138. THE INFLUENCE OF INTERMITTENT CONSUMPTION OF VITAMIN C ON THE DEVELOPMENT OF SCURVY

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It is customary to speak of the daily requirement of a dietetic constituent. This, of course, does not mean that in order to avoid catastrophic results the dose in question has to be consumed with regularity every day, for it is evident that, owing to the uncertainties associated with the struggle for existence, no species could have survived under such conditions. It is comprehensible that the daily dose need not be regular in the case of constituents which are capable of storage in the body, even though our knowledge of all the stages in the mechanism involved in the utilization of food by the body is imperfect. The problem, however, becomes more arresting when consideration is given to those dietetic substances which appear, according to our present knowledge, to be incapable of storage, at least to any appreciable extent. Vitamin C is a case in point. The writer has shown [Zilva, 1936] that vitamin C is not stored by the growing guinea-pig for reserve purposes. Thus he found that fully saturated and unsaturated animals alike developed scurvy on a scorbutic diet; they succumbed to it under similar conditions, the time taken for the first manifestation and for the fatal issue of the disease being of the same order in each case. This observation called for further investigation, particularly of the behaviour of these animals when the minimum dose instead of being administered daily was offered intermittently in amounts which increased in proportion to the number of days between the doses, so that the same total amount was given in each case over the whole period of experiment. This communication deals with a few simple experiments which throw some light on the subject.

It is obvious that in order to obtain the necessary information, it was essential that the minimum dose, or a dose approximating to it, should be used as a standard. The minimum dose does not lend itself to precise definition even in the case of the guinea-pig, in which, unlike the human being, a good opportunity of obtaining more exact results is afforded owing to the possibility of controlled experimentation involving the examination and analysis of tissues at will. In the majority of growing guinea-pigs on a scorbutic diet a daily dose of 1 mg. is sufficient to prevent the appearance of macroscopic signs of scurvy. About double that dose is required to obviate microscopic changes in the pulp of their teeth [Goettsch, 1928], and a daily dose of 5–8 mg. per day is necessary to bring about the deposition of a discernible trace of ascorbic acid in their selective organs [Zilva, 1936].

In this investigation daily doses of 2 and 5 mg. were adopted as standards. The requisite multiple of the former dose was administered to a number of groups of growing guinea-pigs on a scorbutic diet either daily, every other day, every third day or weekly. The equivalent of seven daily doses of the latter

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standard was similarly given once a week (i.e. a dose of 35 mg.). One set of these groups was fully saturated in respect of vitamin C at the beginning of the experiment. Another set was previously maintained for 8 days on a scorbutic diet and these animals thus became unsaturated, containing at the most only traces of ascorbic acid in their tissues. The duration of the experiment was 90 days, at the end of which period the animals were killed by chloroform and autopsied. The constitution of the basal diet and the conditions under which the animals were maintained were the same as those usually employed in this laboratory, details of which have already been given by the writer in previous communications [cf. Kellie & Zilva, 1941].

The results are represented graphically in Figs. 1 and 2. It is immediately evident that the responses of the guinea-pigs in each set of the experiment, namely those which were saturated and those which were unsaturated at the beginning of the tests, were almost identical. The behaviour of the guinea-pigs receiving the 2 mg. dose daily, or its multiple every other day or every third day respectively, was in all cases similar and uneventful; full protection from macroscopic scurvy and good growth were achieved in all these groups. Those, however, which received 14 mg. of ascorbic acid once a week showed definitely poorer growth, and in some of the animals very slight signs of scurvy were observed at the post mortem examination. A striking feature in all these groups was the similarity of the reaction of the saturated and the unsaturated animals. A saturated guinea-pig of about 300 g. contains 15–20 mg. of ascorbic acid in its body (unpublished results), and it would have been expected that if this quantity acted as a store its action would have been reflected at least at some stage of the experiment. This, however, does not appear to have been the case, and the lack of any significant differences between the two groups therefore lends further support to the writer's view that the body ascorbic acid of the saturated animal does not act in any marked degree as a reserve for the vitamin C requirement. When offered 35 mg. once a week, i.e. the equivalent of 5 mg. instead of 2 mg. per day, both the saturated and unsaturated guinea-pigs grew well and were fully protected from macroscopic signs of scurvy. It is, however, of importance to bear in mind that 5 mg. per day approximates to the 'minimum' dose, since it represents the lower limit of a range of daily doses (5–8 mg.) which are just capable of depositing minute traces of ascorbic acid in the tissues.

A clearer interpretation of the above results can be obtained if they are considered in terms of the human being. It must be realized in instituting such a comparison that it can only be of a tentative nature, but everything points to the fact that some conclusions can thus be drawn with a fair degree of probability. A saturated guinea-pig when placed on a scorbutic diet loses its body ascorbic acid rapidly. The greater part of it disappears from the tissues in 3–5 days, and after 8 days the vitamin is in most cases hardly demonstrable by the available methods. Well-declared signs of scurvy are usually found *post mortem* in well-nourished animals after 21–24 days, although this condition may sometimes be demonstrated even after 14 days. Death from the disease ensues after 30–35 days. In the case of the human being on a scorbutic diet the disease runs a similar course. Quantitative data are for obvious reasons not as easily obtained as with guinea-pigs. The body ascorbic acid probably disappears after about 2 months. Crandon *et al.* [1940] found that the plasma ascorbic acid level fell rapidly and reached zero after 41 days. In Rietschel & Mensching's [1939] experiment it fell to 0.17 mg./100 ml. of serum in 58 days. A determination carried out after 100 days gave a zero value. No agreement is found in the literature as to the time taken for the first appearance of the scorbutic symptoms.

Fig. 1. Saturated at beginning of experiment.

N = Normal.

N? = Very small haemorrhage in right knee joint suspected to be due to injury.

VSS = Very slight scurvy.

SS = Slight scurvy.

Fig. 2. Unsaturated at the beginning of experiment.

— = Commencement of administration of dose.

N = Normal.

VSS = Very slight scurvy.

SS = Slight scurvy.

This is not surprising, alike owing to individual variations, to differences in the conditions of existence of the subjects influencing the precipitation of the symptoms, and above all to the uncertainty of the absolute scorbutic character of the diet. We have, however, the data of a recent and exceptionally well controlled experiment available as a standard [Crandon *et al.* 1940]. Crandon placed himself (for a period of 6 months) on a diet entirely deficient in vitamin C, but supplemented by all the other known dietary constituents. The first clinical signs of abnormality, hyperkeratotic papules, appeared after 132 days, but 161 days elapsed before the first specific signs of scurvy, the perifollicular haemorrhages or petechiae over the lower legs, became manifest. By comparing the respective data it will be seen that the ratio between the life span of man and that of the guinea-pig is roughly the same as the ratio between the corresponding times taken for the disappearance of the body ascorbic acid and for the onset of scurvy in these two species. For approximate comparison the factor of 10 may be adopted for converting the data obtained with guinea-pigs into those applicable to human subjects. When this factor is applied to the figures obtained in this investigation with guinea-pigs, taking into consideration at the same time that the vitamin C requirement of man is about double that of the guinea-pig [cf. Kellie & Zilva, 1939], it would appear that 120 mg. every 30 days would be sufficient to protect man from macroscopic scurvy, since 6 mg. every third day is required to produce this effect in the guinea-pig. A multiple of the daily dose of 10 mg., which corresponds to 5 mg. with guinea-pigs, would fulfil this function for man when given at even much longer intervals. Though no precision can be attached to these figures, they nevertheless indicate that doses of the order of 100 mg. given at intervals of a number of days or even weeks can afford at least minimum protection against scurvy. By utilizing the vitamin C requirements of the guinea-pig as a basis for calculation it was found that the dose for the human being could be deduced from them [Kellie & Zilva, 1939], the dose of about 15 mg. per day being found in this way to be necessary to protect man from scurvy. Apart from popular experience recent direct experiments on human beings suggest that this dose is of the right order [Rietschel & Mensching, 1939; Fox & Dangerfield, 1940; Widdowson & Alington, 1941]. There is good reason to believe that the indications obtained in this present investigation are also correct.

If these deductions are correct it may be considered what bearing they have on practical nutrition. As far as the civilian population of this country, leading a normal life, is concerned, the natural supply of vitamin C during the greater part of the year is so superabundant that, even allowing the widest margin for destruction in the cooking and the preparation of the food, the intake is more than adequate to supply the vitamin C requirements. Towards the end of the winter and the early spring, when green vegetables become rather scarce, some individuals, chiefly in urban areas, have to rely on potatoes as their chief source of vitamin C. In all probability this main source alone, considering the quantities that are usually eaten, together with the traces of ascorbic acid in other constituents of the diet, is sufficient to meet the vitamin C requirements of a great many people. When, in addition, it is considered that large quantities of vitamin C are often consumed intermittently during the winter and spring in the form of occasional greens, swedes, turnips, Bramley's Seedling apples, strawberry or black currant jam etc. and, in peace-time, citrus fruits, and when it is further realized that, as the above experiments indicate, a lasting effect can be produced by such irregular consumption of ascorbic acid, confidence is increased in the belief that a general vitamin C deficiency, even at that time of the year, need not

be greatly feared. It is quite probable that saturation may not be attained by many during winter and spring, but, as already pointed out by the author and his colleagues [Johnson & Zilva, 1934; Zilva, 1936; 1937; Kellie & Zilva, 1939], there is no evidence that saturation in respect to vitamin C is a prerequisite of adequate nutrition. The importance of saturation has had a great deal of ventilation in the literature during the last few years, but on close examination it is found that these claims are based on undigested and misinterpreted experiments. Much more convincing are some recent and carefully controlled investigations that militate against the exaggerated importance of saturation. Mensching [Rietschel & Mensching, 1939], after subsisting on a scorbutic diet for about 40 days, developed a cold as a result of exposure to a draught after becoming overheated by performing strenuous storm troop exercises. This infection cleared up normally. Crandon [Crandon *et al.* 1940] throughout an experimental period of 5½ months, which included winter months, on a scorbutic diet enjoyed perfect freedom from respiratory infection, although in previous winters he was subject to such infection. Fox & Dangerfield [1940] also found no indication that a large group of unsaturated subjects was more prone to infection than another group which received more vitamin C. In all three investigations the gums of the subjects remained unaffected. In the last two investigations (in the first the point was not investigated) unsaturation did not influence the healing of wounds; only at the end of 182 days, when scurvy was manifest, did Crandon find that the healing of a self-inflicted wound was impaired. In Crandon's experiment it took 3 months of existence on a completely vitamin C-free diet before signs of fatigue became noticeable. Mensching did not observe any fatigue after living on a scorbutic diet for 100 days, when the experiment ended, nor did tests of physical efficiency in Fox & Dangerfield's investigation reveal any influence of unsaturation on the performance of the experimental subjects. Finally it may be mentioned that the author [Zilva, 1919] some years ago demonstrated that guinea-pigs on a scorbutic diet showed no differentiation in the amboceptor and agglutinin titres and in the complement content of the blood as compared with animals receiving ample vitamin C. All this evidence tends to show that the absence of full saturation, which may possibly occur after intermittent consumption of large doses of ascorbic acid capable of preventing scurvy, need not cause any serious anxiety.

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