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Relation of Vitamin C to infections

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INTRODUCTION

Rubner's law of isodynamic equivalence became obsolete about twenty-five years ago. Before that time the concept, that one food stuff might, with unimportant limitations, be replaced by another so long as the total energy supplied was undisturbed, was orthodoxy (Hopkins, 1923). The study of the vitamins has developed so as to nearly constitute an exact science. On the other hand, with increasing impetus our newspapers, magazines, bill boards and radio, proclaim the value and varied use of the vitamins to all. The word, vitamin, has become a household term. The natural source of these substances is merely food. These conditions have more or less divorced the vitamins from therapeutics and the medical profession. Now, with the more or less recent isolation and synthesis of all of the vitamins except vitamin E, one wonders if the commercialization of these nutritional factors hasn't resulted in loss of interest in this subject by the physician and surgeon.

My purpose, then, in presenting this thesis is an attempt to cover some of the more practical problems concerned with subclinical hypovitaminosis C and thereby stimulate the curiosity of those who may read it. For that reason, you will find some studies in Chemistry, physiology, pathology, immunology and diagnosis followed by some clinical aspects.

CHEMISTRY AND PROPERTIES OF VITAMIN C

While studying the peroxidase systems of tissues Szent-Gyorigyi, (1928) isolated and described a highly reducing substance from bovine adrenal glands. This substance was found by him to be an isomer of glycuronic acid but differed in its high reactivity. To this substance he gave the empirical formula $C_6 H_8 O_6$. This substance, hexuronic acid, was not recognized as an antiscorbutic until 1932 when King and Waugh found it to be identical with crystaline antiscorbutic they had derived from lemon juice. This finding was confirmed by Svirbely and Szent-Gyorgyi in their next paper. Within a short time, by independent investigation, Harris and Ray (1933) and Karrer, (1933) also identified the vitamin and the latter showed that carefully fractioned crystallization prevented production of crystals of varying potency.

One of the earliest questions to arise was whether the antiscorbutic effects were due to the ascorbic acid or due to impurities. Rygh, (1932) expressed the belief that he had isolated vitamin C from orange juice and identified it as menthylnornarcotine, which he claims was antiscorbutic to guinea pigs. Grant, Smith and Zilva (1932) were unable to confirm this. Later in 1932 Rygh and Rygh found that though methylnornarcotine prevented scurvy, it did not enable the guinea pigs to live. They had found that by adding glucuronic acid to the diet the guinea pigs lived and remained healthy. From this work, they concluded that Szent-Gyorgyi's hexuronic

acid was antiscorbutic only because of traces of methylnornarcotine in the substance. In the light of later work this is untrue. Karrer (1933) showed that antiscorbutic properties are present in the most carefully prepared cevatamic acid.

Vitamin C is the lactone of threo-3 keto hexonic acid (Booth and Hansen, 1937). The substance is an odorless, white or yellowish white crystaline powder with a melting point of 192 degrees centigrade (Wright and Lilienfled, 1936 and King 1938) which decomposes at temperatures over 192 degrees. It is soluable in water, acetone, acetic ether and propyl alcohol; insoluable in ethyl ether and purified petroleum benzine (Szent-Gyorgyi 1928 and Svirbly and King, 1931-1932). The dry crystals of ascorbic acid are stable on exposure to air and daylight at room temperatures for over a period of several years. They tend to become buff colored without undergoing appreciable decomposition. Humphrey (1926) found dried orange juice still effective in preventing scurvy after five years. In aqueous solution the rate of aerobic oxidation is greatly accelerated by exposure to light, especially in the presence of flavins (King 1938) and in alkaline solution (Booth and Hansen, 1937). The action of light does not take place in the absence of oxygen (Kon and Watson, 1936). Catalysis greatly increases the volicity of oxidation and destruction of the vitamin in solution (Hess and Weinstock, 1934). Even traces of heavy metals cause rapid oxidation of aerobic acid (Ecker, Pillemer, Wertheimer, and Gradis, 1938).

Vitamin C has very high reducing powers, remarkable because oxidation of the acid is reversible. It is oxidized and reduced alternately giving off and taking up two atoms of hydrogen. Thus it acts as a hydrogen carrier between the different parts of its oxidation system (Haworth, 1933).

Chemical Determination of Ascorbic Acid:

Chemical determination of ascorbic acid as set out by Harris and Ray (1933) is based on a modification of the use of Tillmans indicator, sodium 2,6, di-chlorophenolindophenol. The essential features consist of a preliminary extraction process with trichloracetic acid followed by titration in acid solution.

Emmerie & van Eeklen (1937) found that sulfur compounds, (cysteine, ergothioneine and thiosulfate) interferred with 2,6 dichorophenolindophenol during titration. They advocate the use of mercuric acetate to precipitate these compounds. They also suggest the use of hydrogen sulfide to regenerate the reversibly oxidized ascorbic acid by reduction.

In 1935 Tauber and Kleiner extracted an enzyme from the pericarp of ripe Hubbard squash which they thought to be specific for ascorbic acid. They advocated determination of the reducing capacity before and after destruction of the vitamin by this oxidase as a means of increasing the specificity of the test. Srinivasan (1937) was of the same opinion. This method does not increase the specificity of the test for Johnson and Zilva (1937) showed this oxidase to be nonspecific for the oxidation of ascorbic

acid.

Bessey (1938) is of the opinion that since most interferring substances found in natural systems react with the indicator at a perceptably slower rate than ascorbic acid under the conditions of titration the precision in most cases is still with the range of biologic variation. For this reason he considers the mercuric acetate method of Emmerie as clumsy and also is of the opinion that reversibly oxidized ascorbic acid, dehydroascorbic acid, represents an insignificant portion of the true biologic value of fresh or stored products. Therefore he feels the use of hydrogensulfide is also unnecessary.

PHYSIOLOGY OF VITAMIN C

Vitamin C is necessary to normal physiological activity of the organism. It is apparently one of the oxidation reduction systems of the body, possible acting as a hydrogen carrier in the system. (Hopkins and Morgan, 1936).

The fact that of the animals only man, the other primates and guinea pigs are incapable of synthesizing vitamin C in the body has long been established (Holst-Frolich, 1907; King, Booth and Hansen, 1937). Humans do not excrete ascorbic acid in the urine or excrete it only to a limited extent when ingested by them, unless they are fully saturated (Hess and Benjamin, 1934; Johnson and Zilva, 1934; Harris and Ray, 1935). Taking into consideration the proposed renal threshold of 1.4 mg. percent blood plasma level (Faulkner and Taylor, 1934; 1937) and the depletion of the body tissue concentrations on deficient diets (Zilva, 1936) one sees that the vitamin is either stored to some extent or destroyed in the body. This is also brought out by the fact that massive doses of the vitamin per os do not bring up the excretory level for a varying period of time depending upon the degree of depletion (Johnson and Zilva, 1934). Intravenous administration may confuse this picture, but it must be remembered that this method may result in excretion of ascorbic acid in urine because of the sudden rise of the blood plasma level before the tissues have been fully saturated (Eeklen and Heineman, 1938).

Thus we see that after the body has become depleted of the vitamin the early readministration first cause a restorage in the various tissues and when the deficiency is made up the excess (Hess and Benjamin 1934) vitamin is excreted in the urine. The net loss, or difference between intake and excretion in a saturated person may be taken as the quantity normally used up by the body processes. This value varies with individuals but ranges between 25 and 60 mg. daily (Abbasy and Yudkin, 1936; King, 1938; Abt, and Farmer, 1938). Changing the acid-base balance of the food intake may change the amount excreted (Hawley, Frazer, Button and Stevens, 1936). This alteration may be due to decreased absorption from the intestinal tract due to a changing of bacterial flora accompanying the change of diet (Erderer and Kramar, 1923).

The tissues of the body show a selectivity to Vitamin C in that the contents vary and excessive administration does not increase the concentrations in the tissues of the various organs in a saturated subject (Zilva, 1936). In general, it may be stated that the tissues which contain the highest concentrations of vitamin C are those characterized by high metabolic activity (King 1938). Szent-Gyorgyi (1928) and Koepcke(1937) are of the opinion that the vitamins are essential to normal function of the endocrine system. Deficiencies of vitamin C. reduce the ascorbic acid content of the adrenal glands as much as 50 percent. The

pituitary body contains the greatest amount. In order of descending concentration comes the corpus leuteum, adrenal cortex, young thymus, liver, brain, testes, ovaries, spleen, thyroid, pancreas, salivary glands, lung, kidney, intestinal wall, heart muscle, spinal fluid and blood. The average content of blood plasma is normally about 1.2 mg. of the vitamin per hundred cubic centimeters (Abt, Farmer and Epstein, 1936) but depletion reduces this amount to 0.8 mg. as the prescorbutic state is reached and to approximately 0.5 mg. percent with the appearance of clinical scurvy (Faulkner and Taylor, 1937).

In the absence of vitamin C all cellular functions seem to be injured to some extent. Ascorbic acid is fundamentally important to the formation of normal intracellular substance. In hypovitaminosis C this substance does not have normal properties (Wolbach and Howe 1926). The control of calcium metabolism also comes within the scope of vitamin C activity (King, 1938). Wolbach 1937 states that calcium metabolism is not directly effected but changes in bone and tooth structure are due to arrest of activity of the osteoblasts and adontoblasts and the liberation of calcium salts through the resorption of bone matrix. With these phenomena in mind it is clear that vitamin C is essential for normal growth and development of the organism, (Booth and Handen, 1937).

Normal metabolic activity is moderately lowered during vitamin C depletion as indicated by changes in carbohydrate metabolism and decreased body temperature (Werkman, 1923). Carbohydrate metabolism has been shown to be influenced by vitamin C. Depletion induces a corresponding rise in the fasting blood sugar level and distinctly lowers the glucose tolerance (Sigal and King, 1936).

PATHOLOGY OF VITAMIN C DEFICIENCY

The basis for and explanations of the lesions of Vitamin C deficiency are quite universally agreed upon (Wolbach, 1937; Dalldorf, 1938). The specific physioligical action of ascorbic acid in preventing these lesions however, is as yet unknown and probably involves the chemistry of the living cell.

In 1922 Wolbach and Howe characterized the condition of scorbutus as the inability of the supporting tissues of the organism to produce and maintain the intercellular substance. Dalldorf in 1938 describes this process in relation to the prototype of the mesenchymal structures. The cell type, the fibroblast, normally lies in an amorphous ground substance within which fibrils form. These fibrils become gathered together to form wavy bands of collagen. In this transformation of the reticulum to connective tissue the fibrils seem to be cemented together by a translucent matrix which apparently sets as a gel. He suggests that this is the phase of the formation of intercellular materials which may be controlled by Vitamin C. He has found that in guinea pig experimentation the fibroblasts are present in hypovitaminosis C but that the reticulum and collagen fail to form. The pathologic changes of scurvy as seen in the infant are so similar to those found in a guinea pig experimentally that facts ascertained from experimental study with the guinea pig are applicable to the

human being (Wolbach, 1937).

The intercellular substances concerned in vitamin C deficiency are the collogen of all tissue structures, the matrices of bone, dentin and cartilage, and the nonepithelial cement substances, including that of vascular endothelium (Wolbach, 1937). Dalldorf (1939) agrees with this except that he feels the question as to whether the weakness in the capillaries is in the cement of the endothelium or in the connective tissues surrounding the capillaries is as yet unsettled.

The anatomic manifestations of scorbutic states are modified by two factors, growth and stress. The effects of growth are directly concerned with the fact that osteoporosis resulting from deficiency is more pronounced where growth is more active. It has also been noted that the most rapidly growing bones at a particular age are most affected during scurvy. Bone pains are most frequent in young animals and in children. They seldom occur in the adult.

Stress modifies the site of the lesions and determines the extent and involvement of the structures. Soft tissue changes are hemorrhages in the regions determined by mechancial stresses and trauma; also anasarca and degeneration of skeletal and cardiac muscle. Hemorrhages are due to mechanical weakness, occasioned by the lack of collagenous materials in fibrous tissue structures in bone.

In hemorrhages from the gums and the resorption of alveolar

processes and loosening of the teeth the factors of stress and loss of intercellular materials are apparent. The lesions of the teeth are characterized by the cessation of dentin formation and separation of the pulp from the dentin by liquid. In prescorbutic states the true dentin is replaced by osteodentin, a poorly formed deficient product of migrated or transformed osteoblasts.

At the epidiaphyseal junctures in growing bones the separation of cartilage occurs because of resorption of the matrix and proliferation of osteoblasts. These osteoblasts assume shapes of fibroblasts and in some instances give rise to regions of edematous connective tissue. The cells become surrounded by a liquid which may be a deficient product of continued activity toward matrix formation.

Subperiosteal hemorrhages occur due to loosening of the periosteum by proliferation of osteoblasts of the periosteum in contact with cortical bone. This gives rise to a layer of cell without intercellular material and allows separation of the periosteum from the bone cortex.

The lesion of the skin that is characteristic of scurvy is the perifollicular or petechial hemorrhage. This is commonest on the lower extremities or wherever pressure exposes the weakness of the capillaries.

Histologic repair following the administration of vitamin C in natural foods or as ascorbic acid is dramatic in character. Within eighteen to twenty - four hours (Wolbach, 1937) (Dalldorf, 1938) repairative changes are in evidence. Osteoblasts disguised in morphology as fibroblasts begin again to lay down new matrix. New capillary formation becomes possible, so that repair by granulation tissue formation proceeds in organization of blood clots and subsequent callous formation where the hemorrhages were in contact with the bone. All normal processes of repair are resumed, infractions and fractures heal and subperiosteal hemorrhages become in part ossified.

IMMUNOLOGICAL ASPECTS OF VITAMIN C

Intense interest has been shown concerning the relationship of Vitamin C to the defense mechanisms of the body. Zilva was disappointed in 1919 to find that Vitamin C was not the answer to prevention of infectious processes.

In the experimental work on this phase of vitamin C investigation the guinea pig has been extensively used. As previously stated Wolbach (1937) approves of their use and feels interpreations derived from such experimentation are applicable to human beings.

Zilva (1919) maintained guinea pigs on scorbutic diets. To these he gave low doses of vitamin C, insufficient to protect them from scurvy but enough to prolong the duration of the disease for several months. Control animals were kept in an apparently good state of health by feeding them cabbage ad lib. Both the scorbutic animals and the controls were immunized to typhoid organisms. The scorbutic animals yielded sera with as high amboceptor and agglutinin titres as did the controls. The scorbutic animals also showed no diminution in complement activity of the blood.

Similar experiments were performed by Findlay in 1923. He also found the serum reactions showed little rupture in the defense mechanisms. Findlay's further studies included detailed examination of the blood and hemopoetic organs of normal and scorbutic guinea pigs. In the scorbutic animals he found a

gelatinous degeneration of the bone marrow which proceeded to hyaline degeneration. This picture is also described by Wolbach (1937) as being found in both guinea pigs and children. Findlay also found no significant changes in the blood picture of chronic scurvy and accounts for this in relation to the hemopoetic organs by the fact that the changes merely decreased the number of hemopoetic cells rather than involving their development. However Findlay did observe that the scorbutic guinea pigs succumbed to smaller doses of bacteria injected intraperitoneally and that the symptoms of toxemia were manifested more rapidly.

Werkman (1923) concluded that depression of phagocytic activity in scorbutic animals was not due to failure of the animals to elaborate opsonins, but was due to some other agent effecting phagocytosis. It is known that the phagocytic activity decreases with the lowering of temperatures. This may be the factor here for Werkman also showed that hypovitaminosis C caused subnormal temperatures.

More recently Marsh (1936) depleted the vitamin stores of the guinea pig for seven days and found that the complement as determined by a standardized hemolytic serum either disappeared completely or suffered reduction. Zilva (1936) criticizes Marsh in that no experimental evidence that complement activity suffered no changes in relation to vitamin C.

In an extremely careful experimental study Ecker, Pillemer,

Wertheimer and Gradis (1938) concluded that there is a correlation between the concentration of ascorbic acid in the blood plasma and the complementing activity of the serum in guinea pigs. Their method of determination is worthy of note for very few others found such a difference to exist between normal and scorbutic animals.

The animals acted as their own controls. So as to eliminate all other factors, no greenfood was used. Ascorbic acid was the only source of vitamin C. Knowing that ascorbic acid is rapidly oxidized in the presence of traces of the heavy metals they used triple distilled water and metal free glass. From previous experiments (Ecker, Pillemer and Wertheimer, 1938) it was known that ether anesthesia produces an increased concentration of ascorbic acid in the blood serum of both normal and scorbutic guinea pigs. In view of this fact blood for the complement determinations was taken from the heart without anesthesia. In the complement activity determinations the point of initial hemolysis was used as the basis of evaluation.

The reversible oxidation-reduction behavior of complement, as a single protein substance, was found to be dependent in large part upon the ascorbic acid content of the blood plasma. This correlation held true until a definite blood plasma level of 1 mg. percent ascorbic acid was reached. There was no change in complement activity beyond this level. The normal activity of compelement was contingent on its being in the reduced state.

Although other reducing substances such as glutathione, cysteine and hydrogen sulfide sould bring about reduction and activation, ascorbic acid was found to be the reducing agent of major importance in vivo.

This last citation has caused a renewed interest in this phase of the study of the relationship of vitamin C to physiological processes. Until this time it was quite generally agreed (Werkman, 1923; Robertson, 1934; Pakter and Shick, 1938) that vitamin C content of blood plasma did not effect the immunological processes of the body. In the near future we may have an explanation for the apparently increased morbidity (as found by Findlay, 1923) and susceptability to infectious processes (to be discussed later) in hypovitaminosis C.

DIAGNOSIS OF VITAMIN C DEFICIENCY

The symptoms of frank scurvy are well known. They include increasing pallor, irritability, sore mouth, spongy, bleeding gums, loosened teeth, loss of energy, anorexia, loss of weight, sore and swollen joints, petechiae and large superficial hemorrhages, epistaxis, anemia, edema, fragility of bones and pseudo-paralysis. Of these, as brought out earlier in this paper, the predominate symptom depends upon the stage of the process, the age of the individual and the factors of mechanical stress. However, scurvy is not the first symptom of a lack of vitamin C, on the contrary, it is a very late premortal symptom, and between it and perfect health the boundry is very vague.

In the study then of subclinical scurvy the symptoms suggestive of hypovitaminosis include the following: (Booth and Hansen, 1937).

1. Hemorrhagic tendencies.
2. Dental caries, pyorrhea.
3. Vague aches and pains.
4. Fatigue, pallor, anemia.
5. Abnormal cutaneous pigmentation.
6. Increased susceptibility to infection in general, and to specific cases of diptheria, and tuberculosis.
7. Joint disease strikingly similar to rheumatic fever.
8. Vagus nerve disturbance; increased pulse and respiration.

9. Sensory nerve disorders (paresthesias).

10. Increased capillary fragility.

Laboratory Diagnosis:

Three widely used tests for hypovitaminosis C:

The blood plasma test is the estimation of reduced vitamin C in the blood by chemical test. This consists of extraction followed by titration in acid solution as discussed in the section on Chemistry. Blood plasma values of less than 7.5 to 8 milligram percent of reduced vitamin indicate subnormal vitamin C intake (Abt, Farmer and Epstein, 1936; Faulkner and Taylor, 1937).

In blood determinations of ascorbic acid Heineman (1936) prefers the use of whole blood for he states that the ratio of ascorbic acid content of erythrocytes to that of plasma varies under abnormal conditions, for instance, vitamin deficiencies or anemia. Gabbe (1937) thinks determinations in whole blood is impossible due to adsorption of ascorbic acid by oxhemoglobin during precipitation of the oxyhemoglobin. Emmerie and van Eekelen (1937) disagree with this and show the loss is apparent in the presence of oxyhemoglobin because ascorbic acid is partly reversibly oxidized and can be regenerated by reduction with hydrogen sulfide.

The urinary excretion test is based on the determination by chemical titration with 2, 6, dichlorophenolindophenol of the amount of vitamin C normally excreted in the urine; and the

response to a large test dose of pure cevitamic acid. Abbasy, Harris, Ray and Marrack (1935) arrived at an excretory value of about 10 mg. as representing the borderline between deficiency and adequacy, 20 mg. a moderately low intake and 40 mg. a liberal intake of the vitamin. In 1936, Harris, Abbasy and Yudkin concluded that if a subject excretes less than 13 mg. of ascorbic acid a day and fails to respond by a marked increase in excretion on the first or second day to a test dose of 700 mg. per 10 stone (140 pounds, 63.5 Kg.) of body weight, his diet has contained less than the reputed minimum-optimal quantity of vitamin C. Sendroy and Miller (1939) have shown where abnormally slow excretion of administered ascorbic acid does not necessarily indicate low ascorbic acid content of the body when renal function is impaired, because renal damage retards excretion, even where no ascorbic acid deficit exists. The effect of lowered kidney function on ascorbic acid clearance runs approximately parallel to the effect on urea clearance.

The capillary resistance test was first introduced by Hess and Fish in 1914 and popularized by Gothlin in 1933. This test consists in subjecting the capillaries and vessels of the arm to increased intravascular pressure, by means of an ordinary blood-pressure band, and observing whether this strain results in the escape of blood through the vessels -- the appearance of petechial hemorrhages into the skin. This test is widely used but is not specific for scorbutus.

RELATION OF VITAMIN C TO INFECTIONS

The availability of the concentrated form of vitamin C has made it possible for a number of investigators to determine its effect on infections. Long before this time, however, it was recognized that there was a relationship between scurvy and infections. Hess remarked in his book published in 1920 that:

> " * * * infection is the most important condition which may suddenly and precipitously induce scurvy * * * ."

Experimental evidence has been accumulating that there is an increased demand for vitamin C in infections.

In this connection, Faulkner and Taylor, (1937), estimated the serum ascorbic acid in a group of normal individuals, in patients with vitam C deficiency, and in patients with infectious diseases, and found that in those with infections the ascorbic acid titre reached levels far below the values seen in normal individuals and often reached figures encountered in manifest clinical scurvy.

Rinehart (1936), in discussing the concept that rheumatic fever may be due to the combined influence of vitamin C deficiency and infection states that, experimentally, lesions may develop in the heart and joints and not infrequently subcutaneous nodules occur. He further states that in experimental work, no sharp line can be drawn between a disease picture

resembling rheumatic fever and one characterized by a chronic joint disability with pathological similarities to atrophic (rheumatoid) arthritis. Rheumatic fever is a disease fundamentally characterized by widespread injury to collagen, (Swift, 1936). Thus, vitamin C may play a role in the etiology or progression of the disease as urinary excretion of the vitamin is depressed. Later in 1936, Rinehart, Greenberg, and Baker show that there is an increased demand for vitamin C in active rheumatoid arthritis and rheumatic fever.

In a paper published by Abbasy, Harris and Hill, (1936), results were given for a series of 193 patients suffering from juvenile rheumatism, and for 88 cases of surgical tuberculosis. It was shown that the infected patients had a marked deficit in the amount of ascorbic acid excreted in their urine as compared with 64 control subjects receiving the same dietary intake of vitamin C. The infected subject likewise gave a consistently diminished response to test doses of the vitamin. In a later paper, Abbasy and Harris (1937) presented a report on seventeen active cases of osteomyelitis, seventeen half-healed cases, sixteen healed cases and ten controls. The range of values for the excretions of vitamin C by the active cases was only about one half that of the control cases, that is, from 9 to 15 mg. daily as compared with from 20 to 40 mg. daily. Whereas the control cases were all

well above the minimal standard level of excretion, (20 mg. per day as concluded by Abbasy, Harris, Ray and Marrach, 1935), with an average of 26 mg. daily, 76 percent of the active cases were below the standard averaging 11.6 mg. daily. Healed cases were normal in their range of excretions and half-healed cases were intermediate. The control and healed cases reacted well on the first day to test doses of 700 mg. of ascorbic acid per 140 pounds (63.5 Kg.), while the active cases did not react until the second or third day. They concluded from this data that the degree of subnormality in the vitamin C titre goes roughly parallel with the severity of the infection and, correspondingly, the better healed cases show a more adequate output of vitamin C than those in the earlier stages of healing.

In the same journal appears a report of Abbasy and Harris in which they observed that a group of 25 patients with rheumatoid arthritis, who had been kept on a standardized diet with a normally adequate allowance of vitamin C, all fell below the standard in their excretion of the vitamin. A group of controls were all above this standard. Low excretions in this series were associated with high blood sedimentation rates. These results were considered as confirming the conception of the importance of an infective factor in rheumatoid arthritis.

These reviews have shown that vitamin C is consumed in greater amounts by the body during infections. The following

reports are indicative of the effect of maintaining normal blood levels and standard excretory levels of vitamin C.

Interest has been shown in connection with the functions of vitamin C in its effect on the toxin as well as the clinical course of diptheria. Kumagai, Yamagami, Nikai and Imai (1937) report remarkable effects from the intravenous injections of vitamin C in necrotic diptheria, which has been somewhat prevalent in continental Europe during the last decade. These workers administered vitamin C intravenously and the doses of l-ascorbic acid (Roche), which was the prepartion used, varied according to the severity of the morbid picture between 400 and 600 mg. daily. The effect appeared almost immediately after the injection, the characteristic fetor from the mouth being the first symptom to disappear. In a few days the appetite returned, diuresis increased, albuminura disappeared and the heart action improved progressively. They go so far as to express the belief that in severe cases in which it was used as late as two weeks after the onset, it was lifesaving. Moreover, since the introduction of this form of therapy, the mortality from necrotic diptheria in the hospital in which they work decreased from 50 to 70 per cent down to 30 per cent. It should be distinctly understood that the vitamin C in the form of ascorbic acid does not replace antitoxin, but it is used as an adjunct. It was used successfully in

this particular type of malignant diptheria, which previously had not yielded to antitoxin alone, so it is reasonable to assume that it will prove of equal value to supplement antitoxin in ordinary types of the disease.

In order to find the pharmacologic explanation of the effect, they made a systematic investigation of the protein and carbohydrate metabolism, establishing the following facts; In mild or benign diphtheria, the blood sugar values are as a rule normal, in croupous or severe necrotic diphtheria associated with dyspnea, they may increase to 120 to 150 mg. per cent. Also here, the figures accord with the severity of the case. After an injection of, for example, 600 mg. of ascorbic acid, they drop abruptly and, in the course of further injections and improvement, they return to normal.

Perhaps a better explanation may be found in the contention of Kligler and his associates, Leibowitz, and Berman, (1937), that ascorbic acid inactivates diphtheria toxin under both aerobic and anaerobic conditions at a rate depending on the concentration of the vitamin and the temperature of incubation, though the action on preformed toxin is slower than it is on the toxin during production.

In the study of thirty-seven cases of active tuberculosis patients, Bumbalo and Jetter, (1938) noted an average excretion of 5.7 mg. of ascorbic acid per twenty-four hours, using the dichlorophenolindophenol method. These patients had been on

a daily ration equivalent to 55 to 65 mg. of vitamin C. Twenty-five normal, healthy children on approximately the same diet average 29.2 mg. ascorbic acid excretions per twenty-four hours.

With this as a basis, these workers attempted to determine the approximate amount of synthetic vitamin C required to establish and maintain normal excretory levels of the vitamins in these tuberculous children.

In a controlled study of ten of these patients, the basic excretion on their regular diet average 6.4 mg. over a period of four days. Upon addition of fifty mg. of synthetic vitamin C daily over a period of fifteen days, an average urinary output of ten mg. per twenty-four hours of vitamin C was noted. On the twenty-fourth day, the average excretory level had risen to twenty-three mg. per day. Continued feeding of this ration for ten more days maintained this latter level. On discontinuance of vitamin C supplements, the basic levels returned to from six to seven mg. per twenty-four hours on the sixth day.

Ormerod and UnKauf (1937) reported a series of ten cases in which ascorbic acid was used in the treatment of whooping cough. In each case a diagnosis was made from a history of contact, typical cough, vomiting and nocturnal paroxysms. No cough plates or serologic tests were used as an aid in diagnosis in this investigation. In this series, they found that ascorbic

acid had a definite effect on shortening the period of paroxysms from a matter of weeks to a matter of days. In a later report, Ormerod, Unkauf, and White, (1937), increased the number of treated cases to nineteen, with equally favorable results.

Rinehart, Greenberg, Baker, Mettier, Bruckman, and Choy, (1938) found that the cevitamic acid level of the blood plasma during fasting is almost uniformly and severely lowered in rheumatoid and rheumatoid types of arthritis. In the majority of cases the blood level rises after the administration of vitamin C.

These writers have found that with a high intake of vitamin C, clearcut clinical improvement has occurred in the majority of cases. The only other form of treatment in these cases has been selective physical therapy. It is interesting that in the majority of instances of recurrence, the cevitamic acid content of the plasma was depressed. The most satisfactory clinical responses occurred in the cases in which there was a satisfactory rise of the cevitamic acid content. The results in a few cases in which daily intravenous administration of the sodium salt of cevitamic acid was combined with supplements of vitamin C by mouth were particularly encouraging.

These findings fit in well with the observations of other workers.

Marten and Heise's (1937) findings in a series of one hundred and fifty tuberculosis cases compared to fifteen

controls are in full accord with those of Bumbalo and Jetter in that he found hypovitaminosis C to be the rule with pulmonary tuberculosis. He also shows that the degree of unsaturation parallels the extent and activity of the tuberculous involvement.

Bullowa and Rothstein, (1936) demonstrated reduced excretion of cevatamic acid in penumonia.

Abbasy and Yudkin, (1936), obtained data demonstrating diminished excretion of ascorbic acid during all pyrexias and toxemias indicating loss in the body in these conditions.

SUMMARY

In review of the foregoing, we have seen that vitamin C is a very labile acid with very high reducing powers. Ascorbic acid, $C_6 H_8 O_6$, (Cevatamic Acid) is the active, specific, anti-scorbutic agent. It may constitute one of the oxidation-reduction systems of the body.

Determinations of ascorbic acid in the body tissues and fluids by chemical means are accurate with in range of biological error, however, due to individual variation in absorption and excretion, care must be excercised in interpreting the results thus obtained.

Vitamin C is not stored in the body in appreciable amounts, therefore the supply must be constantly renewed. The tissues of various organs show a definite selectivity to the vitamin which apparently varies with metabolic activity of the organ. The body may become saturated with the vitamin, beyond which point, excess amounts are excreted in the urine. With deficient intake the tissue concentrations and the urinary excretion of the vitamin are diminished.

The fundamental function of vitamin C in the organism is the control of the production and maintainence of normal intracellular materials of all tissues of mesenchymal origin. In scorbutic states, the lesions are dependent upon this loss of power to produce and maintain this substance. The location of the lesions and subsequent

symptoms are dependent upon the degree of depletion, the age of the individual (with regard to the higher metabolic activity in growth centers), and the mechanical stresses applied. All uncomplicated lesions may be reconciled to these fundamental facts.

The recent work of Ecker and his associates is changing the previously accepted view that Vitamin C was not concerned with the organisms defense mechanisms against bacterial infection. The relationship between cevatamic acid and the immune reactivity of the body is far from settled. Much work along these lines is to be expected in the near future.

Clinical evidence has been reported indicating increased body requirements for vitamin C during infection and febrile conditions. If for no other reason, diagnosis of subclinical hypovitaminosis C is important. It is in this respect that infections precipitate manifest scorbutic states when the dietary of the individual has been inadequate. A partial lack of vitamin leaves the organism apparently healthy, until it is taxed by some extraordinary exigency.

Reports have also been cited which indicates that adequate daily vitamin C intake greatly lessens the severity of infections and shortens the convalescent period. Such results appear logical in the light of the studies in physiology and pathology with regard to the control of the

intracellular substances of the organs of the body. However, we should be aware of the fact that the study of therapeutic usefulness of vitamin C is as yet in its infancy.

To evaluate the popularity of vitamin C as a therapeutic adjunct is difficult for we are dealing with a substance which was used for years before its fundamental properties were known. The "antiscorbutic principle" apparently went through a period of excess enthusiasm during the post world war era. Now, following the isolation and recognition of ascorbic acid, the substance has been the basis for numerous experiments, but its increase in use therapeutically has been more or less steady, though progressive.

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