5–10% of patients admitted to geriatric departments remain in long-term hospital care (Andrews 1976, unpublished) such an investigation would be relatively easy to perform. It would be of considerable practical value in elucidating the true significance of low vitamin C white-cell levels found in the institutionalized.

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Nonscorbutic Effects of Vitamin C: Biochemical Aspects

The Ascorbic Acid Molecule

The role of vitamin C (L-xyloascorbic acid, ascorbic acid, AA) in correcting the biochemical lesion of scurvy is reasonably well established. It is required for the proper hydroxylation of collagen lysine and proline; without a sufficiency of AA, collagen formation is impaired (Barnes & Kodicek 1972). Defective formation of collagen could, in the final analysis, account for most – but possibly not all – of the characteristics of classical scurvy.

A collagen-like amino-acid sequence is a characteristic of at least two other structures in the body, namely the $C1_q$ subcomponent of complement (Reid 1974) and the basement membrane (Kefalides 1973). It is conceivable therefore that at least some of the so-called 'extra-antiscorbutic' involvements of AA will prove, in the final analysis, to be explicable in terms of a mechanism not entirely unrelated to the currently accepted mode of action of AA in preventing classical scurvy.

AA is not biologically specific in this respect. Recent studies have shown that iso-AA (D-araboascorbic acid) has an equivalent capacity to prevent scurvy; earlier claims that it possessed only a fraction of the antiscorbutic potency of AA were based on studies where a satisfactory level of iso-AA had not been attained in the tissues (Hughes 1973). AA, after oxidation to dehydroascorbic acid, crosses biological membranes with facility, and most tissues contain a glutathione-based system by which dehydroascorbic acid is reduced back to AA (Hughes 1964, Grimble & Hughes 1967). These mechanisms ensure that ingested AA is quickly absorbed and enters the tissues. In biochemical terms, the AA molecule possesses considerable biochemical versatility (Lewin 1974); theoretically, its properties as a biological reductant could be of significance in maintaining the integrity of tissue thiol (—SH) groups, thereby modifying a whole range of biochemical happenings. In terms of availability, distribution and properties the AA molecule is therefore endowed with considerable potential for involvement in areas other than the simple prevention of scurvy.

Extra-antiscorbutic Involvements

There are indications that the prevention of scurvy – as traditionally defined – is not the only involvement of AA in the mammalian body. Tetrahydrofolic acid, which has 43% of the activity of AA in

promoting protocollagen proline hydroxylase activity, is ineffective in prolonging the life-span of scorbutic guinea-pigs (Davies *et al.* 1976). Evidence of this type, together with other experimental and clinical reports, could be interpreted as indicating that AA has extra-antiscorbutic functions. Its possible involvement in cerebral metabolism has been mentioned by Dr Andrews (p 18). Four other areas where a possible influence of AA is indicated are cholesterol metabolism, detoxication, longevity, and resistance to infection.

Cholesterol metabolism: Guinea-pig studies have produced some evidence of a cholesterol-AA relationship. Chronic hypovitaminosis C resulted in raised tissue levels of cholesterol; loading guinea-pigs with dietary cholesterol increased the excretion of AA metabolites; and tissue saturation with AA protected guinea-pigs against the toxic effects of high dietary cholesterol (Ginter 1974, 1975; Hughes 1976, 1977). These findings are all consistent with Ginter's proposition that AA is involved in the conversion of cholesterol to cholic acid – possibly in one or more of the hydroxylations involved in the conversion.

In man the position is less clearly defined – in part, perhaps, because of theoretical difficulties in relating serum cholesterol changes to tissue concentrations. There have been conflicting claims about the ability of AA (particularly AA megadoses) to influence serum cholesterol. Ginter's suggestion that the current emphasis should, in this context, be on the prevention of chronic hypovitaminosis C (latent scurvy) is probably more meaningful than claims that AA megadoses can lower serum cholesterol in AA-sufficient subjects.

Detoxication: There is evidence that elevated levels of AA can protect experimental animals against toxic substances; the formation of bladder tumours by 3-hydroxyanthranilic acid and the hepatotoxic effect of sodium nitrite plus aminopyrene are areas where it is claimed that AA has a protective influence (Schlegel *et al.* 1970, Edgar 1974). Of possible significance is the finding that factors central to certain detoxication systems – such as the cytochrome-P-450 component of the microsomal hydroxylation system – show a reduced activity in hypovitaminosis C (Degkwitz & Staudinger 1974, Fielding & Hughes 1975).

Longevity: There is evidence of a negative correlation between tissue AA levels and age; whether this is causally related to the ageing process *per se* is not known. Pauling has claimed that AA megatherapy could increase his life span by 4–6 years (Pauling 1970, 1974); this, he implies, would result from a reduction in body 'wear and tear' (such as a reduction of the need constantly to reabsorb tubular AA in order to conserve it) rather than from any direct influence on the ageing process itself. Our knowledge of the ageing process is too incomplete to allow us entirely to discount arguments of this type. Nutritional factors, such as the level of dietary protein, are known to influence the life-span of experimental animals and it may well be that AA could, in a like manner, modify the somatic expression of genetically determined change of the type implied in an 'error-proneness' theory (Burnet 1974). Damage to membranous cell structures by lipid peroxidation would appear to be an important deteriorative expression of cellular ageing, and it is conceivable that AA, either directly or perhaps by modifying tissue thiol groups, could retard changes of this type (Tappel *et al.* 1973).

Resistance to infection: The advocacy of the use of AA megadoses in the prevention or treatment of the common cold is well known (Pauling 1970, 1974). To date some fifteen or so trials have been designed to examine this and similar claims. Critical assessments of the results have provided little support for these claims (Berry & Darke 1968, Dykes & Meier 1975). In the surveys where AA had a beneficial effect the difference between the control and supplemented groups rarely attained a high order of significance. In a recent survey in southern Wales involving 450 subjects in the 18–25 age group, daily supplementation with 80 mg AA produced a just significant ($P=0.05$) reduction in the total number of symptoms recorded (Baird *et al.* unpublished). It would appear that no matter how perfectly one structures a survey of this type the results are unlikely to approach an all-or-none type of response. A number of factors could contribute to this situation: (1) AA could be effective against only a small proportion of the viruses involved in the common cold; (2) a genetic factor(s) could establish a biochemical individuality in terms of any infection-AA relationship; (3) the relationship could be of a secondary or 'derived' nature involving one or more of the AA metabolites – about which very little is known – or a quite separate 'mediator' molecule. Lewin's suggestion that AA may influence the activity of cyclic AMP or cyclic GMP is an example of a possible non-direct influence (Lewin 1974).

Tissue Saturation

Extra-antiscorbutic functions of this type could well require tissue AA concentrations in excess of those necessary to provide protection against scurvy. In such circumstances tissue saturation with AA could well be a physiologically desirable state. It is less certain, however, that megadoses of the order of those implicit in the Stone-Pauling

hypothesis are required to achieve tissue saturation (Pauling 1970, 1974; Stone 1972; Hughes 1977). Studies with labelled AA indicated that only a small proportion of an administered megadose was incorporated in the body pool of AA (Hodges *et al.* 1971). More recently, it has been shown that in adult females a daily supplement of 100 mg AA produced essentially the same concentration of leucocyte AA as a daily megadose of 1 g AA (Baird *et al.* unpublished).

Biochemical Implications of Hypervitaminosis C

Not only are AA megadoses unnecessary to achieve tissue saturation; in certain directions there are indications of possible physiological disadvantages associated with dietary hypervitaminosis C. There have been frequent references to these in the medical press (e.g. Briggs 1974). Three of the more plausible possibilities merit comment.

(1) *Increased susceptibility to hypovitaminosis C on cessation of AA megatherapy:* There is some evidence that in man an abrupt change from AA megatherapy to RDA levels may precipitate AA deficiency (Rhead & Schrauzer 1971). The assumption is that the catabolism of AA has become geared to the rapid rate of breakdown necessary to accommodate the high tissue levels produced by megatherapy. Studies with guinea-pigs have given conflicting results (Hughes 1977) Cochrane, in discussing systemic conditioning of this type, has suggested that infantile scurvy could be a consequence of exposure *in utero* to maternal megatherapy (Cochrane 1965).

(2) *Increased formation and excretion of AA metabolites:* The formation of oxalic acid from AA, possibly with adverse consequences in the urinary tract, has been commented on in the scientific press. On balance, however, it would appear that fairly substantial intakes of AA (4–9 g daily) are necessary to produce any significant change in the urinary oxalic acid (Hughes 1977). Briggs has recently shown that a small proportion of a population produce greatly increased amounts of urinary oxalic acid after the ingestion of 4 g AA for 7 days, and that the condition is apparently familial (Briggs 1976). The existence of AA-induced hyperoxaluria of this order should presumably be regarded as a contraindication for AA megatherapy – assuming, of course, that the oxalic acid is not being produced in place of other, more toxic, metabolites.

Little is known about the formation, excretion and physiological significance of other AA metabolites such as diketogulonic acid, lyxonic acid, xylonic acid and possibly others. Yet considerable amounts of these substances are ingested daily in foodstuffs treated with AA during processing; presumably they are also formed endogenously in the tissues of AA those receiving megatherapy. There are recent indications that AA metabolites have mutagenic properties (Stick *et al.* 1976); a detailed characterization of their formation *vis-à-vis* food processing and AA megatherapy would appear to be long overdue.

(3) *Enhancement of metal toxicity:* There are indications that AA may enhance the uptake of certain metals from the gastrointestinal tract (Hughes 1974). In the case of iron such a relationship is regarded as a beneficial one; but an AA-enhanced absorption of toxic metals would fall into quite a different category. Recent work has indicated that AA megadoses given to guinea-pigs exposed to dietary mercury doubled the deposition of mercury in the tissues and halved the survival time of the guinea-pigs (Blackstone *et al.* 1974, Murray & Hughes 1976). Exposure to high concentrations of metal pollutants should perhaps be regarded as a contraindication for AA megatherapy.

Summary

There is certain presumptive evidence for believing that AA has functions other than the simple prevention of classical scurvy; whether these extra-antiscorbutic functions are attributable to AA itself, or to one or more of its metabolites, is not known. Tissue saturation with AA would appear to provide a good insurance against defects in these extra-antiscorbutic areas. Tissue saturation is attainable by a daily intake of 100–150 mg in man; there are no compelling reasons for using megadoses of AA and the emphasis should be on the avoidance of chronic hypovitaminosis C. There is suggestive evidence that megadoses of AA could be physiologically disadvantageous – particularly with regard to *in-utero* exposure and in persons exposed to high environmental levels of toxic metals.

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Current Status of Vitamin D Metabolism

In the past decade major advances have been made in our understanding of the biochemistry of vitamin D. The information gained has greatly enhanced our understanding of many diseases associated with abnormalities of calcium homeostasis.

Biochemistry

Biologically active forms of vitamin D were thought for many years to be obtained by endogenous skin synthesis and from the diet. The main vitamin D-rich foods were eggs, fish, margarine and cod-liver oil (McCance & Widdowson 1960). However, two important pieces of research stimulated the search for more biologically active metabolites of vitamin D. These were the observation of a time lag between the administration of vitamin D and the resulting physiological response (Carlsson 1952) and, later, that actinomycin D (an antibiotic which, when added to bacterial cultures, inhibits DNA-directed RNA synthesis) inhibited the action of vitamin D (Zull, Czarnowska-Misztal & DeLuca 1965). The discovery of metabolites was initially hampered by lack of availability of nuclear magnetic resonance measurements and mass spectrometry (Napoli 1975). However, these chemical problems were overcome and in the 1960s DeLuca's group demonstrated the existence of biologically active metabolites of vitamin D_3 (Lund & DeLuca 1966). It was established that vitamins D_3 is metabolized to 25 hydroxy vitamin D_3 (25 OH D_3) in the liver (Blunt *et al.* 1968, Bhattacharyya & DeLuca 1973), and in the kidney 25 OH D_3 is further hydroxylated to 1,25 dihydroxy vitamin D_3 (1,25 diOH D_3) (Fraser & Kodicek 1970) or 24,25 dihydroxy D_3 (24,25 diOH D_3) (Ghazarian & DeLuca 1974).

As a result of intense chemical and biochemical research a plethora of vitamin D metabolites has been isolated, and many synthetic analogues have been made. Chemically the vitamin D_3 (cholecalciferol) molecule has the potential for hydroxylation at further positions, so giving opportunities for discovering other biologically active metabolites. Biological studies have shown that an elegant vitamin D metabolic pathway now exists (*see* Fig 1), so that skin and dietary sources of vitamin D

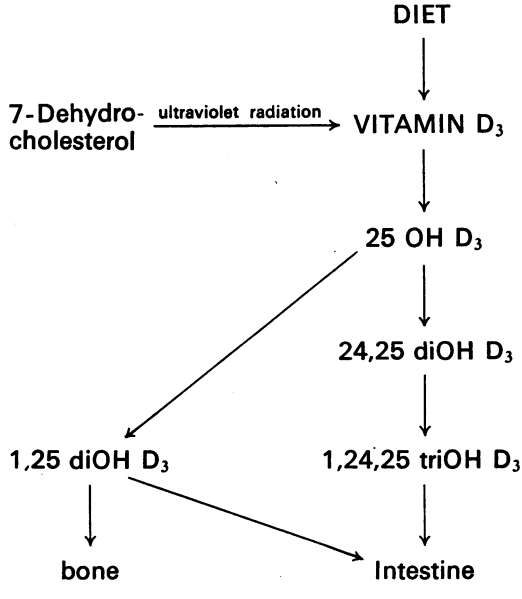

Fig 1 *Vitamin D metabolism*