Comparison of dried milk preparations for babies on sale in 7 European countries

II. Folic acid, vitamin B₆, thiamin, riboflavin, and vitamin E

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Ford, J. E., Porter, J. W. G., Scott, K. J., Thompson, S. Y., Le Marquand, J., and Truswell, A. S. (1974). *Archives of Disease in Childhood*, 49, 874. **Comparison of dried milk preparations for babies on sale in 7 European countries. II. Folic acid, vitamin B₆, thiamin, riboflavin, and vitamin E.** Four vitamins of the B-complex—thiamin, riboflavin, vitamin B₆, and folate—were measured in 30 dried milk preparations for babies, representing products of manufacturers in 7 European countries. 7 of the preparations were also assayed for vitamin E. The milks varied widely in their B-vitamin composition. The content of riboflavin ranged from 2.8 to 11.2 µg/g, thiamin from 2.8 to 13.0, vitamin B₆ from 1.5 to 12.7, and folate from 0.03 to 1.12.

Compared on a basis of equal solids content, all the preparations were considerably richer than mature human milk in thiamin and vitamin B₆, and most were similar to human milk in their content of riboflavin, though a few were markedly higher. With folate the findings were less reassuring. In 3 of the products the content was close to that in goat's milk, which may cause folate-deficiency anaemia in infants. In a further 8 the content was less than 60% of that in human milk.

The 7 products tested showed marked differences in vitamin E content. In 4, which contained cow's milk fat only, the vitamin E content was 0.70–0.83 mg α-tocopherol equivalents/100 g as against 2.33 mg/100 g solids in human milk. The remaining 3 products contained vegetable oils and were considerably richer, with 4.77–10.21 mg/100 g.

The vitamin composition of the diet may be of special importance in the nutrition of the bottle-fed infant. In the preceding paper Widdowson, Southgate, and Schutz (1974) reported on the composition of 32 dried milk preparations; they assessed energy value, total protein, amount and composition of carbohydrate and fat, and the content of sodium, potassium, calcium, magnesium, phosphorus, iron, copper, and zinc. We are grateful to Dr. Widdowson for the opportunity to analyse 30 of these same milks for folic acid, vitamin B₆, thiamin, and riboflavin. 7 of the samples were also assayed for vitamin E.

Materials and methods

The tins of milk were first opened during late 1972 and early 1973 and subsequently resealed and stored in a refrigerator. Samples for assay of the B vitamins were taken in April 1973 and the assays carried out during the following May–July. Two fresh samples of each milk powder were obtained and analysed at once for vitamin E.

Methods of vitamin assay. Folic acid activity was measured microbiologically with *Lactobacillus casei*, by an adaptation of the procedure recommended by Herbert (1961) for the assay of folate in blood serum (Ford, 1967). The milk powders were reconstituted and prepared for test as described by Ford and Scott (1968).

Thiamin was assayed with *Lactobacillus fermenti*, as described by Deibel, Evans, and Niven (1957). Vitamin B₆ was assayed with *Kloeckera brevis*, as described by

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Barton-Wright (1963), except that the test samples were heated with 0.055 mol/l. HCl for 30 min at 100 °C as recommended by Gregory (1959) and were not predigested with Taka-diestase. Riboflavin was assayed with *Lactobacillus casei* as described by Chapman *et al.* (1957).

Vitamin E activity was measured chemically. Test samples were treated with ethanolic potassium hydroxide (Christie, Dean, and Millburn, 1973) and the unsaponifiable fraction was extracted with n-hexane under nitrogen in presence of butylated hydroxytoluene (Bieri, 1969). The vitamin E-active compounds were separated chromatographically (Whittle and Pennock, 1967) and

quantified using bathophenanthroline-ferric chloride reagent (Bieri, 1969). Standard tocopherols and tocotrienols were supplied by Roche Products Ltd, Welwyn, U.K.

Recoveries of added α - and γ -tocopherol were determined, and the values for vitamin E corrected to 100%. Recovery of α -tocopherol averaged 61% (range 55–64%) and of γ -tocopherol 56% (range 54–57%). The vitamin E-activity was expressed as mg α -tocopherol equivalents; taking the biological potency of α -tocopherol as 1.0, the relative potencies of γ -tocopherol, α -tocotrienol, and γ -tocotrienol were taken as 0.08, 0.21, and 0.01, respectively.

TABLE I

Contents (μ g/g) of folic acid, thiamin, riboflavin, and vitamin B₆ in various proprietary milk foods for babies, and in human milk and cow's milk

Product and country of manufacture	Folic acid		Thiamin		Vitamin B ₆		Riboflavin (mean $\pm$ SE)	
Britain								
Ostermilk No. 1	0.29	0.30	2.4	3.5	2.6	2.6	4.0	$\pm$ 0.56
Ostermilk No. 2	0.39	0.36	4.2	5.9	3.3	4.3	7.9	$\pm$ 0.79
Cow & Gate 1	0.27	0.24	3.5	3.4	3.0	3.5	4.6	$\pm$ 0.42
Cow & Gate 2	0.40	0.36	4.2	4.3	4.0	4.4	6.8	$\pm$ 0.67
SMA	0.43	0.39	13.0	13.0	4.7	4.7	5.9	$\pm$ 0.49
France								
Lait Guigoz 1	0.31	0.30	2.9	3.8	2.6	3.0	6.2	$\pm$ 0.24
Lait Guigoz 2	0.27	0.27	2.4	3.3	2.0	2.4	6.8	$\pm$ 0.47
Nativa	0.35	0.32	3.0	3.6	3.7	3.4	7.1	$\pm$ 0.63
Galliasec 1	0.32	0.34	3.4	3.6	3.8	4.0	4.4	$\pm$ 0.12
Milumel 1	0.25	0.25	5.1	5.8	3.6	4.9	4.9	$\pm$ 0.32
Milumel 2	0.31	0.33	5.4	6.4	2.2	3.5	4.3	$\pm$ 0.38
Lemiel 1	0.25	0.26	4.8	6.1	3.1	3.1	2.9	$\pm$ 0.17
Lemiel 2	0.19	0.18	4.7	5.8	2.8	3.2	4.8	$\pm$ 0.39
Holland								
Almiron B	0.09	0.12	4.3	4.0	2.5	2.6	5.8	$\pm$ 0.29
Farilacid	0.23	0.29	5.5	5.6	4.8	6.5	8.3	$\pm$ 0.78
Frisolac	0.03	0.02	8.9	11.6	4.8	4.0	3.4	$\pm$ 0.19
Similac	0.07	0.10	6.1	7.3	1.6	1.4	2.8	$\pm$ 0.14
Germany								
Milumil	0.45	0.49	7.2	6.6	6.4	7.0	3.3	$\pm$ 0.18
Nan	0.49	0.55	8.2	6.5	12.6	12.4	11.2	$\pm$ 0.79
Italy								
Pelargon	0.72	0.63	6.8	7.7	9.9	14.6	4.1	$\pm$ 0.12
Prodieton	0.92	0.86	6.4	7.1	12.4	13.0	6.2	$\pm$ 0.26
Plasmolac	0.18	0.15	3.5	4.0	2.3	3.5	4.8	$\pm$ 0.24
Auxolac	0.25	0.21	3.0	3.0	6.2	—	4.2	$\pm$ 0.32
Denmark								
Eledon	0.78	0.76	6.8	7.9	11.8	11.6	3.8	$\pm$ 0.24
Pelargon	0.73	0.74	7.2	6.6	12.5	11.6	4.8	$\pm$ 0.21
Switzerland								
Eledon	1.12	0.91	4.2	5.8	3.9	3.5	10.5	$\pm$ 1.30
Pelargon	0.56	0.53	2.7	2.9	2.9	2.4	6.8	$\pm$ 0.56
Guigolac	0.32	0.29	2.8	3.0	2.1	3.0	7.4	$\pm$ 0.24
Humana 1	0.23	0.22	9.7	13.1	7.5	11.9	3.4	$\pm$ 0.31
Humana 2	0.23	0.20	9.0	11.3	6.8	8.7	6.2	$\pm$ 0.44
Human milk*	0.40		1.2		0.32		4.0	
Cow's milk*	0.40		3.6		3.2		12.0	

*Typical values for mature milk, calculated to a dry matter basis.

Note: Table I shows results of two independent assays on each sample for folic acid, thiamin, and vitamin B₆. Values obtained from riboflavin in several of the samples were unexpectedly low, and to check them all the samples were assayed four times.

Results and discussion

The results of the B-vitamin assays are set out in Table I, and those for vitamin E in Table II.

Folic acid. Cow's milk and mature breast milk are about equally rich in folate, but the vitamin is labile and may be largely lost in the course of the manufacture of infant foods, or during their subsequent storage and preparation for use. Taking 54 $\mu\text{g/l.}$ as being average for the folate content of human milk (Ford and Scott, 1968), it is evident that several of the present formulas provided considerably less. Thus, on a basis of 12.5% (w/v) total solids in the reconstituted milks, Almiron B provided 13 $\mu\text{g/l.}$, Similac 11 $\mu\text{g/l.}$, and Frisolac only 3 $\mu\text{g/l.}$ These values should be compared with that of 6 $\mu\text{g/l.}$ which is typical for goat's milk, the consumption of which as the sole diet is well known to cause megaloblastic anaemia in infants—the so-called 'goat's milk anaemia'. As a check on these findings fresh samples of Almiron B were obtained from the manufacturer. Their folate content was similar to that recorded above.

In a further 8 of the formulas the folate content was only about half of that in human milk. These findings give some cause for concern and indicate that greater care may sometimes be needed to ensure the conservation of folate during manufacture, or that with certain formulas it may be necessary to provide a supplement of folic acid.

Thiamin. Mature human milk contains only about 150 μg thiamin/l., as against 360–1625 $\mu\text{g/l.}$ measured in the present milk formulas after reconstitution. There was clearly an adequate margin of safety to cover any likely losses during preparation of the foods for use.

Vitamin B₆. In our experience, mature human milk contains about 40 μg vitamin B₆/l., and cow's milk about 400 $\mu\text{g/l.}$ The reconstituted milk formulas varied widely in their vitamin B₆ content—from 190 to 1590 $\mu\text{g/l.}$ Thus, even the poorest of the preparations contained nearly 5 times the concentration present in human milk.

Riboflavin. The riboflavin content of the reconstituted milks ranged from 0.35 to 1.4 mg/l. Some of the values were unexpectedly low, and they were carefully checked in four independent assays on all the samples. As a further check all the samples were again assayed by the fluorescence method of Emmerie (1938), modified as described by Ford *et al.* (1969). The results correlated ($r = 0.95$) with those from the microbiological tests but they were higher by an average of 37%. If we assume that the lower values from the microbiological assays were correct, then 5 of the reconstituted milks contained marginally less than 0.5 mg riboflavin/l. which, from our experience and from surveying values in published reports, is about average for mature human milk. 22 contained 0.5–1.0 mg/l. and 3 contained > 1.0 .

Vitamin E. Table II gives the vitamin E content of 7 of the formulas, and also the ratio of vitamin E to polyunsaturated fatty acid (PUFA). The different products showed marked differences in vitamin E content. The first four products listed contained cow's milk fat only, and the vitamin E activity was predominantly due to α -tocopherol. The remaining 3 formulas contained vegetable oils, and were therefore richer in vitamin E and also in PUFA, which are believed to increase the vitamin E requirement.

TABLE II

Content of vitamin E in selected milk powders and in human milk and cow's milk. For comparison, values are also given for the polyunsaturated fatty acid content (PUFA)

Product	Vitamin E (mg α -tocopherol equivalents/100 g dry matter)	PUFA* (g/100 g dry matter)	Vitamin E PUFA ratio (mg/g)
Ostermilk No. 1	0.70	0.44	1.6
Ostermilk No. 2	0.77	0.37	2.1
Cow & Gate 1	0.83	0.26	3.2
Cow & Gate 2	0.80	0.48	1.65
SMA	8.07	7.17	1.1
Cow & Gate V formula	10.21	3.25	3.1
Almiron B	4.77	12.68	0.38
Human milk	2.33†	4.91	0.47
Cow's milk	0.80	0.47	1.69

*PUFA values from Widdowson and Schutz (personal communication, 1973) except for Cow & Gate V formula where the manufacturer's PUFA value was used. PUFA taken as 18:2 content except for SMA, Almiron B, and human milk where PUFA is taken as the sum of 18:2 and 18:3.

†Value for human milk vitamin E from McCance and Widdowson (1969).

There are two criteria by which the adequacy of the formulas as sources of vitamin E may be judged. We may compare the vitamin E content of the formulas with that of human milk. By this criterion the formulas containing cow's milk fat were inadequate.

Alternatively, we may compare the vitamin E/PUFA ratio for the formulas with that for human milk and with the recommended value of 0.6 mg/g (Harris and Embree, 1963; Witting, 1972). On this basis of comparison all the formulas except Almiron B were adequate.

Davis (1972), in the U.S.A., reported low vitamin E/PUFA ratios in milk formulas, which were apparently attributable to the high content of PUFA. Desai, O'Leary, and Schwartz (1972), in Canada, reported a similar finding, though in this case the vitamin E levels were lower.

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