CHAPTER 5

Recommended Daily Amounts and Biochemical Roles—The Vitamin C, Carnitine, Fatigue Relationship

R. E. HUGHES

University of Wales Institute of Science and Technology, Cardiff, UK

Nutritional thought has developed essentially in terms of deficiency diseases and their prevention. This approach has proved to be a useful analytical tool in revealing the biochemical role of certain essential dietary factors. Furthermore, the prevention of definable deficiency diseases offered a quantifiable, albeit negative, method of defining the recommended daily intake of a nutrient.

But the amount of a nutrient necessary to prevent overt ill health is not necessarily to be equated with the amount consistent with the attainment of optimum health (itself a difficult-to-define state). This is particularly true if nutrients have adjuvant or supportive roles in addition to their functions as essential dietary factors.

Even non-essential nutrients may be of definable nutritional significance. It is now generally accepted that dietary fibre and polyunsaturated fat are of probable dietary significance although no clearly definable deficiency disease can be attributed to their absence from the diet. Current thought is slowly forcing us to replace the traditional simplistic approach, structured around the prevention of deficiency diseases, by the concept of dietary optimisation.

So too in the case of vitamin C (ascorbic acid): the current Recommended Daily Amount (RDA) for the UK is quite clearly based on the amount of the vitamin necessary to prevent the emergence of 'overt' clinical scurvy, as traditionally described:

> 'In the United Kingdom recommendations for ascorbic acid are still based on an amount sufficient to prevent signs of deficiency, with an

> added safety margin, in contrast to other countries where tissue saturation is advocated. The MRC report recommended 30 mg as including a reasonable safety factor for adults. The available data provide no reason to alter this recommendation.' [1].

The 'safety margin' was introduced to compensate for individual variations in requirement and activity and not to accommodate any supposed secondary roles of the vitamin [2]. The 'signs of deficiency' referred to were apparently those associated with classical scurvy:

> 'The first changes were enlargement and keratosis of the hair follicles, beginning after 17 weeks of deprivation. Later the enlarged hair follicles became haemorrhagic and formed the characteristic scorbutic spots. Scorbutic gum changes began to appear after 26 weeks of deprivation.' [2].

The current RDA for vitamin C is thus based upon a single experiment completed some 25 years previously. The thesis presented in this paper is that recent biochemical and nutritional studies suggest that a re-appraisal of the RDA for vitamin C would be not inappropriate.

Many, if not all, of the overt signs of classical scurvy (petechiae, gum deterioration, poor wound healing) are, in the final analysis, theoretically attributable to an impaired mediation of the vitamin in the hydroxylation of collagen prolyl-lysyl residues, now regarded as its primary biochemical role [3,4]. There is, however, accumulating evidence that in certain reasonably clearly definable areas ascorbic acid has metabolic involvements other than the prevention of 'classical' scurvy.

These involvements were previously described as 'extra-antiscorbutic' functions [5,6]—the implication being that they may exist independently of the main role of ascorbic acid in the prevention of scurvy—although one would be forced to admit that any such arbitrary division of function would perhaps be more a matter of semantics than nutrition. Although not necessarily a part of the vitamin's obligatory function, such adjuvant activities could nevertheless be of considerable nutritional significance.

There are five fairly clearly definable areas where there is evidence that ascorbic acid may have an adjuvant or supportive role in metabolism, namely

1. carnitine metabolism
2. cholesterol metabolism
3. detoxication
4. cerebral biochemistry
5. infection

It will be noted that in the first three the role of ascorbic acid is, in essence, in a hydroxylation system, not completely dissimilar, perhaps, from its established role in the biosynthesis of collagen. These have been dealt with elsewhere together with other less clearly defined relationships (e.g. iron metabolism, tumour growth) [7] and other chapters in this book will be describing some of the specific areas in detail. At this stage, perhaps two general comments about these supposed 'extra-antiscorbutic' functions may be made

(i) there is increasing evidence that the involvement of ascorbic acid in these 'extra-antiscorbutic' functions requires amounts greater than those required to prevent the emergence of clinical scurvy
(ii) (which follows from (i)) an involvement of ascorbic acid in one or more of these areas would relate to dietary optimisation but not necessarily to the emergence of clinical scurvy

This paper will discuss evidence for the first of the putative extra-antiscorbutic roles—the involvement in carnitine metabolism and its relationship to physical fatigue. This relationship will be used to illustrate the concept of dietary optimisation and the necessary consequences in terms of a Recommended Daily Amount. It will be suggested that inadequate intakes of ascorbic acid may result in the emergence of fatigue and that this could be a more sensitive index of ascorbic acid deficiency than the currently accepted clinical signs.

There are three types of evidence that relate to the carnitine-ascorbic acid-fatigue relationship: (1) historical, (2) experimental, (3) biochemical.

HISTORICAL EVIDENCE

References to the early emergence of lassitude and fatigue in scurvy were a common feature of the earliest descriptions of the disease. Eugalenus, in 1658, spoke of 'spontaneous debility' [8] and Lister in 1696 of 'weakness of the limbs and considerable fatigue' [9]. Sydenham in 1742 wrote The scurvy is accompanied with (1) spontaneous lassitude (2) heaviness (3) difficulty of breathing especially after exercise' [10]. Medical writings of this period, although often containing a substratum of observational truth, were nevertheless frequently lacking in originality. There can be little doubt that in many cases the descriptions of diseases were unacknowledged borrowings from previous writers. Thus the phrase 'debility, lassitude and difficulty of breathing especially after exercise' used to describe scurvy by

394 OF THE SCURVY.

ſome food; or any diſeaſe which greatly weakens the body, or vitiates the humours.

SYMPTOMS.——This diſeaſe may be known by unuſual wearineſs, heavineſs, and difficulty of breathing, eſpecially after motion; rottenneſs of the gums, which are apt to bleed on the ſlighteſt touch; a ſtinking breath; frequent bleeding at the noſe; crackling of the joints; difficulty of walking; ſometimes a ſwelling and ſometimes a falling away of the legs, on which there are livid, yellow, or violet-coloured ſpots; the face is generally of a pale or leaden colour. As the diſeaſe advances, other ſymptoms come on; as rottenneſs of the teeth, hæmorrhages, or diſcharges of blood from different parts of the body, foul obſtinate ulcers, pains in various parts, eſpecially about the breaſt, dry ſcaly eruptions all over the body, &c. At laſt a waſting or hectic fever comes on, and the miſerable patient is often carried off by a dyſentery, a diarrhœa, a dropſy, the palſy, fainting fits, or a mortification of ſome of the bowels.

Fig. 1. Description of scurvy from Buchan's *Domestic Medicine* 12th edition, 1791 [13].

such diverse authors as Boerhaave in 1738, Home in 1770, Buchan in 1791, and Price and Griffiths in 1849 is of some historical interest but is probably an example of uncritical borrowing of this type [11, 12, 13, 14].

Of somewhat greater significance are the descriptions given by persons who, we have reason to believe, had considerable first-hand experience of scurvy and whose reports were to a great degree based on their own observations. The signes of the Scurvie are many, as namely, a general lazinesse and evil disposition of all the faculties ... shortnesse and difficultie of breathing, especially when they moove themselves...' wrote John Woodall in 1639 [15]. Lind's remarks are particularly revealing 'The first indication of the approach of this disease is... a pale and bloated complexion; with a listlessness to action or an aversion to any sort of exercise ... [which]... degenerates soon into a universal lassitude ... much fatigue and upon that occasion subject to a breathlessness or panting. And this lassitude, with a breathlessness upon motion, are observed to be among the most constant concomitants of the distemper' [16].

Anson, in his voyage around the world (1740-1744) lost some hundreds of men because of scurvy. The official account of the voyage contains the following description of the disease: These common appearances are large discoloured spots dispersed over the whole surface of the body, swelled legs,

putrid gums, and above all, an extraordinary lassitude of the whole body, especially after any exercise, however inconsiderable; and this lassitude at last degenerates into a proneness of swoon on the least exertion of strength, or even on the least motion' [17].

In 1840, George Budd, a careful clinical observer and a pioneer in the formulation of the concept of dietary deficiency diseases, contributed an article 'Scurvy' to Tweedie's *The Library of Medicine* [18]. Budd wrote ' .. one of the earliest indications of scurvy... is a great langour and despondency, and with aversion to every kind of exercise, and the patient is readily fatigued .. .' [19].

Shapter, another careful clinical observer of the same period, in describing an outbreak of scurvy in Exeter in 1847, perhaps put the matter most clearly:

> "... the spongy and swollen gum appears to me to have been erroneously estimated as amongst the primary and most obvious manifestations of the scurvy... I am inclined to say there is a class of well marked symptoms preceding this peculiar indication of the disease ... The first or initiatory stage, and one which may continue for a considerable period, has appeared to me to be characterised by the general and usual indications of debility ... there is complaint of the ordinary feelings of weakness, of listlessness and a disinclination to exercise ...' [20].

There can be little doubt now that members of Scott's ill-fated South Polar expedition had inadequate intakes of ascorbic acid. Their journey overland to the Pole and back took some 19 weeks and it would appear that during this period their ascorbic acid intake was far from adequate. Scott's diary indicates that fatigue and lack of strength were features of the journey; his entry for Monday, January 22, 1912 (after 12 weeks on a virtually ascorbic-acid-free diet) is revealing ' .. I thought we were climbing today, but the barometer gives no change' [21].

One of the Welsh words for scurvy is 'llwg', derived apparently from an earlier form llwyg' [22,23]. It is perhaps not without significance that the corresponding verb llwygaw' is translated in a dictionary of 1688 as 'to fail or be weary' [24].

EXPERIMENTAL SCURVY

The historical evidence is strengthened by descriptions of dietary-induced scurvy in known subjects. William Stark kept detailed notes of dietary

experiments in which he induced in himself a state of scurvy from which he ultimately died. His reference to physical fatigue as a concomitant feature of his condition are, however, few. On September 8, 1769 he reported that he was 'so weak ... that I almost fainted in walking across my room' and on February 15, 1770—a week before he died—he described his condition as 'listless, and was forced, because of the feebleness, to spend most of my time in bed' [25]. It should be realised, however, that the apparent aim of Stark's experiments was to demonstrate that life could be maintained on very simple diets; perhaps, because of this, he had subconsciously conditioned himself to ignore early signs of fatigue and physical weakness.

Of somewhat greater significance are those cases where intentionally induced scurvy has been studied scientifically. In 1936 van Eekelen described ascorbic acid deficiency induced in a male volunteer. He remarked 'It appears noteworthy that at the end of the experiment (after 84 days) no other symptoms of vitamin C deficiency than fatigue and irritability could be observed' [26]. A similar finding was reported in 1940 by Crandon *et al.* [27] in their paper on experimentally induced scurvy in a male adult; they reported that a feeling of fatigue developed from the beginning of the third month of deficiency—a full 6-8 weeks before the emergence of the traditional 'overt' signs such as perifollicular hyperkeratotic papules, petechiae, poor wound healing and softening of the gums. After prolonged deficiency there was a measurable reduction in the ability to perform aerobic work as evidenced by a reduced capacity to run at seven miles an hour [27].

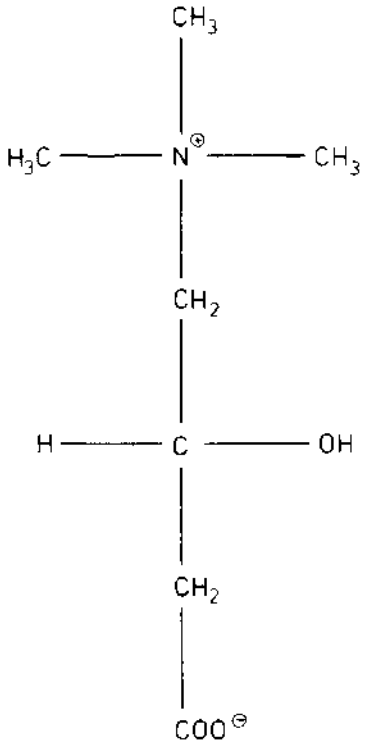
In a more recent study it was found that a group of five subjects 'manifested ... signs and symptoms of fatigue' after only 84-97 days on an ascorbic-acid-deficient diet [28]. Unfortunately, in the otherwise 'definitive' 'Sheffield experiment' little attention was paid to the onset of fatigue; the exercise tolerance tests were poorly executed and it was impossible to analyse the results in any meaningful way [2].

BIOCHEMICAL ASPECTS

Carnitine and muscle metabolism

Recent studies of carnitine metabolism provide a possible biochemical explanation for the early emergence of physical fatigue in scurvy.

Carnitine (β-hydroxy-γ-(trimethylamino)butyric acid (see Fig. 2), sometimes referred to as vitamin B_T) is found for the most part in animal tissues [29]. It has an important role in the transport of fatty acids into the



CARNITINE

Fig. 2. Carnitine (β-hydroxy-γ-(trimethylamino)butyric acid).

mitochondria (Fig. 3) where they may be oxidised to provide energy [30]: skeletal muscle is greatly dependent on fatty acid oxidation for its energy requirements [31,32]. Carnitine is probably synthesised in the liver and stored primarily in the skeletal muscle; its pattern of distribution between tissues would appear to reflect the tissue utilisation of fatty acids as energy sources [29,33]. By modifying the availability of fatty acids for energy

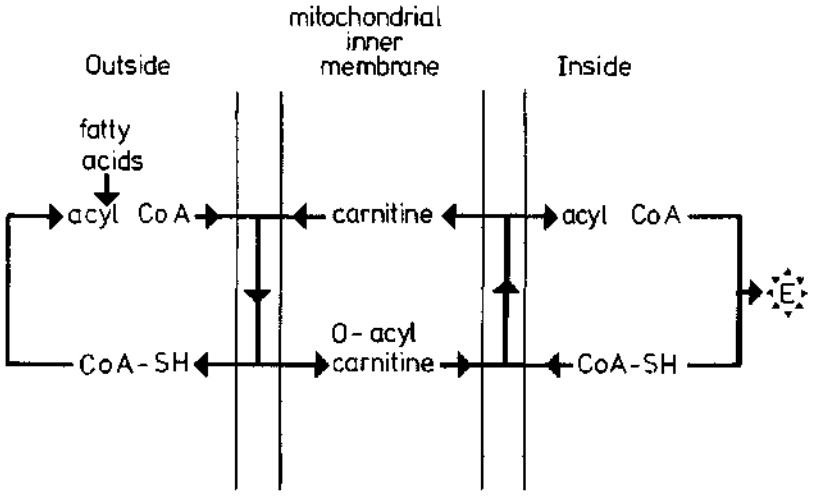


Fig. 3. Role of carnitine as a carrier molecule in the transport of fatty acids into the mitochondria.

production in muscle, carnitine may thereby influence the capacity of the muscle to maintain sustained contraction.

Endogenous production from lysine and methionine probably accounts for the bulk of the carnitine in the body. It has been estimated that in rats ingesting 113μg daily from dietary sources, biosynthesis accounted for a further 486 μg daily [33]. It would appear that a similar relationship exists in man [34].

Mitchell [35] has summarised the salient features of documented cases of systemic carnitine deficiency—a condition where carnitine cannot be produced and/or utilised satisfactorily in the body and where the intake from dietary sources is inadequate to compensate for the deficiency. Muscle weakness and fatigue—particularly after exercise—are invariable features of such cases; some of the case descriptions bear a remarkable resemblance to the prescorbutic state as described by the eighteenth century commentators [34].

Carnitine and ascorbic acid

Recent studies have established the probable nature of the pathway for the biosynthesis of carnitine from lysine and methionine. In the rat liver mitochondria system ascorbic acid has an important role as a co-factor at two hydroxylation points in the biosynthesis—in the conversion of ε-*N*-trimethyl lysine to β-hydroxy-ε-*N*-trimethyl lysine and in the final stage when γ-butyrobetaine is converted to carnitine [36, 37] (see Fig. 4). Lack of dietary ascorbic acid could, therefore, theoretically, reduce the rate of formation of endogenous carnitine.

Our studies have indicated that in guinea pigs such a relationship does in

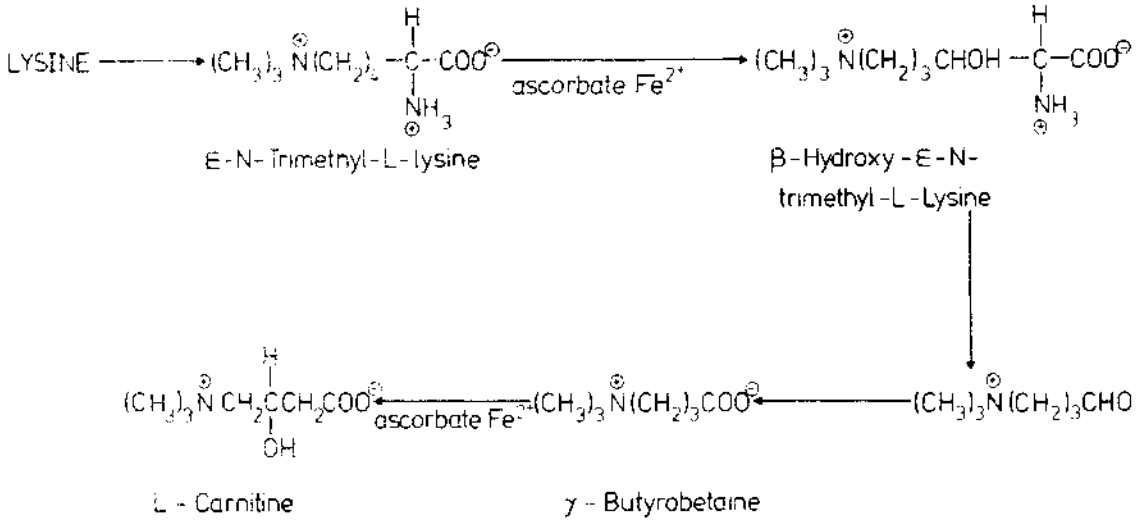


Fig. 4. Simplified scheme showing involvement of ascorbic acid in the conversion of dietary lysine to carnitine (based on Refs. [36] and [37]).

fact exist. By dietary means we produced tissue ascorbic acid concentrations of 12 % and 100 % saturation respectively in two groups of male guinea pigs. In the 'low ascorbic acid' group the mean concentration of skeletal muscle carnitine after 20 days was 0.5 μg/g tissue and in the 'ascorbic-acid-sufficient' group it was l.15 μg/g tissue. There was no concomitant emergence during this period of any of the symptoms customarily regarded as presaging the emergence of scurvy in guinea pigs—such as growth depression and kidney hypertrophy [38] (Table 1).

In a further study it was shown that administration of carnitine (10 mg per animal daily) prolonged significantly the life span of male guinea pigs given a scorbutogenic diet (Fig. 5). This could imply that carnitine may replace ascorbic acid in certain of its functions—a biochemically unlikely explanation. It is more likely that carnitine prolongs the life span by significantly 'sparing' ascorbic acid which would otherwise be used in the formation of endogenous carnitine.

It would therefore appear that the involvement of ascorbic acid in carnitine biosynthesis is a nutritionally significant happening and that muscle carnitine is a highly sensitive indicator of ascorbic acid status. The situation may be compared with human experimental scurvy (see above)

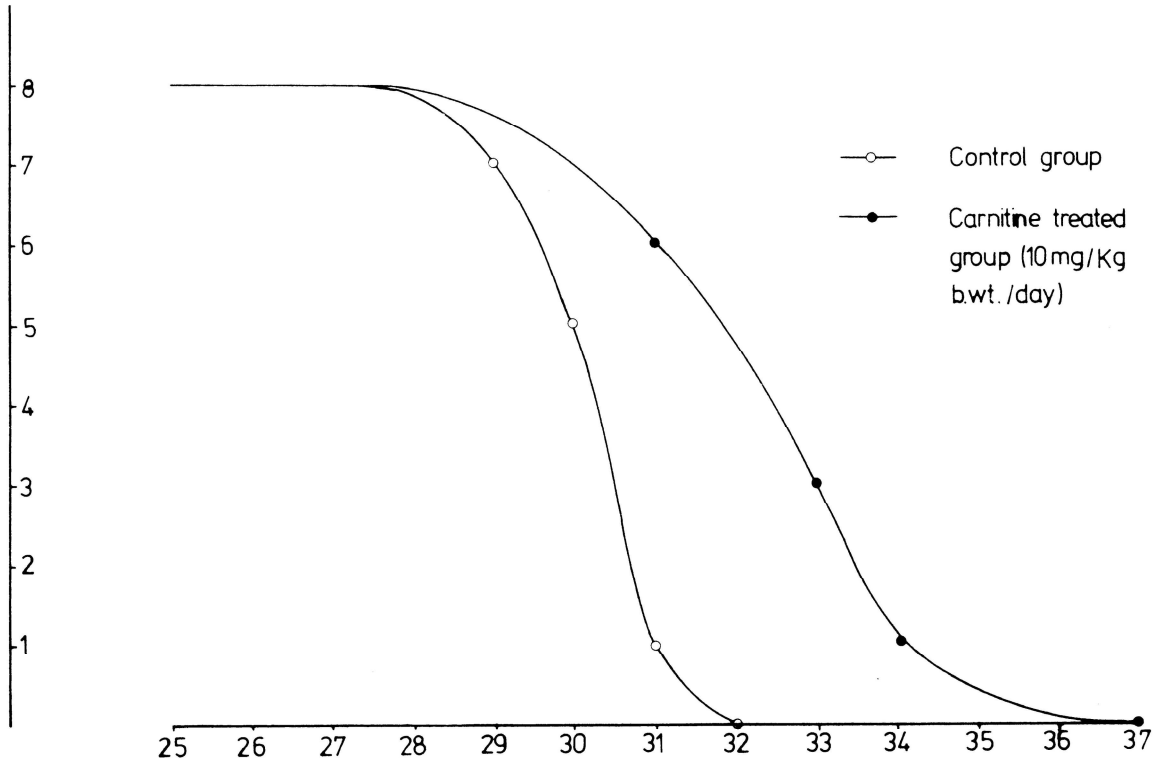


Fig. 5. Effect of a daily carnitine supplement on the survival of guinea pigs given a scorbutogenic diet. (Ordinate, number of animals surviving; abscissa, days on deficient diet.) The difference between the mean survival times for the two groups was statistically significant, $p < 0{\cdot}01$. (Jones, E. and Hughes, R. E., unpublished results.)

Table 1: Ascorbic acid and muscle carnitine in guinea pigs after different dietary intakes of ascorbic acid for 28 days. Mean values with their standard errors for eight animals/groups

Ascorbic acid intake	*Body weight (g)*		*Kidney weight (g/100 g body weight)*	*Ascorbic acid (mg/100 g tissue)*		*Skeletal muscle carnitine† (μg/g fresh tissue)*
	Initial	*Final*		*Liver†*	*Adrenals†*	
2·5 mg/kg body weight	342 ± 5	517 ± 20	0·69 ± 0·20	5·02 ± 0·35	20·3 ± 0·20	0·59 ± 0·10
10 g/litre drinking water	339 ± 6	531 ± 14	0·70 ± 0·21	31·21 ± 0·76	203·2 ± 0·74	1·15 ± 0·11

† Differences between means statistically significant, $p < 0{\cdot}01$ (from Hughes *et al.* [38]).

where fatigue and lassitude emerge well before the more traditional 'overt' signs. Of possible relevance is a report of a reduced incidence of fatigue amongst women with above-average intakes of ascorbic acid [39].

SIGNIFICANCE

It is suggested that the findings collated in this paper provide presumptive evidence of a biochemical involvement of ascorbic acid requiring tissue concentrations in excess of those necessary for the prevention of'classical' scurvy. The implications of this are interesting.

Carnitine deficiency is characterised by an accumulation of triglyceride material in the blood: it would be of interest to learn whether impaired carnitine biosynthesis has a causative role in the possible emergence of hyperlipidaemia in hypovitaminosis C [40].

A recent study has indicated that in rats cardiac muscle is more susceptible to carnitine deficiency than is skeletal muscle. An adequate ascorbic acid intake could therefore be of especial significance for patients whose cardiac performance may be under stress [41].

It is conceivable that large sectors of the population, although ingesting officially acceptable intakes of ascorbic acid, are nevertheless in a state of chronic fatigue because of sub-optimum biosynthesis of carnitine. A reassessment of the recommended daily allowance for ascorbic acid *vis-a-vis* tissue carnitine requirements would not be inappropriate. Certainly a study in depth of the relationships between carnitine, ascorbic acid and physical fatigue or weakness in vulnerable sectors of the population (such as the institutionalised elderly) would be a useful exercise.

Some vegetarians and others whose intake of meat and fish (the main dietary sources of carnitine) is restricted could be particularly disadvan-

taged in this respect, as they are virtually completely dependent upon endogenously produced carnitine [35]. In such cases it would be doubly important to ensure that endogenous formation of carnitine was not limited by an inadequate intake of ascorbic acid. In practice, the simplest answer would be to raise the recommended daily allowance for ascorbic acid to a level commensurate with the maximum rate of carnitine biosynthesis. Measurements of urinary carnitine *vis-a-vis* dietary ascorbic acid intakes in subjects receiving a lysine/methionine-sufficient diet could provide useful guidelines for the definition of any revised recommended daily allowance.

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VITAMIN C
(Ascorbic Acid)

Edited by

J. N. COUNSELL
Technology Centre, Roche Products Limited, Dunstable, UK

and

D. H. HORNIG
Department of Vitamin and Nutrition Research, F. Hoffmann La Roche & Co. AG, Basle, Switzerland

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