whey formula supplied almost as much cysteine per unit volume and energy as breast milk and, unlike the curd formula, meets the estimated cysteine requirements of 23 mg per 100 kcal (45 μ mol per 100 kJ) suggested by Fomon *et al.*, 1973. The differences in growth observed by us were not confirmed by others. Barness *et al.* (1963) found other biochemical differences but felt these had no clear advantage, although Rih *et al.* (1976) concluded that the babies on a curd and whey formula paid a smaller 'metabolic price' to obtain their growth.

Clinical implications. Although the curd and whey formula had nutritional advantages, particularly

in the preterm babies, these advantages were marginal. However, curd formulae containing 15 g/l of unmodified cows' milk protein provide less cysteine than does breast milk. If the protein and, therefore, cysteine content were further reduced to lower the renal solute load, then we suspect the reduction in growth velocity might be more pronounced than we observed. We consider it would be unwise to reduce the protein content of an infant feeding formula based on cows' milk below 15 g/l unless the protein was modified so that it included more whey protein. This caution is emphasised by susceptibility of cysteine to damage in the manufacturing processes (Pieniazek *et al.*, 1975).

Although the protein in the curd and whey formula was modified to resemble that of breast milk, nevertheless, it is still cows' milk protein. For example, the whey fraction contains lactoglobulin, more immunogenic than casein (Lebenthal, 1975), and very little lactoferrin; the curd fraction contains K casein which forms micellar complexes with certain minerals, accounting for their higher content in cows' milk (Jenness and Sloan, 1970). Cows' milk contains very little free taurine.

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