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Symposium on 'Newer aspects of micronutrients'

Symposium 1 Newer aspects of micronutrients in chronic disease

β-Carotene, are we misreading the signals in risk groups? Some analogies with vitamin C

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The gem cannot be polished without friction
nor man perfected without trials

ANON.

New aspects concerning micronutrients in chronic disease is the theme of this symposium and the subject of the present paper is the carotenes. The particular 'gem' I want to 'polish' is the nutritional assessment of β-carotene. How do we interpret β-carotene concentrations in plasma, particularly in those with chronic or acute disease? I think this is particularly pertinent at this time because intervention trials with β-carotene in smokers have appeared to increase the risk of lung cancer and ischaemic heart disease (Finnish Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study; Heinonen & Albanes, 1994). I do not want to speculate about that trial itself, but I do want to consider how we should interpret the low plasma concentrations which are found in subjects with chronic and acute disease or in risk groups within a population. To do this, I will draw your attention to some of the similarities between the behaviour of carotenes and vitamin C in the body in response to stress and then discuss the implications for our interpretation of status.

SIMILARITIES BETWEEN PLASMA β-CAROTENE AND VITAMIN C

There are five very obvious similarities between plasma β-carotene and vitamin C: (1) a direct relationship between dietary intake and plasma concentrations, (2) plasma concentrations in females tend to be higher than those in males, (3) concentrations of both nutrients are depressed in smokers compared with non-smokers, (4) both substances are radical-quenching antioxidants but (5) both substances can be pro-oxidants.

The fact that plasma concentrations of β-carotene have been reported to be higher in women than in men in many countries of the world suggests that there is some measure of functional control over plasma concentrations (Table 1). The difference in plasma

Table 1. *Serum β-carotene concentrations (μmol/l) in men and women in different population groups*

Country		Men	Women	Source
Great Britain:	*n*	944	938	Thurnham & Flora (1988)
	Median	0·24	0·32	
	Range	0·01–6·52	0·02–2·93	
Germany:	*n*	278*	367*	Hesseker *et al.* (1991)
	Median	0·43	0·61	
	2·5, 97·5 centiles	0·10–1·56	0·16–2·19	
Switzerland:	*n*	75	75	Vuilleumier *et al.* (1983)
	Median	0·49	0·63	
	10, 90 centiles	0·25–0·97	0·31–1·33	
Japan:	*n*	1196	618	Ito *et al.* (1990)
	Median	0·35	0·64	
	10, 90 centiles	0·15–0·82	0·28–1·33	
China:	*n*	299	306	Thurnham *et al.* (1988)
	Mean	0·24	0·35	
	SD	0·13	0·18	
USA:	*n*	55	55	Stacewicz-Sapuntzakis *et al.* (1987)
	Mean	0·29	0·42	
	SD	0·18	0·26	

* Numbers shown are those for retinol measurements. Total number of β-carotene measurements was 632 but numbers for individual sexes not given.

concentrations between men and women is even more surprising when it is realized that plasma concentrations directly respond to carotene intake (Willet *et al.* 1983) and intake of dietary carotene tends to be higher in men than women. In the UK, men consume slightly more β-carotene equivalents than women yet have plasma values only three-quarters those of women (Gregory *et al.* 1990). A further factor which, one would imagine, would tend to diminish differences between the sexes is the poor bioavailability of dietary carotenes. Absorption of β-carotene from the carrot is reported to vary between 3 and 97% depending on preparation (World Health Organization, 1967).

In the case of vitamin C, dietary intakes tend to be similar in both sexes (Bates *et al.* 1979; Margetts & Jackson, 1993) and absorption presents fewer problems as the vitamin is water-soluble. However, plasma (PAA) and leucocyte ascorbate (LAA) values tend to be higher in women than in men (Bates *et al.* 1979; Department of Health and Social Security, 1979; Jacob, 1990; Thurnham, 1992).

It is widely reported that smoking depresses plasma β-carotene (Stryker *et al.* 1988; Anderson, 1991; Thurnham, 1994*a*) and vitamin C (Pelletier, 1977; Anderson, 1991). The intake of β-carotene equivalents by smokers is lower than that of non-smokers (Morabia & Wynder, 1990; Margetts & Jackson, 1993) but even allowing for that, plasma levels are lower in smokers by at least one-third (Stryker *et al.* 1988; Thurnham, 1994*a*). The usual interpretation of this phenomenon is that because tobacco smoke contains many free radicals smoking is a pro-oxidant stress (Church & Pryor, 1985; Anderson & Lukey, 1987); thus, radical-quenching nutrients like vitamin C and β-carotene may be consumed in protecting tissues against peroxidation and damage

(Anderson, 1991). Hence, it is argued that the daily nutrient requirements of smokers for β-carotene and vitamin C will be higher than those of non-smokers (Kallner *et al.* 1981; Jacob, 1990) and it is even suggested in the ATBC Cancer Prevention Study (Heinonen & Albanes, 1994) that the amount of β-carotene (20 mg/d) given may not have been enough to have given the necessary protection to the smokers.

β-CAROTENE AND VITAMIN C AS PRO-OXIDANTS

There is an alternative explanation, however, which should be considered. It is that in certain conditions these nutrients are in fact pro-oxidants in the plasma. In relation to this property I suggest that the body may in fact have developed ways of controlling plasma concentrations to minimize the potential damage which could result from pro-oxidant activity. Thus, optimal plasma concentrations of these nutrients to maintain an antioxidant function may not be the maximum possible and differences between the sexes may be due to different levels of pro-oxidant risk, but this is a point which will be discussed later.

The radical-quenching antioxidant properties of β-carotene were first described by Burton & Ingold (1984). They pointed out, however, that radical-quenching properties were only present at low oxygen tensions and that at the oxygen levels found in plasma, β-carotene would act as pro-oxidant. In our own experiments we have also seen this pro-oxidant activity of β-carotene (Chopra *et al.* 1993). Thus, it is difficult to understand how such an antioxidant, which is so sensitive to the presence of oxygen, can protect the lungs against cancer, unless of course it acts as a proxy for something else. Such experimental data, however, do not necessarily indicate that β-carotene will be damaging in tissues and there are recent reports that β-carotene supplements reduce lipid peroxidation *in vivo* (Mobarhan *et al.* 1990; Allard *et al.* 1994). Furthermore, very large amounts of β-carotene have been given to patients with erythropoietic protoporphyria over many years. No adverse effects have been reported in those responding to treatment, but not all patients responded (Mathews-Roth *et al.* 1977; Mathews-Roth, 1986). However, it is possible that the relatively short time-period over which there has been an interest in β-carotene may have precluded the opportunity to examine a sufficient number and variety of patients to detect damage.

In contrast vitamin C, which also has antioxidant and pro-oxidant properties (Stadtman, 1991), is harmful in some circumstances (Herbert, 1993). Where it is harmful it is usually associated with potentially haemolytic conditions such as glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49; G-6-PD) deficiency (Cambell *et al.* 1975), paroxysmal nocturnal haemoglobinuria (Iwamoto *et al.* 1994) etc. In fact, vitamin C will protect healthy, intact erythrocytes exposed to H_2O_2 but will not protect those from patients with chronic haemolytic anaemia associated with G-6-PD deficiency (Lachant & Tanaka, 1986). The likelihood of haem-Fe or unbound Fe being present in a suspension of cells from patients with haemolytic anaemia is obviously high and would explain this observation, since it is also known that vitamin C is a potent reducing agent which will convert Fe^{3+} to Fe^{2+}; Fe^{2+} is capable of catalysing the formation of the hydroxyl radical ($OH^{\bullet}$; reaction 2, see p. 562). In fact, much more is known about vitamin C, as its metabolic functions have been under active investigation for a much longer time than β-carotene. For this reason I propose to concentrate my attention on PAA.

INFLUENCE OF DISEASE OR TRAUMA ON PLASMA ASCORBATE

In common with the effect of smoking on PAA (Pelletier, 1977), disease and trauma have also been reported to be associated with low PAA or LAA concentrations. Low PAA levels are a frequent observation in a wide variety of infections, acute and chronic diseases (Bingol *et al.* 1975; Olusi *et al.* 1979; Sinha *et al.* 1984; Yoshioka *et al.* 1984; Jennings *et al.* 1987; Riemersma *et al.* 1991). Low PAA is also found in the elderly (Thurnham, 1992) in whom constitutive disease is a frequent finding (Department of Health and Social Security, 1979; Joosten *et al.* 1992).

Why should PAA be low in persons with disease or who are exposed to free-radical stresses? Obviously in acute disease anorexia will play a role, and smoking may affect food preferences and appetite, but in chronic diseases it may not be so obvious why PAA should be reduced. For example, in diabetes, it is reported that blood levels are below those of control subjects consuming the same intake of vitamin C (Cunningham *et al.* 1991; Sinclair *et al.* 1991) and patients fail to respond to vitamin C supplements to the same extent as healthy control subjects (Sinclair *et al.* 1991).

In acute trauma such as that following a myocardial infarction, low PAA has also been described (Riemersma *et al.* 1994) but there is also a very characteristic behaviour of LAA levels. Hume & Weyers (1973) reported a 50% drop in LAA levels into the 'deficient' range following the onset of the common cold. Supplementing their volunteers with as much as 3 g vitamin C/d did not prevent the fall but delayed it from the first to the third day and LAA values did not drop into the 'at risk' range. However, with or without supplementation, LAA returned to pre-infection levels within 5 d.

The effects of surgery on LAA is similar to that of the common cold (Crandon *et al.* 1961; Irvin *et al.* 1978). LAA measurements are made on the buffy layer, which is the leucocyte-rich fraction found at the interface between the erythrocyte and the plasma when centrifuging blood. Irvin *et al.* (1978) recognized that the buffy layer is far from being an homogeneous sample and that there was a large increase in leucocytes following surgery. He confirmed the post-operative fall in LAA levels and reported that it was inversely related to the leucocytosis. He found no association with the severity of the surgical procedure and suggested that the changes in LAA concentrations may be a normal part of the metabolic response to trauma.

It is now generally recognized that 50% of the vitamin C in the buffy layer is in the leucocytes and the rest is in the platelets. Following trauma there is a leucocytosis, mainly due to an increase in granulocytes which are relatively low in vitamin C, and platelet numbers remain relatively steady. Leucocyte numbers revert to normal in 3–5 d and LAA levels are restored (Vallance, 1986). No-one appears to have asked the question where do the leucocytes obtain their extra vitamin C to restore LAA levels, although at least two groups of workers have shown that granulocytes can take up vitamin C from the surrounding solution *in vitro* (Evans *et al.* 1982; Moser & Weber, 1984).

VITAMIN C: AN ANTIOXIDANT OR PRO-OXIDANT IN DISEASE

Vitamin C is believed to be of fundamental importance as an antioxidant in tissues (Anderson & Lukey, 1987; Niki, 1991) and the evidence is very persuasive that it regenerates oxidized vitamin E to the active reduced form (Packer *et al.* 1979). The amount needed for this function is not known but if vitamin C can be regenerated

Table 2. *Features of trauma and infection which indicate a role for the acute-phase response in scavenging iron from the circulation and tissues**

Inflammatory changes and acute-phase response	Possible function or consequence
Increased vascular endothelial permeability	May facilitate movement of smaller proteins such as ferritin and transferrin to extracellular compartment to scavenge Fe in inflamed tissues
Lowered serum transferrin	Increased degradation of transferrin reduces Fe carried to haemopoietic tissues
Reduced transferrin saturation	Transferrin saturation initially unaltered but may fall in chronic disease
Raised serum ferritin	Increased levels are a very common feature of acute and chronic infection and may also be a feature of occult disease
Raised tissue Fe uptake	There is increased uptake of Fe principally by the liver, spleen and bone marrow particularly in chronic infection and trauma
Raised serum caeruloplasmin (*EC* 1.16.3.1)	Modest increases in many diseases. Ferroxidase activity may be of particular importance for conversion of Fe^{2+} to Fe^{3+}, the form of Fe taken up by transferrin and ferritin
Raised serum haptoglobin	Haptoglobin binds haemoglobin and is taken up by reticulo-endothelial cells of the liver. Level rapidly becomes undetectable in haemolytic conditions
Appearance of 'unbound' Fe	Normally undetectable but presence of 'bleomycin Fe' reported in malaria and rheumatoid arthritis
Leucocytosis	Raised leucocyte counts reported in chronic (cardiovascular disease, smokers) and acute (e.g. surgery) trauma. Increase in granulocytes may facilitate ascorbate uptake from plasma or destruction by superoxide
Low serum ascorbate levels	Low concentrations in both acute and chronic trauma may function to prevent conversion of Fe^{3+} to Fe^{2+}
Acute fall in leucocyte ascorbate (LAA) levels	Initial fall probably due to dilution by incoming granulocytes but LAA levels restored in 4–5 d. May remain low in chronic trauma

* Based on Koj (1985) and Thurnham (1990, 1994*b*).

following oxidation (Orringer & Roer, 1979) large circulating concentrations may not be necessary.

In contrast, vitamin C can release Fe from ferritin *in vitro* and stimulate lipid peroxidation (Gutteridge *et al.* 1983). The reducing properties of vitamin C are responsible for the conversion of Fe^{3+} to Fe^{2+} and this reaction is extremely important for the absorption of Fe (McLean Baird *et al.* 1974). However, within the tissues this process could be potentially harmful since Fe^{2+} is a potent free-radical catalyst with the potential to produce hydroxyl radicals ($OH^{\bullet}$; Stadtman, 1991). The ability of a mixture of Fe^{2+} salts and H_2O_2 to react with many organic molecules was first observed by Fenton in 1894 who gave his name to the following reaction:

$$Fe^{2+} + H_2O_2 = Fe^{3+} + OH^{\bullet} + OH^{-}. \qquad \text{(reaction 1)}$$

Fe, and Cu, will also catalyse *in vitro* the reaction of superoxide radicals and H_2O_2 producing $OH^{\bullet}$. In both cases the lower valency forms, Fe^{2+} and Cu^{+}, are the more

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active. This reaction was first described by Haber and Weiss in 1934 and has become known as the Haber–Weiss reaction. The net equation is shown below.

$$O_2^- + H_2O_2 \xrightarrow[\text{catalyst}]{\text{Fe salt}} O_2 + OH^\bullet + OH^- . \qquad \text{(reaction 2)}$$

However, in the absence of free transition metal ions, i.e. normal physiological conditions, this reaction is unlikely since within the blood and tissues, Fe is usually bound to transport, storage or tissue proteins and, therefore, the body is protected from potentially damaging reactions. A breakdown of tissue integrity, however, can occur under conditions of sustained stress, for example infection, inflammation, strenuous exercise or other trauma resulting in the biochemical and endocrine changes known as the acute-phase response (Koj, 1985; Thurnham, 1990; Moore *et al.* 1993). Such trauma causes tissue damage, potentially releasing Fe into the circulation, and free Fe has been detected in some disease states (Rowley *et al.* 1984; Buffinton *et al.* 1986). Furthermore, the administration of iron dextran to a patient with long-standing rheumatoid arthritis, exacerbated the synovitis and provided evidence of Fe-promoted oxidant stress and the accumulation of oxidized ascorbate in serum and synovial fluid following treatment (Winyard *et al.* 1987). In response to such trauma, there is a rapid increase in vascular endothelial permeability which may allow transferrin to pass more easily into the extracellular fluid and bind any free Fe. Subsequently, the concentration of serum transferrin is decreased as an acute-phase response possibly by increased catabolism (Aisen, 1984). This effectively reduces the supply of Fe from liver to tissues while ferritin and caeruloplasmin (*EC* 1.16.3.1) increase, to continue the scavenging of free Fe from plasma and extracellular fluids (Koj, 1985). It should be noted caeruloplasmin may counter the potential pro-oxidant effects of vitamin C, since both transferrin and ferritin take up Fe as Fe^{3+} and the ferroxidase activity of the raised caeruloplasmin levels in infection probably facilitates the conversion of Fe^{2+} to Fe^{3+} to assist with this uptake and removal of any free Fe from body fluids to storage (Thurnham, 1990) (Table 2).

MECHANISM BY WHICH PLASMA ASCORBATE IS REDUCED DURING TRAUMA AND SOME IMPLICATIONS

One of the common features of the acute-phase response which may be important in ascorbate metabolism is the increase in the leucocyte count (Sipe, 1985). Leucocyte counts are elevated in smokers compared with non-smokers (Anderson & Lukey, 1987; Gosling *et al.* 1990). Leucocytosis is a risk factor for cardiovascular disease (Yarnell *et al.* 1991) and such patients tend to have low blood ascorbate levels (Riemersma *et al.* 1991). Also, a negative correlation between ascorbate levels and total leucocytes has been reported by several workers (see Bates *et al.* 1979).

The drop in LAA concentrations accompanying trauma has been attributed to a dilution of existing leucocytes by incoming granulocytes which are low in vitamin C. Monocytes contain approximately five times the quantity of ascorbate of the incoming polymorphonuclear leucocytes (Jacob, 1990). The initial drop in LAA, however, is rectified in 4–5 d (Vallance, 1986) and presumably LAA are restored by uptake from the plasma (Evans *et al.* 1982; Moser & Weber, 1984), although some of the fall in PAA concentrations in disease may represent increased utilization of vitamin C to regenerate

vitamin E (Packer *et al.* 1979). In addition, some vitamin C may be destroyed by reaction with superoxide produced by polymorphonuclear leucocytes (Hemila *et al.* 1984).

Studies with isolated human granulocytes showed that ascorbate is actively accumulated, that stimulation of granulocytes increases the uptake and that the rate of uptake is temperature dependent, with a maximum at 40° (Evans *et al.* 1982; Moser & Weber, 1984). That is, fever and infection will promote ascorbate uptake by granulocytes. It has also been pointed out by others that monocytes can achieve intracellular concentrations of ascorbate in the millimolar range from plasma concentrations from 30 to 110 μmol/l (Evans *et al.* 1982).

The uptake of ascorbate by leucocytes from the surrounding plasma may be a purely fortuitous feature of their activity, however: (1) it lowers the concentration in the plasma of a potential pro-oxidant, (2) it removes from plasma and extracellular fluids a very good radical scavenger (Niki, 1991) which might compete with the radical defence being generated by polymorphonuclear neutrophils (PMN; Anderson & Lukey, 1987) and (3) the ascorbate taken up by PMN in no way impairs the radical production and may enhance it (Anderson & Lukey, 1987). Therefore, reductions in PAA and LAA in trauma may be a physiological response to lower the concentration of a substance which antagonizes both the natural Fe-scavenging processes accompanying infection and trauma and the radical-generating capacity of PMN.

IS THERE AN OPTIMAL PLASMA LEVEL OF VITAMIN C TO PROVIDE ANTIOXIDANT PROTECTION BUT LOW ENOUGH TO AVOID PRO-OXIDANT ACTIVITY?

A few years ago we carried out studies in The Gambia to investigate the plasma antioxidant status of children with malaria (Knowles *et al.* 1991). We reported that the plasma levels of ascorbate in children with mild or severe malaria were very little different from those of children with other mild or severe diseases or from those of nurse controls. In all groups, plasma ascorbate was uniformly very low in spite of the fact that all blood samples were centrifuged and preserved with metaphosphoric acid within 15 *min* of collecting the blood (Knowles *et al.* 1991; Table 3).

The study was done in the middle of the rainy season when there is a high incidence of malaria and other parasitic, bacterial and viral infections in The Gambia (Bates *et al.* 1983). The control group of nurses were young adults and because adults acquire

Table 3. *Plasma ascorbate concentrations (μmol/l) in Gambian children with malaria, other mild and severe diseases and young adult controls* (from Knowles *et al.* 1991)

		Malaria		Other disease		
	Cerebral	Severe	Mild	Severe	Mild	Controls
n . . .	31	24	21	45	18	44
Median	13·8[ab]	21·5[a]	11·6[ab]	11·1[b]	17·8[a]	14·3[ab]
Range	0–83·8	0·3–106·8	3·2–64·6	0–88·6	3·9–82·5	3·2–53·9
Percentage of values <11·4	39	33	43	56	28	41

[a,b] Values with different superscript letters were significantly different (Kruskall Wallis followed by Scheffe tests): $P<0·05$.

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immunity, adults do not frequently develop malaria; nevertheless, they are being bitten by mosquitoes and are closely exposed to the other infections in the environment. In such circumstances it would not be surprising for immune and acute-phase responses to be stimulated.

The rainy season has been reported in the past to be associated with low vitamin C status. Bates *et al.* (1982) reported that plasma vitamin C was at its lowest between the months of July and October. It is interesting, however, that when attempts were made to assess the functional vitamin C status of young adult men living in Keneba in The Gambia, no evidence of functional vitamin C deficiency could be detected (Powers, 1991).

Those studies were done by Dr Hilary Powers who devised what looked to be a very promising way of assessing functional vitamin C status (Powers, 1987). Vitamin C has been reported by many workers to influence the activity in the guinea-pig of the liver mixed-function oxidase system which confers the ability to handle xenobiotic drugs. To test the system she used methacetin which has a convenient methyl group that can be labelled. The liver demethylates this compound and the methyl group is excreted in the breath as CO_2. Experiments using [^{14}C]methacetin in guinea-pigs in various states of vitamin C depletion showed an excellent agreement between the level of $^{14}CO_2$ in the breath and plasma vitamin C (Powers, 1987). When, however, [^{13}C]methacetin was given to human volunteers before and after vitamin C supplementation there was no relationship between the level of $^{13}CO_2$ enrichment and the plasma vitamin C (Powers, 1991). However, Dr Powers' studies were done on Gambian men in the rainy season. Plasma levels of vitamin C were low at the outset, but they may have been low because of the high level of infection and other stresses in the environment at that time of the year. The authors suggested that either the dependence of methacetin on vitamin C status is less in man than that in the guinea-pig or that Gambian men are only exposed to a relatively-short-term dietary vitamin C withdrawal which was insufficient to affect functional status. They suggested that the study should be repeated on elderly subjects in the UK where vitamin C depletion may be more persistent; but PAA levels in the elderly may also be a reflection of the high level of constitutive disease found in this group (Joosten *et al.* 1992). However, if the study should be repeated on individuals who are known to be depleted in vitamin C by its experimental withdrawal from the diet, the previous arguments would suggest that the test might prove to be useful in man as well as the guinea-pig.

My own conclusion from this work is that the levels seen in Gambian men, and possibly also in UK elderly subjects, may reflect the PAA concentration which is optimal for providing antioxidant protection and avoiding pro-oxidant damage in subjects exposed to an elevated risk of disease, i.e. 11–20 μmol/l.

INCREASING PLASMA ASCORBATE LEVELS IN PERSONS EXPOSED TO TRAUMA

The difficulty in increasing PAA levels in Gambian subjects during the rainy season had in fact been reported earlier by Bates *et al.* (1982) who found that a supplement of 35 mg vitamin C had no effect on PAA in lactating and pregnant women. They subsequently found that about four times this amount (117 mg) was needed to influence circulating ascorbate levels (Bates *et al.* 1983) in lactating women which compares with the

recommended nutrient intake during lactation of 90 mg for Western women (Department of Health, 1991).

In smokers too, Kallner *et al.* (1981) showed that approximately 30 mg more dietary ascorbate was needed to match PAA levels in smokers with those in non-smokers, as the distribution of ascorbate in smokers between the tissues and the plasma tended in the direction of the tissues. Likewise in diabetes, patients show a lower response to dietary supplements than do controls (Sinclair *et al.* 1991).

Thus, in all these situations, namely, the presence of disease, high exposure to disease and smoking, it is more difficult to raise PAA primarily because the equilibrium of the tissue–PAA distribution would appear to be shifted in the direction of the tissues. Also, it has not been possible to demonstrate evidence of ascorbate deficiency.

IS PRO-OXIDANT ACTIVITY OF β-CAROTENE OF PHYSIOLOGICAL IMPORTANCE?

In contrast to vitamin C, there is very little evidence in the literature to suggest that β-carotene may be handled in the same way as ascorbate in the presence of trauma. In malaria, which is the only disease I have studied personally, levels of β-carotene were lower in patients when compared with matched controls (Thurnham *et al.* 1991), but I could see no evidence of any increase in carotenes in convalescent patients (Thurnham *et al.* 1990). However, values for plasma β-carotene are influenced by dietary intake and such effects may override fluctuations due to trauma, making it difficult in the absence of dietary information to assess plasma values properly. Furthermore, there is much more variation in the bioavailability of carotenes from food than there is with vitamin C and it is possible that carotene levels may be controlled at the level of absorption.

Likewise, we still know little about the relationship between tissue levels of carotenes and the concentrations in the circulation. One recent paper in this area reported that carotenes are present in atherosclerotic plaques in both man and rabbits and that intravenously administered β-carotene is rapidly taken up by the plaque but not by normal vessel wall in rabbits (Mitchell *et al.* 1993). Currently, little is known of the way in which carotenoids penetrate plaque or any other tissue and it will be interesting to establish what mechanism is involved.

The absence of information on control of plasma carotene concentrations does not mean that control does not exist, but it shows that nobody has yet looked to see whether control mechanisms exist. Some control of carotene levels would definitely appear to exist, in view of the consistent sex differences observed in very different population groups. However, the ability to separate and measure carotenes by HPLC is a relatively recent technique, and our experience both in Europe and America in ring tests has shown that there are still considerable problems with methodology. As these problems are overcome no doubt more reliable information will emerge and interesting metabolic features will be demonstrated.

CONCLUDING REMARKS

Falls in serum micronutrient concentrations associated with the acute-phase response, particularly of vitamin C, may benefit host tissues against endogenous and exogenous stresses by removing a potential pro-oxidant. In these situations a 'low normal'

concentration of a circulating nutrient may be adequate or even optimal for antioxidant requirements. Thus, low concentrations of PAA may be adequate to retain the antioxidant properties of the plasma since a mechanism for regeneration of reduced ascorbate has been demonstrated (Orringer & Roer, 1979). No such mechanism for regeneration of any carotene has yet been demonstrated but likewise no oxidized form of the carotenes has yet been demonstrated to exist in tissue. The pro-oxidant functions of carotenes may have very different consequences from those of vitamin C.

Finally, it should be said that during the acute-phase response the host may compromise its own requirements to attain a short-term advantage over a suboptimal situation. For the host, this may not remain an indefinite advantage. Thus, in chronic disease nutritional support may be desirable, but it should not be necessarily assumed that this is the case.

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