VITAMIN C.

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Some Historical Notes.

The clinical features of scurvy were originally noted by the crusader de Joinville in the 13th Century and Vasco da Gama reported that he lost from this scourge 100 out of a crew of 160 men during his historic voyage round the Cape of Good Hope in 1498. It was not, however, until the 18th Century that the association of scurvy with a deficiency in the diet of fresh fruit and vegetables was clearly recognised and recorded by the Austrian army physician Kramer in 1720, by Bachstrom in 1734 and by Lind in 1747.

Modern landmarks in the isolation and identification of vitamin C are (1) The production of experimental scurvy in guinea-pigs by Holst and Frölich in 1907. (2) The discovery by Tillmanns in 1928 that the dye 2:6 dichlorophenol-indophenol could be used as an indicator for the presence of vitamin C. (3) The isolation by Szent-Györgyi in 1928 of a substance from the adrenal gland which was called "hexuronic acid." (4) The isolation independently in 1932 by Szent-Györgyi and by King and Waugh of a crystalline substance from lemon juice, orange juice and cabbage with similar properties to hexuronic acid and which was found from experimental trials in animals to be a potent anti-scorbutic agent. Further experiments on animals and humans by Szent-Györgyi, King and Waugh, Harris, Zilva and others conclusively proved that the material isolated was vitamin C and the name ascorbic acid was given to it.

Chemical Nature.

Ascorbic acid is an acid, white, crystalline substance, related to the hexose sugars, and having the empirical formula $C_6H_8O_6$. It is now prepared commercially from glucose.

Ascorbic acid is moderately stable in solid form if kept in a sealed tube without access of air or moisture. It is soluble in water, and in solution it is much more sensitive to oxidising agents, including atmospheric oxygen, especially when the solution is alkaline. Its decomposition is accelerated by heat and visible light. The first oxidation product of ascorbic acid is readily reduced to ascorbic acid and this oxidation-reduction reaction is believed to be of physiological importance.

Physiological Functions.

Ascorbic acid appears to play a vital part in the maintenance of the normal physical state of certain mesenchymal derivatives. Consequent on a deficiency of ascorbic acid a satisfactory formation of collagen and fibrils in connective tissue fails to occur and degenerative changes in osteoblasts and odontoblasts lead to a production of inferior bone and dentine. Skeletal changes and dental caries follow. The hæmorrhagic tendency, which is the most obvious clinical feature of scurvy, is believed by some

workers to be due to a failure of the endothelial cells lining capillaries to produce inter-cellular substance in amounts adequate for the integrity of the capillary wall. Since morphological changes have not been demonstrated in the capillaries it is not known whether the reduced capillary resistance is due to this cause or to defects in the pericapillary connective tissue and collagen sheath. Ascorbic acid in solution has the property of accepting and then giving up oxygen. This property has led to the belief that it plays an important rôle in the oxidation-reduction reaction by which the cells of the body utilise foodstuffs to provide the heat and energy required for the whole organism. There is, however, no experimental evidence for this view. Of considerable physiological importance is the observation that a correlation exists between the concentration of ascorbic acid in the blood and complement in the serum. In addition, it has been claimed that the production of specific antibodies can be stimulated by the intravenous injection of ascorbic acid. These findings are of intense interest because it has been shown in many diseases that the ascorbic acid content of the blood is low, and because claims have been advanced in regard to the therapeutic value of ascorbic acid in many infective diseases.

The Relationship of Vitamin C deficiency to Disease.

The well-recognised finding that many diseases (e.g., acute and chronic infections, nephritis, peptic ulcer, Addison's disease, diabetes, etc.) are associated with a low ascorbic acid content of the blood and urine has led to an immense amount of work in order to determine whether the deficiency is the cause or the result of the diseased state. Of particular interest in this connection is the relationship of ascorbic acid deficiency to rheumatic fever, a condition in which low plasma ascorbic acid values are consistently found. Rinehart found that if guinea-pigs on a satisfactory diet were injected with extracts of hæmolytic streptococci no cardiac lesions were produced, whereas severe pathological changes regularly occurred when, prior to injection, the animals had received diets deficient in ascorbic acid. It was subsequently shown that the lesions produced were similar to those developing as a result of vitamin C deficiency alone, the effect of the streptococcal injection being to augment and accelerate the pathological picture.

Those who believe that the deficiency of vitamin C is of primary etiological importance in the diseases mentioned above stress the interesting relationship of ascorbic acid to the immunity processes previously discussed. They draw attention to the fact that, as clinical improvement occurs, a corresponding rise in the ascorbic acid content of the blood can be demonstrated and claim that specific therapeutic results are obtained by the administration of vitamin C in adequate amounts.

Those who hold that the deficiency follows the development of disease explain the reduced ascorbic acid content of the blood as being directly due to increased destruction or utilisation consequent on the raised metabolism in fever and to the reduced intake resulting from anorexia and gastro-intestinal upset. They dispute the claims that ascorbic acid has specific effects in any conditions other than scurvy.

It is difficult at the present time to come to any final conclusions when such conflicting views have been advanced by distinguished research workers. It is obviously wise both for the prevention and treatment of disease to put the body in the best physiological position to maintain health. Accordingly, the restoration and maintenance of the ascorbic acid reserves of the tissues to an optimal level during and between illnesses is a wise precaution that can be safely recommended. In general this is best done by giving the vitamin in the form of natural food (fresh fruit and vegetables), but in severe infective diseases it may be necessary to give supplements of ascorbic acid.

It has lately been claimed that ascorbic acid may be of value in the treatment of toxic manifestations developing during treatment with arsenic, bismuth and gold. There is, for example, evidence that the dermatitis which occasionally arises during antisyphilitic treatment may be favourably influenced by the administration of large doses of ascorbic acid.

Clinical picture of Vitamin C deficiency.

The clinical picture will depend mainly on three factors :—

- (1) The degree and duration of the vitamin deficiency.
- (2) The age of the patient.
- (3) The effects of additional factors, e.g., infection, trauma, etc.

When the deficiency of vitamin C is extreme and prolonged manifest scurvy develops with the clinical picture of insidious and progressive weakness and exhaustion, anæmia, sponginess of the gums, hæmorrhage in many situations and pain in the muscles and joints. The fully-developed picture is now rarely seen in European countries, and when it occurs it is usually found in elderly men living alone and cooking their own food (bachelor scurvy). During the past two years we have seen twenty-four cases of the fully-developed disease. This relatively high figure is explained by the fact that we have had the opportunity of investigating the majority of cases entering the medical wards of the hospitals in Edinburgh which serve a population of approximately one million persons. Manifest scurvy still occurs not infrequently in infants and young children. If the hæmorrhagic features are not prominent in early life, the condition may not be recognised, since the presenting manifestations are poor health, slight fever, anorexia, mild anæmia and pain on moving the limbs, features which may occur in infective states or in rickets. As already mentioned, the failure to arrest the pathological changes in the bones and teeth may lead to serious defects in development. In contrast to the rarity of manifest scurvy, latent or subclinical scurvy or, as it is better called hypovitaminosis C, is still common. In some cases of hypovitaminosis C the diagnosis may be suspected from the sponginess of the gums, the symptoms of easily induced debility and exhaustion, and from the dietary history. In many more it can only be made by certain special tests (see below).

It has long been known that the requirements of ascorbic acid are increased in infections. Numerous records are available of outbreaks of scurvy in soldiers in the field and sailors at sea subsequent to epidemic disease. Another factor of great importance in the production of scorbutic hæmorrhages is mechanical injury and undue stress. Thus it has been recognised for many years that the back muscles of scorbutic soldiers frequently show a maximal incidence of hæmorrhage. In our experience the commonest site of hæmorrhage is the lower limbs. This we attribute to the increased venous pressure consequent upon the upright posture and to the liability of the lower limbs to mild trauma. For similar reasons hæmorrhages may be found at the tops of boots, under garters, etc. We have noted the disappearance of large hæmorrhages, particularly of the lower limbs, when the patient is kept in bed even when he is maintained on a diet deficient in ascorbic acid.

Even in the apparent absence of such causes a remarkable variation in individual liability to develop scorbutic hæmorrhages occurs in persons who are taking diets apparently similar in their degree of ascorbic acid deficiency. We know of no satisfactory explanation of this phenomenon. It is probable, however, that in some cases excessive consumption of alcohol is a factor in the development of scurvy in

persons whose diet appears to be only relatively deficient in ascorbic acid. Such an effect is indeed well recognised in vitamin B deficiency. The anorexia, chronic gastritis and hypochlorhydria resulting from alcoholism have a deleterious effect on the intake and absorption of all vitamins.

Lastly, we would stress the difficulty in interpreting clinical findings and laboratory observations in the scorbutic subject since almost invariably multiple deficiencies coexist. Inadequate intake of vitamin B₁, for example, may also be present and the consequent anorexia and defective function of the alimentary tract may augment other deficiencies.

The Anæmia of Scurvy.

Anæmia is usually regarded as a fundamental feature of scurvy. It is rather surprising, therefore, that reference to the literature reveals that anæmia is absent in about 30 per cent. of cases. Of the 24 cases investigated by ourselves 33 per cent. showed no anæmia. We have not been able to establish a correlation between the degree of deficiency of the vitamin, the amount of hæmorrhage and the presence or the degree of anæmia. In our experience hæmoglobin values of under 50 per cent. are rare: only two patients out of 24 had a hæmoglobin level below this value. The anæmia of scurvy may be hypo, ortho, or hyperchromic. It is usually stated to be orthochromic and normocytic, but 7 out of the 16 cases with anæmia in our series had a colour index of 1.1 or over.

The anæmia which develops in scurvy does not respond to iron or to liver extract but, following treatment with ascorbic acid, a reticulocyte response of some 5—10 per cent. occurs followed by an immediate improvement in the blood picture. It is of interest to note, however, that in a survey of many hundreds of cases of nutritional anæmia in women of the poorest classes in Aberdeen we obtained an adequate response to iron therapy, although the dietary histories made it certain that the subjects were in a state of generalised hypovitaminosis. This is not to say that a more rapid improvement would not have resulted had supplements of ascorbic acid been given. There is indeed evidence that certain cases of nutritional anæmia which respond slowly to iron may improve more rapidly if ascorbic acid is given in addition.

Special Methods of Investigation.

The diagnosis of "sub-clinical scurvy" depends essentially upon the use of certain tests. These may be considered in four groups. The principle upon which the majority depend is that the dye 2:6 dichlorophenol-indophenol is reduced to a colourless substance by ascorbic acid. This chemical reaction is so arranged as to form a method for the quantitative estimation of the vitamin.

1. **Tests on the urine.** It has been recommended that the daily urinary output of ascorbic acid be determined by direct titration of the urine against a solution of the dye and it is generally held that healthy adults receiving an adequate daily intake of the vitamin excrete 10—30 mg. or more of ascorbic acid daily. It must be pointed out, however, that the dye is decolourised by a number of other substances normally present in urine and this fact may explain the occasional case of scurvy in which the urinary excretion of ascorbic acid appears to be adequate. A number of procedures have been devised for rendering the estimation more specific but all these are laborious and not suitable for general use.

A more satisfactory procedure is to employ a "saturation test" in which the urinary excretion of ascorbic acid is determined following the administration of a large

quantity of the vitamin. The principle underlying this test is that, if the ascorbic acid content of the tissues is subnormal, a large proportion of the test dose will be retained to produce "saturation" after which excretion of the vitamin will occur. In general it will be found satisfactory to give 500 mg. of ascorbic acid in a single dose by mouth and to estimate the excretion during the following 24 hours. If the subject be fully saturated at least 15 per cent. of the test dose will be excreted. To assess the degree of deficiency, should this response not be obtained, the test dose is repeated daily until a satisfactory excretion is found. Scorbutic patients may require from 5—8 g. of ascorbic acid before a sharp rise in urinary excretion occurs.

2. **Tests on the blood.** Ascorbic acid is approximately evenly distributed in the blood between the plasma and the corpuscles. It is of interest that the leucocytes contain a relatively high concentration of the vitamin. Values of 0.8—1.2 mg. ascorbic acid per 100 c.c. may be accepted as normal for plasma ascorbic acid and a figure below 0.5 mg. per 100 c.c. may be taken as evidence of hypovitaminosis C.

3. **Intradermal test.** This procedure seeks to assess the ascorbic acid content of the skin by noting the time taken to decolourise a solution of the dye injected intradermally. Experience has shown that it is not a test which can be recommended.

4. **Determination of the capillary resistance (fragility).** This test depends upon the hypothesis that, since ascorbic acid is essential for the maintenance of the integrity of capillary walls, an inadequate intake of the vitamin will result in abnormally fragile capillaries which will rupture easily when pressure is applied to them. It is obvious that other conditions which influence the integrity of the capillary walls (thrombocytopenic purpura, leukæmia, toxic and infective states) must first be eliminated by appropriate investigations. Extensive experience has shown, however, that the nutritional state of an individual with respect to ascorbic acid cannot be accurately assessed by determination of the capillary resistance. For example, 9 cases of manifest scurvy, treated with ascorbic acid in amounts adequate to cure all the clinical features of the condition and to restore the plasma ascorbic acid to the normal level, were still found to have abnormally fragile capillaries. The administration of vitamin P restored the capillary resistance to normal.

Vitamin P, the precise chemical constitution of which is at present unknown, was first described by Szent-Györgyi in 1938. It was obtained by him in a concentrated form from paprika, a fruit rich in ascorbic acid. He claimed that it could restore the capillary resistance to normal in certain cases of purpura unaffected by the administration of ascorbic acid. One of the authors has obtained evidence which suggests that vitamin P has a specific effect in controlling capillary resistance. Of equal interest is our finding that three of our cases of manifest scurvy with marked subcutaneous and intramuscular hæmorrhage revealed no evidence of abnormally fragile capillaries.

Dosage and Requirements.

The international unit of vitamin C has the antiscorbutic activity of 0.05 mg. of pure crystalline l—ascorbic acid; 1 mg. therefore equals 20 international units. As ascorbic acid can be estimated quantitatively by chemical methods, the tendency to-day is to speak of ascorbic acid dosage in terms of milligrams rather than of international units.

The daily minimum requirement of the normal adult male is in the region of 30 mg. (i.e., 600 international units) of ascorbic acid. The daily optimum dose is twice this

amount. Infants and young children must receive a relatively abundant supply of vitamin C, and 15 mg. of ascorbic acid is the minimum daily requirement of an infant. When scurvy has developed, much larger doses should be given, say, 100 mg. daily to an infant or 500 mg. to an adult. In conditions of enhanced metabolism—pregnancy, lactation, violent exercise, or fever—the utilization of vitamin C is increased, and accordingly the intake of ascorbic acid in these conditions should be about 100 mg. daily.

It might be suggested that accepted standards of normal ascorbic acid requirements are excessive since many individuals among the poorer sections of the population take in the diet amounts of vitamin C much below the accepted minimum figure given above, and yet appear to be in reasonable health. There is a danger, however, in accepting this view too easily. The majority of women suffering from nutritional iron deficiency anæmia do not come to the doctor complaining of poor health. The anæmia is usually discovered during a routine examination for some other purpose, e.g., pregnancy, etc. It is only after the hæmoglobin level has been restored to normal that they realise what good health really means.

Natural Sources.

The chief natural sources of ascorbic acid are citrus fruits and green leaves. Fruits other than citrus, such as the tomato and black currant, also have a high ascorbic acid content. Although the potato contains only a moderate amount of ascorbic acid, its very wide use among the poorest classes places it high in the scale of foodstuffs of value for the prevention of scurvy. Again and again potato famines in Ireland have been accompanied and followed by outbreaks of scurvy.

Canned foods are generally considered to be of little value as anti-scorbutics, but with modern methods of canning, in which care is taken to exclude factors tending to destroy vitamin C, the loss of the vitamin may actually be less than in the cooking of the fresh products. Table I gives the average ascorbic acid content, in milligrams per ounce, of some of the commoner foods, fresh, cooked and canned.

Most dried foods contain little or no ascorbic acid.

TABLE I.

ASCORBIC ACID CONTENT (mg. per oz.) OF SOME COMMONER FOODS.

<i>Foodstuff.</i>	<i>Fresh.</i>			<i>Cooked.</i>			<i>Canned.</i>
Orange juice	15	..	..	—	..	..	—
Lemon juice	15	..	..	—	..	..	—
Grapefruit juice	15	..	..	—	..	..	12
Apple	1	..	..	—	..	..	—
Plum	1.5	..	..	0.9 (boiled)	..	..	0.7
Black currant	65	..	..	54	..	..	65
Tomato	6	..	..	—	..	..	4
Turnip (root)	10	..	..	—	..	..	—
Cabbage	20	..	..	8 (boiled)	..	..	—
Cauliflower	20	..	..	13 (boiled)	..	..	—
Beans (broad)	9	..	..	—	..	..	5
Brussels sprouts	34	..	..	14 (boiled)	..	..	17
Peas (fresh, green)	7	..	..	—	..	..	4
Peas (dried)	1	..	..	—	..	..	—
Potato	4	..	..	2 (boiled)	..	..	—
Milk	0.5	..	..	—	..	..	—
Liver	10	..	..	6 (fried)	..	..	—

The prevention or treatment of hypovitaminosis C is best achieved by the ingestion of the natural foods containing ascorbic acid. With the help of Table I the family doctor will have no difficulty in giving advice to his patient on the kind of food, and the quantities required daily, to achieve this result.

The leading manufacturing chemists market synthetic ascorbic acid under various names. The vitamin is available in tablets of 25 and 50 mg. and also in solution in sealed ampoules of 100 mg. and 500 mg. for parenteral administration.

Summary.

The chemical nature, physiological functions and the relationship of ascorbic acid deficiency to disease are discussed.

The clinical picture of manifest scurvy is described and attention is drawn to certain factors which influence the course of the disease and the clinical manifestations.

Tests for the diagnosis of latent scurvy are given and their relative value discussed. The physiological requirements for ascorbic acid in health, in disease and in special states of stress are considered and methods of supplying the vitamin in natural foods or as synthetic preparations are given.

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