

## Vitamin E and selenium: contrasting and interacting nutritional determinants of host resistance to parasitic and viral infections

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Beneficial effects of trace amounts of dietary Se were first observed in vitamin E-deficient rats so that a strong metabolic interaction between these two micronutrients was apparent since the initial discovery of the nutritional value of Se (Schwarz & Foltz, 1957). Later work showed that Se and vitamin E tended to spare one another's requirement for the prevention of certain nutritional deficiency diseases (e.g. exudative diathesis in chicks, see Thompson & Scott, 1969). However, there were other conditions that appeared to respond specifically to only one of these nutrients (e.g. resorption gestation in rats, see Harris *et al.* 1958). As our knowledge about Se and vitamin E grew, the complexity of their interactions became clear with some conditions being specific for one or the other nutrient, whereas other syndromes responded equally well to either nutrient (Table 1). Many of the early results could be adequately explained by the

Table 1. *Selenium- and/or vitamin E-responsive nutritional diseases of rats*  
(Adapted from National Research Council, 1983)

	Conditions responsive to dietary:	
	Se	Vitamin E
Liver necrosis	+	+
In the second generation:		
Sparse hair coat	+	–
Poor growth	+	–
Poor sperm motility	+	–
Cataracts	+	–
Discoloration of body fat	–	+
Discoloration of the uterus	–	+
Depigmentation of incisors	–	+
<i>In vitro</i> haemolysis (no glucose)	–	+
Impaired reproductive capacity of females	–	+

The rat was selected as the model since this is the mammalian species for which the Se and vitamin E deficiency diseases are best clarified.

antioxidant properties of both these nutrients: Se as a component of the peroxide-destroying enzyme glutathione peroxidase (*EC* 1.11.1.9) and vitamin E as a lipid-soluble antioxidant (Hoekstra, 1975). More recent work has established metabolic roles for Se that appear to be unrelated to its antioxidant activity (e.g. as a component of iodothyronine 5'-deiodinase (*EC* 3.8.1.4), see Arthur *et al.* 1990).

Nutritional antioxidants such as Se and vitamin E have received a great deal of attention lately with regard to their possible protective benefits against chronic degenerative diseases such as cancer and cardiovascular disease (Collins *et al.* 1994; Riemersma, 1994). On the other hand, our work has shown that Se and vitamin E status can also have a profound impact on the ability of a host to resist acute infectious disease. Here we review effects of Se and/or vitamin E nutrition on two infectious-disease models in mice: malaria and coxsackievirus-induced heart muscle damage (the latter perhaps related to Keshan disease).

#### MALARIA

Our interest in the relationship between nutritionally-induced oxidative stress and malaria was first stimulated by the Chinese traditional antimalarial drug Qinghaosu (QHS; Klayman, 1985; Hien & White, 1993). This compound contains a cyclic endoperoxide group and is thought to destroy the parasite by generating oxy free radicals (Clark *et al.* 1983). QHS and its derivatives represent a potent new class of antimalarials and synthesis of novel QHS analogues is a highly active research area (Posner *et al.* 1994).

If indeed QHS acted through the generation of free radicals, dietary deprivation of vitamin E, a free-radical quencher (Packer, 1991), might be expected to enhance the antimalarial activity of the drug. This in fact was shown to be the case since low doses of QHS have a much stronger effect against *Plasmodium yoelii* in vitamin E-deficient mice than in normal mice (Levander *et al.* 1989a). However, no such enhancing effect of QHS activity was seen under conditions of Se deficiency. This differential effect of vitamin E deficiency and Se deficiency on the antimalarial efficacy of QHS might prove helpful in clarifying the mode of action of the nutrients and/or the drug. For example, the fact that vitamin E status influences the potency of the drug while Se status does not could suggest that the drug exerts its action in a lipid phase of the cell which is inaccessible to the cytoplasmic glutathione peroxidase. Another possible explanation for the results is that the malarial parasite apparently does not possess a Se-dependent glutathione peroxidase (Jung *et al.* 1989), so perhaps one would not expect the parasite's response to the drug to be affected by host Se status.

The successful demonstration that vitamin E nutrition could have an impact on the pharmacological effectiveness of QHS prompted us to test the effect of dietary fish oil, a known tocopherol antagonist (Dam, 1962), on the potency of the drug. An experiment was designed to study three-way interactive effects of QHS, fish oil, and vitamin E deficiency on malarial infection but the vitamin E-deficient mice consuming fish oil were so strongly protected against the disease that it was not possible to ascertain any additional beneficial effect of the drug (Levander *et al.* 1989a). A similar protective effect of fish oil against malaria in vitamin E-deficient mice had been noted about 30 years earlier by Godfrey (1957a) working at Mill Hill in London. Subsequent work by us showed that a variety of fish oils and fish-oil concentrates fed in the diet exhibited this

Table 2. *Contrasting effects of selenium and vitamin E deficiency on the ability of mice fed on diets containing fish oil to resist malarial infection\** (Adapted from Morris *et al.* 1990)

(Mean values with their standard errors for ten animals)

Dietary supplement (mg/kg)		Plasma vitamin E concentration ( $\mu\text{mol/l}$ )		Erythrocyte glutathione peroxidase (EC 1.11.1.9) activity (units/g haemoglobin)		Rate of survival at intervals (d) after inoculation			
Vitamin E	Se	Mean	SE	Mean	SE	12	19	27	60
100	0.5	4.1	0.2	309	5	2/10	0/10	–	–
100	0	4.4	0.4	8	1	2/10	0/10	–	–
0	0.5	<1.2		310	10	10/10	9/10	5/10	5/10

\* Weanling male outbred CD-1 Swiss albino mice were fed on a torula yeast-based diet containing 50 g menhaden (fish) oil/kg with or without vitamin E and/or Se as RRR- $\alpha$ -tocopheryl acetate or sodium selenite respectively. After 4 months, all mice were inoculated intraperitoneally with  $5 \times 10^4$  erythrocytes parasitized by *Plasmodium yoelii* (17X strain). Survival was monitored for 60 d after infection. Survival in the group fed on the vitamin E-deficient diet containing fish oil after 19 d was not as good as that seen in other experiments (Levander & Ager, 1993), perhaps because these mice were older at inoculation (19 weeks *v.* 7 weeks).

antimalarial activity in vitamin E-deficient mice (Levander *et al.* 1990). A plant oil containing *n*-3 fatty acids (linseed oil) also had this antimalarial property in vitamin E-deficient mice as did ground flaxseed and chemically purified ethyl linolenate (Levander *et al.* 1991).

A series of experiments was carried out to determine the practical limitations of this nutritionally-induced antimalarial action. Feeding the pro-oxidant vitamin E-deficient diet containing fish oil for only 2 weeks was sufficient to confer substantial antimalarial protection to the mice, so a severe vitamin E deficiency is apparently not needed to obtain this effect (Levander *et al.* 1989c). Presumably, if the plasma tocopherol levels could be decreased without affecting tissue stores too much, the dietary treatment would still have its antimalarial effect since the erythrocytic form of the parasite 'sees' only the bloodstream. In fact, it was possible to demonstrate antimalarial effects of a fish-oil concentrate fed in diets that resulted in low but finite plasma tocopherol concentrations, although the degree of protection offered by the diet was inversely related to the resultant level of vitamin E in the plasma (Levander & Ager, 1993). Taken together, these observations suggest that human populations marginally deficient in vitamin E might respond favourably to the antimalarial action of fish oil.

As in the case of the experiment with QHS, Se deficiency had little or no effect on the survival of infected mice fed on fish oil, whereas vitamin E deficiency markedly improved the survival of infected mice fed on fish oil (Table 2). This was true despite a prolonged feeding period with the Se-deficient diet (4 months before inoculation) resulting in a severe Se deficiency (erythrocyte glutathione peroxidase activity in the deficient group was less than 3% of that in the control group). Thus, once again host Se deficiency did not have the same deleterious consequences for the parasite as host vitamin E deficiency.

Two difficult problems in dealing with malaria in the field are the emergence of drug-resistant parasites (Bloland *et al.* 1993) and the occurrence of the cerebral form of

the disease (Miller *et al.* 1994). Chloroquine-resistant plasmodia are now a fact of life in many parts of the world and the parasite is developing resistance to several drugs simultaneously. Animal studies have shown that resistance can also develop to drugs of the QHS family (Chawira *et al.* 1986) so that the therapeutic utility of even this class of antimalarials may eventually be lost. In this context, it is of interest to note that feeding the pro-oxidant vitamin E-deficient diet containing menhaden oil protects against chloroquine-resistant lines of three species of murine malaria: *P. yoelii*, *P. berghei*, and *P. vinckei* (Levander *et al.* 1989b; Ager *et al.* 1991; Ager & Levander, unpublished results). Such dietary manipulation was also effective against a sulphone-resistant line of the murine parasite, so that the benefits of nutritionally-induced oxidative stress against the disease were not limited to one type of drug-resistant parasite (Ager & Levander, unpublished results). Even if pro-oxidant nutritional intervention never succeeds as a practical tool for human malaria control, it would seem that phenomena such as those discussed previously might provide useful new theoretical insights into the general problem of drug resistance.

Cerebral malaria is a severe complication of the disease which often results in the death of the patient (Miller *et al.* 1994). Although several factors are thought to determine whether this particular form of the disease will develop (e.g. local endemicity of the disease, age of the patient etc.), often the nutritional status of the infected individual is not considered. Early animal experiments indicated that feeding mice high levels of a synthetic antioxidant (butylated hydroxyanisole; BHA) offered protection against cerebral malaria (Thumwood *et al.* 1989). This finding gave rise to the concept that excess production of free radicals might be involved in the pathogenesis of this form of the disease. Several years later, the somewhat paradoxical observation was made that dietary fish oil, a pro-oxidant stress, also protected partially against murine cerebral malaria (Blok *et al.* 1992). In the latter report, indomethacin, a potent cyclooxygenase inhibitor, had no effect on the course of the infection, thereby ruling out any role of prostaglandins in the protective effect of the fish oil. Moreover, *ex vivo* biosynthesis of tumour necrosis factor, a cytokine presumed to play a key role in the pathogenesis of cerebral malaria, was not decreased by the dietary fish oil treatment. We also found a partial protective effect of dietary fish oil against murine cerebral malaria and that protection could be made virtually complete when the fish oil was fed in a vitamin E-deficient diet (Ager *et al.* 1993). We were also able to show that feeding either of two structurally-unrelated synthetic antioxidants, N,N'-diphenyl-*p*-phenylenediamine (DPPD) or probucol, blocked the protective action of the pro-oxidant vitamin E-deficient diet containing menhaden oil in a cerebral malaria model (Levander *et al.* 1995). Both these observations are most compatible with the hypothesis that the protective effect of dietary fish oil against cerebral malaria is due to dietary-induced oxidative stress. Thus, dietary-induced oxidative stress protects not only against the later sequelae of malarial infection (e.g. anaemia) by suppressing parasite growth, but also against the early manifestations of the disease (e.g. central nervous system consequences) in a model of cerebral malaria. Whether oxidative stress status plays any role in the outcome of human cerebral malaria is not known at this time, although increased levels of lipid peroxidation products have been reported in the cerebrospinal fluid of such patients (Das *et al.* 1991).

The biochemical mechanism by which fish oils exert their beneficial effect against malaria is not certain since these oils are known to have a wide-ranging impact on several

aspects of metabolism (Simopoulos, 1991). Nonetheless, the most reasonable and straightforward explanation for this antimalarial property of fish oil seems to be its ability to exert pro-oxidant stress on the parasite. Indeed, it is difficult to account for many of our experimental observations on any other basis (dependence of fish oil on vitamin E deficiency for full protection, blockage of the protective effect of the fish oil by concurrent consumption of synthetic antioxidants etc.). On the basis of research conducted thus far, one cannot eliminate the possibility that dietary fish oil could have some effect against malaria other than increased oxidative stress. However, it has long been appreciated that plasmodia are remarkably sensitive to oxidative stress and are not able to grow well under normal O<sub>2</sub> tensions (Scheibel *et al.* 1979). Thus, the concept that parasite growth is suppressed by feeding the host a pro-oxidant diet seems eminently plausible. By forcing the parasite to incorporate highly-unsaturated fatty acids into its membranes in the absence of vitamin E (plasmodia cannot synthesize their own fatty acids and must rely on host nutrition to fulfil this need), the already precarious balance between pro-oxidant and antioxidant factors in the parasite is upset and the parasite is killed. Current research does not allow us to conclude whether this chain of events occurs at the level of the parasite or host erythrocyte membrane or both.

One might ask whether the dietary fish oil in the vitamin E-deficient diet acts by stimulating the immune response of the host against the parasite. However, feeding fish oil generally suppresses rather than stimulates innate immune function (Kelley & Daudu, 1993). Acquired immune response, also, does not appear to play a role here either, since the protective effect of the fish oil vitamin E-deficient diet was seen equally well in nude (athymic) or SCID-beige mice (D. W. Taylor, O. A. Levander, V. R. Krishna, C. B. Evans, V. C. Morris and J. R. Barta, unpublished results). Nonetheless, once mice are cured of malaria by the pro-oxidant diet, they remain resistant to subsequent malarial rechallenge no matter which diet is fed to the host (Ager *et al.* 1992). Thus, short-term nutritional protection against malaria by feeding the vitamin E-deficient diet with fish oil appears to confer long-term immunological protection against the parasite. A similar phenomenon has recently been reported in Mg-deficient mice infected with *Babesia hylomyisci* (Maurois *et al.* 1994). *Babesia* belong to the same phylum of protozoan parasites as *Plasmodium* and Mg deficiency is known to exert an increased oxidative stress on erythrocytes in hamsters (*Mesocricetus auratus*; Freedman *et al.* 1992). In his pioneering studies, Godfrey (1957b) showed that fish oil in a low-vitamin E diet protected against *Babesia rodhaini* in mice.

Our success in controlling plasmodial growth by manipulating host oxidative stress status through diet has led us to try this approach with other parasitic diseases. Feeding fish or flaxseed oil in a normal starter ration decreased caecal lesions in chicks infected with the coccidial parasite, *Eimeria tenella* (Allen *et al.* 1994). Like *Babesia*, *Eimeria* is of the same phylum as *Plasmodium*, although its life-cycle and host habitats are quite different. Coccidiosis is an economically significant problem for the poultry industry and the parasite has developed resistance to many commonly used anti-coccidial drugs, so any new leads to the control of the parasite could be useful. On the basis of an earlier report that feeding a diet deficient in vitamin E and Se to mice impaired development of *Schistosoma mansoni* (DeWitt, 1957), we tested the effect of the vitamin E-deficient diet with fish oil on the progression of schistosomiasis in mice (Morris *et al.* 1994). As shown by others (Fusco *et al.* 1992), dietary menhaden oil increased the cercarial penetration by *S. mansoni*. However, we found that feeding menhaden oil in a vitamin E-deficient diet

decreased the liver surface nodule scores and the number of adult worms despite the increased cercarial penetration. Given the fact that schistosomes contain a Se-dependent glutathione peroxidase (Roche *et al.* 1994), it might be profitable to examine the influence of host Se deficiency on the growth and maturation of *S. mansoni*.

Will the dietary manipulation of host oxidative stress status ever find application in attacking human malaria? Malaria is now the leading killer of children under 5 years in sub-Saharan Africa and the World Health Organization estimates that globally the number of persons infected with malaria is increasing at a rate of approximately 5%/year (Kolberg, 1994). *In vitro* addition of *n*-3 fatty acids inhibits the growth of *P. falciparum* (Fevang *et al.* 1992; Kumaratilake *et al.* 1992) so that the phenomena discussed in the present review are apparently not limited only to murine malaria. On the other hand, serum and erythrocytes collected from an individual supplemented with tocopherol-fortified fish oil had no effect on the *in vitro* growth of blood stages of *P. falciparum* (Abu-Zeid *et al.* 1993). Supplementation of normal (laboratory chow) diets with menhaden oil resulted in substantial (though not complete) protection against murine malaria so that vitamin E deficiency does not seem to be required to obtain at least some beneficial effect of the fish oil (Levander & Ager, 1993). A pilot study conducted in China indicated that patients clinically cured of malaria with QHS recrudesced more slowly when given a supplement of fish oil concentrate not containing vitamin E, although all patients eventually redeveloped the disease (Levander *et al.* 1994). Perhaps fish oil might find a use in the therapy of human malaria as an adjunct to treatment with pro-oxidant drugs such as those related to QHS. Low doses of artelinic acid, a derivative of QHS, had a more powerful chemosuppressive action in mice fed on menhaden oil than in mice fed on lard even though both groups were supplemented with vitamin E (Levander & Ager, 1993). Additional research is needed to determine what, if any, role dietary-induced oxidative stress may play in the control of human malaria.

#### KESHAN DISEASE

The discovery that nutritional levels of Se protect against the endemic juvenile cardiomyopathy known in China as Keshan disease has to rank among the greatest triumphs of public health trace element nutrition. Several lines of evidence suggest that low Se status is the basic underlying cause of this condition. For example, Keshan disease has been linked to many indicators of poor Se nutrition including decreased blood and hair Se content and low dietary Se intakes (Keshan Disease Research Group, 1979a). Moreover, the Chinese scientists carried out an extensive Se supplementation trial which provided convincing evidence for the ability of Se to prevent the disease (Keshan Disease Research Group, 1979b).

Despite their obvious success in associating Keshan disease with impaired Se nutriture, the Chinese scientists were always very careful to point out that certain features of the disease could not readily be explained on the basis of poor Se status alone (Yang *et al.* 1988). In particular, there was an appreciable seasonal and annual variation in the incidence of the disease which suggested an infectious component. Several enteroviruses were isolated from Keshan disease victims. One such virus, coxsackie B4, was found to cause extensive damage to heart muscle when injected into mice fed on a low-Se diet based on grains from a Keshan disease area (Ge *et al.* 1987). Supplementation of the

Table 3. *Effect of vitamin E and dietary fat on the heart damage caused by a myocarditic coxsackievirus (CVB3/20) in mice\** (Adapted from Beck *et al.* 1994a)

(Mean values and standard deviations for five or ten animals)

Vitamin E (mg/kg)	Dietary fat (g/kg)			Histopathological score at intervals (d) after inoculation					
	Lard	Menhaden oil	Maize oil	7		14		21	
38.4	40	0	10	0.2	0.4 <sup>a</sup>	1.5	0.8 <sup>b</sup>	1.6	0.7 <sup>b</sup>
0	40	0	10	1.6	0.5 <sup>b</sup>	2.8	0.4 <sup>c</sup>	2.8	0.4 <sup>c</sup>
38.4	0	40	10	0.2	0.4 <sup>a</sup>	1.4	0.8 <sup>b</sup>	1.8	0.4 <sup>b</sup>
0	0	40	10	1.9	0.3 <sup>b</sup>	4.0 <sup>d</sup>		4.0 <sup>d</sup>	

<sup>a, b, c, d.</sup> Means with unlike superscript letters were significantly different ( $P < 0.05$ ).

\* Weanling male C3H/HeJ mice were fed on a torula yeast-based diet containing lard, menhaden oil or corn oil and 0.2 µg Se as sodium selenite/g either with or without vitamin E (RRR- $\alpha$ -tocopheryl acetate). After 4 weeks, all mice were inoculated intraperitoneally with 10<sup>5</sup> tissue culture infectious dose-50 of CVB3/20 in 0.1 ml RPMI-1640 medium. Pathological score: 0, no lesions; 1, foci of mononuclear cell inflammation associated with myocardial cell reactive changes without myocardial cell necrosis; 2, inflammatory foci clearly associated with myocardial cell reactive changes; 3, inflammatory foci clearly associated with myocardial cell necrosis and dystrophic calcification; 4, extensive inflammatory infiltration, necrosis and dystrophic calcification.

mice fed on the deficient diet orally or by adding Se to the drinking water before inoculation reduced the severity of heart lesions caused by the virus.

As a follow-up to those studies, Beck *et al.* (1994a,b,c,d) initiated a series of experiments to determine the effect of Se deficiency on the cardiotoxicity of coxsackievirus B3. In agreement with the Chinese results, Se deficiency increased the histopathological damage caused by a myocarditic strain of the virus, coxsackievirus B3/20 (CVB3/20; Beck *et al.* 1994c). Likewise, vitamin E deficiency also increased the cardiac damage caused by CVB3/20 and substituting menhaden (fish) oil for the lard in the diet resulted in even greater damage (Table 3; Beck *et al.* 1994a). Neither Se nor vitamin E deficiency had any effect on neutralizing antibody response or natural-killer-cell function. However, either deficiency resulted in decreased lymphocyte proliferation as stimulated either by mitogen (Concanavalin A) or by antigen (prepared from membranes of HeLa cells infected with coxsackievirus). This impairment of cell-mediated immune function may have allowed the virus to multiply to a greater extent, thereby accounting for the increased viral titres seen in the hearts of the Se- or vitamin E-deficient mice.

Beck *et al.* (1994a,b) then extended their nutritional studies to a strain of the virus, coxsackievirus B3/0 (CVB3/0), that is amyocarditic (i.e. causes no heart damage under normal conditions). When the host mice were fed on a diet deficient in either vitamin E or Se, the normally benign CVB3/0 was able to cause mild but significant heart damage (Beck *et al.* 1994a,b). Furthermore, when CVB3/0 was isolated from the hearts of either vitamin E- or Se-deficient mice, passed through HeLa cells, and re-inoculated into normal (i.e. non-deficient) mice, the virus retained its capacity to cause heart damage. This observation suggested the possibility that passage of the benign CVB3/0 through a deficient host had somehow altered its genetic make-up such that now the virus exhibited virulence. Similar results were obtained with the normally myocarditic virus (CVB3/20) in that the increased virulence apparent in a deficient host was retained after passage

through HeLa cells and re-inoculation into normal mice. Therefore, inoculation of either a myocarditic or amyocarditic form of the virus into either a Se- or vitamin E-deficient host resulted in an increased virulence that was stable to passage through cell culture and manifested itself as greater heart damage upon re-inoculation into a normal host.

The most plausible, and yet controversial, explanation for these results is that the viral genome mutated as a result of the virus having been passed through an oxidatively-stressed host. It should be emphasized that the viral preparations used here (CVB3/0 and CVB3/20) are homogeneous cloned and sequenced viruses, not the heterogeneous viral population that you might expect to find in a wild-type virus. This would rule out the possibility that the growth of a particular sub-population within the original inoculum might be favoured under conditions of host oxidative stress, thereby leading to an apparent genotypic change. Coxsackievirus is a non-enveloped virus, therefore it is difficult to visualize how such a virus could pick up host factors that might influence virulence and retain them during passage through cell culture.

If indeed a genetic change in the virus is the explanation for our results, these experiments would provide a novel insight into our understanding of host–virus interrelationships. To our knowledge, these studies would provide the first example of an influence of host nutritional status on the genetic constitution of a virus. How could such a change be accomplished? Perhaps dietary oxidant stress compromises the host immune system so that the virus replicates to higher titres thereby increasing the chances of mutation. RNA viruses, such as the coxsackievirus, are characterized by relatively high mutation rates, in the range of  $10^{-3}$ – $10^{-5}$  substitutions per nucleotide copied (Domingo & Holland, 1994). Up to ten genomic changes may account for the difference in cardiovirulence between the CVB3/0 and CVB3/20 described previously (Chapman *et al.* 1994). On the other hand, only a single amino acid residue accounted for the virulent phenotype of a pancreatic variant of coxsackievirus B4 (Caggana *et al.* 1993).

What would be the consequences of any such nutrition-driven mutation in the viral genome? Morse (1993a) has coined the term ‘emerging viruses’ to refer to ‘viruses that either have newly appeared in the population or are rapidly expanding their range, with a corresponding increase in cases of disease.’ Change in host nutritional status was thought to occasionally play a role in the recognition of viruses already widespread in the human population through the precipitation of new or more serious disease. Increased viral traffic (i.e. transfer and dissemination of viruses to new host populations) was considered the major determinant of ‘new’ viral diseases with viral evolution being of lesser relative importance. Our results with dietary-induced oxidative stress, however, suggest that mutation rates in RNA viruses may be accelerated greatly during nutritional deprivation so that evolution of new viruses may assume greater significance under some conditions.

Our dietary antioxidant deficiency–viral infection model has been useful also in exploring other host factors that determine virus pathogenicity. For example, mice are known to develop resistance to the coxsackievirus as they get older so that after about 8 weeks of age they become refractory to viral challenge. If mice were fed on a Se-deficient diet, however, they retained susceptibility to CVB3-induced myocarditis up to 14 weeks of age (Beck *et al.* 1994d). Susceptibility of mice to CVB3-induced myocarditis is also a function of genetic background of the host since different strains of mice vary widely in their ability to resist myocardial disease. The biochemical basis for these differences in susceptibility to viral disease due to host genetics is not completely understood but may be related to several factors such as the presence of viral receptors, interferon production

Table 4. *Contrasting effects of selenium and vitamin E deficiency on two mouse models of infectious disease*

Test diet	Effect of deficiency	
	Malaria	Myocarditis
Vitamin E-deficient (lard)	+	-
Vitamin E-deficient (fish oil)	++	--
Se-deficient	0	--

++, Very beneficial effect; +, beneficial effect; 0, no effect; -, detrimental effect; --, very detrimental effect.

or major histocompatibility complex haplotypes. We were able to render mice genetically resistant to CVB3-induced myocarditis susceptible by feeding them a diet deficient in both Se and vitamin E (Williams *et al.* 1994).

Our results also could have important implications regarding the aetiology of Keshan disease. If an enterovirus such as coxsackievirus is involved in Keshan disease, the mouse studies indicate that vitamin E, as well as Se, could have a role in the disease since deficiency of either nutrient increased the heart damage caused by the virus. Indeed, marginal vitamin E nutriture was reported in residents of Keshan-disease areas (Yang *et al.* 1984; Xia *et al.* 1989). Moreover, rats fed on diets comprised of cereals from an endemic area developed liver necrosis, indicating that the diet was low in both vitamin E and Se (Ge *et al.* 1987). On the basis of changes in rat heart muscle mitochondria, Yang *et al.* (1994) recently concluded that Se deficiency alone is not sufficient to account for the pathogenesis of Keshan disease. Other dietary components, including vitamin E, were suggested as possible complicating factors. Further work is needed to establish the relative role of Se and vitamin E in the aetiology of Keshan disease.

#### CONCLUDING REMARKS

The results reviewed here demonstrate the profound impact of dietary oxidative stress on the outcome of infection in two mouse models of disease (Table 4). In one case (malaria), vitamin E deficiency protected against the disease, whereas in the other (viral myocarditis), vitamin E deficiency made the condition worse. On the other hand, Se deficiency had no effect on the course of murine malaria, but strongly exacerbated viral-induced heart damage. Work by others has indicated that oxidative stress may be involved in a number of other parasitic and viral diseases such as trypanosomiasis (Godfrey, 1958; Docampo & Moreno, 1984), babesiosis (Godfrey, 1957*b*; Maurois *et al.* 1994), HIV/AIDS (Droge *et al.* 1992; Greenspan & Aruoma, 1994) or influenza (Hennet *et al.* 1992). Should our results linking an increase in the pathogenesis of coxsackievirus with the host diet be explained by alterations in the genome of the virus, then the implications for human health of similar genetic changes possibly occurring in other viruses might be profound indeed.

Our studies are the product of effective collaboration of a nutritional biochemist, a parasitologist, and a viral immunologist. Such collaborations can lead to novel, and sometimes unanticipated, results. In the preface to his recent book, Professor S. S. Morse pointed out that 'finding answers to the questions posed by emerging viruses

requires attacking the problem from several different perspectives, often crossing disciplinary lines' (Morse, 1993b). And yet the difficulties confronting such interdisciplinary collaborations are well known (organizational obstacles, funding impediments, etc.; Kahn & Prager, 1994). Nonetheless, it is hoped that others will join in exploring the fascinating frontier delineated by the interface of oxidative stress status and infection.

Note added in proof: Beck *et al.* (1995) have in fact recently provided evidence that the genomic sequence of an avirulent Coxsackievirus B3 is altered as a result of being passed through a Se-deficient mouse.

#### REFERENCES

- Abu-Zeid, Y. A., Hansen, H. S., Jakobsen, P. H., Bygbjerg, I. C. & Dyerberg, J. (1993). No effect of human serum and erythrocytes enriched in *n*-3 fatty acids by oral intake on *Plasmodium falciparum* bloodstage parasites *in vitro*. *European Journal of Clinical Nutrition* **47**, 431–436.
- Ager, A. L., Fontela, R., Morris, V. C. & Levander, O. A. (1993). A menhaden oil vitamin E-deficient (MO-VE) diet protects against cerebral malaria in a mouse model. *FASEB Journal* **7**, A151.
- Ager, A. L., Levander, O. A., Fontela, R., May, R. G. & Morris, V. C. (1991). Chloroquine-resistant (CR) but not -sensitive (CS) *Plasmodium vinckei* cured in mice by menhaden oil (MO) diet deficient in vitamin E (VE). *FASEB Journal* **5**, A1081.
- Ager, A. L., Levander, O. A., Morris, V. C., Fontela, R. & May, R. G. (1992). Once cured of malaria by a menhaden oil-vitamin E-deficient diet, mice become resistant to rechallenge with the parasite regardless of diet fed. *FASEB Journal* **6**, A1212.
- Allen, P. C., Danforth, H. D. & Levander, O. A. (1994). High dietary levels of flaxseed oil and fish oil lower cecal lesion scores in broiler chickens infected with the cecal parasite, *Eimeria tenella*. *Proceedings of the 55th Flax Institute of the United States* **55**, 123–126.
- Arthur, J. R., Nicol, F. & Beckett, G. J. (1990). Hepatic iodothyronine 5'-deiodinase. The role of selenium. *Biochemical Journal* **272**, 537–540.
- Beck, M. A., Kolbeck, P. C., Rohr, L. H., Shi, Q., Morris, V. C. & Levander, O. A. (1994a). Vitamin E deficiency intensifies the myocardial injury of coxsackievirus B3 infection of mice. *Journal of Nutrition* **124**, 345–358.
- Beck, M. A., Kolbeck, P. C., Rohr, L. H., Shi, Q., Morris, V. C. & Levander, O. A. (1994b). Benign human enterovirus becomes virulent in selenium-deficient mice. *Journal of Medical Virology* **43**, 166–170.
- Beck, M. A., Kolbeck, P. C., Shi, Q., Rohr, L. H., Morris, V. C. & Levander, O. A. (1994c). Increased virulence of a human enterovirus (coxsackievirus B3) in selenium-deficient mice. *Journal of Infectious Diseases* **170**, 351–357.
- Beck, M. A., Morris, V. C. & Levander, O. A. (1994d). Mice that normally become resistant to CVB3-induced myocarditis because of aging retain their susceptibility when fed a selenium (Se)-deficient diet. *FASEB Journal* **8**, A541.
- Beck, M. A., Shi, Q., Morris, V. C. & Levander, O. A. (1995). Rapid genomic evolution of a non-virulent Coxsackievirus B3 in selenium-deficient mice results in selection of identical virulent isolates. *Nature Medicine* **1**, 433–436.
- Blok, W. L., Vogels, M. T. E., Curfs, J. H. A. J., Eling, W. M. C., Buurman, W. A. & Van Der Meer, J. W. M. (1992). Dietary fish-oil supplementation in experimental gram-negative infection and in cerebral malaria in mice. *Journal of Infectious Diseases* **165**, 898–903.
- Bloland, P. B., Lackritz, E. M., Kazembe, P. N., Were, J. B. O., Steketee, R. & Campbell, C. C. (1993). Beyond chloroquine: implications of drug resistance for evaluating malaria therapy efficacy and treatment policy in Africa. *Journal of Infectious Diseases* **167**, 932–937.
- Caggana, M., Chan, P. & Ramsingh, A. (1993). Identification of a single amino acid residue in the capsid protein VP1 of coxsackievirus B4 that determines the virulent phenotype. *Journal of Virology* **67**, 4797–4803.
- Chapman, N. M., Tu, Z., Tracy, S. & Gauntt, C. J. (1994). An infectious cDNA copy of the genome of a non-cardiovirulent coxsackievirus B3 strain: its complete sequence analysis and comparison to the genome of cardiovirulent coxsackievirus. *Archives of Virology* **135**, 115–130.

- Chawira, A. N., Warhurst, D. C. & Peters, W. (1986). Qinghaosu resistance in rodent malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **80**, 477–480.
- Clark, I. A., Cowden, W. B. & Butcher, G. A. (1983). Free oxygen radical generators as antimalarial drugs. *Lancet* **i**, 234.
- Collins, A., Duthie, S. & Ross, M. (1994). Micronutrients and oxidative stress in the aetiology of cancer. *Proceedings of the Nutrition Society* **53**, 67–75.
- Dam, H. (1962). Interrelations between vitamin E and polyunsaturated fatty acids in animals. In *Vitamins and Hormones*, vol. 20, pp. 527–540 [R. J. Harris and I. G. Wool, editors]. Orlando: Academic Press.
- Das, B. S., Mohanty, S., Mishra, S. K., Patnaik, J. K., Satpathy, S. K., Mohanty, D. & Bose, T. K. (1991). Increased cerebrospinal fluid protein and lipid peroxidation products in patients with cerebral malaria. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **85**, 733–734.
- DeWitt, W. B. (1957). Experimental schistosomiasis *mansoni* in mice maintained on nutritionally deficient diets. I. Effects of a torula yeast ration deficient in factor 3, vitamin E, and cystine. *Journal of Parasitology* **43**, 119–128.
- Docampo, R. & Moreno, S. N. J. (1984). Free radical metabolites in the mode of action of chemotherapeutic agents and phagocytic cells on *Trypanosoma cruzi*. *Reviews of Infectious Diseases* **6**, 223–238.
- Domingo, E. & Holland, J. J. (1994). Mutation rates and rapid evolution of RNA viruses. In *The Evolutionary Biology of Viruses*, pp. 161–184 [S. S. Morse, editor]. New York: Raven Press.
- Droge, W., Eck, H. P. & Mihm, S. (1992). HIV-induced cysteine deficiency and T-cell dysfunction – a rationale for treatment with N-acetylcysteine. *Immunology Today* **13**, 211–214.
- Fevang, P., Bjorkman, A. & Hostmark, A. (1992). Suppression of *Plasmodium falciparum* *in vitro* by polyunsaturated fatty acids. *XIII International Congress for Tropical Medicine and Malaria*. Jomtien, Thailand. p. 268 Abstr.
- Freedman, A. M., Mak, I. T., Stafford, R. E., Dickens, B. F., Cassidy, M. M., Muesing, R. A. & Weglicki, W. B. (1992). Erythrocytes from magnesium-deficient hamsters display an enhanced susceptibility to oxidative stress. *American Journal of Physiology* **262**, 1371–1375.
- Fusco, A. C., Weaver, S., Salafsky, B. & Shibuya, T. (1992). Influence of a menhaden oil diet on cercarial penetration of *Schistosoma mansoni*. *Journal of Parasitology* **78**, 738–740.
- Ge, K. Y., Wang, S. Q., Bai, J., Xue, A. N., Dang, X. J., Su, C. Q. & Wu, S. Q. (1987). The protective effect of selenium against viral myocarditis in mice. In *Selenium in Biology and Medicine – Part B*, pp. 761–768 [G. F. Combs, J. E. Spallholz, O. A. Levander and J. E. Oldfield, editors]. New York: Van Nostrand Reinhold.
- Godfrey, D. G. (1957a). Antiparasitic action of dietary cod liver oil upon *Plasmodium berghei* and its reversal by vitamin 'E'. *Experimental Parasitology* **6**, 555–565.
- Godfrey, D. G. (1957b). The influence of dietary cod liver oil and vitamin E upon *Babesia rodhaini* in mice. *Experimental Parasitology* **6**, 465–485.
- Godfrey, D. G. (1958). Influence of dietary cod liver oil upon *Trypanosoma congolense*, *T. cruzi*, *T. vivax* and *T. brucei*. *Experimental Parasitology* **7**, 255–268.
- Greenspan, H. C. & Aruoma, O. I. (1994). Oxidative stress and apoptosis in HIV infection: a role for plant-derived metabolites with synergistic antioxidant activity. *Immunology Today* **15**, 209–213.
- Harris, P. L., Ludwig, M. I. & Schwarz, K. (1958). Ineffectiveness of Factor 3-active selenium compounds in resorption-gestation bioassay for vitamin E. *Proceedings of the Society for Experimental Biology and Medicine* **97**, 686–688.
- Hein, T. T. & White, N. J. (1993). Qinghaosu. *Lancet* **341**, 603–608.
- Hennet, T., Peterhans, E. & Stocker, R. (1992). Alterations in antioxidant defenses in lung and liver of mice infected with influenza virus. *Journal of General Virology* **73**, 39–46.
- Hoekstra, W. G. (1975). Biochemical function of selenium and its relation to vitamin E. *Federation Proceedings* **34**, 2083–2089.
- Jung, A., Fritsch, B., Dieckmann, A., Bleiholder, B. & Otchwemah, R. (1989). Selenium-independent glutathione peroxidase in malaria parasites. In *Selenium in Biology and Medicine*, pp. 38–42 [A. Wendel, editor]. Berlin: Springer Verlag.
- Kahn, R. I. & Prager, D. J. (1994). Interdisciplinary collaborations are a scientific and social imperative. *The Scientist* **8**, 12.
- Kelley, D. S. & Daudu, P. A. (1993). Fat intake and immune response. *Progress in Food and Nutrition Science* **17**, 41–63.
- Keshan Disease Research Group (1979a). Epidemiologic studies on the etiologic relationship of selenium and Keshan disease. *Chinese Medical Journal* **92**, 477–482.

- Keshan Disease Research Group (1979b). Observations on effect of sodium selenite in prevention of Keshan disease. *Chinese Medical Journal* **92**, 471–476.
- Klayman, D. L. (1985). Qinghaosu (artemisinin): an antimalarial drug from China. *Science* **228**, 1049–1055.
- Kolberg, R. (1994). Finding 'sustainable' ways to prevent parasitic diseases. *Science* **264**, 1857–1859.
- Kumaratilake, L. M., Robinson, B. S., Ferrante, A. & Poulos, A. (1992). Antimalarial properties of *n*-3 and *n*-6 polyunsaturated fatty acids: *in vitro* effects on *Plasmodium falciparum* and *in vivo* effect on *P. berghei*. *Journal of Clinical Investigation* **89**, 961–967.
- Levander, O. A. & Ager, A. L. (1993). Malarial parasites and antioxidant nutrients. *Parasitology* **107**, S95–S106.
- Levander, O. A., Ager, A. L., Morris, V. C. & May, R. G. (1989a). Qinghaosu, dietary vitamin E, selenium, and cod liver oil: effect on the susceptibility of mice to the malarial parasite *Plasmodium yoelii*. *American Journal of Clinical Nutrition* **50**, 346–352.
- Levander, O. A., Ager, A. L., Morris, V. C. & May, R. G. (1989b). Menhaden-fish oil in a vitamin E-deficient diet: protection against chloroquine-resistant malaria in mice. *American Journal of Clinical Nutrition* **50**, 1237–1239.
- Levander, O. A., Ager, A. L., Morris, V. C. & May, R. G. (1989c). Protective effect of dietary fish oil against malaria in vitamin E-deficient mice. In *Health Effects of Fish and Fish Oils*, pp. 461–467 [R. K. Chandra, editor]. St John's, Canada: ARTS Biomedical Publishers.
- Levander, O. A., Ager, A. L., Morris, V. C. & May, R. G. (1990). *Plasmodium yoelii*: comparative antimalarial activities of dietary fish oils and fish oil concentrates in vitamin E-deficient mice. *Experimental Parasitology* **70**, 323–329.
- Levander, O. A., Ager, A. L., Morris, V. C. & May, R. G. (1991). Protective effect of ground flaxseed or ethyl linolenate in a vitamin E-deficient diet against murine malaria. *Nutrition Research* **11**, 941–948.
- Levander, O. A., Fontela, R., Morns, V. C. & Ager, A. L. (1995). Protection against murine cerebral malaria by dietary-induced oxidative stress. *Journal of Parasitology* **81**, 99–103.
- Levander, O. A., Fu, S., Cai, X., Ren, L. & Morris, V. (1994). Effect of menhaden oil ethyl esters (MOEE) on the recrudescence of malaria in patients previously treated with qinghaosu (QHS). *FASEB Journal* **8**, A438.
- Maurois, P., Delcourt, Ph., Gueux, E. & Rayssiguier, Y. (1994). Magnesium deficiency protects against *Babesia hylomysci* and mice become resistant to rechallenge with the parasite regardless of diet fed. *Parasitology* **108**, 245–248.
- Miller, L. H., Good, M. F. & Milon, G. (1994). Malaria pathogenesis. *Science* **264**, 1878–1883.
- Morris, V. C., Ager, A. L., May, R. G. & Levander, O. A. (1990). Effect of selenium (Se) and synthetic antioxidants on the antimalarial action of menhaden oil (MO) fed to vitamin E-deficient (–VE) mice. *FASEB Journal* **4**, A504.
- Morris, V. C., Hara, I., Levander, O. A., Fusco, A. C. & Salafsky, B. (1994). Effect of dietary menhaden oil (MO) and vitamin E (VE) on *Schistosoma mansoni* (SM) infection in mice. *FASEB Journal* **8**, A438.
- Morse, S. S. (1993a). Examining the origins of emerging viruses. In *Emerging Viruses*, pp. 10–28 [S. S. Morse, editor]. New York: Oxford University Press.
- Morse, S. S. (1993b). *Emerging Viruses*. New York: Oxford University Press.
- National Research Council (1983). *Selenium in Nutrition*, revised ed. Washington, DC: National Academy Press.
- Packer, L. (1991). Protective role of vitamin E in biological systems. *American Journal of Clinical Nutrition* **53**, 1050S–1055S.
- Posner, G. H., Oh, C. H., Webster, H. K., Ager, A. L. & Rossan, R. N. (1994). New, antimalarial, tricyclic, 1,2,4-trioxanes: evaluation in mice and monkeys. *American Journal of Tropical Medicine and Hygiene* **50**, 522–526.
- Riemersma, R. A. (1994). Epidemiology and the role of antioxidants in preventing coronary heart disease: a brief review. *Proceedings of the Nutrition Society* **53**, 59–65.
- Roche, C., Williams, D. L., Khalife, J., LaPresle, T., Capron, A. & Pierce, R. J. (1994). Cloning and characterization of the gene encoding *Schistosoma mansoni* glutathione peroxidase. *Gene* **138**, 149–152.
- Scheibel, L. W., Ashton, S. H. & Trager, W. (1979). *Plasmodium falciparum*: microaerophilic requirements in human blood cells. *Experimental Parasitology* **47**, 410–418.
- Schwarz, K. & Foltz, C. M. (1957). Selenium as an integral part of Factor 3 against dietary necrotic liver degeneration. *Journal of the American Chemical Society* **79**, 3292–3293.
- Simopoulos, A. P. (1991). Omega-3 fatty acids in health and disease and in growth and development. *American Journal of Clinical Nutrition* **54**, 438–463.

- Thompson, J. N. & Scott, M. L. (1969). Role of selenium in the nutrition of the chick. *Journal of Nutrition* **97**, 335–342.
- Thumwood, L. M., Hunt, N. H., Cowden, W. B. & Clark, I. A. (1989). Antioxidants can prevent cerebral malaria in *Plasmodium berghei*-infected mice. *British Journal of Experimental Pathology* **70**, 293–303.
- Williams, D. L., Beck, M. A., Morris, V. C. & Levander, O. A. (1994). Mice genetically resistant to coxsackievirus-induced myocarditis become susceptible when fed a diet deficient in both selenium (Se) and vitamin E (VE). *FASEB Journal* **8**, A435.
- Xia, Y., Hill, K. E. & Burk, R. F. (1989). Biochemical studies of a selenium-deficient population in China: measurement of selenium, glutathione peroxidase and other oxidant defense indices in blood. *Journal of Nutrition* **119**, 1318–1326.
- Yang, G. Q., Chen, J., Wen, Z., Ge, K., Zhu, L., Chen, X. & Chen, X. (1984). The role of selenium in Keshan disease. *Advances in Nutritional Research* **6**, 203–231.
- Yang, G. Q., Ge, K., Chen, J. & Chen, X. (1988). Selenium-related endemic diseases and the daily selenium requirement of humans. *World Review of Nutrition and Dietetics* **55**, 98–152.
- Yang, F. Y., Lin, Z. H., Xing, J. R., Li, S. G., Yang, J., San, S. & Wu, L. Y. (1994). Se deficiency is a necessary but not sufficient factor required for the pathogenesis of Keshan disease. *Journal of Clinical and Biochemical Nutrition* **16**, 101–110.