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Vitamin undernutrition

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Complex scientific problems need to undergo reduction and simplification, to arrive at hypotheses which can be tested experimentally. For scurvy, for instance, the theories ranged from foul vapours to pestilence, until Lind's famous experiment demonstrated the efficacy of a particular dietary cure, thus implying a specific dietary cause.

Unfortunately, the pressing need to simplify a complex problem, always carries with it the danger of over-simplification and, hence, that of misleading conclusions. An example of this from the twentieth century has been the extreme fluctuations of emphasis in the principal focus for 'undernutrition'. During the early decades of the century, the simultaneous discovery of many of the vitamins, and the advances in understanding of their modes of action, engendered great enthusiasm for their curative efficacy. This was then swept aside by 'kwashiorkor' and a strong emphasis on protein deficiency, until McLaren (1974) suggested that another reappraisal was needed, whereupon energy deficiency held centre-stage for many years. However, 'malnutrition' and 'undernutrition' are usually complex phenomena, involving several groups of nutrients plus stresses such as infection, and each situation needs to be assessed by its particular characteristics and components.

In order to be guided by the medical aphorism 'first diagnosis, then treatment', I would advocate the direction of effort towards the development of better *functional* indices of status, which will ensure that intervention effort can be specifically designed and targeted for particular problems, or high-risk groups. This need is evident, not only for the classical situations of undernutrition in the Third World, but also, and perhaps even more urgently, for the more subtle and insidious undernutrition which increases the risk of life-span degenerative diseases, found in all human societies. Here, dietary advice and intervention must take place over a very long time-span if they are to be effective, and the development of reliable 'risk-indicators' and intermediate markers is an important and exciting challenge.

Since 'vitamin undernutrition' is far too vast a subject to be tackled in its entirety in a single review article, I will select just a few areas of current interest, to attempt to illustrate the kinds of challenges which are likely to face future generations of scientists working in the field of vitamin requirements.

THE RESURGENCE OF VITAMIN A

Until very recently, the problem of vitamin A deficiency was equated to that of xerophthalmia, keratomalacia, and blindness in young children. During the past decade, however, evidence has been obtained from several countries that marginal vitamin A deficiency may carry with it an increased risk of morbidity from infectious diseases and of preventable child mortality, which has greatly extended the scale and scope of the problem, and with it, the promise of interventional benefit. Table 1 summarizes the conclusions of these recent studies, most, but not all, of which have concluded that

Table 1. *Recent vitamin A supplementation studies in preschool children* which have used morbidity (MB) and/or mortality (MT) as outcome measures*

Country	Reference	Outcome
Indonesia: N. Sumatra (H)	Sommer *et al.* (1986), West *et al.* (1989)	34% reduction in MT
	Muhilal *et al.* (1988)	40% reduction in MT
Thailand: Rural NE (H)	Bloem *et al.* (1990)	67% reduction in diarrhoea, respiratory disease
Nepal: Lowland (H)	West *et al.* (1991)	30% reduction in MT
Highland (H)	Daulaire *et al.* (1992)	50% reduction in MT
India: Rural south (L)	Rahmathullah *et al.* (1990, 1991)	54% reduction in MT, no effect on MB or growth
Rural centre (H)	Vijayaraghavan *et al.* (1990)	No effect on MT or MB
South Africa: Cape Town (H)	Hussey & Klein (1990)	50% reduction in measles MT or complications
Durban, underprivileged (H)	Coutsoudis *et al.* (1991)	Reduction in measles MB
Tanzania: Rural (H)	Barclay *et al.* (1987)	50% reduction in measles MT
Ghana	Arthur *et al.* (1992)	Reduced MB
Australia (L)	Pinnock *et al.* (1986, 1988)	Variable outcome for children with 'frequent illness'

H, high dose (20–30 mg) given at infrequent intervals, or as a single dose; L, low dose (0·45–3 mg) given at daily or weekly intervals.

* Except where otherwise indicated, the subjects were all members of the general, free-living population of the area studied, aged 0–7 years.

targeted vitamin A supplements can reduce either morbidity or mortality, or both, in communities where vitamin A deficiency is a significant public health problem. Clearly the promising start to this endeavour should be welcomed, but it may be advisable to caution against excessive euphoria, since there remain many unresolved questions. These concern the design and interpretation of some of the studies, their applicability to population groups at lesser risk, and their practicality as public health intervention measures. As noted previously, unitary and simplistic approaches are not always the most effective.

Nevertheless, the lure of a 'magic bullet' has rekindled interest in the mode of action of vitamin A, which remains almost as poorly understood as it was half a century ago. Redoubled efforts to define the critical nutrient-sensitive components of the immune system may, in time, shed long overdue light upon the fundamental action of several micronutrients, including vitamin A, which modulate the activity of this most complex of biological systems.

THE ANTIOXIDANT VITAMINS

More controversial than morbidity and mortality as endpoints for the assessment of nutritional status, is the concept that 'adequate' or even 'optimum' nutrition may depend

on the prevention of subtle types of oxidative damage, which may only become evident in functional terms towards the end of the natural life-span of the individual, i.e. by modifying the incidence of degenerative diseases of old age. Such diseases encompass heart disease, cancers, cataracts, arthritic damage, and similar failures of homeostasis, or accumulations of insults. The concept that there may be a trade-off between short-term survival from infection (for example, by the oxidative burst-activity of a macrophage), and long-term integrity of sensitive macromolecules such as DNA, has opened the door to the relatively new concept of a multi-faceted, integrated defence system against free radical-mediated oxidative damage, whose components depend rather heavily on the antioxidative micronutrients, particularly vitamins C and E.

The nature of the pro-oxidant species, and their biological significance, is gradually being clarified, but we still have a long way to go. The controlled insertion of O into an entity such as peptidylproline in nascent collagen clearly is an essential biological reaction, whose failure is one of the most obvious results of the disease, scurvy. This reaction requires the controlled involvement of ascorbate and Fe^{2+}, a combination which can result in devastatingly uncontrolled oxidative damage, if they occur together *in vitro*, where modulation by protein is absent. This paradoxical dichotomy between *in vivo* and *in vitro* patterns of activity has generated a great deal of confusion in the free radical and oxidant–antioxidant field, which is only gradually being resolved.

There is also a wide range of biological activities among the pro-oxidant entities such as hydroxyl radical, singlet O, thiyl and nitroso radicals, superoxide, H_2O_2, hypochlorous acid etc. Working in conjunction with the antioxidant enzymes, superoxide dismutase (*EC* 1.15.1.1), catalase (*EC* 1.11.1.6) and glutathione peroxidase (*EC* 1.11.1.9), and with endogenously-synthesized antioxidant entities such as glutathione, the antioxidant vitamins E and C and the carotenoids clearly play an essential protective role. While vitamin E and the carotenoids function primarily in a lipidic milieu, perhaps most importantly in the lipid bilayers of cell membranes, vitamin C evidently functions almost entirely in the aqueous phase, in both intracellular and extracellular compartments. A regeneration cycle may link vitamin E with vitamin C (Niki, 1991), although this has been questioned (Burton *et al.* 1990). Vitamin C may be regenerated by glutathione or by reduced pyridine nucleotide, but these regeneration processes are not yet well-characterized *in vivo*.

The circumstantial evidence which links adequate vitamin C status with decreased 'severity' of infections (Hemila, 1992) may be attributable to the removal of pro-oxidant species such as hypochlorous acid, once their localized action on invading micro-organisms has been achieved, at which point their unwanted pro-oxidant side-reactions become a threat to homeostatic mechanisms and to macromolecular integrity. We are beginning to understand that protective, as distinct from catalytic, roles for some micronutrients are biologically very important and should, therefore, influence the calculation of nutrient requirements. But this raises a difficult philosophical and practical question: how can we define 'adequate intakes' and 'adequate status', for nutrients which fulfil an insurance and protective role against *sporadic* insults, which are essentially unquantifiable, as distinct from the quantifiable 'saturation' function of vitamins which act purely as precursors of coenzymes and, therefore, of saturable enzyme–coenzyme complexes? Clearly we cannot necessarily conclude that 'because protection is beneficial, therefore dietary intakes ought always to be maximized'. The possibly deleterious effects of high vitamin C intakes, for instance under conditions of haemochromatosis or of

rheumatoid arthritis, and the uncertainty about high vitamin E intakes possibly altering prostanoid metabolism and other essential pro-oxidant processes, indicates the need for caution and for more research.

VITAMINS AND CATARACT

I have chosen to explore in some detail the recent development of ideas about vitamin involvement in cataract-risk and its prevention, partly because it illustrates some of the paradoxes and pitfalls which beset vitamin and antioxidant research, and partly because the eye lens is a relatively discrete and functionally simple organ, which can be studied and observed *in situ*, with relatively simple equipment. It can also be studied in short-term organ culture systems, which offer the advantages of minimum physiological variables or homeostatic adjustments.

The lens is vulnerable to a wide variety of chemical and physical insults, which frequently result in opacification, i.e. cataract. A summary of insults which have responded to antioxidant vitamin modulation, either *in vitro* or *in vivo*, is given in Table 2.

The first, and perhaps the most important antioxidant vitamin with respect to lens integrity, is vitamin C. It is present at a concentration of about 1 mM in the normal human lens (Varma, 1987), which declines both with age and with cataractogenesis (Varma, 1987; Bates & Cowen, 1988). Protective, presumably antioxidant, effects of ascorbate are described in a wide variety of studies. They have included studies of cultured lenses challenged with pro-oxidant insults (Varma, 1987, 1991), and of lens homogenates exposed to u.v. light (Taylor *et al.* 1987). Ascorbate supplements *in vivo* have also reduced the severity of cataractogenesis caused by galactose-feeding (Kosegarten & Maher, 1978; Vinson *et al.* 1986), by corticosteroids (Nishigori *et al.* 1985), by selenite (Devamanoharan *et al.* 1991) and by the glutathione antimetabolite, buthionine sulphoximine (Mrtensson & Meister, 1991). Leakage of crystallins from the lens of diabetic rats *in vivo* was reduced by ascorbate supplements (Linklater *et al.* 1990). Various other biochemical functions of ascorbate in the lens also support the view that it can protect against cataractogenic insults (Gerster, 1989; Varma, 1991; Bates *et al.* 1992). It is of some interest that protection may be afforded, not only against insults which are unequivocally oxidative in nature, but also against those which appear to be essentially osmotic, such as exposure to sugars which result in intra-lenticular sugar alcohol accumulation.

Several authors have highlighted the direct correlation which exists between ascorbate levels and H_2O_2 levels in aqueous humour (Pirie, 1965; Giblin *et al.* 1984; Riley *et al.* 1986). This apparently paradoxical observation has been incorporated into an interesting hypothesis (Eaton, 1991) that the ascorbate in aqueous humour serves to convert the O_2 diffusing from the atmosphere to H_2O_2 and thence to water, before it reaches the lens. A relatively high level of H_2O_2, therefore, indicates the efficient scavenging of O_2, and it may also enhance the bacteriocidal properties of the aqueous humour. Two previous studies (Schocket *et al.* 1972; Palmquist *et al.* 1984) have suggested that hyperbaric O_2 is cataractogenic.

Paradoxically, ascorbate may also participate in deleterious reactions in the lens (Garland, 1991). These include the formation of calcium oxalate 'retrodots' in the perinuclear region (Bron & Brown, 1986), and of pentosidine–protein cross-links, pigments and precipitates from the oxidation products of ascorbate, some of which

Table 2. *Protection against cataractogenic insults by vitamins in model systems*

Model	Reference	Type of insult	Effect of vitamin supplement
Vitamin C			
Cultured rat lenses	Varma (1987, 1991)	Oxidants from light or xanthine	Protection from Rb leakage; malondialdehyde
Rabbit lens homogenates	Taylor *et al.* (1987)	u.v. radiation	Less protein aggregation
Guinea-pigs (scorbutic)	Kosegarten & Maher (1978)	Galactose feeding	Fewer cataracts
Chick embryos	Nishigori *et al.* (1985)	Corticosteroids	Fewer cataracts
Guinea-pigs	Blondin *et al.* (1986)	u.v. radiation	Less protein aggregation and exopeptidase loss
Rats	Vinson *et al.* (1986)	Galactose feeding	Fewer cataracts
Diabetic rats	Linklater *et al.* (1990)	Diabetes	Less crystallin leakage
Newborn rats	Mrtensson & Meister (1991)	Buthionine sulphoximine	Fewer cataracts
8-d-old rat pups	Devamanoharan *et al.* (1991)	Selenite feeding	Fewer cataracts
Diabetic guinea-pigs	Bates *et al.* (1992)	Streptozotocin	Less sorbitol accumulation
Vitamin E			
Cultured rat lenses	Creighton & Trevithick (1979), Trevithick *et al.* (1981)	Vitamin E deficiency	Fewer cataracts
	Stewart-DeHaan *et al.* (1981)	High temperature	
	Varma *et al.* (1982)	Fluorescent light	Less malondialdehyde
	Creighton *et al.* (1983)	Steroids	Fewer cataracts
	Ross *et al.* (1983, 1990)	Radiation	
	Creighton *et al.* (1985)	Galactose exposure	
	Libondi *et al.* (1985)	Lysophosphatidyl choline	
	Hirai *et al.* (1987)	Glucose exposure	
	Varma *et al.* (1982)	Photoperoxidation	Less malondialdehyde
Turkey embryos	Ferguson *et al.* (1956)	Vitamin E deficiency	Fewer cataracts
Rat fetuses	Bunce & Hess (1976)	Vitamin E + tryptophan deficiency in dams	
Diabetic rats	Ross *et al.* (1982)	Streptozotocin	Prevention of abnormal morphology
Rabbits	Bhuyan & Bhuyan (1984)	3-aminotriazole or galactose	Arrest of cataract; less lipid peroxidation
Rabbits + rats	Gupta *et al.* (1984)	Naphthalene or galactose	Fewer cataracts
Emory mouse	Varma (1991)	Natural (senile) cataract	Reduced
Rats	Ross *et al.* (1990)	Radiation	Less lens damage
Riboflavin			Effect of deficiency
Rats	Day & Darby (1936)	'Vitamin G deficiency'	Cataracts
Pigs	Wintrobe *et al.* (1944)	Riboflavin deficiency	
Rats	Srivastava & Beutler (1972)	Galactose feeding	
	Hasegawa & Yagi (1975)	Riboflavin deficiency	Abnormal morphology
	Bhat (1982–3)		Abnormal crystallins
	Hirano *et al.* (1983)		Peroxidation
	Rao & Bhat (1989)		
	Dutta *et al.* (1990)	Adriamycin	Glutathione loss

resemble substances found in 'brunescent' cataracts and in extracted crystallins (Bensch *et al.* 1985; Ortwerth *et al.* 1988; Nagaraj *et al.* 1991; Nagaraj & Monnier, 1992). In addition, X-irradiation damage to lens DNA *in vitro* can be enhanced by vitamin C (Trevithick *et al.* 1987). These seem to represent either reactions of oxidation products of ascorbate, formed when its antioxidant capacity and functions have been exceeded, or else non-physiological reactions of ascorbate with O_2 and free transition metal ions which are more characteristic of *in vitro* than *in vivo* situations. They are unlikely to imply that ascorbate has pro-cataractogenic actions *in vivo*, although the accumulation of some of its oxidative breakdown products may provide a sensitive indicator of 'lens health'.

There is cogent evidence that vitamin E can also play an important role in protection against cataract (Table 2). This suggests that the integrity of lipid membranes may be important in maintaining lens transparency, especially in the cortex.

The importance of riboflavin status for lens integrity is somewhat more controversial. Like ascorbate, riboflavin can either be a pro-oxidant or an antioxidant, depending on its milieu. Both early and more recent evidence has pointed towards increased risk of cataract in riboflavin-deficient animals (Table 2), but the common assumption that this is usually attributable to impaired glutathione economy, may not be correct (Bates, 1991). There is limited evidence for an association between riboflavin deficiency and increased cataract-risk in humans (Prchal *et al.* 1978; Horwitz *et al.* 1987).

Two recent studies of human populations in Western countries (USA and Canada) have suggested that certain vitamins may exert protective effects against senile cataractogenesis (Jacques & Chylack, 1991; Robertson *et al.* 1991). Intervention studies are needed to test the hypothesis that enhanced intakes of vitamins may be protective, and these are now in progress at several centres. Potential benefits for the quality of life of elderly people and for the costs associated with cataract surgery are considerable.

Lack of space precludes any detailed discussion of the parallel studies on protective effects of antioxidant vitamins against other degenerative diseases such as cancer and heart disease; however, the interested reader is referred to a recent symposium (Slater & Block, 1991).

SOME RECENT CHALLENGES OF FOLIC ACID AND VITAMIN B_{12}

The notably successful outcome of the recently-completed intervention trial for the prevention of recurrence of neural tube defects (NTD) by periconceptional vitamin supplementation of high-risk subjects (MRC Vitamin Study Group, 1991), a 4000 μg daily folate supplement being the unequivocally effective intervention, has several important implications. It provides a clear message for women who have already had at least one affected fetus. If, as seems likely, the risk would also be reduced for women who are at high risk of having a first-affected fetus, then there may be implications for dietary recommendations and for public health policy, directed to all women of child-bearing age.

The recently revised UK recommended dietary allowance (RDA), now renamed DRV (dietary reference values; Department of Health, 1991), show an estimated average requirement of 150 μg/d for folate, and a reference nutrient intake (RNI) of 200 μg/d, for women who are not yet pregnant. These values are broadly in line with other recent revisions by World Health Organization and elsewhere, and with mean population folate intakes in most Western societies, where overt folate deficiency is rare. The NTD inter-

Table 3. *Might vitamin B_{12} economy be disturbed in some women at risk of neural tube defects (NTD)?*

Observation	Reference
In some affected mothers, erythrocyte folate or plasma vitamin B_{12} levels were low, but plasma folate was normal. Vitamin B_{12} deficiency lowers erythrocyte folate, but not plasma folate	Schorah *et al.* (1980), Yates *et al.* (1987), Scott *et al.* (1990), Schorah & Smithells (1991)
Vitamin B_{12} transport and economy (but not folate economy) were abnormal in the amniotic fluid of mothers carrying, or having previously carried, spina bifida fetuses	Magnus *et al.* (1986), Gardiki-Kouidou & Seller (1988), Economides *et al.* (1992), Weekes *et al.* (1992)
High doses of folate are known to mask some of the functional effects of vitamin B_{12} deficiency	Chanarin (1979)
Severe vitamin B_{12} deficiency usually prevents normal pregnancy	Chanarin (1979)
Vitamin B_{12} was not included in any of the recent NTD-prevention trials	Scott *et al.* (1990), Schorah & Smithells (1991), MRC Vitamin Study Group (1991)

vention study, however, used a daily supplement of 4000 μg pteroyl glutamate (the commercially-available form of folic acid), which is twenty times the RNI, and considerably more than the amount of food folate that can be obtained from the diet.

Before we conclude that the new DRV are inappropriate it should be noted that less than 0·3% of women in the UK are at risk of bearing an affected fetus. RNI are designed for the majority (generally at least 97·5%) of normal healthy people in the community. However, they can never cover the vastly increased needs of small and abnormal subgroups, as is clearly illustrated by the case of pernicious anaemia. Here, the oral vitamin B_{12} intake would have to be several orders of magnitude greater than the usual requirement, so the only reasonable option is diagnosis and special treatment. The same applies to many other vitamin-responsive inborn errors of metabolism (Bartlett, 1983).

For women at risk of bearing NTD fetuses, many can benefit from folate supplements, but we do not know for certain whether this benefit is derived from the correction of an existing diet-related deficiency, or to an increased requirement resulting from 'abnormal' metabolism. Although 4000 μg/d seems to be effective, it may be unnecessarily high. We do not yet know whether the risk could be decreased by attention to diet alone, or whether a pharmacological supplement is needed. It may prove to be possible to devise a screening test which will determine whether individual women have abnormal folate economy, and should, therefore, take extra folate during the periconceptional period. Finally, although folate supplements clearly are effective, they may not necessarily reveal the primary cause of the problem in every case. For instance, the close metabolic interrelationship between folate and vitamin B_{12} (Scott & Weir, 1981) implies that each vitamin can influence the other, since they share common functional pathways. The observations in Table 3, particularly the elegant study by Gardiki-Kouidou & Seller (1988), seem to imply that an impairment of vitamin B_{12} economy may occur in at least some of the affected subjects. It is noteworthy that none of the interventional studies so far have included vitamin B_{12} as part of the supplement. Likewise, abnormalities involving Zn, or possibly other micronutrients, may predominate in some cases, or situations (Scott *et al.* 1990; Schorah & Smithells, 1991).

Vitamin B_{12} has recently received publicity for another reason, namely its possible role in some kinds of neurological degeneration. Although it has long been known that prolonged, untreated pernicious anaemia can result in irreversible neurological degeneration and hence ataxia, this is generally thought to be a rare complication nowadays, because of improved diagnostic procedures. However, it has been claimed that subtle and unrecognized vitamin B_{12} deficiency states, not accompanied by anaemia, may affect neurological function (Lindenbaum *et al.* 1988; Carmel, 1992). This could be important for preventable neurological disease, especially of elderly people. These claims have been criticized (Chanarin *et al.* 1992) and are not yet proven, but they serve to remind us that the ever-increasing cohort of very elderly people at risk of or affected by cognitive decline represent a major risk-group with respect to micronutrient deficiencies, including vitamin deficiencies.

A link may likewise exist between thiamine economy (Gibson *et al.* 1988; Haas, 1988) and senile dementia states. Even if not causative, such an association needs to be explored in terms of interventional possibilities aimed at reducing functional deterioration. There is also some promising evidence of a link between requirements for folate or other water-soluble vitamins and certain affective disorders of the brain (Abou-Saleh & Coppen, 1986).

Such observations clearly imply that there may exist a number of important pockets of functional vitamin deficiency within high-risk Western population subgroups which need to be identified, studied and, where appropriate, offered intervention. The recent notable success of the NTD intervention trial ought now to pave the way for imaginative studies in this field. The possibilities of new tools for the exploration of vitamin requirements, such as the development of stable isotope-labelled vitamins to study absorption, body-pools and turnover and of stable-isotope labelled, metabolic pathway precursors to study functional requirements at the detailed biochemical level, promise major advances in the near future.

Limitations of space preclude more than a glimpse of the opportunities for research on vitamin undernutrition in future years. However, this prospect does not imply that the elixir of life necessarily exists in a vitamin pill, as indeed we are reminded by the timeless admonition of an ancient text, 'Regimen Sanitatis':

> 'Use three physicians still, the first doctor Quiet,
> Next doctor Merry-man and doctor Dyet,
> They that in physicke will prescribe you food,
> Six things must note, we here in order touch,
> First what it is, and then for what tis good,
> and when, and where, how often, and how much,
> Who note not this, it cannot be withstood,
> They hurt, not heal, yet are too many such.'

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