Vitamin requirements for term infants: considerations for infant formulae

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Abstract

Objective. To provide the informed health professional with an up to date evaluation of the current thinking regarding requirements for vitamins in infant feeds.

Establishing criteria for adequacy. Vitamin adequacy in the neonate is currently defined in terms of circulating levels of a vitamin or of the activity of a vitamin dependent enzyme in the erythrocytes. Although these measurements have their value there is a need to develop biochemical, physiological or clinical markers of well defined specific function. For some vitamins there is a risk of deleterious effects of very high intakes: risk of toxicity needs to be taken into consideration when making recommendations for inclusion in infant formulae.

Breast milk as the 'gold standard'. Breast milk concentrations of vitamins have been used as the criteria of adequate intake by neonates. This may not always be justified. Greater consideration needs to be given to differences in bioavailability of vitamins from breast milk compared with formula feeds, of the influence of season, and of stage of lactation, on the stated composition.

Experimental approaches. Animal studies have provided limited information regarding effects of different levels of intakes on current status indices in the neonatal period. There are few reports of randomized controlled studies into the effects of different levels of vitamins and these rely heavily on biochemical criteria of adequacy.

Recent developments. The inclusion of β-carotene into formula feeds for premature babies is an issue of current interest. What is the justification for this? Are there potential benefits for the term infant? Riboflavin deficiency in the period around weaning may affect the normal structural and functional development of the gastrointestinal tract; some of these effects may be permanent.

Research to be done. A greater understanding of the absorption and metabolism of vitamins during infancy is required in order to help establish dietary requirements. The relative bioavailability of vitamins in human milk and formulae needs to be investigated. Criteria for vitamin adequacy should be extended to include measures of function. Information regarding the conversion factor from tryptophan to niacin in infancy would allow us to set niacin requirements with greater confidence. There is a particular lack of information about concentrations of biotin and pantothenic acid in breast milk and the relative biochemical status of infants receiving breast milk and formulae. Benefits of including β-carotene into infant formulae need to be evaluated. The role of individual micronutrients in the structural and functional development of the gastrointestinal tract should be explored.

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Introduction

After birth there is a period of intense developmental activity of many of the body's systems, and the energy and micronutrient requirements of the newborn infant are correspondingly high. Breast milk concentrations of micronutrients are considered to be adequate for normal growth and development, for only in unusual circumstances do frank clinical deficiencies occur in breast fed infants. In well nourished populations biochemical markers of vitamin status appear to reflect adequate intakes of vitamins during early infancy in most studies of babies born at term, with the exception of vitamins D and K. Even when maternal vitamin intakes are low, transport into the milk often occurs at the expense of maternal status. Also, with the exception of vitamins D and K, concentrations of vitamins in the milk are generally fairly unresponsive to very high maternal intakes.

Setting the criteria for adequacy

The most recent Government publication covering Dietary Reference Values (DRV) includes values for infants for little more than half of the vitamins (Department of Health, 1991). A fundamental difference between this and earlier reports lies in the very use of the term DRV, in recognition of the continuing potential for misuse and misinterpretation of a single value for recommended daily intakes of any nutrient. Consequently current guidelines for daily vitamin intakes take the form of a range of values describing the distribution of requirements for individual vitamins. By and large, in the literature however, recommendations for enteral intakes of vitamins by infants are published as single values, a departure from the spirit of the COMA Report on DRV (Department of Health, 1991).

The value of the DRV as the basis of recommendations for the inclusion of vitamins in infant formulae is limited by the quality of the information on which the DRV have been set. A further issue of continuing debate is the criteria by which to assess adequacy of intake for individual vitamins. The lower reference nutrient intake, set at a level 2 SD below the estimated average requirement, is considered to meet the needs of some individuals in a population group, and is notionally comparable to the minimum intake required to prevent clinical symptoms of a deficiency. The Reference Nutrient Intake (RNI), set at a level 2 SD above the estimated average requirement, is considered to cover the requirements of most of a population and therefore is comparable to the early recommended daily intake. A further consideration is whether intakes greater than the upper limit of the normal range could offer any particular benefits.

Defining vitamin deficiency

Daily intakes of a vitamin that are so low as to lead to clinical signs of deficiency are unequivocally inadequate. Higher intakes may be sufficient to prevent clinical signs of deficiency but may nevertheless be associated with altered physiological function or abnormal biochemistry. In the early stages of vitamin depletion there will be a reduction in tissue stores and/or a lower concentration of the vitamin in the circulation, changes which can be identified biochemically. As depletion progresses the reduced availability of the vitamin may disrupt cellular function, the activity of specific vitamin dependent enzymes may fall, and abnormal metabolites may appear in the plasma and urine. Physiological disturbances (reduced grip

strength, impaired night vision), may accompany or succeed such biochemical changes. What level of depletion constitutes deficiency? It is conventional to refer to vitamin deficiency as clinical or subclinical. Subclinical deficiency is a broad term embracing values for biochemical and physiological markers of vitamin status deemed to fall outside normal limits in the absence of clinical or pathological signs of abnormality. Although the measurement of plasma or erythrocyte concentration can provide useful information for some vitamins, biochemical or physiological measurements that are clearly related to some functional abnormality can provide information that is more useful. These are usually referred to as 'functional indices'. Table 1 summarizes the main functional indices currently in use for the assessment of vitamin status. It can be seen that for some vitamins there is no functional index of status, which is therefore assessed by measuring the circulating concentration of the vitamin or a metabolite.

The information on which the estimates of adult requirements for vitamins have been based comes from a variety of sources, the most commonly used being: intakes required to maintain specified circulating levels of a vitamin, tissue concentrations, or the activity of a vitamin dependent enzyme; intakes by a group deemed to be free of deficiency disease; intakes associated with a functional marker of adequacy. Estimates of infant vitamin requirements are based for the most part on calculations of intakes by breast fed babies, among whom clinical signs of vitamin deficiency are rare. These estimates have not always been supported by biochemical evidence of adequacy. Only a few studies in infants have used functional indices of vitamin status, and these are given in the Table.

It can be argued that unless data are available to suggest that deficiency or toxicity occurs in breast fed infants, recommendations for intakes that approximate to the average amounts consumed by breast fed infants should be adequate. The onus is on nutritionists to establish functional indices of vitamin inadequacy and thereby to demonstrate that vitamin deficiency is not a feature of exclusive breast feeding in a generally well nourished population.

Breast milk as a 'gold standard'

The consensus approach to the estimation of infant vitamin requirements is to calculate approximate intakes in breast fed infants. This approach takes the reasonable view that breast milk provides an adequate source of vitamins and circumvents the practical and ethical constraints to more experimental approaches to the estimation of requirements. There are however drawbacks to this approach.

Measurement of vitamins in breast milk

Differences in composition of milk collected from the left or the right breast, differences in composition of foremilk from hindmilk, diurnal variation, seasonal variation, and stage of lactation may all introduce variation in measured concentration of vitamins (Greene, 1982; Ford *et al.* 1983; Butte *et al.* 1984). These factors should therefore be taken into account when publishing values for milk composition. There are, in addition, technical difficulties in measuring the concentration of certain vitamins in milk. For some vitamins stability can be a problem, as for vitamin C and riboflavin (Bates *et al.* 1985; Fritz *et al.* 1987). For others, such as folate and vitamin A, the method of extraction and detection can influence the accuracy of the measurement. A number of reviews have been written about the concentration of vitamins in human milk; the reader is referred to Lönnerdal (1986), George & de Francesca (1989) and

Table 1. Functional indices of vitamin status

Vitamin	Main functional indices*	Comments	Example of application in infants
Thiamin	Erythrocyte transketolase activation coefficient	Specific for thiamin and reasonably sensitive	DeBuse 1992
Riboflavin	Erythrocyte glutathione reductase activation coefficient	Specific for riboflavin and sensitive to moderate depletion. Not suitable for subjects with glucose-6-phosphate dehydrogenase deficiency	Bates *et al.* 1982
Pyridoxine	Activation coefficient for erythrocyte transaminases	Specific for pyridoxine; may not respond to short term acute depletion	Moore *et al.* 1986
	Xanthurenic acid excretion following a tryptophan 'load'	Not very sensitive to short term depletion. Flux through this pathway is disturbed by a number of factors other than vitamin status	Bessey *et al.* 1957
Folate	Formiminoglutamic acid excretion after a histidine load	Not very sensitive to short term depletion. Abnormal also in vitamin B_{12} deficiency	Hoffbrand, 1970
	Plasma total homocysteine	Sensitive, but also influenced by vitamin B_6 and vitamin B_{12} status	
Vitamin B_{12}	Methylmalonic acid excretion with or without a valine load	Specific to vitamin B_{12}. Reasonably sensitive to moderate depletion	Specker *et al.* 1990
	Plasma total homocysteine	(see folate)	
Biotin	Abnormal excretion of various organic acids	Probably not sensitive to moderate depletion; not entirely specific	
Vitamin A	Relative dose response: percentage increase in plasma retinol following an oral dose	Specific, although not sensitive to moderate depletion	
	Urinary excretion of glycosaminoglycans	Not yet clear	
Vitamin E	Red blood cell haemolysis on exposure to hydrogen peroxide	Neither sensitive nor entirely specific	Miyake *et al.* 1991
Vitamin K	Plasma PIVKA (protein induced by vitamin K absence)	Specific for vitamin K but may not be sensitive to moderate depletion	Corrigan & Kryc, 1980
	'One-stage prothrombin time': time taken to form a fibrin clot	Neither specific nor sensitive	Greer *et al.* 1991

* The activation coefficient is the ratio between the activities of the enzyme measured in the presence and the absence of its cofactor (either the vitamin or a compound derived from the vitamin).

Bates & Prentice (1994). These last authors have also written an excellent review which considers the effects of maternal vitamin supplements on levels in human milk (Bates & Prentice, 1996).

Estimation of volumes of milk consumed

If concentrations of vitamins in breast milk are to be translated into estimates of infant vitamin requirements information is needed about the average volume of breast milk consumed by infants of different ages. Test weighing of babies before and after feeds has formed the basis for estimates of milk consumption by infants and if performed with due care is reported to give values accurate to 3·0 g (SD 3·1) (Butte *et al.* 1984). In contrast with cross-sectional studies and some longitudinal studies with high attrition rates (Wallgren, 1945; Whitehead & Paul, 1981; Dewey & Lönnerdal, 1982), Butte *et al.* (1984) showed no steady increase in milk intake over 4 months of study of 41 breast fed infants, and an overall mean of 733 (SD 89) ml/d. The mean coefficient of variation in milk intake among infants of the same age was 17 % (SD 1 %). Milk intakes over the first six months of life in infants in well nourished communities are reported to range between 600 and 900 ml/d (Lönnerdal *et al.* 1976; Picciano *et al.* 1981).

In this article, calculations of vitamin consumption from breast milk make the assumption that 750 ml is an average volume of milk produced during the first six months of breast feeding.

Bioavailability of vitamins from breast milk

Of greater practical importance than simply the concentration of a vitamin in breast milk or formulae is the proportion that is available for use in the body, i.e. the bioavailability. A major determinant of nutrient bioavailability is the efficiency of absorption, the measurement of which forms the basis of several approaches to the estimation of vitamin bioavailability. Factors such as current nutrient status, nutrient digestion and gastrointestinal integrity can have an effect on the rate of vitamin absorption. There are virtually no comparative studies of the bioavailability of vitamins in formulae and human milk but there are some *in vitro* data that suggest there may be differences for some vitamins.

Studies of the form in which vitamin B_{12} occurs in breast milk have revealed the presence of vitamin binding proteins, other than transcobalamin, which may facilitate absorption (Sandberg *et al.* 1981; Trugo & Sardinha 1994).

Folate polyglutamates have about half of the biological activity of the monoglutamates; some reports show that the monoglutamates predominate in human milk which may therefore offer a source of folate with high availability (Halsted *et al.* 1978; Cooperman & Lopez, 1991). A further suggestion is that folate binding proteins in breast milk may protect the folate from bacteria and facilitate absorption, thus making folate in human milk more available than from formulae (Antony *et al.* 1982; Ek & Magnus, 1982). In addition a bile-salt stimulated lipase in human milk hydrolyses retinyl esters, thereby facilitating absorption of vitamin A (Fredrikzon *et al.* 1978).

Differences in availability of vitamins from breast milk and formulae have not been properly evaluated but may result in misleading estimates of infant vitamin requirements. It is tempting to assume that the availability of vitamins from formulae is less likely to be limited by other constituents than for human milk but iron is particularly well absorbed from human milk in comparison with formulae and the same may be true of some vitamins.

Practical considerations

Even assuming that consensus can be reached regarding daily vitamin requirements for infants, there are further considerations to be made by the manufacturers of infant formulae. Certain vitamins, notably vitamins A, C and thiamin, are degraded during the manufacture of formulae and during shelf life storage. There may also be a varying vitamin concentration in the raw materials used in the manufacturing process.

The basis for making recommendations

Published values for concentrations in breast milk will be presented (see Tables 2*a*, *b*) followed by a discussion of experimental data pertaining to requirements. The current levels in formulae will be evaluated in the light of these data. See also Tables 3 and 4.

Water-soluble vitamins

Water-soluble vitamins have no storage tissue and therefore tissue depletion occurs rapidly during a period of low dietary intake. In general the maternofetal transport favours active accumulation in the fetus throughout gestation and circulating levels are often higher in the infant at birth than in the mother. Levels in the circulation fall relatively rapidly in the neonatal period if intakes are not maintained (Greene, 1982).

Vitamin B_1—Thiamin

Thiamin is active metabolically in the form of thiamin pyrophosphate, as the cofactor for several enzymes important for oxidative decarboxylation of intermediary metabolites, and in the transketolation of some pentoses. Severe deficiency in infancy (infantile beriberi) is characterized by hypotonia, vomiting, and abdominal pain, and may progress to cardiac failure. Other than anaphylactic shock in response to high parenteral doses there are no known toxic effects of thiamin in humans. The saturability of the absorption process (no more than 2·5 mg at a time) offers a useful guideline for an upper limit of intake (Morrison & Campbell, 1960).

Levels in breast milk. Average breast milk levels of thiamin show relatively little variation among women from developing countries and those from the more industrialized countries. Despite some evidence for lower thiamin intakes among women from India, the Gambia, and Kenya, mean levels of mature breast milk thiamin are relatively constant at around 0·15 mg/l, and comparable to mean levels in breast milk from well nourished women, between 0·14 and 0·18 mg/l (Macy, 1949; Department of Health & Social Security, 1977). This suggests a relative insensitivity of breast milk thiamin to maternal intakes although it is possible to increase levels by maternal supplements early in lactation (Kon & Mawson, 1950). Levels increase as lactation progresses. Thiamin deficiency has not been reported in breast fed infants born to well nourished mothers although beriberi has been documented in a breast fed infant born to a malnourished mother (DeBuse, 1992). Clinical thiamin deficiency has also been reported in a partly breast fed infant receiving supplemental nondairy formula (Wyatt *et al.* 1987). The calculated thiamin content of the formula was $<10\,\mu g/l$.

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Table 2*a*. Water-soluble vitamin levels in milk collected from mothers in industrialized countries (beyond 15 days *post partum*)

Vitamin	Range of mean values	References	Level achieved with oral supplementation	Reference
Thiamin (mg/l)	0·14–0·22	Kon & Mawson, 1950; Chanda *et al.* 1951; Nail *et al.* 1980; Thomas *et al.* 1980; Schanler & Nichols, 1985	0·28	Kon & Mawson, 1950
Niacin[a] (mg/l)	1·8–2·0	Coryell *et al.* 1945; Pratt *et al.* 1951; Ford *et al.* 1983	3·9	Pratt *et al.* 1951
Folate (μg/l)	11–110[b]	Pratt *et al.* 1951; Thomas *et al.* 1980; Sneed *et al.* 1981; Cooperman *et al.* 1982; Ek, 1983; Smith *et al.* 1983; Eitenmiller *et al.* 1984; Udipi *et al.* 1987; Van Zoeren-Grobben *et al.* 1987; O'Connor *et al.* 1991	137	Tamura *et al.* 1980
Ascorbic acid (mg/l)	33–85	Munks *et al.* 1945; Kon & Mawson, 1950; Irwin & Hutchins, 1976; Thomas *et al.* 1979, 1980; Sneed *et al.* 1981; Karra *et al.* 1986	110	Irwin & Hutchins, 1976
Riboflavin (mg/l)	0·24–0·48	Roderuck *et al.* 1945; Kon & Mawson, 1950; Nail *et al.* 1980; Thomas *et al.* 1980; Ford *et al.* 1983; Van Zoeren-Grobben *et al.* 1987	1·87	Pratt *et al.* 1951
Pyridoxine (mg/l)	0·015–0·2	Roepke & Kirksey, 1979; Thomas *et al.* 1979, 1980; Sneed *et al.* 1981; Ford *et al.* 1983; Styslinger & Kirksey, 1985; Van Zoeren-Grobben *et al.* 1987; Moser-Veillon & Reynolds, 1990; Kang-Yoon *et al.* 1992	0·4	Kang-Yoon *et al.* 1992
Vitamin B_{12} (μg/l)	0·23–0·97	Thomas *et al.* 1979, 1980; Sneed *et al.* 1981; Ford *et al.* 1983	1·8	Trugo & Sardinha, 1994
Pantothenic acid (mg/l)	2·6–6·7	Johnston *et al.* 1981; Ford *et al.* 1983; Song *et al.* 1984		
Biotin (μg/l)	5·2–11·0	Ford *et al.* 1983; Hirano *et al.* 1992; Mock *et al.* 1992		

[a] not including the contribution from tryptophan.
[b] reports of very low values may not be reliable (see text).

Table 2*b*. Fat-soluble vitamin levels in milk collected from mothers in industrialized countries (beyond 15 days *post partum*)

Vitamin	Range of mean values	References	Level achieved with oral supplementation	Reference
vitamin D[b](μg/l)	not detectable–2·1	Greer & Tsang 1983; Hollis, 1983; Ala-Houhala *et al.* 1988; Hollis *et al.* 1988; Hoogenboezem *et al.* 1988; Takeuchi *et al.* 1988; Vieth, 1990	180	Hollis *et al.* 1988
vitamin E[c] (mg/l)	1·1–8·0	Jansson *et al.* 1981; Thomas *et al.* 1981; Chappell *et al.* 1985; Lammi-Keefe *et al.* 1985; Chappell *et al.* 1986; Harzer *et al.* 1986; Moffatt *et al.* 1987; Van Zoeren-Grobben *et al.* 1987	11	Anderson & Pittard, 1985
vitamin A (mg/l)	0·49–0·77	Thomas *et al.* 1981; Chappell *et al.* 1985; Kim *et al.* 1990		
carotenoids (mg/l)	0·08–0·23	Thomas *et al.* 1981; Chappell *et al.* 1985; Kim *et al.* 1990		
vitamin K (μg/l)	1·0–9·2	Motohara *et al.* 1984; Von Kries *et al.* 1987; Fournier *et al.* 1987; Canfield *et al.* 1991; Pietschnig *et al.* 1993	130	Greer *et al.* 1991

[b] vitamin D equivalent.
[c] α-tocopherol equivalent.

Table 3. Range of concentrations of vitamins in formula for term infants from birth to six months compared with levels in human milk

	formula[a] (per litre)	human milk[b] (per litre)
Thiamin (mg)	0·39–1·00	0·14–0·28
Riboflavin (mg)	0·53–1·50	0·4–2·87[c]
Niacin (mg)	3·4–9·00	1·8–3·9
Pyridoxine (mg)	0·33–0·65	0·23–0·4[d]
Folic acid (μg)	33–110	30–137[e]
Vitamin B_{12} (μg)	0·33–2·2	0·23–1·8
Ascorbic acid (mg)	66–90	33–110
Pantothenic acid (mg)	2·0–4·0	2·6
Biotin (μg)	10–20	5·2
Vitamin A (mg RE)	0·6–1·0	0·49–0·77[f]
Vitamin E (mg α-TE)	4·6–10	1·1–8·0[g]
Vitamin D (μg)	10–11	13–17[h]
Vitamin K (μg)	26–100	13–130[i]

[a] Information supplied by the following companies, whose formulae are available in the UK: Boots, Cow and Gate, Farleys, SMA, Milupa. (The energy content of these formulae ranges between 65 and 70 kcals/100 ml; the protein content between 1·3 and 1·6 g/100 ml).

[b] lower limit: the lowest average concentration reported in human milk from apparently healthy women, ideally where biochemical or clinical evidence of healthy baby; upper limit: the highest concentration in human milk (after supplementation, in the absence of any biochemical evidence of excess in the baby.

[c] lower limit based on intakes in Gambian infants sufficient to normalize biochemical deficiency.

[d] lower limit based on average in human breast milk of mothers consuming the reference nutrient intake.

[e] folate may be less available from formula than human milk; this intake in formula is associated with normal erythrocyte morphology in term infants.

[f] lower limit based on recommendations by Olson (1987*b*).

[g] α-tocopherol equivalents.

[h] intake sufficient to maintain plasma 25-hydroxyvitamin D_3 above 20 ng/ml.

[i] based on upper level in breast milk in unsupplemented women from industrialized countries.

RE, retinol equivalent (mg retinol (usually as an ester) + 1/6 mg β-carotene + 1/12 other biologically active carotenoids; TE, α-tocopherol equivalents.

Other factors. Other than levels in human milk, very few data are available on which to base requirements for thiamin in infancy. Requirements are closely related to energy intake and therefore a useful manner in which to express these for infants is μg/100 kcal. Accordingly, and basing requirements for infants almost exclusively on levels reported in breast milk, the RNI for thiamin from birth up to 12 months has been set at 30 μg/100 kcal (0·2 mg/l), by the most recent COMA panel (Department of Health, 1991). This is generous compared with the DHSS (1981) guidelines of 19 μg/100 kcal (0·14 mg/l) for the composition of artificial feeds. There is no clear rationale for this discrepancy, but in practical terms the difference is unlikely to have any biological significance. Of greater interest is the substantial discrepancy between these values and the actual content of thiamin in infant formulae (Table 3). Levels in commercial formulae for term infants range from 0·39 to 1·00 mg/l (55–142 μg/100 kcal), providing about 0·3–0·75 mg/d. One very old study suggested that infants between 7 and 12 months receiving 0·12–0·20 mg/d showed normal levels of urinary metabolites (Holt *et al.* 1949). Unfortunately there are no more recent comparable data, but the indication is that current levels of thiamin in some formulae may be well in excess of needs (Table 4). The lack of reported toxic effects of

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Table 4. Estimated daily vitamin intakes from formulae compared with current recommendations

Vitamin	Intake from formulae[a]	UK RNI[b]	USA RDI[c]
Thiamin (mg)	0·29–0·75	0·2	0·3
Riboflavin (mg)	0·40–1·13	0·4	0·4
Niacin (mg)	2·55–6·75	3	6
Pyridoxine (mg)	0·25–0·49	0·2	0·3
Folic acid (μg)	25–83	50	25
Vitamin B_{12} (μg)	0·98–1·65	0·3	0·3
Ascorbic acid (mg)	50–68	25	30
Pantothenic acid (mg)	1·5–3·0	1·7*	2*
Biotin (μg)	7·5–11·3	NA	10*
Vitamin A (μg RE)	450–750	350	375
Vitamin E (mg)	3·5–7·5	> 2·35*	3
Vitamin D (μg)	7·5–11	8·5	7·5
Vitamin K (μg)	19·5–75·0	NA	5

[a] Estimated from levels in formula (Table 2) and a consumption of 750 ml daily.
[b] Department of Health (1991).
[c] National Research Council (1989).
* Estimated safe and adequate intake.
NA, none available; RDI, recommended daily intake; RE, retinol equivalents; RNI, reference nutrient intake.

oral doses of this vitamin will have presumably encouraged the use of high levels in formulae to provide a generous safety margin. There are likely also to be concerns about loss of the vitamin during manufacture, and during the shelf life of the product.

Vitamin B_2—Riboflavin

Riboflavin is a component of flavin mononucleotide and flavin adenine dinucleotide, both of which serve as electron acceptors or donors in reactions ubiquitous throughout metabolism. Clinical signs of deficiency include angular dermatitis, seborrheic dermatitis and corneal vascularization. Impaired growth rate is a feature of riboflavin deficiency and there is evidence that iron absorption and metabolism are disturbed (Powers *et al.* 1991; Fairweather-Tait *et al.* 1992; Powers, 1995). Except when very high parenteral doses (300–400 mg/kg) have been given to preterm infants there have been no reports of toxicity in humans.

Levels in breast milk. The concentration of riboflavin in human milk is considerably lower than in cows' milk and is fairly sensitive to maternal intake (Deodhar *et al.* 1964; Nail *et al.* 1980; Bates *et al.* 1982). Average levels in mature breast milk from mothers in industrialized countries are reported to lie around 0·3 mg/l, but can range between 0·2 and 0·8 mg/l (Macy, 1949; DHSS, 1977; Ford *et al.* 1983). Mothers whose riboflavin intakes are low may produce breast milk with reduced riboflavin content (mean 0·20 mg/l) and give birth to babies with biochemical evidence of riboflavin deficiency (Bates *et al.* 1982). Under these circumstances the breast milk levels are often insufficient to correct the riboflavin deficiency in the infant. Higher levels (up to 1·87 mg/l) are achievable if mothers are supplemented with riboflavin, with no adverse effects to the baby.

Other factors. Controlled supplementation studies among Gambian infants have provided additional data on which to base requirements for riboflavin over the first year of life. When intakes from breast milk, estimated to be about 0·2 mg/d, were increased to 0·4 mg/d by supplementary feeds, erythrocyte glutathione reductase activation coefficient was normalized (Bates *et al.* 1982). The RNI for formula fed infants has accordingly been set at this level (Department of Health, 1991). This level is generous compared with an average intake of 0·23 mg/d calculated from the concentration in breast milk appearing in the DHSS (1977) report on the composition of human milk, and 0·26 mg/d calculated from a broader range of studies conducted in the West and reported by Schanler (1989).

Babies receiving phototherapy for hyperbilirubinaemia are known to be at risk of developing transient biochemical riboflavin deficiency (Gromisch *et al.* 1977; Sisson, 1987). There is evidence that a transient riboflavin depletion may not be restricted to this group of babies but may be a feature of some term babies receiving human milk exclusively (Hovi *et al.* 1979). Unfortunately it is not known whether the riboflavin concentration in the breast milk fed to these babies was especially low.

Recent developments. Evidence is accumulating from animal studies to show that a transient riboflavin deficiency at around weaning results in morphological and kinetic changes in the duodenal epithelium which are not readily reversible (Powers *et al.* 1993; Williams *et al.* 1995, 1996). Early changes include crypt hypertrophy, a reduction in the crypt bifurcation rate and a failure to produce the normal density of duodenal villi. These changes would be expected to reduce the absorptive surface area of the duodenum. As the depletion progresses an increased rate of crypt cell proliferation, together with an increased rate of transit of enterocytes along the villi, may reduce the absorptive lifespan of enterocytes. An observed lengthening of the villi may be an adaptive response to a reduced absorptive surface area. The response to riboflavin depletion is remarkably fast; a recent study showed morphological abnormalities after only 70 hours of feeding a deficient diet (Yates *et al.* 1997). This work provides a clear demonstration of the vulnerability of gastrointestinal development to nutritional insults in infancy. Riboflavin deficiency at a critical time in gastrointestinal development may result in permanent arrest of normal development of the gastrointestinal tract.

Levels in formulae for term infants range between 0·53 and 1·5 mg/l, which are comparable with levels in breast milk and provide about 0·4–1·23 mg/d (Table 3). The possibility that even a transient riboflavin deficiency during infancy has long term consequences requires further study. Further developments in this area of research should be taken into account when considering recommendations for levels in infant formulae. Although toxicity is not likely to pose a problem an upper limit could be set based on levels achieved in the milk of supplemented mothers (Table 3).

Vitamin B_6—Pyridoxine

The term vitamin B_6 refers to three pyridines; pyridoxine (also called pyridoxol), pyridoxal and pyridoxamine, which are interconvertible. The phosphorylated derivatives of pyridoxal and pyridoxamine act as coenzymes in reactions especially prominent in amino acid metabolism. Some pyridoxal phosphate dependent enzymes are important in neurotransmitter synthesis and myelination and a deficiency of vitamin B_6 has been shown to disturb normal brain development in rats. Deficiency in infants is reported to be associated with anaemia, failure to thrive, diarrhoea and vomiting, and in severe deficiency to lead to seizures. Very high intakes of pyridoxine have been associated with sensory neuropathy, but treatment of inborn metabolic errors with 500–1500 mg/d are without reported ill-effects.

Levels in breast milk. Maternal intake of vitamin B_6 (as pyridoxine, pyridoxal or pyridoxamine and their phosphorylated derivatives) influences levels in breast milk (Kirksey & Udipi, 1985). Reported levels of vitamin B_6 in human milk vary greatly depending in part on the vitamin intake of the mother, the bioavailability of the vitamin source and the stage of lactation. Average concentrations reported in mature milk from unsupplemented women in industrialized nations range between 0·1 and 0·18 mg/l with a wide individual variation between 0·07 and 0·22 mg/l (Macy, 1949; Kon & Mawson, 1950; Ford *et al.* 1983). Levels at the lower end of this range suggest inadequate maternal intakes. Requirements for this vitamin are linked to the protein intake and because of this the concentration in human or formula milk is sometimes expressed relative to the protein content. Results from 5 studies show values for the ratio of vitamin B_6 : protein in milk from mothers having different vitamin B_6 intakes, between 7 and 30 μg/g protein, which would be expected to provide between about 68–300 μg/d (Thomas *et al.* 1979, 1980; Sneed *et al.* 1981; Styslinger & Kirksey, 1985; Borschel *et al.* 1986). The mean value for pooled expressed mature human milk given in the DHSS report of 1977 is significantly lower, at 5 μg/g protein, providing only about 45 μg/d.

Other factors. Accidental feeding of a vitamin B_6-deficient formula to infants led to clinical deficiency; an intake of 0·3 mg/d was sufficient to correct the clinical deficiency, intakes in excess of 1 mg/d normalized the biochemical deficiency (Molony & Parmelee, 1954; Bessey *et al.* 1957). It has been pointed out that the pyridoxal-lysine which formed in the overheated formula has antipyridoxine activity; these values are therefore likely to be an overestimate of requirements (Gregory, 1980). However, it is not clear whether lower intakes would have corrected the clinical deficiency and, as discussed earlier, vitamin B_6 intakes much lower than this have been calculated for apparently healthy infants.

A study carried out among Egyptian mothers and their infants documented behavioural abnormalities in 3–6 month old infants whose mothers had vitamin B_6 levels in their milk of below 0·085 mg/l, which would have provided about 0·065 mg/d (McCullough *et al.* 1990). Recent work in animal models suggests that inadequate intake of vitamin B_6 during early life alters the postnatal development of the function of *N*-methyl-D-aspartate receptors, important in neurotransmission and thought to play a role in learning and memory (Guilarte, 1993).

The American Academy of Paediatrics (1985) has recommended generous intakes of vitamin B_6 of 0·3 mg/d, or 35 μg/g protein over the first 6 months of life. These recommendations accommodate the highest levels measured in human breast milk and are comparable with intakes reported to correct the clinical deficiency associated with the consumption of a damaged formula. In contrast the current RNI for infants in the UK has been set at a conservative 0·2 mg/d (23 μg/g protein) for infants up to 6 months of age, which is higher than the intake calculated from the pooled milk data used by the DHSS in an earlier report (1981) but similar to values calculated from the data of Paul & Southgate (1987).

Intakes at the lower end of the distribution in human milk from mothers in the UK do not appear to be associated with clinical deficiency but there are limited data on which to base comments concerning the biochemical status of these infants. Such data might strengthen the case for the relatively conservative RNI.

Levels of vitamin B_6 currently included in infant formulae range from about 0·33 to 0·65 mg/l (about 18–36 μg/g protein) (Table 3), which would provide intakes similar to those babies receiving breast milk at the upper end of the concentration range.

Vitamin B_{12}

Vitamin B_{12} is defined as a group of cobalt-containing corrinoids that have biological activity in humans. The two cobalamins known to be coenzymically active in humans are methylcobalamin, important in folate metabolism, and 5-deoxyadenosylcobalamin, important to carbohydrate and lipid metabolism. Deficiency is associated with neurological changes and disturbance of cell turnover, especially in the bone marrow (Garewal *et al.* 1988).

Levels in breast milk. The dominant form of vitamin B_{12} in human milk is methylcobalamin. The vitamin is protein bound but not to transcobalamin II, which is also present in milk (Trugo & Sardinha, 1994). The total vitamin B_{12} content of human milk only weakly reflects levels in maternal serum. Mean concentrations of 0·2–1·3 μg/l in mature breast milk of unsupplemented well nourished mothers are reported, but with a wide range for individuals and a higher concentration in colostrum (Macy, 1949; Kon & Mawson, 1950; Ford *et al.* 1983). The response to maternal oral supplements is minimal, with levels reaching 1·88 μg/l, lower than some values reported in the milk of unsupplemented women (Thomas *et al.* 1980; Styslinger & Kirksey, 1985). Clinical deficiency of vitamin B_{12} has been reported in breast fed infants of strictly vegan mothers and of mothers with pernicious anaemia (Hoey *et al.* 1982; Kuhne *et al.* 1991). Levels of vitamin B_{12} in the milk fed to these infants can be as low as 0·06 μg/l (Jathar *et al.* 1970).

Other factors. The daily requirements for vitamin B_{12} are considered to be small relative to other water-soluble vitamins, partly aided by an efficient enterohepatic circulation, accounting for its long biological half-life, and significant synthesis by flora in the gastrointestinal tract. The mechanism by which vitamin B_{12} is absorbed is sufficiently different from other water-soluble vitamins to deserve particular attention. At physiological concentrations (1–5 μg), the vitamin is absorbed by an intrinsic factor dependent route. Intrinsic factor (IF) is a glycoprotein secreted by the parietal cells of the gastric mucosa. The vitamin binds initially to a so-called R protein in the stomach and subsequently, in the upper part of the small intestine, cobalamin binds to IF, in which form it is protected against proteolytic digestion, and the complex moves down the small intestine. In the ileum the complex binds to mucosal receptors which facilitate cobalamin transport into and across the mucosal cell and into the bloodstream. Cobalamin is carried in the bloodstream bound to specific carrier proteins, the transcobalamins. Cobalamin that is not bound to IF is absorbed throughout the length of the small intestine but at approximately one tenth of the efficiency of IF-mediated absorption and only then at intakes higher than physiological.

Usually babies are born with stores of vitamin B_{12} which are gradually depleted over the first six months of life (Luhby *et al.* 1961); the introduction of solid feeds normally permits the maintenance of adequate stores after this period. Deficiency due to dietary inadequacy is rare and in the majority of cases is due to a lack of IF. There are however many documented cases of exclusively breast fed infants of vegan or vegetarian mothers presenting with serious neurological abnormalities attributable to frank vitamin B_{12} deficiency (Higginbottom *et al.* 1978; Gambon *et al.* 1986). Where levels of vitamin B_{12} in the breast milk were measured these were low. Although immediate treatment usually includes the intramuscular injection of a relatively high dose of vitamin B_{12} (about 100 μg), daily intakes of 0·1 μg are reported to be adequate in some instances (Jadhav *et al.* 1962). The administration of 1·0 μg/d to infants receiving nutrients parenterally was sufficient to maintain plasma levels above those of healthy controls (Moore *et al.* 1986). Babies who are unable to produce IF because of gastric surgery, ileal resection or other gastrointestinal problems will develop cobalamin deficiency if not treated.

The size of tissue stores will determine the regularity and size of the dose of cobalamin, which can be given intravenously or by intramuscular injection.

A study of 62 healthy infants between 0·8 and 6·8 months of age showed lower serum vitamin B_{12} and higher urinary methylmalonic acid (see Table 2*a*) among breast fed infants of omnivorous mothers than formula fed infants (Specker *et al.* 1990). The significance of these differences in biochemical values has not been evaluated.

The current lower reference nutrient intake has been set at 0·1 μg/d based on the apparent effectiveness of this dose in correcting a megaloblastic anaemia in infants receiving inadequate amounts from breast milk (Jadhav *et al.* 1962). The RNI of 0·3 μg/d is based on a study showing normalization of abnormal methylmalonate excretion in breast fed infants of vegetarians (Specker *et al.* 1990). By comparison with breast milk levels achieved in healthy mothers (see Table 3) these are fairly modest reference values.

Commercial formulae for term infants commonly used in the UK contain between 0·33 and 2·2 μg/l, providing between about 0·25 and 1·65 μg/d. These intakes are generous compared with intakes calculated for healthy infants, but allow for a less efficient absorption than from breast milk, which contains B_{12} binding proteins which are thought to facilitate absorption (Trugo & Sardinha, 1994).

Folate

Folate is important as a coenzyme for reactions leading to the transport of single carbon groups, many of which are involved in amino acid metabolism and nucleic acid synthesis. Low intakes can thus impair cell division and protein synthesis and are associated with poor growth and structural and functional changes in the gastrointestinal tract, although abnormalities in the peripheral blood picture are not a consistent feature of deficiency. There is no evidence of toxicity from high intakes.

Levels in breast milk. Bovine milk contains considerably more folate than human milk. The mode of analysis has generated some disagreement regarding the form in which it is found in human milk. Cooperman & Lopez (1991) state that in bovine milk over half the folate is present as the polyglutamate of methyltetrahydrofolate whereas the monoglutamate of this folate predominates in human milk. Others, however, have found polyglutamates to comprise up to 60 % of the total folate activity in human milk (Tamura *et al.* 1980; O'Connor *et al.* 1991). Folate polyglutamates have about half of the biological activity of the monoglutamates; human milk may therefore offer a source of folate with high availability. The concentration of folic acid in breast milk from well nourished women is reported to vary between 11 and 110 μg/l, as mono- and polyglutamates. It has been pointed out that the values at the lower end of this range are likely to be underestimates, resulting from suboptimum assay conditions (Bates & Prentice, 1996). During prolonged lactation milk folate levels increase significantly, which is a pattern generally characteristic of the water-soluble vitamins (Halsted *et al.* 1978; Ford *et al.* 1983). Levels in breast milk seem to be relatively resistant to maternal supplements although the response is somewhat better if the mother is malnourished (Deodhar *et al.* 1964; Thomas *et al.* 1980; Sneed *et al.* 1981). Neither clinical deficiency nor low values for biochemical indices of folate status have been reported for breast fed infants born at term (Ek & Magnus 1979; Salmenperä *et al.* 1986).

Other factors. Folate binding proteins in breast milk may protect the folate from bacteria and assist absorption, thus folate in formula milks may be less available than from human milk (Antony *et al.* 1982). The RNI, set at 50 μg/d, has taken into account the levels in human milk

and a study of small for gestational age babies born at term, in whom haematological indices were normal at an intake of 50–60 μg/d from formulae (Foged *et al.* 1989). This same study showed that at the same calculated folate intake plasma levels were higher in breast fed babies than babies receiving formula milk, supporting the suggestion that folate is less available in formulae than from breast milk. The suggested 4 μg/100 kcal, approximating to a daily intake of 20–25 μg over the first month of life, made by the American Academy of Pediatrics (1985) is by comparison rather conservative, but an intake at this level was reported to result in normal erythrocyte morphology in full term infants (Ek & Magnus, 1982).

Commercial formulae for term infants contain 33–110 μg/l (about 4·5–16 μg/100 kcal or 25–85 μg/d). At the lower end of this range intakes are unlikely to offer a very significant margin of safety should requirements be elevated, particularly if absorption is relatively inefficient compared with folate in breast milk. Factors that increase the requirement for folate include haemolytic anaemia, which may occur as a result of inadequate vitamin E intake, and the use of certain drugs such as methotrexate and 5-fluorouracil. Folate supplements have proved useful in treating subjects with homocysteinaemia of various aetiology including carriers of the relatively common labile variant of methyltetrahydrofolate reductase. If infant screening for this disorder were to be introduced there would be implications for folate intakes.

Niacin

Niacin is a generic term for nicotinic acid and nicotinamide, both of which are substrates for the synthesis of the nicotinamide nucleotide coenzymes, nicotinamide adenine dinucleotide (NAD) and the phosphorylated derivative (NADP). They are essential throughout metabolism as proton and electron carriers in a wide range of oxidation/reduction reactions. Most of the niacin present in tissues is as the nicotinamide nucleotides. Although niacin is conventionally classified as a vitamin, endogenous synthesis from tryptophan occurs. The efficiency of conversion of tryptophan to niacin (as nicotinic acid) is low (it has been estimated that only 1 mg nicotinic acid is synthesized from 60 mg tryptophan), but this pathway can make a substantial contribution to the niacin 'pool'. To accommodate the value of tryptophan as a source of niacin, requirements may be expressed in terms of niacin equivalents.

A deficiency of this vitamin is characterized by a photosensitive dermatitis, inflammation of mucous membranes, diarrhoea and vomiting. Severe deficiency can be fatal. At intakes greater than 1 g/d there is hepatotoxicity in humans.

Levels in breast milk. The average preformed niacin content of mature milk from healthy mothers is reported to lie between about 1·8 and 2·3 mg/l, which would provide about 1·4–1·8 mg/d (Ford *et al.* 1983). Values show a wide range and are lower in colostrum (Macy, 1949; Ford *et al.* 1983). The use of maternal supplements can raise low breast milk levels in malnourished mothers (Deodhar *et al.* 1964; Prentice *et al.* 1983). Early studies of the dietary niacin requirement in infancy showed that this depended on the proportion of dietary energy supplied by protein (Holt, 1956). If present in excess of needs the tryptophan content of the milk will provide a further source of niacin. It has been estimated that tryptophan contributes about 70 % to the niacin equivalents in human milk. An average tryptophan content of human milk is given as 220 mg/l which could supply 3·67 mg niacin/l (Butte *et al.* 1984; American Academy of Pediatrics, 1985). The appropriate conversion factor from tryptophan to niacin in infants, and the proportion of the milk tryptophan that undergoes this conversion, are not known with any certainty.

Other factors. Requirements for niacin are linked to energy expenditure, and intakes in infants may therefore be usefully expressed as niacin equivalents/100 kcal. The current lower reference nutrient intake for niacin equivalents in infancy has been derived from the average level of niacin (0·33 mg/100 kcal) in pooled expressed human milk reported twenty years ago (DHSS, 1977). As this was the same order of magnitude as the estimated average requirement for adults (0·55 mg/100 kcal), calculated from repletion studies, the DRV were set at the same level as those for adults (Department of Health, 1991). As the 1977 human milk composition data reported niacin rather than niacin equivalents, the use of this value leads to an underestimate of the intake of niacin equivalents in breast fed infants. The DRV for niacin in infancy may actually be closer to the average concentration in breast milk than is first apparent. It is arguable that because the efficiency of conversion of tryptophan to niacin is not known in infants, DRV for this group should be expressed as preformed niacin.

Commercial formulae contain 3·4–9 mg niacin/l (about 0·75–1·25 mg niacin/100 kcal) which, if compared with preformed niacin in breast milk, even after maternal supplementation, appears to provide a large margin of safety even for high requirements. Toxic effects of very high intakes (in excess of 100 times the RNI) in adults have been reported for both nicotinamide and nicotinic acid. There are no data relating to possible toxic effects in infancy on which to base an upper limit of intake of this vitamin from infant formula. A useful reference might be the level in human milk after supplementation of the mother (Table 3).

Vitamin C—Ascorbic acid

Vitamin C is present principally in the body as ascorbic acid and dehydroascorbic acid; ascorbic acid is the active form, although transport into some cells is easier for the oxidized form and mechanisms exist to reduce dehydroascorbic acid to ascorbic acid intracellularly. Biologically it functions predominantly in oxygen dependent hydroxylation reactions such as in the synthesis of carnitine, collagen and noradrenaline. Deficiency manifests itself clinically as scurvy, which is characterized by petechial haemorrhage, swelling of the joints, bone abnormalities, and mental changes.

Levels in breast milk. The mean concentration of vitamin C (oxidized and reduced forms) in breast milk from well nourished mothers is reported to lie in the range 33–110 mg/l (Bates & Prentice, 1994). This would supply about 25–75 mg daily. Vitamin C supplements given to malnourished women have been shown to increase levels in milk (Deodhar *et al.* 1964; Bates *et al.* 1983) but effects of supplements of up to 1000 mg/d for two days to healthy women are minimal (Byerley & Kirksey, 1985). Mechanisms may be in place to regulate the level of vitamin C in human milk, suggesting that there may be disadvantages of very high intakes in infancy. Neither clinical deficiency nor plasma levels of vitamin C associated with biochemical changes have been reported in breast fed infants.

Other factors. Plasma levels of vitamin C fall in the few days immediately after birth, a pattern that has been described in babies born at term and those born prematurely (Irwin & Hutchins, 1976; Silvers *et al.* 1994). As intakes from breast milk or formulae become established levels rise again to values seen in adults.

There is little information about the body pool and turnover rates for vitamin C in infants. Intakes of 7–12 mg/d have been shown to be sufficient to protect infants against scurvy (Rajalakshmi *et al.* 1965). An intake of 20 mg/d in formulae is sufficient to achieve plasma levels at those observed in term infants (Irwin & Hutchins, 1979).

It has been argued that the vitamin C requirements of preterm infants are greater than those for term infants. This argument stems from the fact that prematurity is associated with an immaturity of the hydroxylation pathway for tyrosine, and vitamin C influences the rate of flux through this pathway. The functional significance of transient neonatal tyrosinaemia has never been satisfactorily evaluated however (Menkes *et al.* 1972; Mamunes *et al.* 1976). Also the protein content of infant formulae is not as high as was recommended in the past which will have a bearing on the incidence of transient neonatal tyrosinaemia. A stable isotope study of the effect of supplemental vitamin C on the rate of tyrosine catabolism in premature infants showed no benefit of increasing vitamin C intakes above 20 mg/d although at this level of intake tyrosine catabolism was increased over that measured at an intake of 8 mg/d. The functional significance of the difference is however likely to be minimal (Powers *et al.* 1994).

Of some concern though is the possibility that at high concentrations in extracellular fluids vitamin C could exert an effect that is pro-oxidative under some circumstances, thereby increasing the likelihood of oxidative tissue damage. Concentrations of plasma vitamin C observed in some premature babies are high enough to interfere with the antioxidant ferroxidase activity usually exhibited by plasma caeruloplasmin (Powers *et al.* 1995). High levels of circulating vitamin C in some premature babies are associated with low plasma antioxidant activity and this in turn has been identified as an important predictor of poor outcome (Silvers *et al.* 1993, 1994). The relevance of these findings to term babies has not yet been evaluated. Other hazards of high intakes have also been documented in infants but this is an area that has not been fully explored. Infants who receive total parenteral nutrition receive much higher intakes of vitamin C than breast fed infants; for infants born prematurely, on intakes averaging 52 mg/d, this results in plasma levels up to three-fold those seen in term babies (Moore *et al.* 1986). Intakes eliciting this response range from 24 to 79 mg/kg daily.

The RNI has been set at 25 mg/d; as exclusively breast fed babies show no obvious sign of deficiency at this level of intake it seems to be adequate.

As the rationale for high levels of vitamin C in infant formulae is weak but there is a demonstrable disadvantage of high plasma levels, the setting of safe upper limits of intakes in infancy may be warranted. For babies born prematurely this seems particularly pertinent. Plasma levels of 300 μmol/l (about 3 times the upper limit in adults) were reached in premature babies receiving a total of 60 mg/kg vitamin C daily (about 70 mg/d). At this concentration of vitamin C ferroxidase activity of caeruloplasmin *in vitro* was impaired suggesting it posed some disadvantage. On this basis a safe upper limit for total vitamin C intake in premature babies of 50 mg/kg over the first week of life is suggested. Babies born at term are less vulnerable to the potentially pro-oxidative effects of vitamin C, as their more mature renal system makes such high plasma levels unlikely.

Commercial formulae for term infants contain vitamin C at levels comparable with those in breast milk (66–90 mg/l or about 8 mg/100 kcal), which would provide an average of 60 mg/d, but some formulae for infants born prematurely provide up to 25 mg/100 kcal. If this were the sole source of the vitamin there would be no cause for concern but many special care baby units give additional daily supplements of water-soluble vitamins, including vitamin C, which may not be appropriate over the first week of life.

Pantothenic acid

Pantothenic acid is the functional component of coenzyme A, ubiquitous in intermediary metabolism as a two-carbon carrier. Despite its central role in metabolism there have not been any well documented specific effects of deficiency or excess in man.

Levels in breast milk. The average concentration of pantothenic acid in the breast milk of unsupplemented Caucasian mothers, measured using a microbiological technique, was 6·7 mg/l (Johnston *et al.* 1981). There was however a large variation between individuals, between 1·8 and 18·5 mg/l. These values are higher than those reported by other groups, but Song and others (1984) have shown that the methods used to free bound forms of pantothenic acid will influence results of analytical studies. Supplementation of Indian women with 50 mg/d increased breast milk levels from an average of 1·0 mg/l to 3·0 mg/l (Deodhar *et al.* 1964).

Other factors. There is currently no satisfactory biochemical method for determining pantothenic acid status in man and no experimental data from human studies on which to base recommendations for infant formulae. Commercial preparations contain 2·0–4·0 mg/l (about 0·2–0·3 mg/100 kcal), comparable with breast milk.

Biotin

Biotin acts as an essential co-factor for enzymes important in carboxylation steps in gluconeogenesis, lipogenesis and amino acid metabolism. A range of symptoms have been reported in experimental depletion of biotin including anorexia, glossitis, and dermatitis. Patients receiving long term parenteral nutrition have developed similar symptoms but otherwise deficiency in humans is rare.

Levels in breast milk. There have been only a few studies of the biotin content of human milk, and these indicate mean values in the range 5·2–11 μg/l (Prentice *et al.* 1983; Hirano *et al.* 1992; Mock *et al.* 1992). Maternal intake influences breast milk levels if these are initially low. Breast milk levels in Gambian women showed no increase from an average of 8·97 μg/l in response to maternal supplements of 7·4 μg/d (Prentice *et al.* 1983). Supplementation of Indian mothers with biotin up to 0·25 mg/d increased milk levels from 1·6 μg/l to 5·0 μg/l (Deodhar *et al.* 1964).

Other factors. Biotin status can be estimated by measuring the excretion of organic acids which accumulate as a result of a reduction in the activity of certain carboxylases. Lactate and pyruvate levels in the blood may rise as carbohydrate and lipid metabolism is disturbed, but these changes are not specific.

Infant formulae contain 10–20 μg/l which is generous compared with levels in human milk, (Table 3) but as biotin does not pose a toxicity problem it seems satisfactory.

Fat-soluble vitamins

The absorption, transport, storage and excretion of the fat-soluble vitamins exhibit fairly marked differences from the water-soluble vitamins. Some of these differences are pertinent to the recommendations for concentrations in infant feeds and will be included in discussion of levels in breast milk. Although reports of toxicity are not restricted to this group of vitamins there are more documented cases than for the water-soluble vitamins, albeit rarely in infants, and therefore symptoms are better characterized. Safe upper limits for intakes during infancy should be a consideration when setting levels in infant feeds.

Vitamin A

Vitamin A is a generic term for a group of retinoids with similar biological activity, including retinol, retinal and retinoic acid. The diet provides retinoids, usually as retinyl esters and some carotenoids with provitamin A activity. Quantitatively and in terms of provitamin A activity the most important of these is β-carotene. Vitamin A has a role in vision, reproduction and the maintenance of epithelial cell integrity. Classical symptoms of vitamin A deficiency include night blindness and, eventually, xerophthalmia. A less severe deficiency has been associated with anaemia; aspects of immune function may also be impaired. Carotenoids are now considered to have a role other than as precursors of retinoids, as antioxidants. Inadequate intakes of carotenoids may compromise antioxidant defence.

Prolonged intakes above 7500 μg/d have led to hepatomegaly, alopecia, hyperostosis and hypercalcemia (Mahoney *et al.* 1980; Bendich & Langseth, 1989). Symptoms of toxicity have been associated with plasma retinol levels greater than 1 mg/l (3·5 μmol/l). Vitamin A overdose has been linked with intracranial hypertension in infants and children (Dhivavibulya *et al.* 1991).

Levels in breast milk. Vitamin A levels in the diet, and dietary reference values, can be expressed as retinol equivalents and it is assumed that 6 μg β-carotene is equivalent to 1 μg retinol. Retinoids have been the focus of a great deal more attention than carotenoids, with respect to vitamin A activity. Very little work has been conducted into the concentration and availability of carotenoids in human milk and reports of vitamin A concentration in breast milk usually mean retinoids, the majority of which may be present as esters. The concentration of β-carotene in human milk is in the range 10–220 μg/100 ml depending on the days *post partum* (Chappell *et al.* 1985) and seems to reflect maternal dietary intake (Gebre-Medhin *et al.* 1976), although even where concentrations of retinoids are low β-carotene may not make a very significant contribution to the vitamin A activity in the milk. Levels in milk do not seem to be a function of gestational age in contrast to α-tocopherol and retinol. The reported range for the level of preformed vitamin A in breast milk is extremely wide (0·15–2·26 mg/l) which, it is suggested, reflects differences in maternal intake (Moran *et al.* 1983; Schwarz, 1989). Restricting considerations to studies of milk collected from unsupplemented well nourished women after the first two weeks of lactation reduces the reported range to 0·49–0·77 mg/l (see Table 2*b*). The strength of the influence of maternal intakes of vitamin A on levels in breast milk is uncertain as the majority of supplementation studies have been carried out in communities where maternal intakes are low but in which other factors, such as infection, may have influenced the association (Venkatachalam *et al.* 1962; Kim *et al.* 1990). Long term supplementation of Gambian women at levels close to current recommended nutrient intakes elicited only a modest increase in breast milk levels of this vitamin (Villard & Bates, 1987).

Other factors. The availability of vitamin A in ester form in breast milk depends on bile-salt stimulated lipase; the absorption of the products of hydrolysis is influenced by the fatty acid content of the diet, possibly by the ratio of polyunsaturated (PUFA) to saturated fatty acids (Gaull *et al.* 1982).

Taking an average value of 0·6 mg/l for breast milk levels and an estimated 750 ml milk consumed daily by the infant, one can calculate an average 0·45 mg taken daily. This is certainly well in excess of the requirements of some infants as breast fed babies receiving only 0·1 mg/d showed no signs of vitamin A deficiency (Butte & Calloway, 1981). The COMA Panel for setting DRV has adopted the recommendations made by the recent FAO/WHO Expert Group, which arrived at 0·35 mg/d retinol equivalents (Food & Agriculture Organization,

1988). Consequently the RNI currently stands at 0·35 mg retinol equivalents for the first year of life. This compares well with a calculated average 0·375 mg retinol equivalents daily provided by Olson (1987*b*). Premature infants at risk of developing chronic lung disease who received intramuscular doses of 350 μg showed an increase in plasma levels of retinol to those seen in healthy children and adults (Shenai *et al.* 1987).

Consideration of daily requirements of vitamin A for infants is limited by the lack of a sensitive index of vitamin A status. Plasma retinol levels are insensitive to changes in intake over a broad range of intakes being regulated by export from the liver, at least until hepatic stores are very severely depleted. Plasma retinol values are lowered by infection, independent of vitamin A status. Urinary levels of glycosaminoglycans have been shown to correlate with plasma retinol levels in children, suggesting that this may offer an alternative approach to the assessment of vitamin A adequacy (Hussein *et al.* 1985). It has yet to be seen whether this has application to infants. The relative dose response has received some attention as a putative marker of vitamin A status. This measures the ratio of the difference in the concentration of plasma retinol over a 5 h period from the administration of a dose of vitamin A to the value after 5 h; low vitamin A status raises the relative dose response (Amédée-Manesme *et al.* 1984).

Commercial formulae available for term infants in the UK provide 0·6–1·0 mg retinol equivalents/l which would provide about 0·45–0·75 mg retinol equivalents daily. This range is comparable with that provided in the milk of well nourished but unsupplemented mothers and therefore seems reasonable (Table 3). Safe upper limits of vitamin A intake in infancy have not yet been properly evaluated. Beta-carotene is included in some formulae for preterm infants. The rationale behind this is that carotenoid concentrations are low in the plasma of premature babies, which contributes to reduced protection against oxidation damage in this group. However, the absorption and utilization of β-carotene are not understood and benefits from its inclusion in infant formulae have yet to be demonstrated.

Vitamin E

There are eight naturally occurring compounds with vitamin E activity. Alpha-tocopherol is the most active isomer, and usually the most abundant in the plasma. Low circulating levels can lead to a premature haemolysis of red blood cells, an observation that has formed the basis of the biochemical threshold for normality. Deficiency in children has been associated with neuromuscular disturbances. Other symptoms attributed to vitamin E toxicity include thrombocytopenia and hepatomegaly (Karp & Robertson, 1986).

Levels in breast milk. The breast milk concentration of α-tocopherol in healthy mothers falls in the range 1·1–8·0 mg D α-tocopherol equivalents/l, being dependent on the time during lactation as the concentration of D α-tocopherol is higher in early than late milk. The reliability of the reported levels of tocopherol in breast milk will be influenced by which isomers of tocopherol are measured. Beta and gamma forms have been measured in human milk and found to be higher in mature milk compared with colostrum or transitional milk (Jansson *et al.* 1981; Haug *et al.* 1987; Moffatt *et al.* 1987; Boersma *et al.* 1991). Whereas β-tocopherol represents only about 2 % of the total tocopherol content of human milk, γ-tocopherol can constitute up to 30 % in mature milk. Although γ-tocopherol has long been considered to have only about 7 % of the biological activity of α-tocopherol, Moffat and others (1987) have suggested that it may be as high as 30 %, in which case it could make a substantial contribution to the vitamin E activity in milk.

Other factors. Extremely low levels of α-tocopherol in cord blood relative to the maternal circulation led to the assumption that the transport of vitamin E from mother to fetus is very poor; certainly there has been only limited success in the use of maternal supplements during pregnancy to increase levels in the newborn (György *et al.* 1952; Mino & Nishino, 1973). More recent studies have failed to show a relationship between cord or infant levels of plasma vitamin E and those in maternal plasma (Hågå & Cran, 1982; Dison *et al.* 1993). Conclusions drawn from studies of circulating levels of tocopherol in infants compared with adults can be misleading if tocopherol levels are not expressed relative to circulating lipids (Horwitt *et al.* 1972; Department of Health, 1991). Current guidelines for interpreting plasma levels of tocopherol are based on the observation that erythrocytes from adults haemolyse on exposure to oxidizing agents when plasma α-tocopherol levels fall below 11 μmol/l or the ratio α-tocopherol : cholesterol falls below 2·25 μmol/mmol. A recent study of vitamin E status among infants born prematurely reported 86 % of 144 babies born between 22 and 39 weeks gestation with plasma levels of α-tocopherol less than 11·2 μmol/l at birth but after correcting for plasma cholesterol the percentage of babies falling below the threshold for biochemical normality fell to 5 % (Silvers, 1996). Reports of low plasma levels of tocopherol in newborn babies probably reflect relatively low lipid intakes and low levels of plasma lipid. Interestingly, Miyake and coworkers (1991) have shown that although erythrocyte tocopherol levels in premature infants are similar to those in adults their erythrocytes are more susceptible to oxidative stress.

The vitamin E requirement is highly influenced by the PUFA content of tissues, which is in turn influenced by dietary intake. In a free-living population the PUFA intake varies widely and this poses a problem for the setting of DRV with any practical value. In consequence the most recent panel on DRV for the UK population chose to limit their recommendations for this vitamin to a range of acceptable intakes (Department of Health, 1991). It should be less difficult to calculate requirements on the basis of the PUFA content of the diet for infants receiving feeds of known composition. However, it is not at all clear what factor should be used in expressing tocopherol requirements relative to dietary PUFA for adults, and there is even less certainty with regard to infants. The total tocopherol content of human milk correlates with the total lipid content and with linoleic acid. The ratio α-tocopherol equivalent : linoleic acid falls in the range 0·8–2·4 mg/g (Jansson *et al.* 1981). The HMSO publication *Artificial Feeds for the Young Infant* (DHSS, 1981) adopted the value of 0·4 mg α-tocopherol equivalents/g PUFA for infant milk formulae, a figure arrived at from dietary studies among American adults (Bieri & Evarts, 1973). This value has since been endorsed by the most recent Department of Health (1991) report on DRV. Recommendations for the tocopherol content of infant feeds are unlikely to be improved upon until controlled studies of intakes and biochemical markers of adequacy are carried out.

Difficulties in establishing requirements for vitamin E in infant feeds are compounded by the fact that although the main form, like human milk, is D α-tocopherol acetate, γ-tocopherol may form a significant component of the total tocopherol content of formula feeds. This would explain the contribution that γ-tocopherol made to total plasma tocopherol in a large study in premature babies (Silvers, 1996) and highlights the importance of expressing vitamin E requirements in α-tocopherol equivalents.

Although there is evidence that excess vitamin E in infancy can be harmful, all such data come from supplementation studies in preterm infants. The 1970s and 80s saw considerable interest in the possible prophylactic use of vitamin E for the prevention of bronchopulmonary dysplasia, retinopathy of prematurity and necrotizing enterocolitis. Of the many supplementation studies carried out in premature infants very few provided convincing evidence for a

beneficial effect of vitamin E and current opinion seems to be that supplemental vitamin E for infants receiving human milk or infant formulae is not justified and may be harmful (Committee on Fetus and Newborn, 1985; Aranda *et al.* 1986; Neal *et al.* 1986). Intravenous administration of 15–30 mg vitamin E as tocopherol acetate in a preparation known as E-Ferol resulted in liver and kidney failure and death (Martone *et al.* 1986). These effects have subsequently been attributed to the polysorbate vehicle used. Other documented effects of pharmacological intakes include decreased platelet aggregation, creatinuria, impaired fibrinolysis, and soft tissue calcification (Lemons & Maisels, 1985; Barak *et al.* 1986). Increased risk of sepsis in premature infants has been associated with plasma levels of tocopherol greater than 3·5 mg/100 ml (82 μmol/l); daily intakes less than 25 mg/kg have only rarely achieved this level. Plasma levels below this threshold are therefore likely to be safe for term infants. The association between intake and plasma level on the other hand may be quite different for term and preterm babies, as turnover may be greater in the latter group. Plasma levels of 80 μmol/l may be achieved at lower intakes in term babies than in preterm babies. The upper limit of vitamin E in infant formulae for term infants may not need to be set as high as that proposed by Bell (1989), about 70 mg/l providing about 53 mg/d, but more information is required about the relative turnover of this vitamin in term and preterm infants.

The form in which vitamin E appears in formula feeds is usually tocopheryl acetate, which is more stable than the unesterified form, and well absorbed by infants. Levels in commercial formulae for term infants currently range between 4·5 and 10 mg/l which would provide between about 3·4–7·5 mg/d. This compares well with the upper part of the range of intakes (0·8–6 mg/d) calculated for healthy breast fed infants and therefore seems sensible (Table 3).

Vitamin D

Vitamin D serves as a precursor of 25-hydroxyvitamin D and 1,25-dihydroxyvitamin D, the latter being the main active form *in vivo*. Requirements for this vitamin are inextricably linked with the dietary availability and absorption of calcium. The two main forms of the vitamin are ergocalciferol (vitamin D_2), available mainly from plants, and cholecalciferol (vitamin D_3), the main source of which is through its synthesis by the action of ultraviolet light on 7-dehydrocholesterol in the skin. The hydroxylated derivatives are also available from the diet. Vitamin D status is conventionally expressed as the plasma concentration of 25-hydroxyvitamin D. Deficiency leads to growth disturbances, bone abnormalities, muscle weakness and hypotonia. Intakes of mg amounts of vitamin D over weeks result in hypercalcaemia and associated calcification of tissues; effects may be mediated by increased availability to its sites of action (Vieth, 1990).

Levels in breast milk. Vitamin D is present in human milk mainly as ergocalciferol (about 0·3 μg/l), cholecalciferol (0·04 μg/l), and 25-hydroxyvitamin D (about 0·16 μg/l). In terms of biological activity the hydroxylated form represents the majority (Hollis, 1983; Hollis *et al.* 1986). It has been suggested that this is of particular value to premature infants as their rate of conversion of vitamin D to the hydroxylated form may be immature (Hoff *et al.* 1979). A more recent study demonstrated normal hepatic hydroxylation of vitamin D in very low birth weight infants (Koo *et al.* 1989). The 25-hydroxyvitamin D increases in concentration during lactation in contrast to vitamin D *per se*, which shows a decrease (Kunz *et al.* 1984). Trace amounts of 1,25-dihydroxyvitamin D and 24,25-dihydroxyvitamin D have also been measured but in amounts that make no significant contribution to vitamin D activity in milk (Reeve *et al.* 1982). There is a seasonal variation in the levels of vitamin D and metabolites in breast milk and this

in turn is associated with a seasonality in the plasma levels of 25-hydroxyvitamin D in breast fed infants (Greer *et al.* 1987). This is especially evident in Northern latitudes (Markestad *et al.* 1984). The use of average values for the levels of vitamin D and its metabolites in breast milk is thus limited unless it is clear at what time of the year the milk samples were collected. Values reported for milk from apparently well nourished mothers give a range from almost undetectable to 2 μg/l (Bates & Prentice, 1994). The relative contribution that the diet and the pathway for endogenous synthesis make to vitamin D status varies according to age and season (McLaughlin *et al.* 1974). Because of the low concentrations in breast milk, newborn infants who are breast fed are particularly dependent on the stored vitamin D obtained before birth to meet requirements in early life, even though sunlight does make some contribution (Bachrach *et al.* 1979; Edidin *et al.* 1980).

Hypocalcaemia and rickets secondary to vitamin D deficiency have been found in exclusively breast fed infants who are nonwhite or who receive inadequate exposure to sunshine (Chang *et al.* 1992). Biochemical deficiency is reported relatively frequently in exclusively breast fed babies (Markestad *et al.* 1984, 1987). Hypocalcaemic seizures have been reported in breast fed infants of mothers with less than 5 μg/l of 25-hydroxyvitamin D in their plasma (Ahmed *et al.* 1995). For this vitamin breast milk levels are not seen as the gold standard and the consensus view seems to be that lactating mothers should receive a supplement of vitamin D (DHSS, 1988; Department of Health, 1991). The importance of vitamin D supplements in pregnancy should not be overlooked. Supplements of vitamin D to women during pregnancy can not only increase breast milk vitamin D concentrations (Ala-Houhala *et al.* 1988) but are also reported to lead to increased birth weight and a reduction in neonatal hypocalcaemia (Watney *et al.* 1971).

Other factors. The period of greatest demand for vitamin D in infancy is between six months and three years of age, when the rate at which calcium is laid down in bone is highest (Belton, 1986). Plasma 25-hydroxyvitamin D is the most abundant circulating vitamin D metabolite and its concentration is sensitive to endogenous and exogenous vitamin D and the ability of the liver to hydroxylate vitamin D (Haddad, 1983). Measurement of the level of osteocalcin in plasma has been developed as a marker for bone turnover; this may prove to be useful as a functional index of adequate vitamin D status (Kruse & Kracht, 1986). The introduction of relatively noninvasive methods for measuring bone mineral density offers a useful measure of vitamin D (and mineral) adequacy.

On the basis of plasma levels of 25-hydroxyvitamin D and alkaline phosphatase activity, human milk appears to be inadequate as an all the year round source of vitamin D compared with infant formulae (Roberts *et al.* 1981; Greer & Tsang, 1983). In a randomized double-blind study Greer *et al.* (1982) demonstrated a shortfall in bone mineral content in babies fed human milk alone compared with those receiving a supplement of 10 μg daily. Oral supplements of vitamin D of 25 and 50 μg/d to lactating mothers elicited a significant but wide range of responses in breast milk levels (Hollis *et al.* 1988). Higher intakes by breast feeding mothers (up to 2·5 mg/d) have resulted in idiopathic hypercalcaemia in the infant (Greer *et al.* 1984). The margin of safety between adequate and excessive intakes seems to be rather small, and this should be taken into account when formulating infant feeds. A longitudinal study in term infants receiving 10 or 20 μg vitamin D daily for 16 weeks showed that 10 μg daily was sufficient to maintain plasma 25-hydroxyvitamin D_3 values above 20 ng/ml and all values fell in the range considered normal for both infants and adults (Pittard *et al.* 1991). When supplementation was in the form of 25-hydroxyvitamin D_3, plasma concentrations of this form were maintained in the normal range at intakes of 1·5 μg/d but not of 0·85 μg/d.

The RNI for vitamin D in infancy has been set at a level (8·5 μg/d) which exclusively breast fed babies may not achieve. An average level for total vitamin D in breast milk, calculated from 14 studies published since 1980, is reported to be 0·71 μg/l. Using an estimated milk intake of 750 ml/d this level would provide 0·53 μg vitamin D, which is far lower than the current Department of Health (1991) recommendation of 8·5 μg daily for children up to 6 months and lower than the 10 μg/d that Pittard and colleagues (1991) considered to be adequate. Horwitt and coworkers (1972) reported total vitamin D in breast milk at about 0·54 μg/l, most of which was present as 25-hydroxyvitamin D. The American Academy of Pediatrics (1985) calculated an average intake by breast fed infants of 0·38–0·75 μg/d.

The possibility that formulae are overfortified with vitamin D has been the subject of recent discussion, prompted by an outbreak of vitamin D intoxication among infants in a North American city. Holick and others (1992) reported concentrations of vitamin D in infant formulae more than 200 % of that stated on labels and more than 170 IU/l (42·5 μg/l), which contrasts with the current legal maximum in the USA of 17 μg/l. The reliability of the data has been disputed by the Infant Formula Council (Gelardi, 1992). Those familiar with the very early literature regarding vitamin D requirements in infancy will be reminded that overfortification of milk with vitamin D led to vitamin D toxicity in some children (Jeans & Stearns, 1938).

Commercial formulae available in the UK for term infants are remarkably consistent in their vitamin D levels, which fall between 10 and 14 μg/l; this would provide 8–11 μg daily, similar to that available from human milk (Table 4). Data on which to base the upper limits in infant formulae are scarce. Markestad *et al.* (1987) reported hypercalcaemia in some infants receiving 600 000 IU every 3–5 months, even though levels of vitamin D metabolites were not abnormally high. They concluded that this dosage is unsafe. Jeans & Stearns (1938) showed that intakes above 1800 IU daily interfered with growth. Intakes approaching this level cannot be reached with current infant formulae. The American Academy of Pediatrics (1985) reported that toxicity occurs in infants receiving 2000–4000 IU (50–100 μg) daily over several months.

Vitamin K

Vitamin K is a generic term for a group of 2-methyl-1,4-naphthoquinone derivatives. The group includes phylloquinone (vitamin K_1), from plants and animals, and menaquinones. Humans have the ability to synthesize a menaquinone from the precursor menadione. Vitamin K is required for the synthesis of certain clotting proteins including prothrombin. There is increasing interest in the role that vitamin K plays in the synthesis of osteocalcin which is important in the formation of new bone. Osteocalcin contains three γ-carboxylated residues, the formation of which is vitamin K dependent. Humans can convert a synthetic precursor of vitamin K, menadione, to biologically active menaquinones, through alkylation in the liver. Menaquinones and phylloquinone can be absorbed by humans although details of relative efficiency of absorption are not available for infants. Excess menadione, the synthetic form of vitamin K, is potentially toxic, leading to haemolytic anaemia and hyperbilirubinaemia of the newborn. Phylloquinones are without toxic effects, even at high doses.

Inadequate intake of vitamin K leads to defects in the clotting system. Newborn infants are susceptible to haemorrhagic disease. Effects of vitamin K depletion on bone metabolism in newborn infants have yet to be evaluated.

Levels in breast milk. There is little information concerning the concentration of vitamin K (mainly phylloquinone) in mature human breast milk. Reports suggest values between 1 and

10 μg/l (Bloch *et al.* 1984; Von Kries *et al.* 1987; Canfield & Hopkinson, 1989; Greer *et al.* 1991) and average values lower than those in cows' milk. In some cases the concentration in breast milk has proved to be insufficient; intracranial haemorrhage secondary to vitamin K deficiency has been responsible for deaths among exclusively breast fed infants (Lane *et al.* 1983; Hanawa *et al.* 1988). Pietschnig *et al.* (1993) were unable to show an influence on levels in milk of long term supplementation of the maternal diet with 88 μg. In contrast, other workers have shown that single doses of 0·5–3 mg can produce an increase in breast milk vitamin K (Haroon *et al.* 1982; Von Kries *et al.* 1987; Greer *et al.* 1997). Gastrointestinal flora provide an endogenous source of this vitamin which, it has been suggested, make a greater contribution in babies fed formula than in breast fed babies (Keenan *et al.* 1971; Conly *et al.* 1994).

Other factors. The concentrations of plasma vitamin K dependent clotting factors are lowest at birth but increase without intervention to reach adult values at about 6 weeks of age. It cannot be stated with certainty that these low levels at birth represent anything other than a normal physiological state. The development of a functional measure of vitamin K status has helped to resolve this question. Vitamin K deficiency leads to an increase in the concentration of an antigenically intact but functionally ineffective prothrombin (non-carboxylated). Vitamin K deficiency is associated with a low ratio of coagulant to antigen; expressed differently, vitamin K deficiency can be characterized by the presence of 'protein-induced in vitamin K absence' (PIVKA) II. Several reports have described the presence of PIVKA II in newborn infants (Muntean *et al.* 1979; Corrigan & Kryc, 1980); McNinch *et al.* (1983) showed a fall in PIVKA II in response to vitamin K administered intramuscularly at birth.

The need for intramuscular vitamin K at birth remains a subject of some discussion. Although there are conflicting reports about the prevalence of abnormal plasma prothrombin in babies fed formula milks compared with those who are breast fed (Büller *et al.* 1986; Garrow *et al.* 1986), late haemorrhagic disease is still reported in some exclusively breast fed babies, who seem to be at greatest risk at 3–6 weeks of age when their plasma levels show a marked fall, often to values below those seen in formula fed infants (Hogenbirk *et al.* 1993).

There seems to be little doubt that classic haemorrhagic disease of the newborn, which usually occurs at 2–5 days, does not occur in babies given 1 mg vitamin K intramuscularly at birth. Administration of vitamin K in this manner may not, however, be as safe as an oral dose. A retrospective controlled study in the UK suggested that intramuscular administration of vitamin K (but not oral vitamin K) at birth is associated with an increased risk of later malignancy (Golding *et al.* 1992). These authors show that after intramuscular administration of vitamin K, the plasma can reach levels 5000-fold greater than levels reported in breast fed infants. The implications of these findings are still being debated; a large nested case-control study subsequently conducted in the USA failed to demonstrate an increased risk of childhood cancer in association with vitamin K prophylaxis (Klebanoff *et al.* 1993). Recent developments include the formulation of an orally available vitamin K_1 preparation as a micellular solubilization in glycocholic acid and lecithin (Schubiger *et al.* 1993).

As breast milk seems to offer limited protection against vitamin K deficiency, the recommendation for intakes in infancy should be set close to the highest intakes calculated from milk of unsupplemented mothers, which would be about 8 μg/d.

A review of data led Olson (1987*a*) to recommend a dietary intake of about 10 μg for the first 12 months of life. The American Academy of Pediatrics (1985) recommends 5 μg daily in infancy. These recommendations assume a single dose given prophylactically at birth, which would presumably provide some hepatic stores. Vitamin K deficiency bleeding is not a feature

of babies receiving bottle feeds which, it should be argued, provide enough available vitamin K to meet infants' needs. Current formulae provide about 25–50 μg vitamin K daily.

Conclusions

Appropriate levels of vitamins in infant formulae have been discussed in the light of current recommendations and recent research findings. Current recommendations are based largely on levels of vitamins in breast milk but this approach has limitations. Wherever possible, studies which explore the association between calculated daily vitamin intakes and biochemical or other functional markers of adequacy in the infant should be used in the setting of lower and upper limits for levels in infant formulae.

There is a need for a better understanding of the way in which infants absorb and metabolize vitamins and any special roles that vitamins may have during this period. Stable isotope studies of the availability of vitamins, body pools and turnover rates offer great potential in this context. The use of breast milk constituent concentrations as the gold standard for the preparation of formulae should be supplanted by outcome assessments.

There needs to be greater consistency in the way recommendations for vitamin intakes during infancy are expressed. For consistency across the range of vitamins, mg/d is probably the most appropriate. For preterm babies it is generally more useful to express daily intakes as mg/kg. The vitamin content of infant formulae is most simply expressed as mg/l as long as an indication is given of the energy and protein content. Where possible, recommendations for minimum and maximum intakes (lower and upper limits in infant formulae) should be made. More information is required to enable vitamin E requirements to be linked to PUFA.

Recommendations for daily intakes of vitamins during infancy are, on balance, generous. Where levels in formulae exceed these substantially there may be cause for concern. More information is required regarding possible adverse effects of prolonged high intakes of vitamins. For those vitamins for which there are claims of special benefits of very high intakes these considerations are especially pertinent.

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