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Vitamin C

Volume II

C. Alan B. Clemetson

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FOREWORD

While frank scurvy is rare nowadays, subclinical vitamin C deficiency is common and is now known to be associated with elevated blood histamine levels, which rapidly return to normal when ascorbic acid is administered. Epidemiological and experimental evidence suggests that our common metabolic defect, the inability to synthesize ascorbic acid from simple sugars, may be largely responsible for the development of subendothelial hemorrhage, thrombosis, atheroma, and degenerative vascular disease. This book is more concerned with factors affecting ascorbic acid metabolism, such as aging, smoking, infection, trauma, surgery, hormone administration, heavy metals, pregnancy, hemolysis, ionizing radiation, aspirin, alcohol, and other drugs which cause a disturbance of ascorbic acid metabolism and may thereby lead to vascular disease, than it is with simple dietary deficiency of ascorbic acid. The clinical, pathological, and chemical changes observed in ascorbic acid deficiency are discussed in detail; several diseases and disorders associated with abnormalities of ascorbic acid metabolism are described. Possible toxic effects resulting from the oxidation of ascorbic acid are noted, and reasons for the use of D-catechin or other chelating fiber to prevent or minimize the release of ascorbate free radical are detailed.

PREFACE

About 60 years ago, and before the isolation of ascorbic acid, Mme. L. Randoin (1923)* found the number of research studies on the antiscorbutic vitamin so great as to make it impossible for her to review them all.

"Jai maintenant à parler des recherches de toute nature faites sur le facteur antiscorbutique. La tâche est bien ingrate, car le nombre de ces recherches est si grand qu'il m'est évidemment impossible de les passer toutes en revue et, au surplus, elles présentent, par défaut de convergence, de telles lacunes, qu'il est vraiment difficile d'en donner une idée d'ensemble."

Now I must speak of all kinds of studies of the antiscorbutic factor It is a thankless task because the number of research studies is so great that it is clearly impossible to review them all; moreover, by failure of agreement, they present such gaps that it is truly difficult to present a consistent thesis.

Today, the profusion of the literature on this subject is even more overwhelming. It is growing so fast that it is impossible to do justice to all the work that had been done in this field. Moreover, having written 57 chapters in 36 months, it is inevitable that the chapters written first will not be as up-to-date as those written last.

Undoubtedly, some important works have been omitted, either because they have escaped my notice or because they were written in a language that I cannot read. Any workers whose contributions have been omitted must accept my assurance that it was not by intent.

It is hoped that this book presents a consistent thesis and that its main message is clear. It does not so much concern the amount of vitamin C in the diet, as it does the factors affecting ascorbic acid metabolism, the diseases that may result from abnormalities of ascorbic acid metabolism, and some suggestions as to what we may be able to do to prevent them.

Although the title of this book is *Vitamin C,* it could equally well have been entitled *Vitamin C, Heavy Metals, and Chelating Fiber.*

C. Alan B. Clemetson, M.D.
Pineville, Louisiana
February, 1987

THE AUTHOR

C. Alan B. Clemetson, M.D., was born in England. He attended the King's School, Canterbury, Magdalen College, Oxford, and Oxford University School of Medicine, graduating as a physician (B.M., B.Ch.) in 1948. He is an obstetrician and gynecologist, with fellowships in British, Canadian, and American colleges (F.R.C.O.G., F.R.C.S.C., and F.A.C.O.G.), but he has devoted most of his life to research and has published papers on many diverse subjects.

His career has included academic positions at London University, the University of Saskatchewan, the University of California at San Francisco, the State University of New York, and at Tulane University in Louisiana, where he is currently Professor at the School of Medicine.

He has challenged many conventional ideas and believes that, "certainty of knowledge is the antithesis of progress." Thus, every statement in this book is backed by reference to experiments and observations in the literature; contrary findings are cited, weighed, and given due credence.

VITAMIN C

Volume I

Volume II

Volume III

Chemical Changes Associated with Vitamin C Deficiency

Histamine Metabolism
Proline and Lysine Metabolism
Carbohydrate Metabolism
Folic Acid Metabolism
Cholesterol Metabolism
Tyrosine and Phenylalanine Metabolism
Tryptophan Metabolism
Adrenal Corticoid Metabolism
Uric Acid Clearance

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Habitual Abortion
Abruptio Placentae
Prematurity and Premature Rupture of the Fetal Membranes
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Clinical and Pathological Findings in Ascorbic Acid Deficiency

Chapter 1

VASCULAR CHANGES

I. CLINICAL SIGNS

Bleeding gums, swollen discolored interdental papillae, bruises, large ecchymoses, subperiosteal hematomata, and painful swollen joints are the classical signs of naturally occurring human scurvy. The same signs are seen in guinea pigs after 2 or 3 weeks on an ascorbic acid-deficient diet, but for some reason, these signs are very late to develop in experimentally produced human ascorbic acid deficiency. All are manifestations of "capillary fragility", which is supposedly the characteristic defect in scurvy; yet clinical examination of the ten volunteers of the Sheffield experiment described by Krebs (1953) revealed none of these signs during the first 17 weeks of total ascorbic acid deprivation. Moreover, no evidence of "capillary fragility" was detectable during that time. After 21 weeks, six of the ten deprived volunteers had developed hyperkeratosis and plugging of the hair follicles of the arms and legs; these enlarged hair follicles subsequently became red, developed petechial hemorrhages, and turned purple.

In fact, after 7 and 8 months of total ascorbic acid deprivation, when two volunteers developed precordial pain and electrocardiographic changes indicative of serious cardiac emergencies (Chapter 24, Volume III), multiple skin petechiae and acne were the main clinical manifestations of scurvy, and the general fitness of the other eight subjects still appeared to be fairly good. Perhaps ascorbic acid deficiency finds our weak spots; certainly the hearts seem to have been the weak spots in these two individuals.

The observations that scurvy does not seem to affect the gums of edentulous infants or old people, but does sooner or later affect the gums of those with teeth, suggest that focal sepsis arising from particles of decaying food between the tooth and the gum may precipitate local signs of scurvy. The foul mouth of scurvy is seen sooner in those with carious teeth and later in those with good dental hygiene.

While we know that classical scurvy can be cured with vitamin C, the evidence that it is usually caused by vitamin C deficiency alone is less obvious. Fox et al. (1940) found plasma ascorbic acid levels in a group of healthy South African miners to be just as low as in a group of miners with frank scurvy. Moreover, Prunty and Vass (1943) conducted urinary saturation tests and found that some nonscorbutic subjects had an ascorbic acid deficiency greater than that of a patient with scurvy. Could it be that a residual trace of ascorbic acid, if it is in the reduced form, can maintaian a normal redox potential in the tissues, but a residual trace of ascorbic acid all in the oxidized form means scurvy?

As so many early writers have noted, scurvy is often precipitated by infection or by other adverse conditions, many of which have been discussed in Volume 1. For instance, infants who are but mildly ascorbic acid deficient may quite rapidly develop scurvy as a result of an infection. A simple method of detecting the prescorbutic state would be very valuable, for petechial hemorrhages are so late to develop, and it seems to be the existence of preclinical scurvy over an extended period of time that predisposes to atherosclerosis and amyloid disease with all their attendant problems, such as coronary thrombosis, cerebral degeneration, and cerebral thrombosis or hemorrhage.

II. MICROVASCULAR FRAGILITY

There seems to be no question that the petechial hemorrhages and the ecchymoses in the skin and the periarticular and subperiosteal hemorrhages of scurvy are due to fragility of

the small blood vessels, actually the small venules. However, there does not seem to be any really reliable skin test for measuring what is variously known as capillary fragility, capillary strength, or capillary resistance which can be used to detect the prescorbutic state.

Rumpel (1909) suggested the idea of diagnosing scarlet fever by means of a capillary fragility test; a compression band placed around the upper arm caused the appearance of petechiae at the usual location of the scarlet fever rash, in the antecubital fossa. This test was further elaborated by Rumpel's assistant, Leede (1911), but there was no means of obtaining an exact pressure.

Hess (1913) and Hess and Fish (1914) introduced the idea of using a sphygmomanometer cuff and described the appearance of many petechiae in the forearm skin of children with scurvy after the application of 80 or 90 mmHg pressure to their upper arms for 3 min. This led to the development of the "Hess test" for capillary fragility, in which a cuff is applied to the upper arm at 100 mmHg for 5 min and a count is made of the number of petechial hemorrhages subsequently appearing in a circle of skin 2.5 cm in diameter in the antecubital fossa.

Stephan (1921) observed positive capillary fragility tests during menstruation and in many diseases besides scurvy; these included scarlet fever, measles, influenza, smallpox, and acute polyarthritis. Nevertheless, Öhnell (1928) found the Hess test to assist in the diagnosis of subclinical scurvy.

According to Göthlin, it was Boye (1929) who introduced the idea of inflating the sphygmomanometer cuff to a pressure midway between the systolic and diastolic arterial pressures, but Göthlin (1931) gave his reasons for preferring to use an infradiastolic pressure, thus avoiding any impairment of arterial blood flow, occluding only the venous return. He usually used a 50-mmHg pressure for 15 min and counted the petechiae subsequently developing in a 6-cm circle in the bend of the elbow (or 4 cm for children). Seven or more petechiae were considered as an abnormal result.

Using his test on a group of 50 children, 11 to 14 years of age, from the free municipal schools of Uppland in Sweden, Göthlin found abnormal capillary fragility in 11; test results of 9 of them were restored to normal within 3 weeks by the simple means of adding an orange a day to their diets. So it would seem that 18% of the children had been ascorbic acid deficient in April and May when this study was conducted. Studying a man aged 47 and a woman aged 38 in a mental institution, he showed that capillary fragility developed after 2 months on reduced ascorbic acid intakes provided by 0.2 and 0.4 cc of orange juice per kilogram of body weight per day, or 13 and 24 ml of orange juice a day, respectively, and that their capillary strengths returned to normal within a few weeks when 0.7 and 1.0 cc of orange juice per kilogram of body weight per day, or 45 and 61 ml of orange juice per day, were provided as minimum antiscorbutic doses.

Hess (1932) stated that, "there are decided individual variations in regard to the reaction to this test, so that although it is true that petechial spots are far more numerous in individuals suffering from latent or active scurvy, the reaction cannot be used as evidence of a deficiency in vitamin C intake." This is certainly true as thrombocytopenic purpura and acute infectious fevers, such as measles and scarlet fever, also cause capillary fragility; moreover, individual variation is a major factor affecting the results.

Dalldorf (1933) used a small suction cup for a negative pressure test. The cup had a 1-cm inside diameter and a broad lip to allow firm adhesion to the skin; it was applied to the outer surface of the arm at a set pressure for a period of 1 min; the resistance of the capillaries was expressed as the least negative pressure required to produce visible petechiae. Using this method, he estimated that 35 to 66% of children from poor homes in Valhalla, NY, had subclinical scurvy, but he stated that a "consistent feature of the measurements of capillary resistance has been the difference between persons." He observed that the character of the skin itself partly determines the results of the tests. Dalldorf and Russel (1935) studied

both old and young residents of a county home in Valhalla and found that those with capillary fragility all showed an increase in capillary resistance following intravenous injection of 100 mg of ascorbic acid and that this was maintained for several days. They offered these findings as evidence that subclinical scurvy was the most common cause of capillary fragility.

Wright and Lilienfeld (1936) described their own modification of the Göthlin test, requiring a tourniquet on the upper arm to be inflated to a pressure midway between the systolic and diastolic blood pressures for 15 min; petechiae were counted 5 min later in an outlined circular area 2.5 cm in diameter with its upper margin 4 cm below the elbow crease. A normal petechial count is rarely above ten. These authors wrote that, "No laboratory or physical finding has been nearly as helpful in following the clinical course of this group of patients (scorbutics) as the capillary fragility test standardized as described."

Using Dalldorf's suction cup method, Roberts et al. (1937) made 5 observations during 1 year on each of 85 children living in an institution near Chicago. The mean capillary strength fell from a high of 46 cmHg in August to a low of 33 cmHg in April; these values coincided closely with the vitamin C intakes of the children, but the authors could not prove a cause and effect relationship.

Göthlin (1937) described a modification of his infradiastolic positive pressure test in which an upper arm cuff is inflated, first to 35 mmHg for 15 min and then an hour later to 50 mmHg for 15 min; petechiae counted within a 6 cm circle in the antecubital fossa after the lower pressure test are scored as 2 each and all petechiae which appear only after the higher pressure test are scored as 1; the two scores are added together. Scores above 8, after inspection with a 5-diopter lens in daylight, are considered as indicating capillary fragility; if the count observed in healthy persons was decreased by 30% or more following 6 d of treatment with ascorbic acid, 300 mg daily, then Göthlin would diagnose the capillary fragility as having been due to vitamin C deficiency.

Bell et al. (1940) used the positive pressure test of Göthlin (1931); the petechial count was found to be affected by the area of skin selected and also on the intensity of the illumination used for inspection. Another problem, common to all positive pressure cuff tests, is that one cannot repeat the test on the same arm for a period of time; one must wait to be sure that all local vascular damage has had time to heal. Göthlin (1933) allowed 2 to 4 weeks between tests. Wright and Lilienfeld (1936) allowed 8 d, but Eddy (1972) has suggested that more than 6 months may be required for complete healing of petechial hemorrhages in elderly vitamin C-deficient individuals.

Negative pressure tests using a 2-cm suction cup, as in the Brown (1949) modification of the Dalldorf resistometer, have the advantage that one can carry out repeated tests on neighboring areas of skin using the thin skin of the inner aspect of the upper arm or the antecubital fossa. However, the results obtained are sometimes contradictory; one may obtain a positive test result at 10 cmHg followed by a negative result at 20 cmHg a few minutes later.

Lavollay and Sevestre (1945) found ascorbic acid to have a positive action on the capillary resistance of both scorbutic and normal guinea pigs, but many workers, including Abt et al. (1936), Farmer (1940, 1944), Crandon et al. (1940), and Rapaport et al. (1940), have found capillary fragility measurements to be unreliable as an index of vitamin C deficiency in human subjects. Reviewing the literature on this subject, Munro et al. (1947) noted that negative responses to capillary fragility tests have been observed even in scurvy.

Robson and Duthie (1952), using a negative pressure method to study the "capillary resistance" of patients with rheumatoid arthritis, ankylosing spondylitis, disseminated lupus erythematosus, periarteritis nodosa, idiopathic thrombocytopenic purpura, and hypopituitarism, observed that the skin capillary strength could be increased by the administration of adrenocorticotropic hormone (ACTH) or cortisone. In fact, there are reasons for believing that cortisone somehow increases the ascorbate to dehydroascorbate ratio (Figures 1 and 2,

Chapter 13, Volume I) and that this acts in compensation for ascorbate deficiency in scurvy (Figure 5, Chapter 8, Volume III).

Scarborough (1953), studying experimentally induced ascorbic acid deficiency in human volunteers, used the high-pressure, short-duration test of Hess (100 mmHg, 3 min) and the low-pressure, long-duration test of Göthlin (50 mmHg, 15 min), as well as a negative-pressure suction cup test. He found the Göthlin test to be more reliable than the Hess test. The results were more regular and followed more closely the negative-pressure values, but inexplicable fluctuations were noted with all methods. Parrot (1954) devised a simple, light, and portable apparatus for measuring capillary resistance and found it to be a useful clinical tool. It consisted of a pump which could be operated by one hand, connected to a combined ventouse and manometer for placement in the subclavicular region; he credited Hecht as the originator of the suction method in 1907.

Kramár et al. (1956) found that even minor and temporary emotional changes can cause arteriolar vasoconstriction, leading to a negative "capillary fragility" test result in someone who had shown a positive test a few minutes earlier. Thus, it seems that a negative "capillary fragility" test result may be unreliable and only positive results are meaningful.

Kramár (1962) found a good correlation between a pressure and a suction method for determining "capillary resistance" in a study of 132 healthy normotensive adults; he used a small 7-mm-diameter suction cup with an outside diameter of 13 mm at a set negative pressure for 1 min on the proximal volar aspect of the forearm. He modified the positive pressure method of Wright and Lilienfeld, marking out a 3-cm-diameter skin area and using a 5-min upper arm compression time, because the 15 min recommended was too uncomfortable. Positive results were obtained by the pressure method in all subjects whose capillary resistance values were less than 8 cmHg by the suction method. Moreover, the pressure method never yielded positive findings in any subject with capillary resistance values greater than 21 cmHg by the suction method (Figure 1).

Krasner and Dymock (1970) made a comparison of the positive pressure test of Hess (1913), a modification of the Dalldorf (1933) test, and the buffy layer ascorbic acid content as measured by the method of Denson and Bowers (1961). The overall correlation between the two tests of capillary resistance was good, although discrepant results were found in both the normal and the abnormal ranges, but neither of these tests showed any correlation with the ascorbic acid levels. Although 51 out of 111 subjects had subnormal buffy coat ascorbic acid (TAA)* levels (<18 $\mu g/10^8$ WBC) and 20 patients had levels below 8 $\mu g/10^8$ cells, there was no relationship between either test of "capillary resistance" and the ascorbic acid level in the leukocytes of these people.

However, Eddy (1972), in a reevaluation of the appendix by Scarborough (1953) to the report by Bartley et al. (1953) on the Sheffield study of experimental scurvy, observed that all but one of ten scorbutic young subjects showed abnormal results to Scarborough's Hess tests, even though the main report of the same investigation found no such relationship. Moreover, in a reevaluation of the original records of the double-blind controlled study reported by Griffiths et al. (1968), Griffiths (1968), and Taylor (1968), Eddy noted that the petechial counts in the Hess tests of the 40 elderly patients of the control group increased much more during the year than did those of the 40 patients receiving ascorbic acid, 200 mg daily, along with other vitamin supplements (Table 1). Nevertheless, Eddy concluded that "tests for capillary resistance appear to have a very limited value in the diagnosis of specific vitamin deficiency." Capillary permeability is also increased in scurvy, but it is even more difficult to measure, and estimations of it have been mostly restricted to animals.

The writer is therefore convinced that an automated method for estimating plasma ascorbic acid (AA)** and whole blood histamine levels needs to be developed, so that the fasting

* TAA—total ascorbic acid, reduced and oxidized forms.

** AA—ascorbic acid, reduced form.

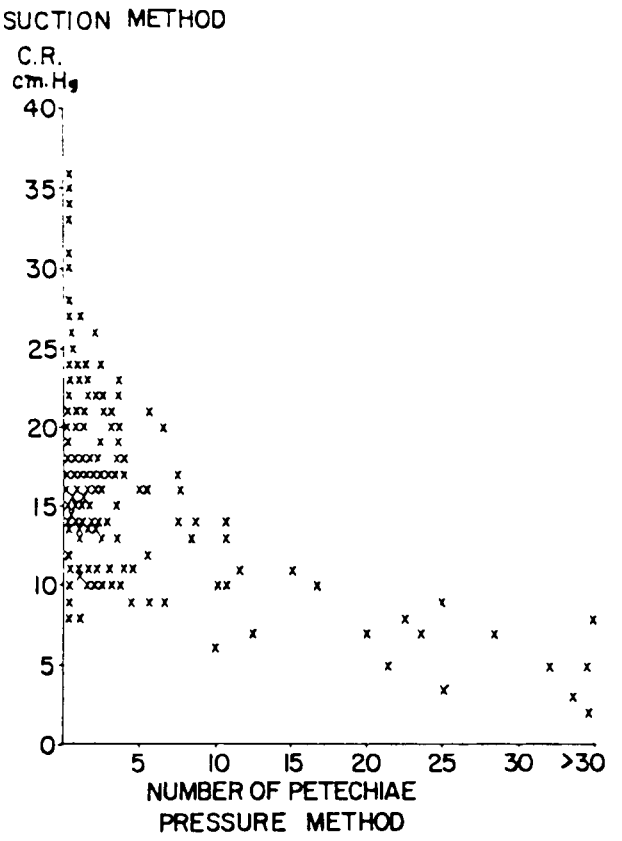

FIGURE 1. Comparison of the pressure and suction techniques for the determination of capillary resistance on 132 healthy normotensive adults of both sexes. Each plotted cross represents simultaneous results obtained by both methods. In the pressure method, the number of petechiae and in the suction method, the least degree of suction (in cmHg) required to elicit one or more petechiae served as the indictator. (From Kramár, J. [1962], *Blood*, 20, 83. With permission.)

levels of these blood components can become routinely available for all patients admitted to hospital. One learns much more from these blood levels than one ever would from a study of skin "capillary strength".

Actually what we may eventually need to measure is the ratio of reduced to oxidized ascorbic acid, for the integrity of the small blood vessels may well depend more on this ratio than on the actual quantity of ascorbic acid in the blood. Indeed, it may be the ability of healthy persons to utilize reduced glutathione, corticosteroids, and other systems to keep ascorbic acid in the reduced form that protects them from scurvy even when their ascorbic acid levels are low. Unfortunately, the techniques for accurate chemical estimation of very small quantities of ascorbic acid and dehydroascorbic acid do not yet exist, but electrical measurement of the redox potential of the blood is possible and polarographic methods of analysis will be further developed.

III. MICROVASCULAR PATHOLOGY

Weakness of the small blood vessels in the skin and other tissues is the most obvious manifestation of scurvy, but opinions seem to differ as to whether the primary defect lies

Table 1
MEAN VALUES WITH THEIR STANDARD ERRORS FOR LEUKOCYTE ASCORBIC ACID CONCENTRATIONS ($\mu g/10^8$ WBC) AND COUNTS OF CAPILLARY HEMORRHAGES IN HESS TESTS IN ELDERLY PATIENTS[a]

	Control group			Treated group		
Examination	No. of subjects	Ascorbic acid conc	Hess count	No. of subjects	Ascorbic acid conc	Hess count
Pilot study	31	—	6.1 ± 1.1	35	—	4.6 ± 0.9
1 (0 months)	40	15.2 ± 1.2	12.3 ± 1.2	39	15.5 ± 1.9	8.4 ± 1.3
2 (3 months)	35	19.9 ± 1.7	12.8 ± 1.5	39	43.5 ± 2.6	10.9 ± 1.2
3 (6 months)	33	27.9 ± 2.3	21.8 ± 2.0	34	56.8 ± 3.3	10.3 ± 1.2
4 (9 months)	22	24.6 ± 2.6	28.5 ± 2.8	24	53.8 ± 3.4	10.8 ± 2.4
5 (12 months)	27	26 6 ± 3.1	32.3 ± 2.1	31	54.7 ± 4.4	9.0 ± 2.0
After trial (18 months)	26	—	24.3 ± 2.8	22	—	12.0 ± 2.9

Note: There were 11 deaths in the control group and 7 deaths in the treated group between the first and fifth examinations.

[a] A reevaluation of the data of Griffiths et al. (1967).

From Eddy, T. P (1972), *Br. J. Nutr.*, 27, 537. Cambridge University Press. With permission.

in the cells of the vascular endothelium, the "intercellular cement", or the supporting tissues of the vessel wall.

Studying experimental scurvy in guinea pigs, Jackson and Moore (1916) noted thinning of the walls of some small veins, with the escape of red blood cells; they judged these lesions to be responsible for the hemorrhages seen in the muscles, around the joints, in the bone marrow at the ends of the diaphyses, in the tooth pulp, at the costochondral junctions, and occasionally in the skin and lymph glands.

Hess (1921) stated that the lesion of the lining of the blood vessels is one of the most characteristic signs of scurvy. He noted that "the coagulability of the blood is almost normal in this disorder, the escape of blood from the vessels being due to a weakening of their walls, or a lesion of the endothelial cells or their cement substance." Observations by Findlay (1921), in Edinburgh, revealed definite pathological changes in the capillaries and smaller venules of guinea pigs with scurvy. These were listed as follows: (1) degenerative changes in the lining endothelium; (2) extreme congestion; (3) fine edema of the tissues surrounding the vessels; and (4) hemorrhagic areas in close relation to the capillaries.

1. Degenerative changes were extremely common in the endothelial cells lining the capillaries. At many points the cells were found to be swollen and granular, while in frozen sections stained with Sudan III some of the cells showed small fat granules.
2. Congestion of the capillaries and smaller venules was a marked feature in all organs. The degree of congestion was often such as to lead to considerable dilation of the capillaries, a condition specially noticeable in the sinusoids of the liver and in the intertubular vessels of the kidney.
3. In guinea-pigs dying from scurvy there was almost always noticeable a degree of fine oedema in the connective tissue in relation to the capillaries. [This condition was especially well seen in the lungs, the kidneys, and in the heart.] The oedema would appear to be due to increased transudation of fluid through the damaged capillary wall.
4. In many sections there was noted an appearance which strongly suggested that the red blood corpuscles were escaping by diapedesis through the intercellular cement substance.

Findlay cited evidence that the intercellular substance is produced by the endothelial cells;

he suggested that degenerative processes affecting these cells could also interfere with the active formation and repair of the intercellular substance and could therefore be responsible for its increased permeability.

Höjer (1924) confirmed Jackson and Moore's observation that the vascular wall seemed to be thin both in the arteries and in the veins of guinea pigs with scurvy. He attributed this weakness to a considerable atrophy of collagen and stated that, "As collagen fibrils partly constitute the walls of even very small vessels and are likely essential to give them their firmness, this partial disappearance of the collagen fibrils must be considered an important factor in the pathogenesis of the haemorrhages."

"Besides the direct weakening of the vascular wall in the manner indicated, a certain influence ought perhaps also to be ascribed to the general atrophy with shrinking of the organ cells under the influence of normal connective-tissue reaction. Through this organ atrophy, the vessels will lack the outer support for their atrophic wall, without which the pressure from the inside must have a still greater effect."

Wolbach and Howe (1926) characterized "scorbutus as inability of the supporting tissues to produce and maintain intercellular substances;" they suggested that this included the failure of the endothelial cells to form cement substance, and advanced the theory that, "the failure of cells to produce intercellular substance in scorbutus is due to the absence of an agent common to all supporting tissues which is responsible for the setting or jelling of a liquid product."

The basic pathology of scurvy was summarized by Perla and Marmorston (1937) as follows. "In scurvy, there is an inability of the supporting structures to produce and maintain intercellular substances. Direct proof of this conclusion has been obtained in the study of teeth in regard to the dentin, in the study of growth and repair of bone in regard to the bone matrix and in the study of repair of soft tissue in regard to the collagen of connective tissues."

"The failure of capillary formation is due to the failure of endothelial cells to form cement substances. The proliferative power of epidermis, endothelium, fibroblasts and osteoblasts is not diminished in scorbutus. The osteoblasts, in spite of morphologic change, preserve their chemical potentialities and produce a bone matrix. The odontoblasts continue to produce a liquid material which lacks jelling properties. A failure of the cement substance in blood vessels accounts for the hemorrhage."

Dalldorf (1938) concurred in the opinion that fibroblasts lose the ability to form collagen in scurvy and noted that collagen bundles reappear within 18 h when the vitamin deficiency is satisfied. However, discussing microvascular fragility, he stated, "Whether the effect of the deficiency is on the sheath or on the endothelial cement substance has never been settled."

Chambers and Zweifach (1940), studying the interendothelial cement substance of the capillaries in the mesentery of the frog, noted that, "the inter-endothelial lines can be stained, under viable conditions, by spraying 10% Ag NO_3 on the outer surface of the capillaries with a micropipette." It was noted that the blackened substance of the cement was gradually washed away and was replaced by fresh cement. As a result of many observations under different conditions, these workers reached the conclusion that, "an important function of the endothelial cell is the continual secretion of an intercellular cement, the chemical stability of which controls the permeability of the blood capillary." Wolbach and Bessey (1942) agreed with Dalldorf and stated that, "the capillary bleeding so common in scurvy is probably the result of structural weakness, either the result of changes in the cement substance binding the endothelial cells together, or in collagen fibrils immediately adjacent to the capillaries."

Chambers and Cameron (1943), studying epithelial sheets from guinea pig and chick embryos in cell culture, noted that the cells would float apart in a calcium-free medium and would readhere to one another when calcium was added to the medium. They found that ascorbic acid promoted cell proliferation, but was not essential for cell-cell adhesion; while

conceding that ascorbic acid is essential for the formation and maintenance of collagen, they interpreted their own findings as indicating that ascorbic acid was not needed for the formation and integrity of the mucopolysaccharides of the intercellular cement.

In further studies of the small blood vessels of the mesentery of the frog, Chambers and Zweifach (1947) obtained evidence for the presence of mucopolysaccharides in the connective tissue sheath of the capillaries and also evidence that they are essential to the strength of these vessels; they applied hyaluronidase to the surface of the capillaries and noted the abrupt development of microscopic petechial hemorrhages: these occurred through spots in the wall weakened by the softening of the supporting connective tissue sheath and were therefore easily ruptured by the internal blood pressure.

Lee and Lee (1947), studying guinea pigs, observed that, "an especially prominent feature of the scorbutic state was the dilation and sluggish flow observed in the small collecting venules." Penney and Balfour (1949), studying wound healing in guinea pigs, observed the production of mucopolysaccharide surrounding the capillaries of normal animals. There was a failure of the production of this substance in vitamain C-deficient guinea pigs, but mucopolysaccharide appeared within 12 h after the administration of ascorbic acid; it was often associated with fine reticulin fibers which came to surround the growing vascular buds. The mucopolysaccharides appeared to correspond very closely to the "amorphous collagen" of Wolbach.

Gersh and Catchpole (1949) observed condensations of glycoprotein forming the basement membrane beneath epithelial surfaces and also surrounding capillaries and venules. They studied guinea pigs with scurvy and concluded that there was depolymerization of these glycoproteins, or mucopolysaccharides in scurvy, and that this weakened the vessels and caused the tendency to hemorrhage. Reppert et al. (1951) demonstrated that ascorbic acid inhibits the hyaluronic acid-hyaluronidase reaction, both *in vivo* and *in vitro;* this inhibition certainly supports the idea that hyaluronic acid might be destroyed, or depolymerized, in scurvy and that this could account for the decreased strength of the capillaries and venules.

Zweifach (1955) described five components of the capillary wall as follows:

1. An adsorbed layer of protein lining the inner surface (presumably a plasma-constituent and/or platelets enmeshed in the pores of the intercellular cement)
2. The endothelial cells
3. The intercellular cement substance — probably a calcium proteinate — which is hardened by calcium and dissolved by trypsin
4. A condensation of fine tissue fibrils enmeshed in a dense amorphous ground substance, referred to as the pericapillary sheath, which is sensitive to hyaluronidase
5. A layer of connective tissue about 25 to 50 μm in depth

Zweifach noted that the perivascular sheath was deficient in scurvy.

Successive studies have gradually added to our knowlege of capillary and venular structure. Curran (1957) provided evidence that the mucopolysaccharides of the capillary sheath are secreted by the vascular endothelial cells. Marchesi and Florey (1960), in an electron microscopic study of the endothelium of venules in the rat mesentery, gave clear illustrations of the electron-dense zones, known as "attachment belts" or "adhesion plates", at the luminal ends of the junctions between the endothelial cells, but they saw no evidence of a sticky cement substance. Electron microscopic studies by Peach (1962) led him to conclude that the primary defect in scurvy lies in the fibroblasts which are incapable of manufacturing striated collagen. Other observers have noted that existing collagen also becomes weakened in many tissues in scurvy. Movat and Fernando (1964) observed that collagen fibers are numerous around venules in normal tissues, so it seems likely that the weakness of the venular walls in scurvy may be due in part to weakness of the perivascular collagen.

Friederici et al. (1966) wrote an extensive review of the literature concerning microvascular hemorrhage in scurvy and conducted electron microscopic studies of the capillary endothelium in the skin and muscle and in the synovial membrane of the joints of scorbutic guinea pigs. They found significant alterations in the ultrastructure of the capillary endothelial cells and the pericytes and suggested that defective secretion of intercellular mucopolysaccharides and perivascular collagen by these cells might account for the capillary or venular fragility. The intercellular junctions were possibly less well aligned than normal and sometimes appeared less tight.

While hydroxyproline is a major component of collagen, it is also a minor component of basement membrane protein (or mucoprotein). Priest (1970) measured the incorporation of tritium-labeled proline into hydroxyproline in the protein of the basement membrane produced by cells in culture. Hydroxyproline incorporation was found to be stimulated by ascorbic acid and to be restricted by ascorbic acid deficiency; this provided evidence in support of earlier suggestions that the hemorrhages of scurvy may be due to defective synthesis of basement membrane.

IV. HISTAMINE EFFECTS

Now that it has been established that the blood histamaine level is elevated in ascorbic acid deficiency, both in guinea pigs and in human beings (Chapter 1, Volume III), it is appropriate to consider the effects of histamine intoxication as part of the pathology of scurvy. Studying the effects of histamine on the arms of human subjects before there was any knowledge of the histaminemia of scurvy, Stead and Warren (1944) demonstrated that intraarterial injection of histamine increased the permeability of the capillaries supplied by the artery. The rapid loss of protein from the plasma was detected by comparing the blood draining the part before and after injection. The hemoglobin concentration and the hematocrit reading increased markedly, while the protein concentration rose only slightly.

Majno et al. (1961) acknowledged that histamine and serotonin increase the permeability of blood vessels and showed by electron microscopic studies that this was achieved by separation of the endothelial cells, so that discrete intercellular gaps were formed. They found that plasma escapes through these gaps and filters through the basement membrane. Studying the effect of histamine on rats whose plasma had been labeled with black carbon or mercuric sulfide particles, too large to pass through the basement membrane, they observed the black particles accumulating within the walls of the leaking vessels. In this way they were able to establish that histamine and serotonin caused leakage in small blood vessels which always belonged to the venous side of the circulation. The heaviest deposits were found in venules 20 to 30 μm in diameter. The deposits decreased toward the larger venules up to a maximum diameter of 75 to 80 μm and toward the finest vessels, 4 to 7 μm in diameter.

Studying inflammation, Marchesi (1964) noted that first the leukocytes and platelets adhered to the inner surface of the endothelium; then, not only the leukocytes, but also some erythrocytes traversed the intercellular junctions. However, the few erythrocytes usually remained within the periendothelial space beneath the perivascular sheath. It was suggested that the red cells passed between the endothelial cells through openings made by preceding leukocytes in inflammation; but in scurvy, presumably, the histaminemia causes the intercellular junctions to open wide and, no doubt, the weakness of the collagen of the perivascular sheath allows the red cells to proceed through the sheaths of the venules, causing hemorrhage in the tissues or in the subendothelial layers of the larger blood vessels.

Clearly, there are important similarities between histamine intoxication and scurvy, for both conditions affect primarily the venules and involve widening of the spaces between the endothelial cells, but the microvascular changes of scurvy are more extensive. Scurvy involves weakness of the basement membrane beneath the endothelial cells and also weakness

Table 2
SUMMARY OF OBSERVATIONS MADE BY MICROSCOPIC EXAMINATION OF SMALL BLOOD VESSELS IN THE MESENTERY OF NORMAL AND SCORBUTIC GUINEA PIGS DURING LIFE

Observation	Control animals	Scorbutic animals
Epinephrine sensitivity of larger arterioles[a] (100 μm in diameter)	1:500,000 (1:100,000—1:5,000,000)	1:450,000 (1:100,000—1:4,000,000)
Epinephrine sensitivity of smaller arterioles[a] (75 μm in diameter)	1:1,000,000 (1:300,000—1:5,000,000)	No responses ever noted using 1:100,000
Epinephrine sensitivity of precapillary region[b]	1:2,000,000 (1:500,000—1:35,000,000)	No response ever noted using 1:100,000
Epinephrine sensitivity of small venules[b] (75 μm in diameter)	1:500,000 (4 animals)	No response noted using 1:100,000 (3 animals)
Capillary external diameter (μm)	7.0—10.5	7.0—11.0
Presence of vasomotor activity in arterioles and precapillaries	+ − + + + +; usually in "closed" phase	None observed: precapillaries usually opened widely
General nature of blood flow in the arterioles, capillaries, and venules	Rapid, varying with vasomotion; vessels "tonic"	Sluggish, vessels usually dilated, especially in small collecting venules
Presence of petechiae in small venules following trauma	3 in 2 of 20 animals	Present in 11 of 23 animals; numerous

Note: The epinephrine studies were made by direct application of this substance to the tissue under observation.

[a] Epinephrine concentration necessary to produce narrowing to approximately 50% of internal diameter.
[b] Epinephrine concentration necessary to produce complete closure of the vessel at this site.

From Lee, R. E. (1961), *Ann. N.Y. Acad. Sci.*, 92, 295. With permission.

of the perivascular sheath due to absence of mature collagen, and these changes are not produced by histamine intoxication.

V. PERIPHERAL VASODILATION, CONGESTION, AND STASIS

Lee et al. (1955) studied the arterioles, capillaries, and venules of the mesentery in living guinea pigs by low-power microscopy and found that vitamin C deficiency was associated with dilation of all these vessels and with reduced velocity of blood flow throughout the capillary bed. Moreover, the arterioles and precapillary sphincters showed decreased responsiveness to epinephrine and an almost complete absence of the splanchnic vasomotor response to fright. Increased venular fragility was noted as the cause of petechial hemorrhages in scurvy when the vessels of the mesentery were traumatized by stroking with a camel-hair brush; there was no evidence of increased arteriolar or capillary fragility.

VI. LOSS OF VASOCONSTRICTOR RESPONSE

Lee (1961) confirmed his earlier findings concerning the nonresponsiveness of the smaller arterioles, capillaries, and venules of scorbutic guinea pigs to the direct application of epinephrine (Table 2) and also demonstrated the consequent inability of scorbutic guinea pigs to compensate for blood loss. Figure 2 shows the relationship between the total volume of blood removed from vitamin C-deficient and vitamin C-supplemented guinea pigs and the duration of hypotension they were able to withstand before death. "It is evident that avitaminosis C is associated with a prominent reduction in the ability of the animal to withstand blood loss or to survive hypotension in a manner that is encountered in the control animal."

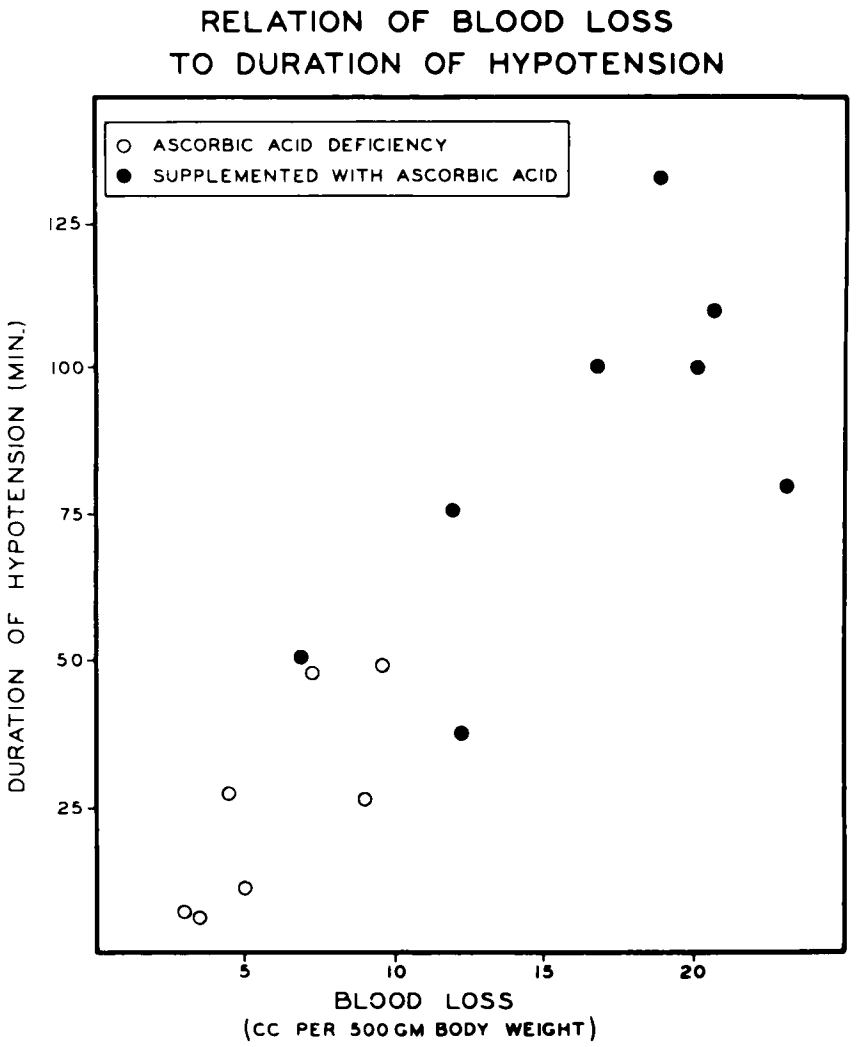

FIGURE 2. The relationship between blood loss and survival during hypotension of normal and vitamin C-deficient guinea pigs. (From Lee, R. E. [1961], *Ann. N.Y. Acad. Sci.*, 92, 295. With permission.)

In contrast, Thoa and Booker (1963) reported that the hypertensive response and the myocardial contractile response to norepinephrine were increased and the responses to dopamine were decreased in scorbutic guinea pigs. These observations are of considerable interest because the works of Goldstein et al. (1965) and of Friedman and Kaufman (1965) showed that ascorbic acid is necessary for the beta-hydroxylation of dopamine to form norepinephrine; they suggested that the cardiac muscle and the arterioles are more sensitive to the pressor amine that is wanting.

Thoa et al. (1966) confirmed the earlier finding that scorbutic guinea pigs were hyperresponsive to injected norepinephrine, but the hearts of scorbutic guinea pigs were found to have a significantly lower level of endogenous norepinephrine (0.90 ± 17 μg per heart) than did those of normal guinea pigs (2.09 ± 19 μg per heart, $p < 0.01$).In studies using tritiated norepinephrine, they observed a 20% reduction in the ability to take up and bind norepinephrine in the hearts of scorbutic guinea pigs. They suggested that the hypersensitivity to norepinephrine was likely due in part to a reduced ability to inactivate catecholamines

by uptake and binding, but it now seems more likely that there is actually a decreased production of norepinephrine (Chapter 6, Volume III).

Bhagat et al. (1966) found isolated atria from scorbutic guinea pigs to be normal in their response to norepinephrine, but confirmed the suggestion by Thoa et al. (1966) concerning reduced retention of norepinephrine by the cardiac muscle in scurvy.

Electron microscopic studies by Sulkin and Sulkin (1967) revealed degenerative changes in the autonomic ganglia of scorbutic guinea pigs. Studies of five human subjects with borderline scurvy after 3 months on an ascorbic acid-free diet and 3 to 10 d of modest repletion were reported by Abboud et al. (1970). They concluded that there was decreased vascular responsiveness to intraarterial norepinephrine and tyramine and to lower body negative pressure during ascorbic acid deficiency, and that this was caused by a defect in the ability of resistance vessels to constrict in response to adrenergic stimuli.

VII. ARTERIAL AND VENOUS PATHOLOGY

Martin (1942) studied the capillary strength of 25 pregnant women with varicose veins and of 25 pregnant women without varicosities and found a strong association between varicose veins and ascorbic acid-responsive capillary fragility. He postulated that normal veins can withstand the strain of increased venous pressure, but the collagen-poor veins of people with chronic subclinical ascorbate deficiency are weak and dilate under stress so that their valves may become incompetent.

Studies of sponge-implanted guinea pigs by Stolman et al. (1961) revealed profound changes in the arteries and veins of scorbutic guinea pigs in areas distant from the implants. "Both arteries and adjoining veins had a significant moth-eaten type of disintegration of many portions of their adventitial layers, which appeared to represent resorption of the interfibrillar substance. This left loose collagen fibers floating almost freely in the fluid-soaked perivascular areas. The endothelial cells were sometimes enlarged and protruded into the lumens of the vessels. There was a notable absence of organized connective tissue in the perivascular areas, with a fluid-soaked ring-like appearance persisting around the vessels. Silver staining of serial sections of similar specimens also showed disorganization of the reticulin-like fibers in the walls of the arteries and veins, especially in the adventitial layers. Examination of sections stained for elastic tissue from similarly depleted specimens revealed discrete elastic fibers that were also floating free in the adventitial and perivascular areas" (Figures 3 and 4). In the control animals on a full diet, the connective tissue of the vessel walls and the perivascular areas was compact and organized and the cellular components were well oriented.

"The predominant defect that occurred in blood vessels under conditions of ascorbic acid depletion was a breakdown or degeneration of the connective tissue within the vessel walls and perivascular areas. This occurred at first as edema of the vessel walls with separation of the collagen bundles, individual fibers, fibroblasts, and smooth-muscle cells. The fibroblasts went through stages of cellular edema followed by cellular shrinkage and retraction of their fibrillar processes. The smooth-muscle cells lost their spindle shapes and became tortuous, as if they were no longer being held in a state of tension. The endothelial membranes in later stages also became edematous and separated from the vessel wall proper. These characteristic changes were seen in vessels of all sizes following ascorbic acid depletion."

VIII. AORTIC ENDOTHELIAL PATHOLOGY

The atherosclerotic changes observed by Willis (1953) in the aortas of guinea pigs on a chronic scorbutic diet and especially in those on a scorbutic diet with added cholesterol are described in Chapter 8 of this volume, which is devoted to atherosclerosis, and are shown

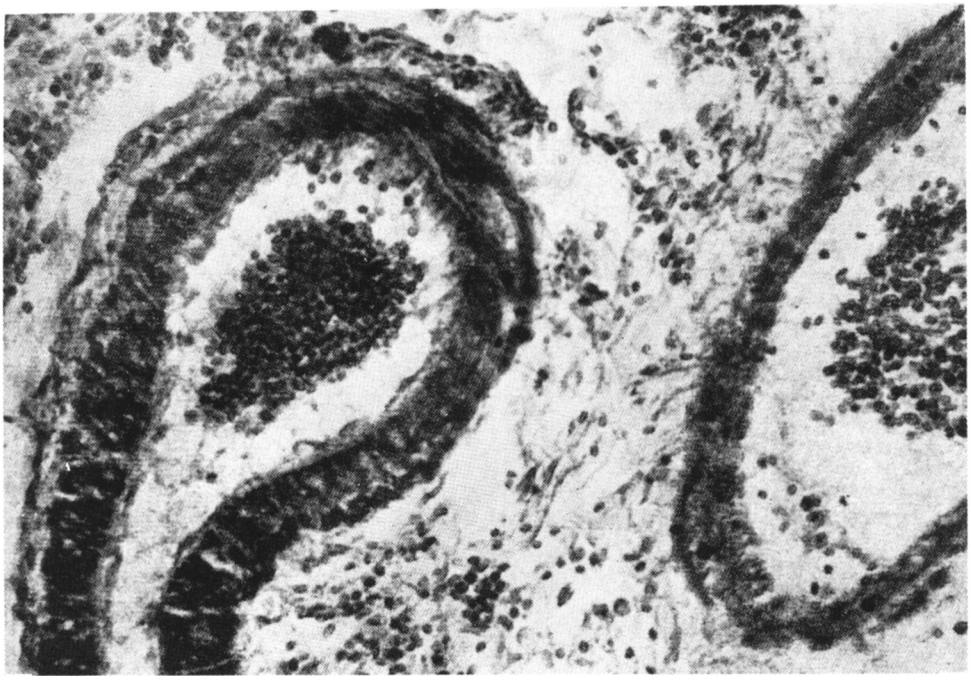

FIGURE 3 Section of an artery (to the left) and a vein (to the right) in tissue from a guinea pig after 20 d of ascorbic acid deprivation Note the breakdown of the vessel walls, especially the adventitial layer, vacuolation of the arterial wall, and lack of connective tissue in perivascular areas (Mallory-Heidenhain connective tissue stain) (From Stolman, J M., Goldman, H. M., and Gould, B. [1961], *Arch Pathol* , 72, 535. ©1961 American Medical Association. With permission)

in Table 1 of that same chapter, so the reader is referred to that section for details of these important observations. Similarly, the important works of Ginter et al. (1969) and of Sulkin and Sulkin (1975) will be found in Chapter 8 of this volume.

In order to obtain a face view of the endothelium, Gore, et al. (1965a) studied "en face" or "hautchen" preparations of the aortic endothelium from normal and from ascorbic acid-deficient guinea pigs. After staining with silver nitrate, there was uniformly intense black silver-staining of the junction lines in the normal animals. In contrast, the cell junctions stained faintly and irregularly in the ascorbic acid-deficient animals (Figure 5).

"It was noted that a small proportion of endothelial cells in normal animals, and a somewhat greater number in C-deficient animals, displayed swollen nuclei more than twice normal size. Occasionally such giant nuclei were distorted and misshapen, but mitoses were not observed. Intranuclear vacuoles were several times as frequent in scorbutic animals as in controls. At times the vacuoles, which stained neither for fat, glycogen, nor acid mucopolysaccharides, communicated with the cytoplasm leaving a pyknotic nucleus" (Table 3).

Electron microscopy of the normal aortic endothelium revealed that, "adjoining endothelial cells were closely opposed; often a short finger-like process originating from the margin of one cell covered the junction and part of the surface of its neighbor. There was not a distinct basement membrane. A rather wide space separating the endothelium from the internal elastic lamina contained small numbers of collagen fibers [Figure 6]. In the scorbutic animal, endothelial cells displayed decreased numbers of ribosomes, both free and attached. Nuclear deoxyribonucleic acid (DNA) was visibly reduced in some cells. Loosening and occasional gapping of the intercellular junctions could be seen as well as a distinct depletion of collagen between the endothelium and the internal elastic lamina" (Figure 7).

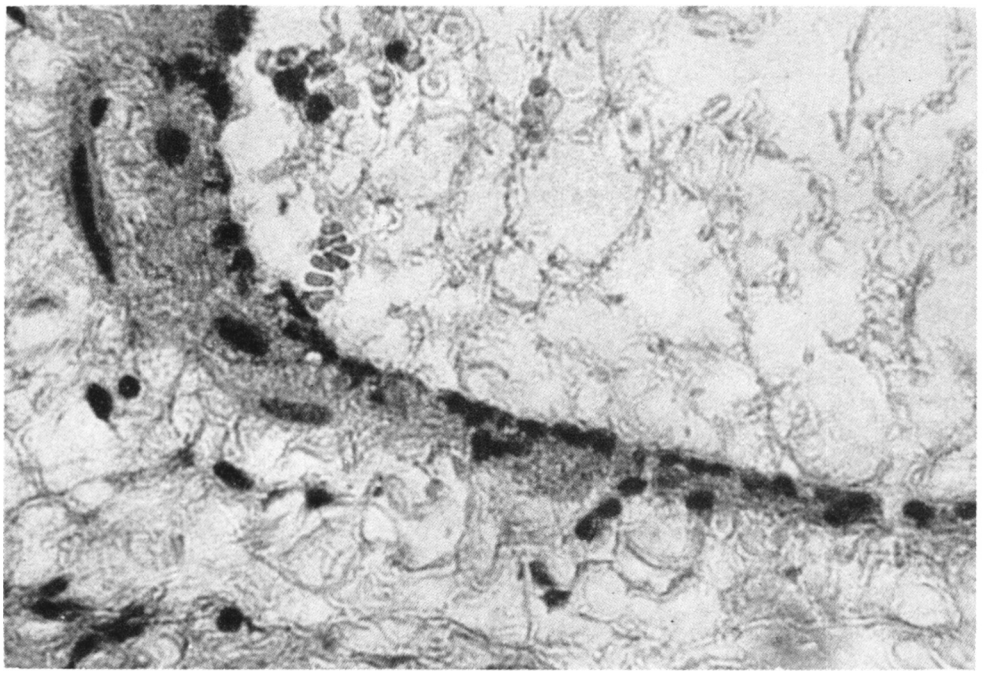

FIGURE 4 Section from the same scorbutic animal as in Figure 3, showing the irregularity of the shape and orientation of cells in the vein wall and the decrease in perivascular tissue. (From Stolman, J M., Goldman, H. M , and Gould, B. [1961], *Arch. Pathol* , 72, 535 ©1961 American Medical Association. With permission)

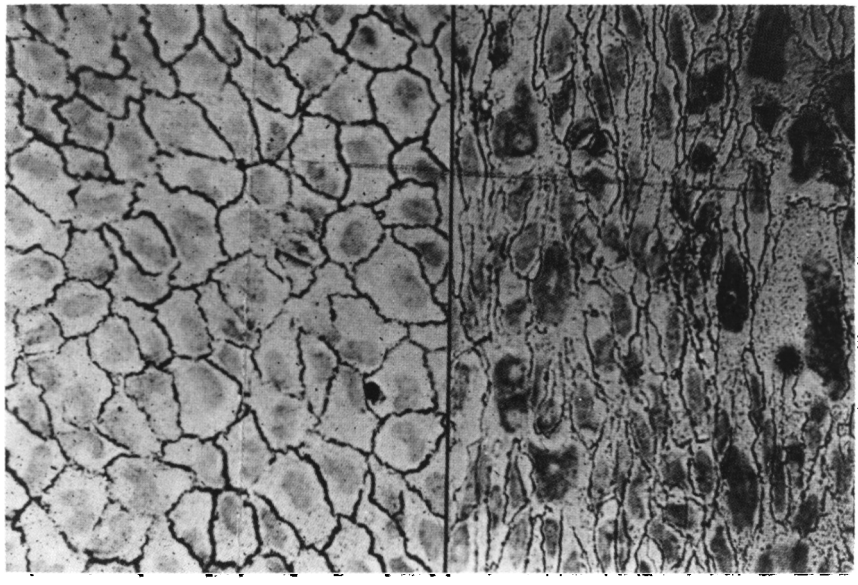

FIGURE 5 *En face* preparations of the endothelium of normal and scorbutic guinea pigs The junction lines between the cells are well stained by silver nitrate in the normal tissue on the left, while the cell junctions stain faintly and irregularly in scurvy, as seen on the right (From Gore, I , Fujinami, T., and Shirahama, T [1965a], *Arch. Pathol* , 80, 371 ©1965 American Medical Association. With permission.)

Table 3
QUANTITATIVE ESTIMATIONS OF THE SILVER NITRATE STAINABILITY OF THE INTERCELLULAR CEMENT LINES OF THE ENDOTHELIUM FROM THE ARCH, THE THORACIC, AND THE ABDOMINAL PORTIONS OF THE AORTAS OF NORMAL AND SCORBUTIC GUINEA PIGS

Endothelial Changes in Ascorbic Acid Deficiency

Group	Guinea pigs	Ascorbic acid level (mg/100 cc)	Cement line stainability[a]			Nuclear abnormalities		
			Arch	Thoracic	Abdominal	Pyknosis	Vacuoles	Enlargement
Control 3 weeks	8	0.66	2.0	2.5	2.5	0.16	0.46	0.14
Scurvy 3 weeks	4	0.2	1.4	1.3	1.3	0.16	3.11	0.49
Scurvy 4 weeks	4	0.2	2.0	1.1	1.1	0.34	2.67	0.75
Chronic scurvy[b]	4	0.2	0.5	1.3	1.8	0.35	1.07	0.21

Note: Nuclear abnormalities are also recorded.

[a] Scored from 1 to 3+: a value of 0.5 was assigned to very faint lines.

[b] Scorbutogenic diet for 6 weeks interrupted during the third and fourth weeks by the addition of ascorbic acid to the deficient diet.

From Gore, I., Fujinami, T., and Shirahama, T. (1965a), *Arch. Pathol.*, 80, 371 ©1965 American Medical Association. With permission.

These authors concluded that, "endothelial cell disjunction must be the essential structural basis for the occurrence of hemorrhage in scurvy." Loss of the fine perivascular web of collagen may also cause weakening of the small venules, which become so markedly dilated and fragile in scurvy.

Gore et al. (1965a), noting that Majno and Palade (1961) had observed a similar widening of the intercellular junctions as the result of histamine administration and that Marchesi (1964) had observed emigration of erythrocytes and leukocytes from venules through widened intercellular junctions in the traumatized mesentery of the rat, commented that, "the prompt and reversible production of the same change by pharmaceutical stimuli or by inflammation clearly must have some other explanation." Indeed it does, for we now know that histamine accumulates when ascorbic acid levels are low.

Kishikawa et al. (1971) confirmed the widening of the endothelial cell junctions in scurvy in a similar study of "en face" preparations of the intima of the aorta of scorbutic and normal guinea pigs.

Scanning electron photomicrographs of the aortic endothelium of normal guinea pigs and of guinea pigs at progressive stages of ascorbic acid deficiency have been taken by Fujinami (1980). In the normal guinea pig, the cells of the endothelium are seen to be closely packed together to form an intact quilt-like surface, but first wrinkles, then desquamation of the cells, and finally disruption of the endothelial barrier are seen to occur at progressive stages of ascorbic acid deficiency.

IX. AORTIC WALL CHEMISTRY

Chemical analyses of guinea pig aortas, from which the adventitia had been removed, were reported by Gore et al. (1965b). The hydroxyproline content of ascorbic acid-deficient aortas (25 mg/g) was found to be significantly lower than normal (34 mg/g, $p < 0.05$) (Figure 8). The hyaluronic acid content was significantly increased ($p < 0.05$) and the chondroitin sulfate B level was significantly reduced in scurvy ($p < 0.05$).

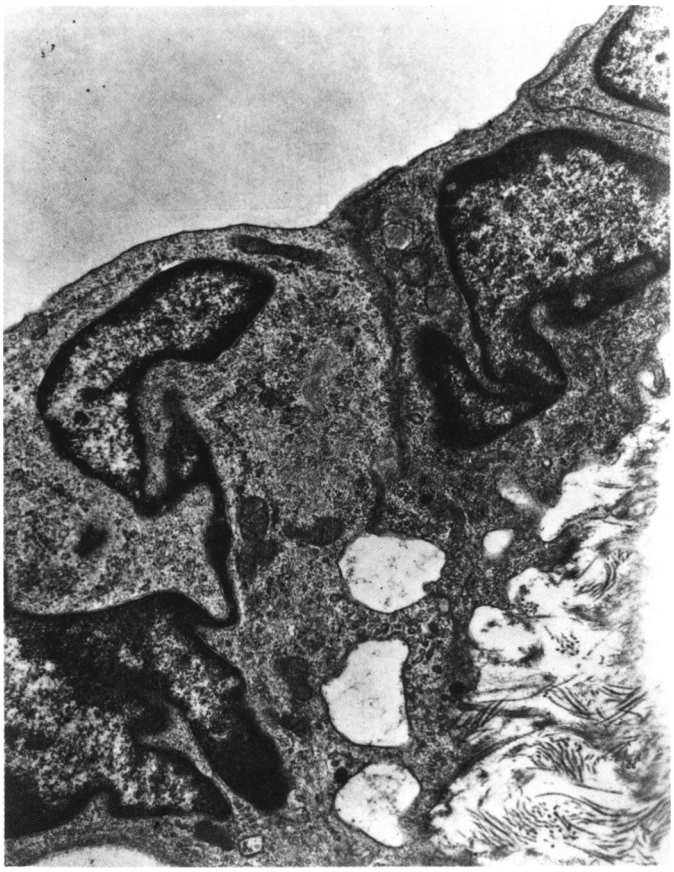

FIGURE 6 Electron micrograph of normal guinea pig endothelium Observe the narrow intercellular junction at the upper right, the finger-like overlapping cell process, and the meshwork of collagen fibers between the endothelium and the unstained internal elastic lamina at the right. (From Gore, I., Fujinami, T , and Shirahama, T. [1965a], *Arch. Pathol* , 80, 371 ©1965 American Medical Association. With permission)

Barnes (1975) studied the incorporation of ^{3}H-proline into hydroxyproline in the aortas of normal and scorbutic guinea pigs and observed a very marked increase in the proline to hydroxyproline ratio of the elastin from 1.5:1 to 20.3:1 after 12 d of ascorbic acid deficiency, but no change in the proline to hydroxyproline ratio in the collagen. Higuchi et al. (1975) observed a decrease of aortic collagen, measured as hydroxyproline, in scurvy, but Ginter and Bobek (1981) failed to observe any significant change in the total hydroxyproline concentration in the aorta in long-term marginal vitamin C deficiency. The most significant chemical changes noted by the latter workers were the accumulation of cholesterol and triglycerides in the arteries and in the liver in long-term marginal vitamin C deficiency, as discussed in Chapters 8 and 13 of this volume and in Chapter 14, Volume III.

X. SUMMARY AND CONCLUSION

It seems that the histaminemia of scurvy causes widening of the gaps between the endothelial cells of the capillaries and venules, which is responsible for the increased "capillary permeability" which occurs both in inflammation and in scurvy. Depolymerization of the

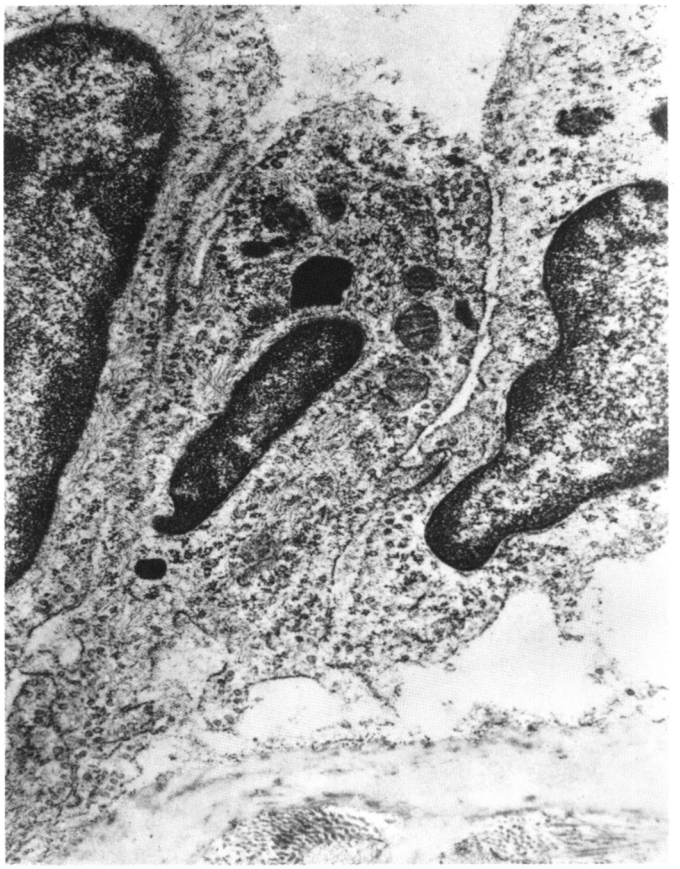

FIGURE 7. Electron micrograph of scorbutic guinea pig aortic endothelium. Note the widened intercellular junctions, the depletion of subendothelial collagen, and the reduction in cytoplasmic organelles. (From Gore, I., Fujinami, T., and Shirahama, T [1965a], *Arch. Pathol.*, 80, 371. ©1965 American Medical Association. With permission.)

mucopolysaccharides of the basement membrane and weakening of the perivascular collagen seem to be the additional changes that lead to increased microvascular fragility and petechial hemorrhages in scurvy. Similar changes in the capillaries beneath the intimal lining of the larger arteries and veins can cause subintimal hemorrhages which may disrupt the continuity of the endothelium and lead to subendothelial cholesterol deposition or to local thrombus formation.

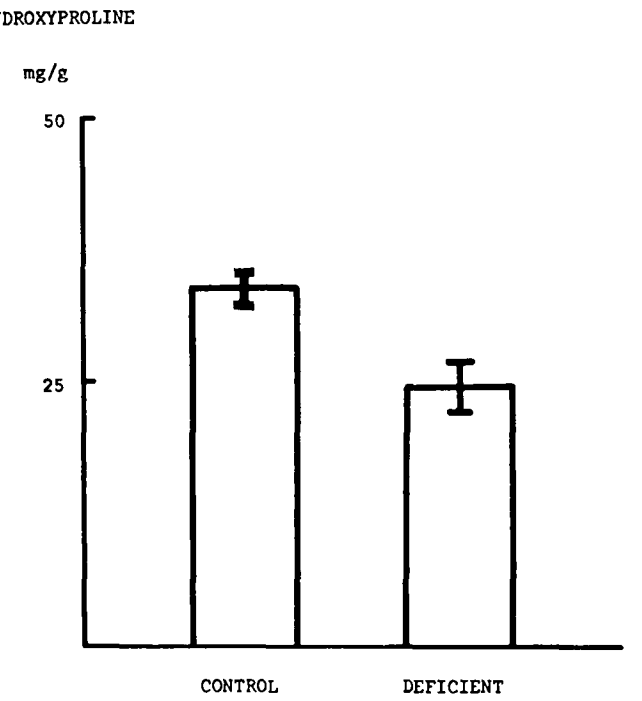

FIGURE 8. Hydroxyproline content of normal and scorbutic guinea pig aortas. (From Gore, I., Tanaka, Y., Fujinami, T., and Goodman, M. [1965b], *J. Nutr.*, 87, 311. ©American Institute of Nutrition. With permission.)

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Chapter 2

DIABETES MELLITUS

I. REVIEW

Observations by Stepp et al. (1935) revealed that ascorbic acid can be useful in the treatment of diabetes mellitus. They noted that the administration of 300 mg of ascorbic acid produced a fall in the blood sugar levels of both normal and diabetic subjects. Stoïcesco and Gingold (1936) made similar observations in normal men; they concluded that i.v. or oral administration of ascorbic acid reduced the fasting blood sugar level and also diminished the hyperglycemia following glucose administration; its action was slow and prolonged.

The diabetogenic effect of ascorbic acid deficiency was demonstrated by Sigal and King (1936) at the University of Pittsburgh. They used the standard type of oral glucose tolerance test to study mature guinea pigs at progressively more advanced stages of ascorbic acid deficiency. It was found that not only scurvy, but also the prescorbutic state decreased the glucose tolerance of these animals, as shown in Table 1. It may be noted that the glucose tolerance rapidly returned to normal on repletion with ascorbic acid; by the 10th day, recovery was fairly satisfactory and by the 15th day, the response was practically normal. In a statistical analysis of their data, these workers observed that after 10, 15, and 20 d of depletion, the calculated probability that successive increases of blood sugar at the 120-min period were real would be 140:1, 2000:1, and 25:1, respectively.

Pfleger and Scholl (1937) observed vitamin C deficiency in diabetics and reported that ascorbic acid administration reduced the amount of insulin needed to control the blood sugar. Söderling and Hamne (1937) described a 10-year-old child who had been treated for diabetes for 2 1/2 years. She responded poorly to insulin treatment until her vitamin C deficiency had been corrected. Observations by Oshima et al. (1938) and by van der Loo (1938) confirmed that the blood sugar and the urine sugar levels of patients with moderate diabetes mellitus were improved by the administration of ascorbic acid, 300 mg daily. Bartelheimer (1938) reported a marked decrease in the blood sugar levels of diabetics after they had been saturated with ascorbic acid. Moreover, Hamne (1938) observed that the glucose tolerance curves of two infants were reduced by the administration of ascorbic acid. Dienst (1939) also reported that the administration of vitamin C improved the glucose tolerance of diabetics, but Ijiri (1939) reported that the blood sugar levels of diabetic patients were not significantly affected by ascorbic acid. Slavich and Torrini (1940) found that sensitivity to insulin increased when ascorbic acid (250 mg) was given intraveously, and this finding was more pronounced in diabetics than in normal subjects.

Secher (1940) demonstrated that in patients with no ascorbic acid detectable in the serum, the blood sugar curve after glucose loading is far higher than when the serum ascorbic acid level has been raised by the ingestion of ascorbic acid. He did not, however, find any significant improvement in the curve of diabetics after administering vitamin C. Hjorth (1940) obtained similar but less definite findings when he studied four patients with different disorders, who had low serum ascorbic acid levels (0.06 to 0.20 mg/100 ml). There was a slight improvement in the glucose tolerance of each patient following the intravenous administration of ascorbic acid (200 to 500 mg daily) for several days. Hosokawa and Sikinami (1941) observed that vitamin C caused a reduction of both sugar and glycogen in the blood and urine of diabetics; they concluded that ascorbic acid seemed to have an action like that of insulin.

Studying 11 patients with cirrhosis or other forms of liver disease, Turchetti and Schirosa (1941) observed that the administration of ascorbic acid, after a test dose of glucose, caused

Table 1
EFFECT OF VITAMIN C DEPLETION AND RECOVERY ON GLUCOSE TOLERANCE OF GUINEA PIGS

Depletion period (d)	mg glucose/100 cc blood after				No. of animals	Average weight (g)	Minimum and maximum weight (g)
	Fasting	40 min	80 min	120 min			
Controls (2 mg/d)	101 ± 0.9	149 ± 3.9	141 ± 3.7	95 ± 1.4	15	583	480—718
10	104 ± 1.4	169 ± 3.3	161 ± 4.7	108 ± 3.0	14	615	470—740
15	109 ± 1.6	176 ± 4.3	177 ± 4.7	131 ± 2.9	15	593	437—747
20	112 ± 1.3	185 ± 4.2	191 ± 5.1	150 ± 5.4	15	507	337—695
Recovery period							
10	101 ± 1.5	163 ± 4.4	144 ± 6.0	109 ± 3.4	13	499	327—707
15	99 ± 0.9	156 ± 2.1	139 ± 1.6	100 ± 1.4	13	528	347—720

From Sigal, A. and King, C. G. (1936), *J. Biol. Chem.*, 116, 489. With permission.

a smaller increase in the blood sugar level than did the administration of glucose alone. Owens et al. (1941a, b) studied the ascorbic acid status of 125 diabetic patients and found no correlation between the ascorbate level and the diabetic state of the patients. Neither did they find any significant difference in the insulin requirement of 16 diabetic patients receiving a daily dose of 300, 600, or 1200 mg of ascorbic acid.

Sylvest (1942a) found only a modest fall in the fasting blood sugar of normal, diabetic, and scorbutic patients following the intravenous injection of ascorbic acid, but did not report any glucose tolerance tests. Secher (1942), in Copenhagen, on the other hand, conducted glucose tolerance tests before and after treatment of ascorbic acid deficiency in human subjects and reached the following conclusions.

1. Ascorbic acid deficiency in the blood is a frequent condition in normal individuals as well as in patients suffering from infectious diseases.
2. Abnormally high blood sugar curves and curves of the diabetic type may be changed to normal curves in individuals with ascorbic acid deficiency by removing the ascorbic acid deficiency of the blood.
3. Judgement of the blood sugar curve is consequently insufficient, if the ascorbic acid content of the blood is not known. This is of special practical significance from an insurance point of view, as the blood sugar curve in certain cases determines the assessment and thereby the payment of premiums.
4. The hyperglycemia in arthritis described by Pemberton, and in infectious diseases as described by numerous researchers, may be deduced from the deficiency in ascorbic acid.

Examples of Secher's findings in patients with temporary diabetes mellitus due to ascorbic acid deficiency associated with infection, etc. are shown in Table 2. Secher also observed that the glucose tolerance of some patients with frank diabetes mellitus did not improve after treatment with ascorbic acid; the glucose tolerance of one diabetic patient even worsened. This becomes understandable when we consider the evidence that it is the residual dehydroascorbic acid (DHAA) of scurvy, or the ratio of DHAA to AA*, that is important in causing diabetes mellitus and that certain individuals (with hemosiderosis, thalassemia, etc.) cannot keep ascorbic acid in the reduced form.

Banerjee (1943a) confirmed the existence of a decreased glucose tolerance in vitamin C-deficient guinea pigs and also (1943b) observed a profound reduction of liver glycogen from a mean of 2240 to a mean of 50 mg/100 g. In further studies (1943c, 1944a), he also

* AA — ascorbic acid, reduced form.

Table 2
DIABETOGENIC EFFECT OF INFECTIONS AND OTHER ILLNESSES; REVERSAL BY ASCORBIC ACID TREATMENT

Illness	Blood ascorbic acid (mg/100 ml)	Blood sugar levels (mg/100 ml)							
		Fasting	1/4 h	1/2 h	3/4 h	1 h	1 $^1/_2$ h	2 h	2 $^1/_2$ h
Pleurisy	0.00	96	180	180	186	170	137	137	126
	0.72	97	157	161	143	128	128	116	85
Rheumatic	0.20	106	143	161	163	200	192	137	145
fever	1.00	85	92	86	141	133	153	127	110
Pneumonia	0.00	108	150	193	225	225	209	172	148
	1.00	99	137	155	157	143	124	128	124
	0.72	75	135	153	161	149	141	114	127
Bronchial	0.24	108	153	222	250	280	228	145	95
asthma	0.84	117	123	154	197	197	167	105	89
	1.04	89	92	159	166	188	178	143	89

Note: From the data of Secher (1942), showing that many people whose glucose tolerance tests give diabetic curves while they are ascorbic acid deficient as a result of infections or other illnesses show normal glucose tolerance curves after treatment of their vitamin C deficiency.

Table 3
THE RELATION OF SCURVY TO HISTOLOGICAL CHANGES IN THE PANCREAS

Group	No. of animals	Number (mean) of the islets in each section	Total (mean) size of the islets in each section (mm^2)
Normal guinea pigs	10	7.6	273.52
Scorbutic guinea pigs	10	11.2	1168.25

Note: Scorbutic guinea pigs were found to have a significantly increased number and size of islets of Langerhans in the pancreas. However, the ratio of alpha to beta cells was increased, and most of the beta cells were degranulated.

From Banerjee, S. (1944), *Nature (London)*, 153, 344. With permission. ©1944 Macmillan Journals, Ltd.

demonstrated a decrease in the insulin content in the pancreas of scorbutic animals to one eighth of the normal value. It was therefore surprising when the same worker, Banerjee (1944b), found a highly significant increase in the number and size of the islets of Langerhans in vitamin C-deficient guinea pigs (Table 3). However, the number of alpha cells was found to be increased relative to the number of beta cells, and the beta cells were found to be markedly degranulated.

It is interesting in retrospect to note that the work of Banerjee (1943a) showed not only that a scorbutogenic diet decreased the glucose tolerance of guinea pigs, but also that high-dose ascorbic acid administration (100 mg per guinea pig per day by injection) also caused a tendency towards a decrease in glucose tolerance, as shown in Figure 1. This must have been quite incomprehensible at the time, but it is understandable now in view of the increased DHAA/AA ratio which can occur both in scurvy and also following large doses of ascorbic acid under certain circumstances.

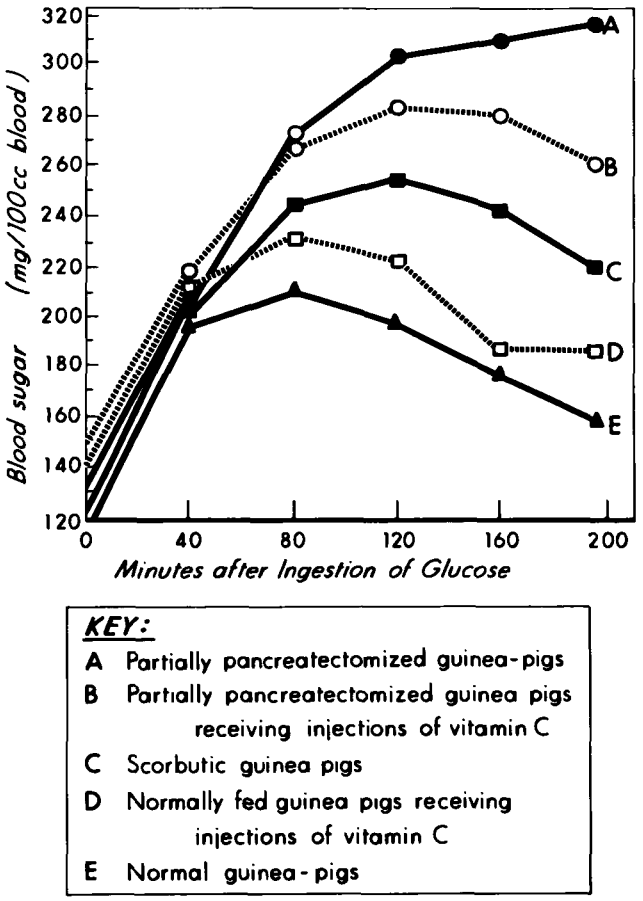

FIGURE 1. Guinea pigs (5 groups, 15 per group). Not only did a scorbutogenic diet (C) cause a decrease in the glucose tolerance of the guinea pigs, but also high-dose ascorbic acid administration (D) caused a tendency in the same direction (see text). (From Banerjee, S. [1943a], *Ann. Biochem. Exp. Med.*, 3, 157. With permission.)

Banerjee (1945) demonstrated an increase in the adrenaline content of the adrenal glands of scorbutic guinea pigs. It was therefore postulated that the disturbance of carbohydrate metabolism might be due to an imbalance between adrenaline and insulin. However, Banerjee and Ghosh (1946) found that scorbutic guinea pigs with demedullated adrenals showed lowered glucose tolerance just like that observed in scorbutic guinea pigs with intact adrenals, so clearly adrenaline is not responsible for the decreased glucose tolerance that is observed in scurvy.

Murray and Morgan (1946) confirmed that ascorbic acid-deficient guinea pigs do have decreased glucose tolerance. In order to rule out any effect of inanition, Banerjee and Ghosh (1947) repeated their experiments using pair-fed control animals to ensure that the test and control diets were identical in quantity as well as quality, except for their vitamin C content. The results shown in Table 4 confirmed the previous findings and clearly demonstrated the diabetogenic effect of vitamin C deficiency. However, in this study, unlike the earlier works of Sigal and King, the fasting blood sugar level was not affected.

Murray (1948) confirmed that scorbutic guinea pigs have high blood sugar levels after feeding glucose; she also stated that intraperitoneal injection of insulin (20 units) did not materially alter the disturbance of carbohydrate metabolism, but this latter conclusion is

Table 4
PANCREATIC INSULIN, LIVER GLYCOGEN, AND ORAL GLUCOSE TOLERANCE LEVELS OF SCORBUTIC AND PAIR-FED GUINEA PIGS[a]

Mean Values ± SE

Group	Fasting blood sugar (mg/100 ml)	Blood sugar levels after feeding glucose (mg/100 ml)			Liver glycogen (g/100 g)	Pancreatic insulin (IU/g)
		45 min	90 min	150 min		
Control guinea pigs, pair fed 21 d	118 ± 3.3	221 ± 16.4	193 ± 10.6	154 ± 9.9	1.936 ± 0.282	0.45
Vitamin C-deficient guinea pigs, 21 d	120 ± 10.2	284 ± 13.6	272 ± 10.8	266 ± 19.4	0.063 ± 0.014	0.11

Note: The fasting blood sugars did not differ, but the blood sugar values of the scorbutic guinea pigs at 45, 90, and 150 min after the feeding of glucose were significantly higher than the corresponding values of the pair-fed controls. The glycogen content of the livers of the scorbutic guinea pigs was significantly lower and the insulin content of their pancreatic tissue was markedly lower than that of the controls.

[a] Compiled from the data of Banerjee and Ghosh (1947).

questionable, as it was based on blood samples drawn at only one time (6 h) when the effect of the insulin may have been dissipated.

Banerjee and Ghosh (1950) reported that ascorbic acid administration caused a reduction in the blood sugar levels of both normal and diabetic subjects. The glucose utilization of all 16 diabetic patients was definitely improved following the administration of ascorbic acid (5 mg/ lb of body weight per day) for 3 weeks, but the glucose tolerance curves remained in the diabetic range. In ten patients, the urine was sugar free 3 weeks after the commencement of ascorbic acid therapy; four others required insulin to abolish the glycosuria.

Arendt and Pattee (1956) conducted insulin-glucose tolerance tests on 21 obese men and women before and after 3 weeks of treatment with oral ascorbic acid, 500 mg every 6 h. The subjects were fasted overnight. Venous blood was drawn and 0.1 unit of regular insulin per kilogram of body weight was given intravenously. At 30 min, or earlier if signs of hypoglycemia were evident, a second sample of blood was drawn and 0.8 g of glucose per kilogram of body weight was given orally; subsequent blood samples were withdrawn at 60, 90, 120, and 180 min.

Blood ascorbic acid determination prior to the study did not show any evidence of hypovitaminosis; the values were all greater than 0.6 mg/100 ml. All of the 21 obese patients had abnormal insulin-glucose tolerance tests before treatment, i.e., one or more blood glucose values exceeding the mean by more than 2 SD. Eight of them also had abnormal glucose tolerance tests. The results of these studies (Table 5) showed that 3 weeks of ascorbic acid treatment was followed by a significant increase of insulin-glucose tolerance, especially in those initially having abnormal insulin-glucose and normal glucose tolerance tests, but also to some extent in those in whom both the initial insulin-glucose and the glucose tolerance tests had been abnormal. It was suggested that the return of the insulin-glucose tolerance curve to normal or near normal in those patients who had normal oral glucose tolerance curves (Figure 2) meant that (1) the insulin-glucose tolerance test is the more sensitive one for the detection of early carbohydrate intolerance and (2) that an abnormal curve is reversible at this stage by the administration of large doses of ascorbic acid.

One patient (No. 2), whose insulin-glucose tolerance had not shown a satisfactory response

Table 5
THE EFFECT OF ORAL ASCORBIC ACID ON THE INSULIN-GLUCOSE TOLERANCE OF 21 OBESE PATIENTS

Patient No.	Age and sex	Weight change	% initial blood sugar level					Insulin reaction
			30 min	60 min	90 min	120 min	180 min	
Group I: Obese Patients with Abnormal Oral Glucose Tolerance Curve								
1	32F[a]	−9	46	101	170	203	165	+
			51	123	169	183	134	+
2	32M	−14	72	161	197	169	143	−
			37	107	155	174	87	?
			48[b]	114	128	88	88	
3	40F[a]	0	52	98	166	147	141	−
			39	108	164	164	150	−
4	43M	−16	62	133	184	152	112	−
			53	132	183	168	109	−
5	44M	−12	50	123	202	225	98	−
			43	115	182	189	140	−
6	47M	?	74	114	178	187	135	−
			70	106	151	166	136	−
			73[b]	117	166	175	151	−
7	53M	−6	47	113	185	211	124	+
			52	100	153	198	191	+
8	53M	0	37	112	229	216	137	−
			50	100	169	150	87	−
Average	43	−8	Before 55	120	189	189	132	
			After 49	111	166	174	129	
SD of differences			10.2	16.4	17.4	19.0	25.5	
SE			3.59	5.8	6.16	6.7	9.0	
Value of *p*			<0.02	<0.05	<0.02	<0.01	<0.01	
Group II: Obese Patients with Normal Oral Glucose Tolerance Curve								
9	15M[a]	0	64	163	163	152	113	−
			40	128	122	118	107	−
10	19F[a]	+1	32	139	150	127	116	−
			46	105	123	128	116	−
11	21F[a]	−3	58	84	101	130	138	−
			67	122	153	153	129	−
12	32F[a]	−6	47	136	167	163	110	+ +
			49	99	139	139	112	+
13	32F[a]	−5	53	142	217	182	124	−
			28	113	141	147	97	−
14	33M	−10	44	175	219	201	91	−
			42	112	152	117	75	−
15	36M	−5	52	132	193	198	127	−
			59	103	89	88	70	−
16	36F[a]	−1	41	93	140	166	166	−
			34	103	135	152	152	−
17	37F[a]	?	42	79	119	144	123	−
			32	75	112	145	121	−
18	38F[a]	0	58	115	157	151	125	−
			54	86	146	139	125	−
19	44M	−4	30	79	157	171	142	−
			44	80	90	131	112	+
20	45F[a]	−8	40	88	161	167	153	+ +
			33	96	119	127	129	+

Table 5 (continued)
THE EFFECT OF ORAL ASCORBIC ACID ON THE INSULIN-GLUCOSE TOLERANCE OF 21 OBESE PATIENTS

Patient No.	Age and sex	Weight change	% initial blood sugar level					Insulin reaction
			30 min	60 min	90 min	120 min	180 min	
Group II: Obese Patients with Normal Oral Glucose Tolerance Curve								
21	49M	−1	53	100	137	167	125	−
			57	103	115	106	115	−
Aver- age	33 6	−4	Before 47	117	160	163	127	
			After 46	102	126	130	112	
SD of differences			23.8	18.3	30.2	32.0	16.3	
SE			2.09	5.07	8.37	8 86	4 51	
Value of *p*			<0.01	<0.01	<<0.01	<0.01	<0.01	

[a] Outpatients.
[b] After ascorbic acid i.m

From Arendt, E C. and Pattee, C. J (1956), *J. Clin. Endocrinol Metab.*, 16, 653. With permission.

following 4 weeks of oral ascorbic treatment, nevertheless showed an excellent response following intramuscular injections of the same dose of ascorbic acid, 500 mg every 6 h, for 2 d; however, this effect lasted for only 5 d after cessation of parenteral therapy. Banerjee and Ghosh (1960) noted that scorbutic guinea pigs display an increased total body cholesterologenesis, apparently due to hypoinsulinism, for on administration of insulin, this alteration is corrected.

Dice and Daniel (1973), of Stanford University in California, reported that large and increasing doses of ascorbic acid progressively reduced the insulin requirement of a juvenile-onset diabetic. The insulin requirement of this 20-year-old subject (Dice) had been constant (32 units of NPH U80 administered as a single morning injection) for 4 years, but this dose was halved by a daily intake of 10 g of ascorbic acid (Figure 3). Unfortunately, these workers did not provide any plasma ascorbic acid data, so one does not know whether there was any defect in ascorbic acid absorption.

The capillary fragility of the skin of 24 normal subjects and 12 diabetics was studied by Cox (1975). The diabetics showed petechiae at much lower negative pressures than the controls (Figure 4) and all diabetics with retinopathy had very fragile capillaries. For treatment, the diabetics were assigned to two groups in a crossover study of (1) placebo for 1 month, then vitamin C (1 g/d) for 2 months, and (2) vitamin C for 2 months, then placebo for 1 month; the capillary strength of all diabetics improved during vitamin C treatment, but the capillary strength of four of the six in group 2 deteriorated toward the end of the month on placebo and one subject developed a fresh crop of retinal hemorrhages after withdrawal of treatment.

Scarlett et al. (1976) conducted intravenous glucose tolerance tests (IVGTT) on normal and nondiabetic obese subjects, first during infusion of isotonic saline and second 2 or more days later, during infusion of ascorbic acid (1 to 2 g every 3 h). There was very little difference between the results of the two sets of tests as regards the plasma glucose, serum insulin, glucagon, or growth hormone levels, except that the serum insulin levels of the normal subjects following glucose infusion were significantly lower during ascorbate infusion. This elegant series of experiments might well have been more instructive if

1. Subjects with low ascorbate levels had been selected for the study.

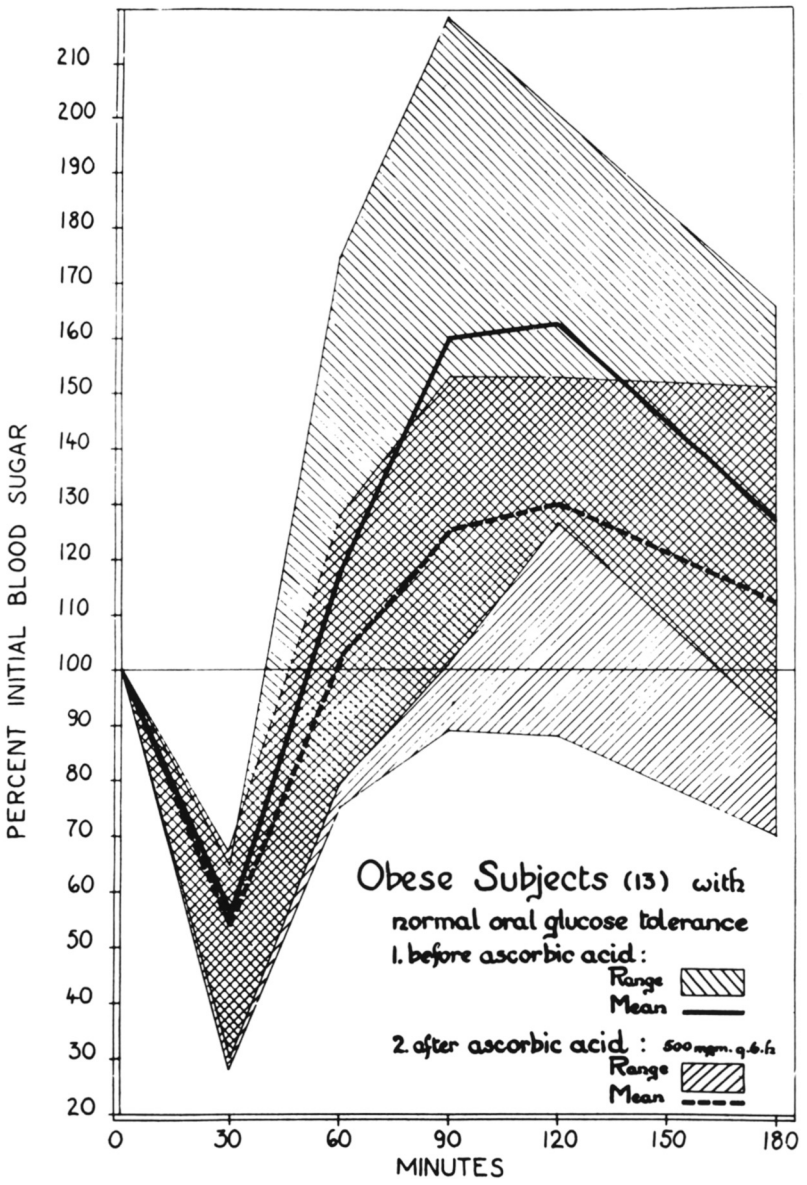

FIGURE 2. The effect of 500 mg of ascorbic acid by mouth every 6 h on the insulin-glucose tolerance curve in obesity. (From Arendt, E. C. and Pattee, C. J [1956], *J. Clin. Endocrinol. Metab.*, 16, 653. With permission.)

2. The second IVGTT had been conducted after ascorbic acid had been administered for several days.
3. More modest doses, such as 200 mg three times a day, of catechin-coated ascorbic acid had been used, instead of massive doses of ascorbic acid alone, which can sometimes have the opposite of the desired effect.

Horrobin et al. (1979) have reported the finding that ascorbic acid enhances the conversion of dihomogammalinolenic acid (DGLA) to prostaglandins (PG), particularly PGEI, in human platelets. Moreover, they point out that PGEI is important in the regulation of responsiveness to insulin. These authors therefore suggest that ascorbic acid may be of value only in conjunction with an adequate supply of the essential fatty acid DGLA.

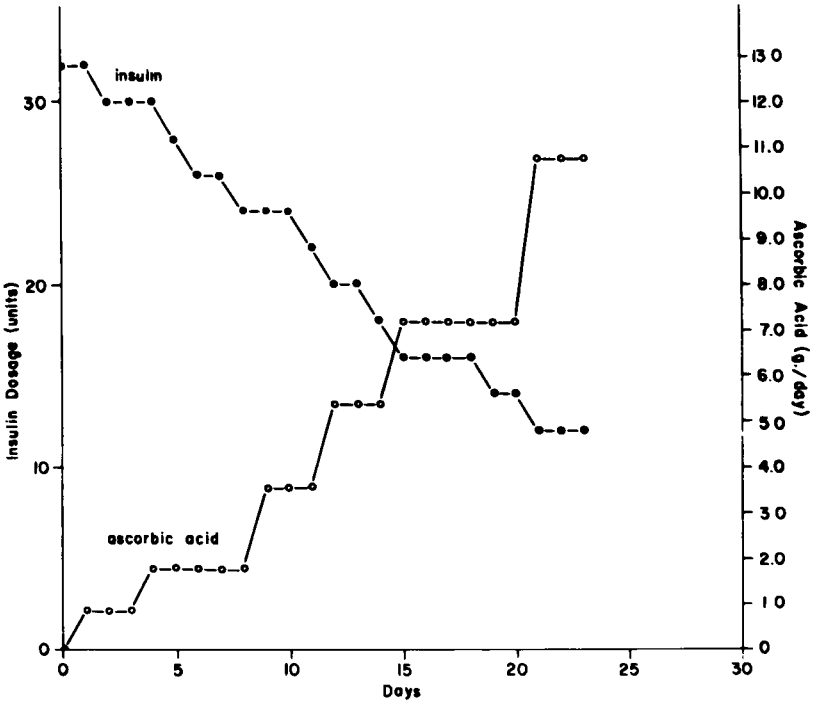

FIGURE 3. The relationship between decreasing daily insulin need (●–●) and increasing daily consumption of ascorbic acid (○–○), while maintaining control of the diabetes. (From Dice, J. F. and Daniel, C. W. [1973], *IRCS* 1, 41. With permission.)

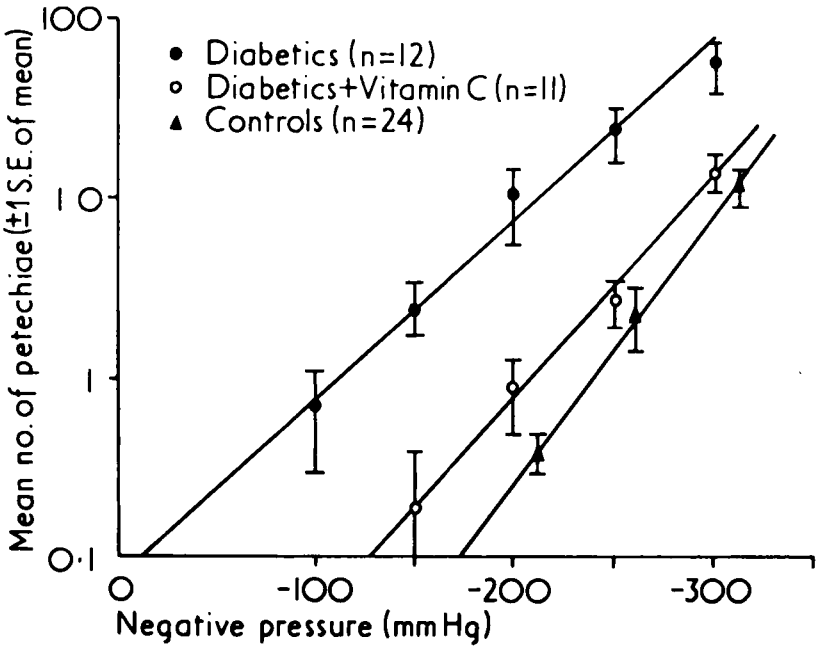

FIGURE 4. Effect of various negative pressures on mean number of petechiae seen in diabetics before and after vitamin C supplements and in a group of nondiabetics. (From Cox, B. D. [1975], *Br. Med. J.*, 3, 205. With permission.)

Table 6
ASCORBIC ACID (AA) AND DEHYDROASCORBIC ACID (DHAA) LEVELS OF BLOOD AND PLASMA FROM NORMAL AND DIABETIC HUMAN BEINGS

Condition	Number of subjects	Sex	Blood		Plasma	
			AA[a]	DHAA[a]	AA	DHAA
Normal[b]	25	Male	0.56 ± 0.20[c]	0.03 ± 0.03	0.37 ± 0.02	0
Normal	12	Female	0.61 ± 0.24	0.03 ± 0.03	0.38 ± 0.27	0
Diabetic[d]	25	Male	0.15 ± 0.20	0.69 ± 0.16	0.13 ± 0.16	0.21 ± 0.03
Diabetic	12	Female	0.19 ± 0.13	0.63 ± 0.13	0.17 ± 0.11	0.22 ± 0.04

[a] In mg/100 ml of blood or plasma.
[b] Age 40 to 70 years; without family history of diabetes and without vitamin C therapy; 2-h postprandial blood glucose between 50 to 90 mg/100 ml. The subjects did not have any history of organic disease.
[c] Mean ± SD.
[d] Age 40 to 70 years; confirmed diabetic patients from different diabetic clinics in Calcutta; diagnosed from 2-h postprandial blood glucose levels (160 to 330 mg/100 ml) and other clinical parameters. The patients were without any complication and did not have vitamin C therapy.

From Chatterjee, I. B. and Banerjee, A. (1979), *Anal. Biochem.*, 98, 368. With permission.

There is no doubt that ascorbic acid deficiency causes temporary diabetes mellitus due to hypofunction of the beta cells of the islets of Langerhans of the pancreas, but the question as to whether prolonged periods of ascorbic acid deficiency can permanently damage the beta cells and lead to permanent diabetes has not been settled. Of course, the development of diabetes in ascorbic acid-deficient patients with hemosiderosis (Chapter 10, Volume I) or thalassemia major (Chapter 15, Volume I) is highly suggestive of this possibility. It is known that the ratio of DHAA to ascorbic acid increases in ascorbic acid deficiency until at last only DHAA is found in the blood and tissues in scurvy. Moreover, it is known that injection of small doses of DHAA into rats causes temporary damage to the beta cells of the islets of Langerhans and that larger doses cause permanent damage (Chapter 3, Volume III). The question as to whether diabetes mellitus can result from small amounts of DHAA in the blood, acting over a longer period of time, cannot be answered at the present time.

Studies of the DHAA levels of blood samples from normal and from diabetic subjects have led to conflicting results. Cox and Whichelow (1975) observed no significant difference between the ascorbate, dehydroascorbate, and diketogulonate levels in plasma from normal and diabetic subjects, but Chatterjee et al. (1975), Chatterjee and Banerjee (1979), and Som et al. (1981) have observed significantly increased levels of DHAA in the blood and plasma of diabetic subjects.

Chatterjee and Banerjee (1979) utilized carbon monoxide to arrest oxidation of ascorbic acid to DHAA in blood samples, both before and after addition of metaphosphoric acid; having arrested oxidation, they were then able to identify DHAA in the blood of diabetic subjects by isolating it as the 2,4-dinitrophenylhydrazone derivative. They found no DHAA in the plasma of normal subjects, but appreciable quantities in the plasma and especially in the blood of diabetics, as shown in Table 6. Their results obtained from six diabetic patients indicate that out of a mean whole blood DHAA content of 0.66 mg ± 0.11 (SD)/100 ml, erythrocytes contained 0.49 mg ± 0.09, and plasma contained 0.13 mg ± 0.05.

Som et al. (1981) reported that diabetic patients were found to have lower than normal plasma ascorbic acid and higher than normal DHAA levels ($p < 0.001$) irrespective of age, sex, duration of disease, type of treatment, and blood sugar control. Their results (Table 7) show that, in contrast to normal subjects, the mean plasma AA levels of their maturity-onset

Table 7
ASCORBIC ACID (AA) AND DEHYDROASCORBIC ACID (DHAA) LEVELS IN THE BLOOD PLASMA OF NORMAL AND DIABETIC SUBJECTS

S1 No.	Subjects	Age (years)	No. of subjects and sex	Treatment	Blood glucose	Plasma AA	Plasma DHAA
1	Normal[a]	18—70	96M/17F	—	74 ± 8	0.52 ± 0.25	0.04 ± 0.06
2	Normal[a]	21—23	19M/7F	—	71 ± 6	0.54 ± 0.31	0.08 ± 0.10
3	Normal[b]	21—23	18M/17F	—	76 ± 5	0.53 ± 0.32	0.08 ± 0.14
4	Diabetic[c]	21—67	12M/8F	Before treatment	183 ± 49	0.11 ± 0.16	0.18 ± 0.07
5	Diabetic[c]	21—67	12M/8F	After treatment	121 ± 29	0.10 ± 0.14	0.17 ± 0.10
6	Diabetic[d]	20—62	13M/13F	Insulin	261 ± 88	0.07 ± 0.11	0.19 ± 0.09
7	Diabetic[d]	21—75	39M/18F	Oral[e]	198 ± 64	0.07 ± 0.14	0.19 ± 0.10
8	Diabetic[f]	37—75	6M/5F	Insulin/oral[e]	366 ± 46	0.09 ± 0.14	0.21 ± 0.08
9	Diabetic[g]	36—74	10M/10F	Oral[e]	109 ± 20	0.07 ± 0.17	0.16 ± 0.07

Note: Values represent mean ± SD. In the case of insulin-treated diabetic patients, blood was drawn in the fasting condition. In all other cases, 2-h postprandial values are reported.

[a] Without family history of diabetes and without prehistory of organic diseases.
[b] Normoglycemic offsprings of diabetic parents (either one or both), but without prehistory of organic diseases.
[c] Newly diagnosed; S1 No. 4, untreated; S1 No. 5, same patients, after 3 months of treatment with insulin or oral sulfonylureas.
[d] Blood samples were collected at random; glycemic control of the patients not known.
[e] Tolbutamide or chlorpropamide.
[f] Uncontrolled diabetics, mean blood glucose value 3 months earlier was 332 ± 51 mg/dl.
[g] Controlled diabetics, mean blood glucose value 3 months earlier was 151 ± 42 mg/dl.

From Som, S., Basu, S., Mukherjee, D., Deb, S., Roy Choudhury, P., Mukherjee, S., Chatterjee, S. N., and Chatterjee, I. B. (1981), *Metabolism*, 30, 572. With permission.

diabetics was very low. In 93 cases out of 134 diabetics, the plasma AA level was nil. The mean plasma AA level of normal subjects (aged 8 to 70 years) was 0.52 mg/100 ml ± 0.25 SD. In spite of these low plasma ascorbic acid levels, these workers observed normal leukocyte ascorbic acid levels in diabetic patients. They therefore concluded that the low plasma ascorbate level must represent a high turnover rate of the vitamin, rather than a vitamain C deficiency, and they considered this to be a metabolic defect associated with diabetes; its cause has not been elucidated. These workers did not find any difference between the erythrocyte reduced glutathione levels or the glucose-6-phosphate dehydrogenase activities of normal and diabetic subjects. Contrary to reports by others, they found that ascorbic acid supplementation did not lower the 2-h postprandial blood glucose level of diabetic patients. High doses of ascrobic acid caused a temporary increase in both the ascorbic acid and the DHAA levels of diabetics.

Unpublished work conducted by Clemetson et al. (1977), at the Methodist Hospital, Brooklyn, using the Hughes (1956) homocysteine method for analysis of blood plasma samples (Table 8), did not provide such clear-cut results; there was considerable overlap between the findings in the diabetic and the normal groups. The DHAA levels of the diabetics and the normal subjects did not differ significantly. Nevertheless, the mean AA/DHAA ratio was somewhat reduced in diabetics, suggesting a disturbance of the oxidation-reduction or redox potential of the blood, as discussed in Chapter 3, Volume III.

Mann (1974) postulated that ascorbic acid may enter human tissues by an insulin-dependent process and that diabetic angiopathy might be due to tissue ascorbic acid deficiency. Subsequent studies by Mann and Newton (1975) demonstrated that D-glucose inhibits the uptake

Table 8
ASCORBIC ACID (AA) AND DEHYDROASCORBIC ACID (DHAA) LEVELS AND AA/DHAA RATIOS IN NORMAL AND DIABETIC SUBJECTS

			Serum	Plasma		
Subjects	n	Age	Sugar (mg/100 ml) (range)	AA (mg/100 ml) (mean ± SD)	DHAA (mg/100 ml) (mean ± SD)	AA/DHAA (ratio of means)
Normal subjects—no family history of diabetes	8	23—63	90—119	1.045 ± 0.5541	0.063 ± 0.0463	16.6/1
Normal subjects—with family history of diabetes	10	24—38	83—115	1.168 ± 0.1861	0.099 ± 0.0839	11.8/1
Diabetics	16	19—70	134—427	0.833 ± 0.4105	0.130 ± 0.0877	6.4/1

Note: Previously unpublished work conducted by Clemetson, Nahm, Kozhiashvili, De Carlo, and Cafaro (1977) at the Methodist Hospital, Brooklyn, NY Fasting blood plasma samples were analyzed for AA and for DHAA by the Hughes (1956) homocysteine method. Unfortunately, the average age of the diabetic patients was greater than that of the hospital personnel who acted as controls, so no definite conclusions can be drawn, but the tendency towards a lower AA/DHAA ratio in the diabetics is evident. Statistical analysis showed no significant differences between the AA, DHAA, or AA/DHAA ratios of the three groups.

of dehydroascorbate by red blood cells. Moreover, Ginter et al. (1978) found the vitamin C concentration in the blood of maturity-onset diabetics to be significantly lower than in age-matched controls at the same season of the year (diabetics, 0.39 ± 0.03; healthy controls, 0.58 ± 0.04 mg/100 ml, $p < 0.001$). The mean leukocyte ascorbic acid concentration in the diabetics (9.8 ± 0.7 mg/100 g) was also substantially lower than that of healthy controls (22.7 mg/100 g, $p < 0.001$).

Sarji et al. (1979) reported that ascorbic acid levels in washed platelets from insulin-dependent diabetics were significantly lower than those obtained from normal subjects (25.5 vs. 45.2 $\mu g/10^8$ platelets, $p < 0.001$). Moreover, Verlangieri and Sestito (1981) demonstrated that the transport of ^{14}C-labeled ascorbic acid into cultured fetal bovine cardiac endothelial cells is markedly increased (2.5 times, $p < 0.005$) by insulin (4 μm/ml) and is decreased fivefold in a medium containing glucose (180 mg/100 ml). Likewise, Norkus et al. (1982), studying pregnant guinea pigs, compared the effects of infusing L-ascorbic acid alone, or with D-glucose, and found that maternal glucose levels exceeding 200 mg/100 ml impaired the placental transfer of DHAA to the fetus.

Studies of human cell preparations by Bigley et al. (1982) and by Moser and Weber (1984) provided *in vitro* evidence that indeed glucose and DHAA do enter human leukocytes by the same pathway and that high glucose levels impair the entry of ascorbic acid into the white blood cells. This may well explain the poor resistance to infection by diabetics. Bigley et al. suggested that inhibition by glucose of dehydroascorbate uptake into cells may contribute to the high plasma dehydroascorbate levels found in diabetic subjects. Furthermore, they pointed to the work of Sherry and Ralli (1948) who showed that administration of insulin to dogs caused a fall in the plasma total ascorbate and a rise in the leukocyte ascorbate level. Aleo (1981) followed this line of reasoning; noting that diabetes mellitus and bacterial endotoxin both have the effect of impairing ascorbic acid access to cells, they suggested that the resulting decreased ascorbic acid and increased histamine levels in the alveolar tissues may well account for the increased prevalence and severity of periodontal disease in diabetics.

A detailed study by Chen et al. (1983) has confirmed these findings *in vivo,* for glucose infusion into normal human volunteers caused not only a rapid rise in the plasma insulin level, but also a rapid fall in the ascorbic acid content of the mononuclear leukocytes (Figure 5). Moreover, the ascorbic acid content of the monocytes of five diabetic subjects was found to be significantly lower than that of nine normal individuals ($p < 0.05$).

Yew (1983a) observed that the plasma, liver, and kidneys of rats made diabetic by injection of streptozotocin had significantly lower (40 to 50% lower) concentrations of ascorbic acid than the same tissues of normal control animals. The DHAA level was found to be increased in the plasma of the diabetic rats, but was unchanged in the liver and was decreased in the kidneys. Furthermore, Yew (1983b) reported that ascorbic acid supplementation (1 g/kg) provided some protection against the diabetogenic effect of streptozotocin in rats. The mean body weights at the end of the study for rats given ascorbic acid and for controls were 580 and 496 g, respectively, plasma glucose levels were 192 and 291 mg/100 ml, and the water intakes were 91 and 165 ml/24 h. The ascorbic acid levels in the plasma, liver, testes, and kidneys of rats given ascorbic acid were significantly increased, but the DHAA concentrations of the plasma, testes, and kidneys were not. The ascorbate/dehydroascorbate ratio in the plasma of the rats given ascorbic acid was increased.

The observations of Cox and Whichelow (1975), in London, and those of Chatterjee et al. (1975), Chatterjee and Banerjee (1979), and Som et al. (1981), in Calcutta, might at first seem to be contradictory; indeed, there have been suggestions that estimation of DHAA in the method used by the Indian group of research workers might have been affected by the blood glucose levels. However, this argument cannot be used to explain the high DHAA levels found in diabetes, for Som et al. (1981) observed them to be just as high in treated as in untreated diabetics.

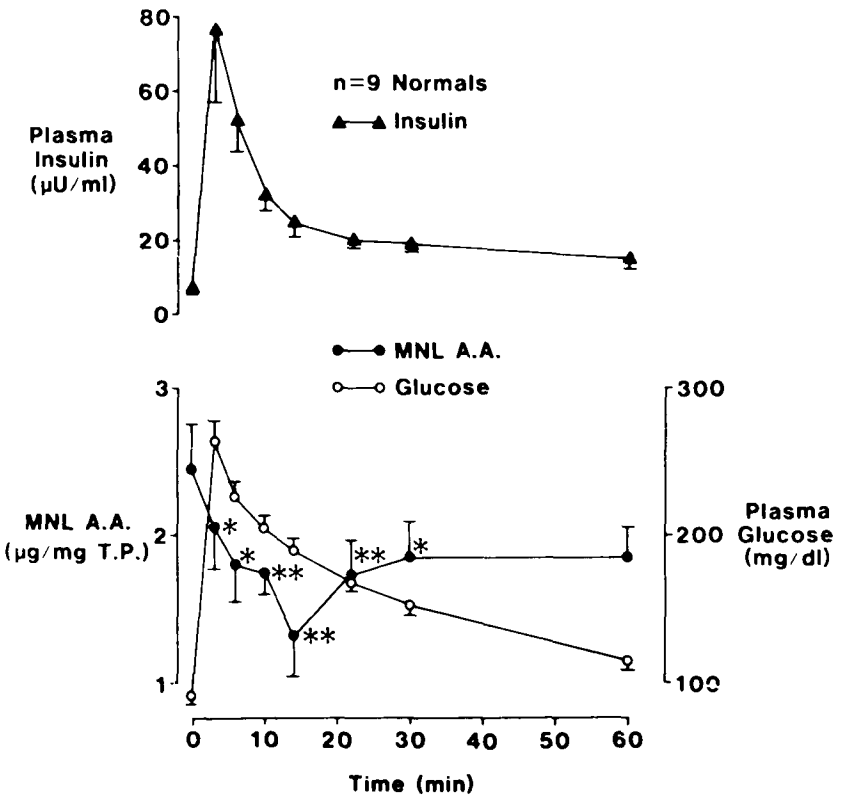

FIGURE 5. The effect of a rapid 20-g intravenous infusion of glucose on mononuclear leukocyte (MNL) ascorbic acid, plasma glucose, and insulin levels (mean ± SEM) of nine normal individuals (*p <0.05 and **p <0.01, ascorbic acid content before vs. after glucose infusion). (From Chen, M. S., Hutchinson, M. L., Pecoraro, R. E., Lee, W. L., and Labbé, R. [1983], *Diabetes*, 32, 1078. With permission from the American Diabetes Association, Inc.)

The most likely explanation for the different findings in London and in Calcutta is that there are several different types of diabetes due to different causes, and that certain types are more common in certain regions of the world. The high-cereal, low-protein diets of many people in India undoubtedly present special problems which are not seen so commonly in some other parts of the world.

Even if we set aside pituitary and adrenal causes of diabetes mellitus, there are several different causes of pancreatic diabetes. Moreover, the hypothesis that DHAA or an increase in the DHAA/AA ratio could cause permanent damage to the islets of Langerhans in human beings does not require that an elevated plasma DHAA level should continue to be present after the damage has been done.

Among the many causes of diabetes, one is surgical removal of the pancreas, which does not directly involve prior disturbance of ascorbic acid metabolism. Indeed, one such patient, a 38-year-old man who was diabetic following pancreatectomy 6 years previously, was studied by Clemetson et al. and is not included in Table 7; he was found to have a plasma AA level of 0.96 mg/100 ml and a DHAA level of −0.006 or zero. Clearly, there can be different causes of diabetes mellitus and several spring to mind without any search of the literature:

Temporary diabetes mellitus

1. Dietary ascorbate deficiency (see Table 1)

2. Borderline AA + infection (see Table 2)
3. Excessive AA + low cereal diet (see Table 1, Chapter 3, Volume III)
4. Birth control pills in prediabetics — hypercupremia
5. Pregnancy in prediabetics — HPL + hypercupremia

Permanent diabetes mellitus

6. Cushing's syndrome — pituitary-adrenal
7. Pancreatectomy — absent islets
8. Thalassemia + AA — hemochromatosis
9. Autoimmune damage to islets — beta cell damage
10. Senile amyloidosis of islets — beta cell damage
11. Radiation of pancreas — beta cell damage
12. Insulin-resistant diabetes — insulin antibodies
13. Insulin-resistant diabetes — insulin receptor failure

We cannot and should not expect to find the same metabolic changes in all forms of diabetes mellitus, a disease which has so many causes. We should, however, try to differentiate between the different kinds of diabetes and try to learn more about the cause of each, paying special attention to diet, heavy metal storage, and genetic predisposition.

It seems to the present writer that the beta cells of the islets of Langerhans may be E_h-sensitive cells, responding to the oxidation-reduction potential of the milieu interieur. Both ascorbic acid and glucose are reducing agents and they stimulate insulin production, while ascorbic acid deficiency and hypoglycemia have the opposite effect and decrease insulin production. Moreover, certain strong oxidants like DHAA, alloxan, X-irradiation, and also aging seem to damage the beta cells. No doubt this is an oversimplification, but it does seem to have a foundation in fact, for Patterson and Lazarow (1950) have shown that sulfhydryl compounds protect against DHAA diabetes in rats.

II. CONCLUSIONS

Deficiency of ascorbic acid, or a disturbance of ascorbic acid metabolism, can cause temporary or permanent damage to the β-cells of the islets of Langerhans of the pancreas, resulting in temporary or permanent diabetes mellitus. Some mild diabetics can actually be rendered nondiabetic by correction of an ascorbic acid deficiency, but ascorbic acid administration clearly cannot repair permanently damaged islets.

One would not have expected ascorbic acid to provide much benefit in insulin-resistant diabetes due to insulin antibodies or to insulin receptor failure. However, there is now a body of evidence that insulin activity is required to promote the entry of ascorbic acid into leukocytes, platelets, vascular endothelial cells, and other tissues. Moreover, high glucose levels have been shown to compete with ascorbic acid for entry into the cells.

Furthermore, the work of Ginter et al. (1978) has shown that long-term administration of ascorbic acid, 500 mg daily for 12 months, to maturity-onset diabetics removed their tissue ascorbate deficiency and caused a highly significant decline of blood cholesterol ($p < 0.001$) and a moderate decline of blood triglycerides, presumably by aiding conversion of cholesterol to bile acids in the liver and by increasing the lipoprotein lipase (LPL) activity in the blood.

Thus, there are three reasons for giving ascorbic acid to diabetics:

1. If the diabetes is due to a deficiency of ascorbic acid, it may be cured.
2. Even diabetics with normal plasma ascorbate levels have low leukocyte, tissue, and platelet ascorbic acid levels, so they need ascorbic acid to retard the development of microangiopathy, to reduce the risk of deep vein thrombosis, and to increase their resistance to infection.

3. Ascorbic acid supplements aid the conversion of cholesterol to bile acids and cause the release of lipoprotein lipase, which may help to reduce the risk of atherosclerosis.

However, there is a caveat, in that high-dosage ascorbic acid may be toxic to people with bronzed diabetes due to hemochromatosis in β-thalassemia and presumably in other forms of iron storage disease (Chapter 8 of this volume). These people need ascorbic acid because they are ascorbic acid deficient and because the vitamin helps to liberate iron as ferritin from the liver, but the ascorbic acid is oxidized too rapidly in the process, with the release of ascorbate free radical and DHAA into the bloodstream. It is general policy, therefore, to give modest doses of ascorbic acid with desferrioxamine and vitamin E to such patients. For similar reasons, all patients may be better served by a modest dose of ascorbic acid with a chelating fiber such as D-catechin (200 mg of each), or as catechin-coated ascorbic acid pills, to bring about a slow and safe chelation of heavy metals, than by a large 1-g dose of ascorbic acid.

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Chapter 3

ANEMIA

I. HUMAN OBSERVATIONS

Mettier et al. (1930) of Harvard University studied nine adults with scurvy and found normochromic anemia with anisocytosis to be a common occurrence. They noted that infection or blood loss might sometimes play a role in causing the anemia of scurvy, but gave reasons for believing that vitamin C has a specific effect on erythropoiesis; neither iron nor the "PA" factor from liver was effective in curing this anemia, but orange juice caused a prompt reticulocyte response and a subsequent rise in the hemoglobin level.

Jennings and Glazebrook (1938) described two patients with scurvy presenting with pain, swelling, and effusions in the knee joints; one had an orthochromic normocytic anemia, but the other had megaloblastic macrocytic anemia associated with histamine-fast achlorhydria and gastric mucosal atrophy. The megaloblastic anemia failed to respond either to liver or to iron, but in both cases, the anemia was cured solely as a result of vitamin C therapy.

Parsons (1938) reported having seen 14 children with scurvy; 3 had orthochromic normocytic anemia and 2 had macrocytic anemia; all were cured by ascorbic acid. Crandon et al. (1940) reported their findings in one adult male who placed himself on a vitamin C-free diet for 6 months: his plasma ascorbic acid and later his leukocyte and platelet ascorbic acid (AA)* levels fell to zero; he developed hyperkeratotic papules and ingrown hairs over the buttocks and the backs of his legs and also failure of wound healing, but he did not develop any changes in his gums and he did not develop anemia.

Ralli and Sherry (1941), writing from New York University, had seen five people with scurvy, of whom three were anemic. All three responded to treatment with ascorbic acid, but these authors suggested that other nutritional deficiencies may have played a role in causing the anemia, for it did not recur in two of these patients when they were subsequently placed on an experimental vitamin C-deficient diet for 45 and 52 d, respectively. Israels (1943) reported three cases of adult scurvy: two had normocytic anemia and one had a mild microcytic anemia, but all three showed a good erythropoietic response to ascorbic acid; no iron or liver was given with the ascorbic acid.

McMillan and Inglis (1944) reported their findings in 53 patients with scurvy, seen in Edinburgh between 1937 and 1944; they were mostly elderly men; 40 of them were anemic and their anemias were classified as macrocytic (2), normocytic (18), simple microcytic (14), and hypochromic microcytic (2). Sternal marrow samples were obtained from six of these patients; five were normoblastic and one was megaloblastic. It was concluded that the anemia of scurvy is due to a complex deficiency, with vitamin C deficiency acting only as an adjunct. After reviewing the literature, Wade et al. (1946) concluded that anemia is a common clinical manifestation of scurvy in adults and that it occurs in two thirds of children with Barlow's disease.

Vilter et al. (1946) reported their findings in 19 adults who were seriously ill with scurvy on admission to the Cincinnati General Hospital between January 1935 and June 1945; all but two were anemic. Moderate icterus with increased urobilinogen excretion and reticulocytosis in the peripheral blood suggested hemolysis as the cause of the anemia. Many of these patients undoubtedly had other nutritional deficiencies, and the anemias ranged from normochromic and normocytic to slightly macrocytic, but all showed excellent hematopoietic responses to treatment with ascorbic acid. These authors suggested that other nutritional

* AA — ascorbic acid, reduced form.

deficiencies play a role in causing the anemia of scurvy, but the other deficiencies are not serious enough to prevent a remission when ascorbic acid is supplied.

Zuelzer and Hutaff (1949) reported 36 cases of infantile scurvy and concluded that the anemia of scurvy can arise from hemorrhage, infection, liver principle deficiency, or iron deficiency. They had observed cures of megaloblastic anemia of infancy by folic acid alone, without any ascorbic acid. Brown (1951) observed that the anemia of scurvy may be microcytic, normocytic, or macrocytic and suggested the operation of additional factors, but the fact that the anemia nearly always responds to ascorbic acid alone made other factors seem irrelevant. This presented an interesting conundrum, the answer of which is only partially understood today.

The work of Welch et al. (1951) and of Gabuzda et al. (1952) demonstrated that one of the functions of ascorbic acid in man is to aid in the conversion of folic to folinic acid (i.e., the reduction of folate to tetrahydrofolate). Two patients with scurvy studied by Gabuzda et al. needed folic acid and ascorbic acid before excreting large amounts of folinic acid in the urine; this occurred following the administration of folic acid alone in a nonscorbutic patient. Thus, the megaloblastic anemia sometimes seen in scurvy was found to be the result of an ascorbate-dependent abnormality of folic acid metabolism (see Chapter 4, Volume III). This was confirmed by the observations of Jandl and Gabuzda (1953) who studied two men with scorbutic megaloblastic anemia; there were reticulocyte responses following the administration of folic acid, and these were potentiated by the subsequent administration of ascorbic acid.

In an experiment reported by Krebs (1953), six out of ten volunteers on a vitamin C-free diet developed hemorrhagic hair follicles on their legs after 6 months and later still they developed signs of scurvy, such as swollen gums and protuberant discolored interdental papillae, but anemia did not occur, even though some continued on this diet as long as 38 weeks.

The absence of anemia in pure experimental ascorbic acid deficiency in human volunteers raises the question as to whether it is hemorrhage, stress, infection, debility, accompanying folic acid deficiency, other dietary deficiencies, or the greater severity and duration of the naturally occurring disease that causes the anemia which is so commonly seen in association with scurvy. Certainly anemia is common in naturally occurring scurvy; moderate or severe anemia was present in 82 of 100 patients with scurvy admitted to the Stobhill General Hospital in Glasgow during a 15-year period, as recorded by Thomson (1954).

Brown (1955) described a 57-year-old laborer with multilobular cirrhosis of the liver associated with frank scurvy and megaloblastic macrocytic anemia. His folic acid and vitamin B_{12} levels were found to be normal. Both the scurvy and the anemia responded to the administration of ascorbic acid alone, and his bone marrow picture rapidly reverted to normoblastic erythropoiesis. Presumably, the hepatic function of converting folic to folinic acid was impaired by the cirrhosis, and the residual hepatic function was further impaired by ascorbic acid deficiency in this man. Other patients with scurvy sometimes need both folic acid and ascorbic acid, but even a small amount of folic acid seems to be sufficient if ascorbic acid supplies are adequate.

Cox et al. (1958) reported that the low serum vitamin B_{12} levels of patients with pernicious anemia, adult celiac disease, and regional ileitis were associated with low plasma ascorbic acid (AA) levels and a disturbance of ascorbate metabolism. This disturbance was partially rectified by the administration of cyanocobalamin, but the connection, if any, between these B_{12}-responsive anemias and the ascorbate-responsive anemias of scurvy is not evident. These authors also observed a highly significant inverse relationship between the seromucoid levels of patients with regional ileitis (signifying disease activity) and plasma ascorbic acid (AA) levels (-0.7211, $p < 0.001$).

Neilson (1960) reported the occurrence of severe megaloblastic anemia and achlorhydria in a 71-year-old man with frank scurvy; the serum B_{12} level was normal and he showed an

excellent erythropoietic response to treatment with ascorbic acid. Unlike Brown's patient, this man had no evidence of liver disease apart from a slightly raised serum bilirubin which was most likely due to extravascular hemolysis. Presumably, he had a disturbance of folate metabolism due to ascorbic acid deficiency.

Fourteen cases of adult scurvy were described by Lind (1960); all were anemic; the survival time of autologous erythrocytes by the ^{51}Cr method was abnormally decreased in one patient who also had a low serum folic acid level. It was suggested that hemolysis may be an important factor in some patients with scurvy. Three further cases of megaloblastic anemia associated with adult scurvy were reported by Will and Murdoch (1960); all three had normal B_{12} levels and showed excellent responses to treatment with ascorbic acid.

Cox et al. (1962) saw seven patients with classical scurvy during a period of 3 years at The General Hospital in Birmingham, England. Ascorbic acid was absent or barely detectable in the plasma of these patients; all had typical ecchymoses and perifollicular hemorrhages, six of the seven had hemarthroses, and five were anemic. Two had low serum iron levels, one had achlorhydria, and four were found to have a simultaneous deficiency of vitamin B_{12}. However, it seems that other disease states including pernicious anemia, adult celiac disease, and partial gastrectomy complicated the pictures. These authors thought it unlikely that vitamin B_{12} deficiency normally plays any role in the megaloblastosis of scurvy. The associations were understandable, i.e., the patient with pernicious anemia had a typical glossitis which had caused a distaste for acid fruits. We must never assume that scurvy is a pure condition. We must always conduct a full investigation of any anemia and consider all the factors that can contribute to ascorbic acid deficiency.

In contrast, there was no evidence of vitamin B_{12} deficiency in any of the seven patients with pauper's scurvy seen by Chazan and Mistilis (1963) during 1 year on the Tufts Medical Service at the Boston City Hospital. All showed moderate to severe anemia: four, normochromic macrocytic; two, normochromic normocytic; and one, hypochromic normocytic. Serum folic acid levels were low in the four patients with megaloblastic macrocytic anemia. The authors attributed this to inadequate dietary intake of folic acid, but they did not attempt a therapeutic trial of ascorbic acid alone.

Hyams and Ross (1963) described the occurrence of megaloblastic anemia, osteoporosis, and scurvy in a 54-year-old London housewife, who presented with severe low back pain due to collapse of L1 vertebral body and ballooning of the intervertebral disks of L1, 2, 3, and 4. There was a slow but complete resumption of normal erythropoiesis as a result of treatment with ascorbic acid, 500 mg daily, and her back problem was markedly improved.

Hart et al. (1964) conducted extensive studies of three patients with scurvy and anemia. The anemia of two of them showed a rapid response to treatment with ascorbic acid alone, but the third, with overt megaloblastic anemia, required folic acid as well to achieve complete restoration of normal red cell maturation.

Vilter (1964) advanced an interesting hypothesis to explain the relationships between ascorbic acid, folic acid, and vitamin B_{12} in the synthesis of nucleic acids and in the treatment of megaloblastic anemias. He suggested that one effect of vitamin B_{12} is the formation of folic acid coenzymes, while ascorbic acid is involved with the protection of folic acid reductase.

Kahn et al. (1966) of Philadelphia suggested that vitamin B_{12} is needed to correct the abnormal ascorbic acid metabolism of some patients. They reported persistently low serum ascorbic acid (TAA)* levels (0.1 mg/100 ml) in a 72-year-old man with scorbutic macrocytic anemia, even after the administration of ascorbic acid, 500 mg three times a day for 16 d and 100 mg three times a day for another 12 d. Although his serum vitamin B_{12} level was consistently within the normal range, they attributed this failure of vitamin C absorption to an abnormality of B_{12} metabolism because there was methyl malonic acid in his urine. This

* TAA — total ascorbic acid, reduced and oxidized forms.

man had markedly increased iron stores in the liver, spleen, and bone marrow, so the impaired absorption of ascorbic acid may well have been due to intestinal hemosiderosis (see Chapter 10, Volume I).

Lloyd et al. (1972) studied five patients with megaloblastic anemia and found them to have markedly reduced platelet ascorbic acid levels in spite of normal plasma levels. Three of these patients had pernicious anemia, one had a folate-responsive anemia, and the other had had a partial gastrectomy and was being treated with phenytoin for epilepsy. In view of the normal plasma ascorbate levels, it was suggested that there may be a defect in the ascorbic acid storage mechanism of the platelets in megaloblastic anemia. This certainly seems reasonable, for both the conversion of folic to folinic acid and tissue storage of ascorbic acid require a low oxidation-reduction potential in the tissue. Beattie and Sherlock (1976) observed a significant negative correlation between the leukocyte total ascorbic acid level and the mean corpuscular volume in their study of liver disease ($n = 95$, $r = 0.45$, $p < 0.01$), so demonstrating an association between ascorbic acid deficiency and macrocytic erythropoiesis in these patients.

II. ANIMAL STUDIES

Aron (1939) observed that young guinea pigs on a scorbutic diet often died before developing anemia, but older guinea pigs on a scurvy diet, with iron pyrophosphate in their drinking water, showed a distinct reduction in the hemoglobin level of the blood within 20 d. Guinea pigs made anemic in this way were cured by the administration of ascorbic acid in large amounts, either orally or subcutaneously. This cure, however, was successful only in animals which had lost no more than about 25% of their body weight or one third of their hemoglobin. The rise in hemoglobin took place much faster than the gain in body weight, so the anemia was cured before the repair of the other body tissues was accomplished.

Sigal (1939), working in King's laboratory at the University of Pittsburgh, confirmed that vitamin C deficiency causes a decrease in the erythrocyte count and in the hemoglobin level of the blood of guinea pigs. Moreover, his data revealed that animals receiving injections of diphtheria toxin developed more severe anemia than others on the same borderline vitamin C intake (0.5 mg daily) or after the same number of days of total vitamin C deficiency. This kind of synergism of deleterious factors may well explain why anemia is so much more common in naturally occurring than in experimental human scurvy.

Saha et al. (1941) observed a progressive fall in the hemoglobin level of guinea pigs during 3 weeks on a scorbutic diet and saw it return to normal when ascorbic acid was provided. They observed no such anemia in pair-fed control animals, so they concluded that, "vitamin C may have some direct role in the formation of hemoglobin."

Megaloblastic anemia was consistently produced in monkeys by May et al. (1950, 1951), who fed them milk diets deficient in ascorbic acid. Monkeys fed the same diets supplemented with ascorbic acid remained in good health, without any anemia or bone marrow changes. This experimentally produced anemia was similar to the megaloblastic anemia which occurs in human infants. It could be cured with folic acid or folinic acid, without the aid of ascorbic acid; it could also be cured with ascorbic acid alone. Vitamin B_{12} did not cure it.

Proehl and May (1952), reporting work on 55 monkeys, concluded that a megaloblastic type of anemia develops during scurvy in monkeys on a diet devoid of ascorbic acid and containing only a small amount of folic acid. Supplementary folic acid prevented megaloblastosis during scurvy, and then a normoblastic anemia developed. As scurvy progressed, hemoglobin and plasma iron decreased, while erythrocyte protoporphyrin and coproporphyrin increased. Fecal urobilinogen became elevated with the onset of tissue hemorrhage, but no change was noted in urinary urobilinogen.

May et al. (1952a) found that conversion of folic acid to folinic acid in monkeys might

be less efficient in scurvy, but reported that ascorbic acid was not necessary for this conversion to take place. They concluded that folic acid deficiency in scurvy appeared to be due to an increased requirement in that disease. May et al. (1952b) reported that megaloblastosis in the marrow did not develop in the scorbutic monkey unless the level of free folinic acid in the liver was low. Low levels of free folinic acid in the liver were found only with low levels of total folic acid, but the level of free folinic acid was sometimes found to be normal although the level of total folic was low.

III. *IN VITRO* STUDIES

The work of Nichol and Welch (1950) and Nichol (1952), 1953) clarified this matter to a great extent. Studying rat liver slices incubated with folic acid, they found that ascorbic acid not only accelerates the conversion of folic acid to its reduced form, folinic acid, which is the essential need, but also protects folinic acid from enzymatic destruction.

IV. MEGALOBLASTIC ANEMIA OF INFANCY

There were in the past many instances of megaloblastic anemia in infancy due to the practice of boiling cows' milk to destroy the tubercle bacillus. Unfortunately, both ascorbic acid and folic acid are heat-labile vitamins; thus, boiling effectively destroys ascorbic acid and lowers folic acid levels to the point of borderline deficiency. Indeed, Kato (1932) reported erythrocyte counts below 4 million in 6 infants and below 3 million per cubic millimeter in 3 others among 13 infants with scurvy. May et al. (1950) showed that premature infants and monkeys fed a boiled milk diet developed both ascorbic acid deficiency and megaloblastic anemia. They found that the anemia could be cured by administration of folic acid, by very small doses of folinic acid, or by the administration of ascorbic acid alone.

However, in most infants, megaloblastic anemia develops after a series of infections, even though the intake of ascorbic acid may have been adequate. Clearly, infection can cause a disturbance of ascorbic acid metabolism and can deplete ascorbic acid stores. This could interfere with folic acid metabolism and lead to megaloblastic anemia; but May et al. (1952) gave reasons for believing that infection can act, even in the presence of normal ascorbic acid levels, to cause a deficiency of folic acid, with megaloblastosis, leukopenia, and fatty degeneration of the liver. They classified megaloblastic anemia of infancy into three broad groups: those responding to vitamin B_{12}, those failing to respond to B_{12}, but effectively treated with folic acid, and those acquiring a borderline deficiency of folic acid in association with a lack of ascorbic acid, in which case the megaloblastosis may be eliminated by either folic acid or ascorbic acid; they gave examples of all three kinds.

Zuelzer and Ogden (1946) reported 25 infants under 18 months of age with severe megaloblastic anemia, associated with various kinds of infection. They found that the anemia, thrombocytopenia, relative leukopenia, and bone marrow changes of the 17 surviving infants responded well to treatment with folic acid, but not to ascorbic acid alone. Six of the infants had clinical scurvy with osteoporosis and seven others had osteoporosis and/or hemorrhages, suggesting vitamin C deficiency, but their anemia did not respond to ascorbic acid. This resistance to ascorbic acid treatment may have been related to the "histamine fast" achlorhydria found in nine out of ten of these infants, as this can cause losses of ascorbic acid before absorption (Chapter 20, Volume I). Nearly all of these infants were receiving sulfonamides which have been reported to cause a disturbance of folic acid and ascorbic acid metabolism in rats (Chapter 24, Volume I), so the infection, the sulfonamides, and a poor diet may have combined in these infants to cause such a deficiency of folic acid that they could not respond to ascorbic acid alone.

V. MEGALOBLASTIC ANEMIA OF PREGNANCY

In addition to the slight debility due to the anemia itself, megaloblastic anemia of pregnancy connotes more serious potential problems, for it has been found by several authors to be associated with an increased incidence of placental separation, which can kill the fetus and also places the mother's life in serious jeopardy from disseminated intravascular coagulation, renal and hepatic failure, hypofibrinogenemia, and hemorrhage.

Iron deficiency microcytic anemia is much more common in pregnancy in Britain, Canada, and the U.S., but megaloblastic anemia also occurs. It is nearly always due to a disturbance of folic acid metabolism or a cellular deficiency of folinic acid and not to a deficiency of vitamin B_{12}. The B_{12} deficiency of pernicious anemia occurs mainly in an older age group, and when it does occur in younger women, they rarely get pregnant or carry the fetus past the early months. An association between megaloblastic anemia of pregnancy and abruptio placentae was postulated by Hourihane et al. (1960) when they found that abruptio placentae had occurred in 13 out of 95 patients with megaloblastic anemia. Coyle and Geoghegan (1962) found megaloblastic erythropoiesis on marrow biopsy in 35 out of 77 patients with abruptio placentae. This association was confirmed by Hibbard and Hibbard (1963) who found evidence of megaloblastic erythropoiesis in 46% of 73 cases of abruptio placentae, as compared with 5% of 121 controls. They also found folic acid deficiency, as evidenced by formiminoglutamic acid excretion tests, in 98.6% of patients with abruptio placentae and in only 10.7% of controls. Also, an increased incidence of preeclampsia (23% as compared to 12% was found by Goodall in women with megaloblastic anemia of pregnancy.

Most writers on this subject have assumed that megaloblastic anemia of pregnancy must be due to a dietary deficiency of folic acid, because it can be cured by folic acid, and have expressed the hope that increasing the amount of folic acid in prenatal vitamin supplements would not only prevent megaloblastic anemia, which it does, but also reduce the incidence of abruptio placentae, which it does not. Few have appreciated that it might be a disturbance of folic acid metabolism, rather than a deficiency of folic acid, and that this might be due to a deficiency of ascorbic acid or a disturbance of ascorbic acid metabolism. In fact, an extensive review of folic acid and pregnancy published in 1967 in *Nutrition Reviews* failed even to mention ascorbic acid. Pregnancy does represent an additional demand for folic acid, which is needed for purine synthesis by the rapidly dividing cells of the fetus, but it is unlikely that anyone has a folic acid-deficient diet; it would have to be devoid of all meats and vegetables or else it would have to be boiled to a pulp.

However, Holly (1951) appreciated the need for ascorbic acid in five patients with megaloblastic anemia whom he treated. Plasma ascorbic acid levels were not obtained before treatment in two patients, but were found to be 0 in two others and 0.3 mg/100 ml in another. The anemia of these pregnant women did not respond to treatment with vitamin B_{12} alone, but showed a good response to a combination of B_{12} and ascorbic acid.

Boscott and Cooke (1954) treated five patients with megaloblastic anemia of pregnancy. All excreted large amounts of parahydroxyphenylacetic acid in their urine, which is characteristic of ascorbic acid deficiency, and all ceased to do so after administration of ascorbic acid.

In 1958 Holly wrote that, "a low hemoglobin and hematocrit, leukopenia, thrombocytopenia and hyperferremia are pathognomonic of megaloblastic anemia. Examination of the peripheral blood smear may be helpful, but can be misleading. The diagnosis of megaloblastic anemia in pregnancy demands recognition of the abnormal megaloblast in the marrow. Therapy consists of the administration of folic acid 10 to 15 mg per day, or the combined use of ascorbic acid and vitamin B_{12}. Vitamin B_{12} alone does not induce a remission. Ascorbic acid acts slowly, but in combination with vitamin B_{12} it produces a maximal response." He says these patients with megaloblastic anemia of pregnancy are all vitamin C deficient and

many of them have chronic infections which must be treated. Holly mentioned a hemolytic component in megaloblastic anemia of pregnancy. Pritchard (1962) reported hyperbilirubinemia (mostly the indirect reacting variety), increased urinary urobilinogen, and decreased erythrocyte survival times using radiochromium-labeled red cells, all indicating increased hemolysis in megaloblastic anemia of pregnancy. He used ascorbic acid and folic acid in his therapeutic regimen, with iron, vitamin B_{12}, and antibiotics when indicated.

It might not seem to be very important which of two successful treatments is used in most disorders, but in this condition it is of the utmost importance that supplementary ascorbic acid be given to these patients along with folic acid and not folic acid alone. Clemetson and Andersen (1964) reported very low mean reduced ascorbic acid (AA, 0.16 mg/100 ml) and total ascorbic acid (TAA, 0.25 mg/100 ml) levels and a markedly decreased mean AA/DHAA ratio (1.6:1) in four women following abruptio placentae. Furthermore, Clemetson and Cafaro (1981) reported an increased incidence of abruptio placentae (19%) in women who had plasma ascorbic acid (AA) levels below 0.4 mg/100 ml during the latter half of pregnancy, compared with an incidence of 2% in those with plasma ascorbic acid levels above 0.4 mg/100 ml.

Clearly, there can be a disturbance of ascorbic acid metabolism due to many causes besides the pregnancy itself which involves the active transfer of ascorbic acid to the fetus. These include infection, hyperestrinism, hypercupremia, hemolysis, hemosiderosis, smoking, and all the other factors already discussed in earlier sections of this book (Volume I). Such a disturbance of ascorbic acid metabolism can cause a disturbance of folate metabolism leading to megaloblastic anemia, but ascorbic acid metabolism can also cause capillary and venular fragility in the decidua basalis and a tendency to abruptio placentae, which will not be corrected by folic acid alone.

When we know more about the causes of abnormal ascorbate metabolism in pregnancy, we may find that the chelating flavonoids, tannins, catechins, and other chelating fibers of food also have an important role to play, both as antioxidants for ascorbic acid in the GI tract and as chelators for the removal of excessive stores of heavy metals. The idea of trying to prevent, or at least reduce, the incidence of abruptio placentae by increasing the intake of ascorbic acid and *d*-catechin may seem rather optimistic, but then if we were not hopeful, we would never progress.

VI. IRON DEFICIENCY ANEMIA

Albers (1951) confirmed the clinical impression of others that when iron preparations are given with vitamin C there appears to be a greater absorption of iron into the blood and serum than when iron is given alone. Furthermore, he noted that in those women who were not fasting, there was an initial rise in blood iron which was followed later by a secondary rise. He felt, therefore, that vitamin C not only increased iron absorption, but also acted upon stored deposits of iron in the body. He reached the conclusion, therefore, that ascorbic acid acts in three ways in the treatment of iron deficiency anemia:

1. By action upon the iron preparation itself, in maintaining the divalent form in the intestine
2. In some manner being able to cause the reduction of the ferric to the ferrous form
3. Besides this reducing effect on the iron, the ascorbic acid having effect on the organism itself which results in a marked increase in serum iron, independently of iron absorption, and being related to the liberation of iron from storage depots in the body.

Most information about iron absorption has been obtained from experiments in which inorganic iron salts were fed to patients or animals, but Moore (1955) studied protein-bound

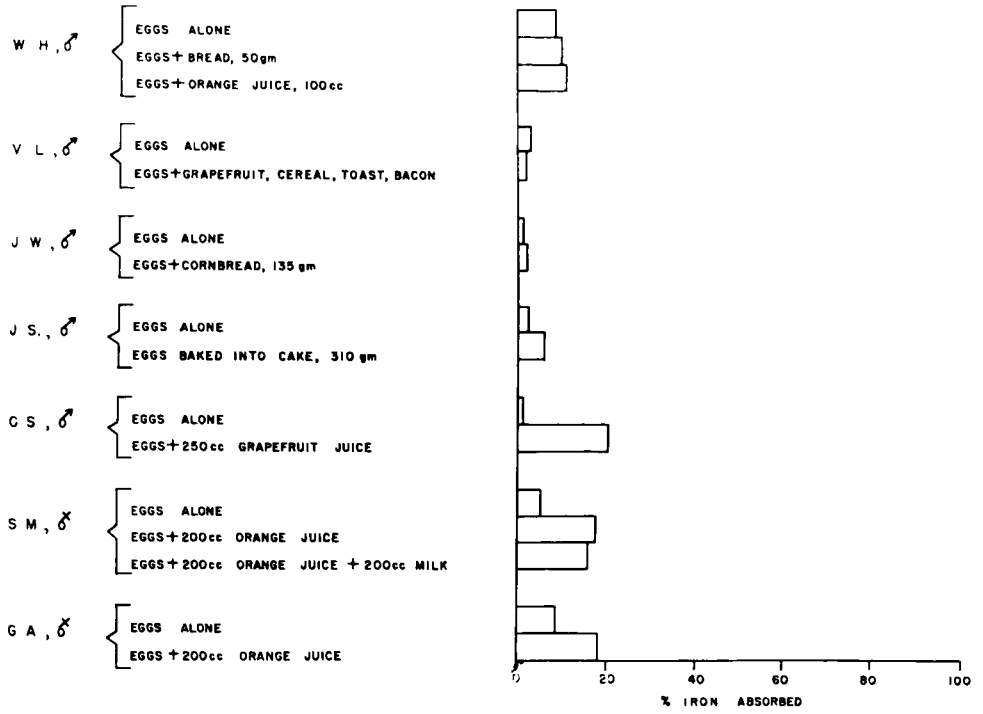

FIGURE 1. Effect of foods on absorption of ^{59}Fe from eggs — normal subjects. (From Moore, C. V. [1955], *Am. J. Clin. Nutr.*, 3, 3. ©American Society for Clinical Nutrition. With permission.)

iron which was part of the food. He injected radioactive iron into hens so that the eggs they produced contained the isotope. These eggs were then fed to healthy individuals, either alone or with other foods or dietary supplements, and the amount of radioactive iron subsequently incorporated into circulating hemoglobin was used as a measure of the quantity absorbed. The results of his initial studies, shown in Figure 1, suggested that a sufficient quantity of orange juice or grapefruit juice taken with the egg markedly increased iron absorption. Subsequent experiments shown in Figure 2 clearly demonstrated that a large dose of ascorbic acid (1 g) given orally with the egg caused a very great increase in iron absorption.

As mentioned in earlier sections of this book, *in vitro* studies by Goldberg (1959) showed that ascorbic acid may play an important role in the incorporation of iron into protoporphyrin, which is a step in the synthesis of heme. Also, Mazur et al. (1960) found that ascorbic acid and adenosine triphosphate (ATP) are required for the incorporation of iron into hepatic ferritin in the rat.

Wilson and Loh (1971) compared the effects of ascorbic acid (500 mg daily), slow-release iron (containing 525 mg of ferrous sulfate), daily, or a combination of these doses of iron and ascorbic acid as dietary supplements for elderly men and women. Unfortunately, they did not select anemic or ascorbic acid-deficient test subjects. In the men, iron alone, and iron with vitamin C, caused elevations in the hemoglobin levels during the trial period. In the women, all three types of supplements caused an elevation in the hemoglobin levels, but the most consistent and uniform rise occurred in the group receiving iron with vitamin C.

Jacobs et al. (1971) found that patients with iron-deficiency anemia have significantly higher leukocyte total ascorbic acid (TAA) levels (55.4 $\mu g/10^8$ cells) than normal subjects (38.6 $\mu g/10^8$ cells) ($p < 0.001$) and suggested that this may be a result of reduced ascorbate

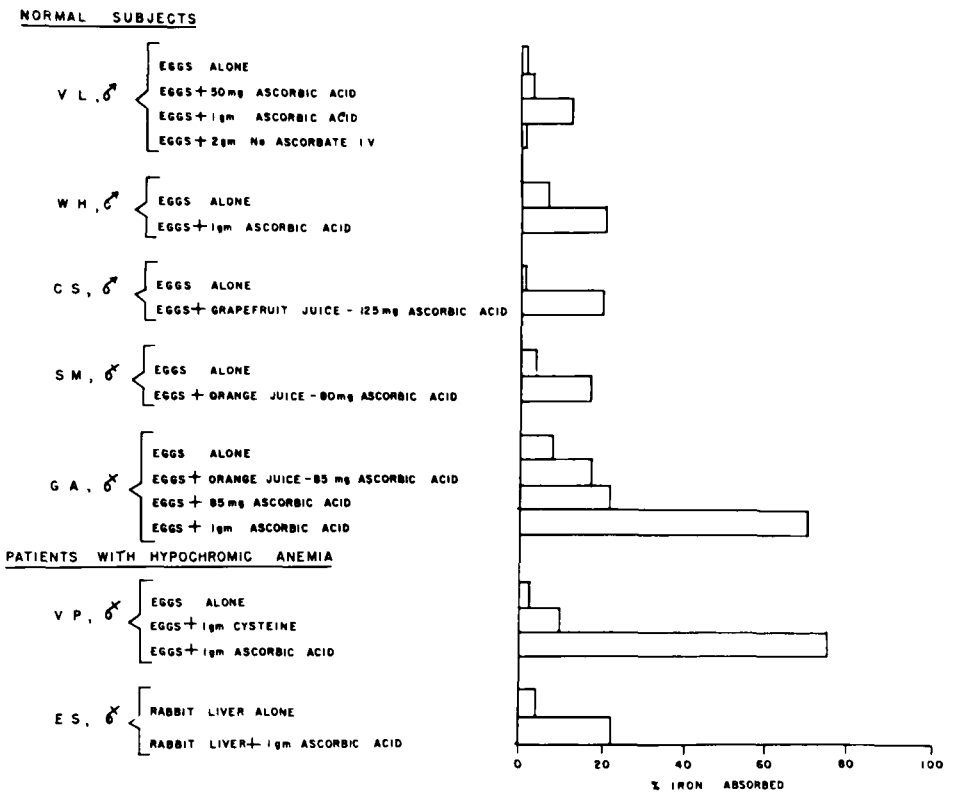

FIGURE 2. Effect of reducing substances on absorption of food iron. (From Moore, C V [1955], *Am J. Clin. Nutr.*, 3, 3. ©American Society for Clinical Nutrition With permission.)

catabolism in iron deficiency anemia. The mean plasma ascorbic acid (AA) level of patients with iron deficiency anemia (0.59 mg/100 ml) did not differ significantly from that of normal subjects (0.83 mg/100 ml). Human studies by Cohen et al. (1981) have demonstrated that ascorbic acid aids the release of iron from storage as ferritin in patients with hemosiderosis undergoing desferrioxamine chelation therapy; this suggests that ascorbic acid may normally help to mobilize iron, as predicted by Albers 30 years earlier.

VII. THE ANEMIA OF HEMOSIDEROSIS

As mentioned in the section of this book on heavy metals and water supplies (Chapter 10, Volume I), there is a serious problem among some male Bantu in South Africa who develop hemosiderosis from drinking too much homemade beer containing too much iron, because the beer is fermented at a low pH in iron pots. Not only do they develop frank scurvy with bleeding swollen gums, extensive ecchymoses, and painful, stiff joints, they also develop osteoporosis, scleroderma, and anemia.

These men have accelerated catabolism of ascorbic acid, as reported by Lynch et al. (1967), who demonstrated accelerated plasma ascorbate clearance in spite of markedly decreased renal excretion of this vitamin. The nature of their anemia was thoroughly investigated by Merskey (1953), Bronte-Stewart (1953), and Bothwell et al. (1964). These investigators found no impairment of iron absorption or transport and no major impairment of hemoglobin synthesis in these scorbutics. In fact, they showed a reticulocytosis, with increased urinary urobilinogen and decreased red cell survival, indicating hemolysis. It seems that the red cells of the scorbutics do not show increased fragility *in vitro* or when transfused

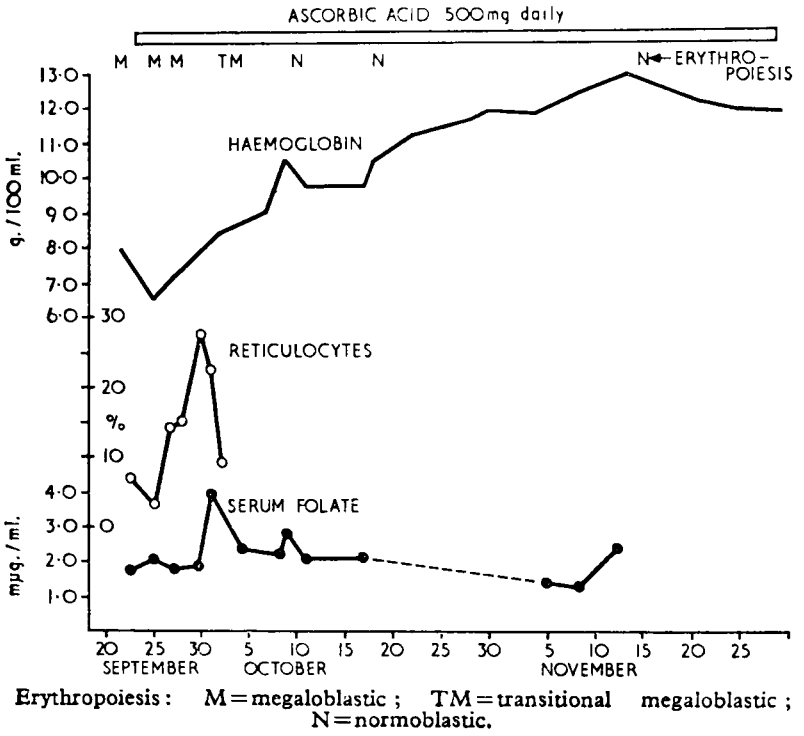

FIGURE 3. Megaloblastic anemia in a 70-year-old man with scurvy, who was successfully treated at the Crumpsall Hospital in Manchester, using ascorbic acid alone. (From Asquith, P., Oelbaum, M. H., and Dawson, D. W. [1967], *Br. Med. J.*, November, 402. With permission.)

into normal individuals, but red cells from normal people have a shortened life when transfused into scorbutic patients.

One might suspect from this that it might be only extravascular hemolysis, occurring in the ecchymoses and intramuscular hematomata, which causes the decreased red cell survival, but several investigators have observed evidence of hemolysis in the absence of large extravasations of blood and are convinced that intravascular hemolysis also occurs in the anemia of hemosiderosis and scurvy. Jacobs et al. (1971), working at the Welsh National School of Medicine at Cardiff, have also found the mean leukocyte (TAA, 22.22 $\mu g/10^8$ cells) and the plasma (AA, 0.26 mg/100 ml) ascorbate levels of patients with iron overload to be lower than normal ($p < 0.001$ and $p < 0.005$, respectively).

VIII. THE ANEMIA OF ADULT SCURVY IN TEMPERATE REGIONS

Adult scurvy is a rare disease nowadays, occurring mostly in elderly men who live alone, but also in women, as reported by Walker (1968). Sporadic occurrences often result from multiple nutritional deficiencies, along with other factors, such as trauma or infection, and so present a varied picture. Thus, there are reports of scorbutic megaloblastic anemia responding to ascorbic acid alone (Asquith et al., 1967) (Figure 3) and of scorbutic megaloblastic anemia which failed to respond to ascorbic acid alone, but responded to folic acid with ascorbic acid (Zalusky and Herbert, 1961). There are also reports of combined ascorbic acid and vitamin B_{12} deficiency in pernicious anemia (Wallerstein et al., 1953). Nevertheless, the majority of adult scorbutics have a normochromic and normocytic or moderately macrocytic anemia with a reticulocytosis and increased urinary urobilinogen, indicative of

hemolysis, as reported by Vilter et al. (1946) and by Goldberg (1963) who reported on 55 patients admitted to the Western Infirmary in Glasgow between 1938 and 1960. The anemia in all of Vilter's patients responded to ascorbic acid alone. One of Goldberg's patients had frank iron deficiency and required iron as well as ascorbic acid, but the anemia of all the others, including two with macronormoblastic and megaloblastic bone marrows, respectively, was cured with ascorbic acid alone.

Cox et al. (1962, 1967) conducted detailed hematological studies on 12 patients with scurvy, and Cox (1968), reviewing the results of these investigations, concluded that the majority of scorbutic patients have a normocytic or macrocytic anemia associated with a normoblastic or macronormoblastic marrow. They found that ascorbic acid therapy alone was effective in the majority of cases, but folic acid was needed occasionally. He felt that extravascular hemolysis, iron deficiency, and vitamin B_{12} deficiencies could be secondary or contributory factors.

IX. CONCLUSIONS

In view of the observations that ascorbic acid aids iron absorption, that ascorbic acid aids the incorporation of iron into protoporphyrin, which is a step in the synthesis of heme, that ascorbic acid is concerned with the combination of iron with apoferritin to form ferritin, and that ascorbic acid and other reducing agents release iron from ferritin, one might expect to find some impairment of iron absorption, transport, or incorporation into hemoglobin in scurvy. However, Bothwell et al. (1964) found no major impairment of iron absorption or transport in human subjects or guinea pigs with scurvy. Moreover, the erythropoietic rate, as judged by the plasma iron turnover, was normal in scorbutic patients with anemia. They did find that the plasma iron level increased after administration of ascorbic acid to scorbutic patients, but concluded that the low plasma iron levels found in scurvy are clearly adequate to supply the needs of the bone marrow, since there was no change in the plasma iron turnover when the plasma iron level had been raised by treatment with ascorbic acid. In view of the reticulocytosis and the decreased survival time of red cells in scurvy, as demonstrated by Merskey (1953) and others, they concluded that the anemia (mean hemoglobin level, 9.7 g/100 ml in their 25 scorbutic patients) must have been the result of hemolysis and/or hemorrhage greater than the capacity of the bone marrow to respond.

Ascorbic acid deficiency also causes a disturbance of folic acid metabolism, which causes a megaloblastic type of anemia when folic acid supplies are borderline. Likewise, an iron deficiency anemia may occur when iron stores are low. A leading article in the *British Medical Journal* (1979) reminds us that the combination of aspirin ingestion and ascorbic acid deficiency may cause gastrointestinal hemorrhage and that this may be a cause of anemia. Indeed, ascorbic acid deficiency must always be considered whenever there is gastrointestinal hemorrhage and no lesion is found.

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Chapter 4

DEFECTIVE WOUND HEALING

Aschoff and Koch (1919) are credited with being the first to observe the defects of intercellular connective tissues in scurvy. They carried out post-mortem studies on soldiers who had died of scurvy during World War I and demonstrated constant pathological changes in all the supporting tissues of the body. Characteristic changes were noted in the bone and cartilage at the costochondral junctions; there was rarefaction of existing bone, both cortex and spongiosa, irregularities and disappearance of cartilage columns, yielding of bone under strain, and a zone of fragmentation of bone trabeculae adjacent to the line of junction with cartilage; fracture may occur at this site; hemorrhages are seen; osteoblasts assume elongated shapes and apparently disappear from regions of bone formation. Höjer (1924) duplicated these findings in guinea pigs on a scorbutogenic diet and noted that the changes were largely due to atrophy of connective tissue fibers in all parts of the body.

Studies by Wolbach and Howe (1926) and Wolbach (1933, 1937) confirmed that there is a defect in the formation of reticulin and collagen by the fibroblasts of ascorbic acid-deficient guinea pigs. The fibroblasts are surrounded by liquid, but within 24 h of the administration of antiscorbutics, there is a jelling of the liquid, and deposits of a fine reticulum (the earliest form of collagen) are seen around the fibroblasts. Wolbach and Bessey (1942) concluded that collagen forms the intercellular substructure of all the supporting tissues — bone, cartilage, fibrous tissue, and dentin — and it is either not produced or is produced in a defective form in scurvy.

Saitta (1929) observed delayed healing of surface wounds in guinea pigs that had been kept on a vitamin C-deficient diet for more than 15 d. He determined the rate of healing by daily measurements of the length and breadth of the wounds. When a vitamin C-containing extract was applied directly to the wound, the healing time was appreciably diminished, regardless of whether the animals were maintained on a normal or scorbutogenic diet. Smith and McConkey (1933) provided similar evidence when they demonstrated that mechanical injury to the mucosa of the duodenum in guinea pigs fed an adequate diet was followed by rapid and complete healing, while similar injury to guinea pigs fed on a diet deficient in vitamin C resulted in the formation of peptic ulcers.

Lanman and Ingalls (1937) showed that guinea pigs maintained on one fifth of the minimal protective dose of ascorbic acid showed wound healing which was inferior to that of controls receiving a full diet. Moreover, the abdominal wounds of scorbutic guinea pigs ruptured at a pressure one third of that required to rupture the wounds of normal animals.

Using silver nitrate staining, Lauber and Rosenfeld (1938) showed in guinea pigs, on a vitamin C-deficient diet, that many organs lost all their vitamin C after the animals had been wounded, but the vitamin could still be demonstrated in the tissues around the healing wounds.

Taffel and Harvey (1938) reported weakness in the healing of stomach wounds in partially scorbutic guinea pigs. Studying guinea pigs on an absolutely scorbutic diet, they found markedly inferior wound strength; they also made the following observations: "The costochondral junctions and the epiphyses of the long bones, especially at the wrist and the ankle, were thickened, bulbous and tender. Although only rare spontaneous fractures were noted, the long bones were so fragile that frequently even gentle handling was sufficient to crack them. Scattered small and large haemorrhages were observed in the subcutaneous tissues of the limbs and particularly on the abdominal wall where the animals rubbed themselves on the floor of the cage. At first irritable and easily excitable, the guinea pigs later became listless and apparently avoided moving about in the cage — none of them had haemorrhagic gums."

The clinical picture is very similar in infants, so we must always suspect infantile scurvy or Barlow's disease in any child with open sores, bruises, and unexplained fractures. There are now laws in many countries requiring that such children be reported to the "child abuse authorities"; it is therefore absolutely essential that ascorbic acid deficiency should be ruled out by blood analysis before the parents or guardians are blamed for any abuse. The parents will be sufficiently distressed by their own poverty and by the sickly, whining child with fractures and skin sores that will not heal. They may evey be suspecting each other of having abused the child. Barlow's disease calls for help and dietary advice, not accusation, vilification, and rejection or imprisonment of the parents. Even Mr. Squeers should have his day in court.

Bouton (1939), working at the Hastings State Hospital in Nebraska, noted extremely poor progress in the healing of decubitus ulcers and other lesions involving loss of skin, despite apparently insufficient clinical grounds for such delay, and observed that some of these decubitus ulcers improved promptly and markedly after the administration of large quantities of tomato juice. Likewise, Wolfer and Hoebel (1939) recognized that many surgical patients are debilitated and vitamin C deficient. These authors routinely provided large doses of ascorbic acid, 1 g daily, both before and after surgery, to reduce the risks of wound disruption, evisceration, or postoperative hernia. They also suggested that this dietary supplement reduces the risk of peritonitis arising from a leaking intestinal suture line and is valuable to promote the union of fractures.

Holman (1940) studied the blood ascorbic acid levels of 70 patients of low economic levels admitted to the wards of the Stanford-Lane Clinic in San Francisco prior to surgery. In spite of the ready availability of citrus fruits in California, 44% of the patients showed values of 0.15 to 0.30 mg/100 ml, indicating vitamin C deficiency. In nine patients, values fell below 0.15 mg/100 ml, so they were on the verge of scurvy and would certainly have suffered wound dehiscence if they had not been supplemented with vitamin C. Holman also found 26% of his patients to be deficient in vitamin A. He recommended that all patients due to undergo elective surgery should be admitted to the hospital several days beforehand and should be prepared with a high-caloric, high-protein diet and should be provided with high-dosage vitamin supplements. He also recommended that this dietary supplementation should be continued as soon as possible after surgery.

In an experiment conducted on himself and reported by Crandon et al. (1940), Dr. John Crandon of Boston showed that a wound made in his back after 3 months on a vitamin C-deficient diet was well healed after 10 d, but a similar wound made after 6 months on this diet showed no signs of healing after 10 d; intercellular substance was deficient and the wound was filled with unorgainzed blood clot. Clearly, experimental scurvy is very slow to develop in a healthy human subject, but it probably develops much more rapidly in clinical situations, where its onset may be accelerated by infection or by any of the other factors discussed in Volume 1.

Hunt (1941) summarized the essentials of wound healing as migration and proliferation of epidermal and mesodermal cells, production of an intercellular matrix, and the formation of new blood vessels. Studying scorbutic and subscorbutic guinea pigs, he found that fibroblastic proliferation was normal, but collagen formation was not; in scorbutic wounds, weak argyrophil precollagen persisted and firm van Gieson-staining collagen did not form. He also studied the effect of scurvy on healed wounds and found that the new collagen of the scar reverted to an argyrophil precollagenous state. Hunt expressed the opinion that all forms of intercellular matrix are deficient in scurvy, including reticulum, collagen, bone, enamel, dentin, and possibly also cartilage and elastin.

Bourne (1942a) found a close correlation between the tensile strength of skin wounds in guinea pigs and the amount of vitamin C injected daily as a supplement to a scorbutogenic diet; his results are shown in Table 1. He postulated that ascorbic acid might in some way

Table 1
THE TENSILE STRENGTH OF GUINEA PIG SKIN WOUNDS, 1 WEEK AFTER INCISION, WHILE ON DIFFERENT ASCORBIC ACID INTAKES

	Number of guinea pigs				
	3	3	3	3	3
Daily dose of vitamin C injected subcutaneously (mg)	2	1	0.5	0.25	0
Tensile strength of wound in grams	283	162	154	60	46

From Bourne, G. H. (1942a), *Lancet*, December, 661. With permission.

be responsible for linking the amino acid chains of precollagen to form collagen. Bourne also pointed out that ascorbic acid is essential for the healing of bones. He stated that, "In the first stages of the repair of bone, cellular proliferation and the production of fibres similar to collagen are essential preliminary processes to calcification, and it is to be expected, therefore, that a deficiency of vitamin C would delay healing in bone." Moreover, Bourne (1942b), studying guinea pigs with injured femurs, showed that the degree of healing was proportional to the amount of vitamin C given. He cited several authors who had noted nonunion of human fractures in scurvy, but he doubted the stories of old healed fractures becoming disunited.

Guinea pig studies by Hartzell and Stone (1942) showed that a vitamin C-deficient diet profoundly affected wound healing. Abdominal wounds in guinea pigs receiving an adequate ascorbic acid intake (5 to 10 mg/d) were almost universally clean and well healed by the sixth to tenth day, but the wounds of those on an ascorbic acid-deficient diet (0.2 mg/d) were edematous, and bloody crusts, formed as a result of oozing soon after the operation, were still present. The tensile strength of transverse strips of the wounds of these animals were measured and found to be about half normal on the 6th postoperative day and about a quarter normal on the 8th to 14th day (Figure 1).

Bartlett et al. (1942) studied the ascorbic acid (AA)* levels of blood plasma, skin and fascia at the time of surgery and again, 10 d later, in six men undergoing operations for inguinal hernia. They also studied the tensile strength of specimens of skin and fascia. Although the data were necessarily limited they did support the conclusion that, "A sufficient depletion of vitamin C produces a decreased ascorbic acid content and tensile strength in healing wounds in the skin and fascia of human beings," and also that, "A fasting plasma ascorbic acid level below 0.2 mg/100 ml must be reached before these changes occur."

The work of Jones et al. (1943) confirmed that the tensile strength of healing wounds in guinea pigs varies with the intake of ascorbic acid and with the resulting ascorbic acid content of the scar. Experimentally induced local wound sepsis was particularly marked in scorbutic animals and was a potent factor interfering with wound healing and reducing tensile stength.

A definitive study of wound healing in ascorbic acid-depleted human subjects was reported by Wolfer et al. (1947). Nine male medical students were placed on a diet containing less than 10 mg of ascorbic acid a day and continued on this diet for 7 months, until the mean ascorbic acid content of their leukocyte-platelet layer had fallen to 0.75 mg/100 g. Five control students on a full diet had a mean leukocyte-platelet ascorbic acid level of 32 mg/100 g. Experimental wounds in the skin and fascia of the lateral aspects of the thighs of the

* AA — ascorbic acid, reduced form.

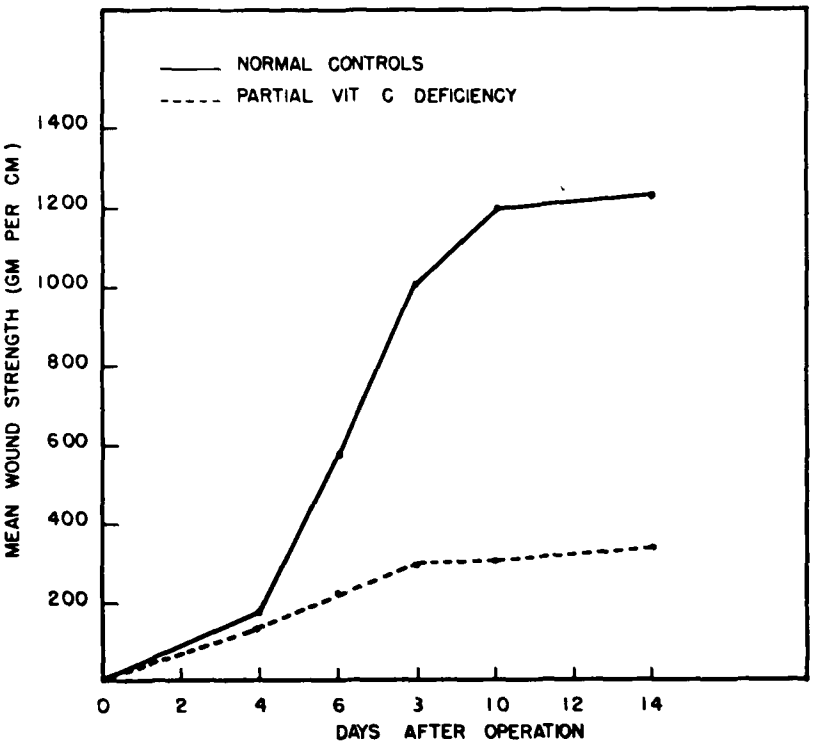

FIGURE 1. Effect of partial vitamin C deficiency on wound healing in guinea pigs. The unbroken line represents the average tensile strength of 1 cm of wound in 60 guinea pigs fed adequate amounts (5 to 10 mg) of ascorbic acid daily. The broken line represents the average tensile strength of 1 cm of wound in 40 guinea pigs fed deficient amounts (0.2 mg) of ascorbic acid daily (From Hartzell, J. B. and Stone, W. E. [1942], *Surg. Gynecol. Obstet.*, 75, 1. With permission.)

ascorbic acid-depleted and control subjects were studied by excising specimens at various stages of healing. The vitamin deficiency was found to be associated with approximately a 50% diminution in the tensile strength of the wounds at 7 to 10 d after incision and resulted in a delay in the attainment of comparable wound strength in uncomplicated wounds from 7 to 11 d. Moreover, wound complications developed in four of the vitamin-depleted subjects following either the incision or the later biopsy: they were local skin necrosis (1), infection (2), and ecchymosis (1).

Bosse and Axelrod (1948) observed that pyridoxine deficiency, and also riboflavin deficiency, delayed wound healing in rats. Both deficiencies resulted in increased precollagen and decreased collagen, somewhat similar to the situation in ascorbic acid deficiency, but the animals had lost weight to such an extent that it was not possible to know whether the changes were not due to inanition.

Localio et al. (1948) demonstrated the effectiveness of methionine in restoring the wound healing capacity of protein-depleted rats; this and other similar studies have suggested an important role for –SH groups, –SS– bonds, or –SH, –SS– redox systems in the healing process.

Studies by Galloway et al. (1948) showed that lack of ascorbic acid delayed the healing of skin wounds in the thigh and in the ear pinna of guinea pigs, but had no effect on the rate of repair of small wounds of the corneal epithelium or the gum mucoperiosteum. It was suggested that regeneration of epithelium alone can take place with normal speed in ascorbic acid-deficient guinea pigs, but when healing of the wound demands new collagen formation,

then lack of ascorbic acid delays epithelialization. Indeed, this has been proven to be true, for larger and deeper corneal wounds need ascorbic acid for healing (Chapter 18, Volume III).

Crandon et al. (1952) reported the results of analysis of samples of blood, plasma, buffy layer, and fascia from 70 surgical patients. They found that the plasma, rather than the buffy layer, more accurately reflected the tissue levels. They stated, "Ascorbic acid deficiency in the fascia of surgical patients is better reflected by plasma than by buffy layer levels." When the fascial ascorbic acid (TAA)* levels were grouped as high (6 to 15 mg/100 g), medium (1.5 to 5 g/100 g), or low (0 to 1.4 mg/g), there were 26 in the low group; only 3 of these had plasma ascorbic acid levels of over 0.18 mg/100 ml, and each of them had recently been receiving an ascorbate supplement.

Pirani and Levenson (1953) made a histological study of the effect of vitamin C deficiency in healed guinea pig wounds. They made midline laparotomy incisions and allowed them to heal for 6 weeks, during which period the animals were fed a nutritionally complete diet. After 6 weeks, when the wound scar was reduced to a thin line and was often hardly visible, they were divided into three groups — ascorbate deficient, pair-fed plus ascorbate, and full diet. In 3 of the 13 instances where scurvy was produced, herniation developed at the site of the wound; however, complete dehiscence did not occur. All of the wounds of the 13 control guinea pigs of groups 2 and 3 were well healed and none developed herniation. Histological observations on the scar tissue of the scorbutic guinea pigs whose laparotomy wounds had healed normally 6 weeks prior to the initiation of the scorbutic diet revealed fibroblastic proliferation and regression of connective tissue elements and hemorrhages. In no case did the collagen fibers stain brilliant red with van Gieson's technique, as did the normal collagen of the controls. The color instead varied from pale red to pink and, in a few animals, to either a diffuse or patchy yellow-pink color. Reticulum was present in much greater amount than in the controls and appeared as a fine network or as groups of coarse, irregular fibrils. The degree of metachromasia, as judged by the toluidine blue stain, was markedly increased when compared with that of the controls. These changes which developed in the connective tissue of a healed wound when ascorbic acid was removed are of the same type as those that occur in healing wounds of scorbutic animals or man. Thus, it is evident that an adequate ascorbic acid intake is essential, not only for normal healing in the period immediately following surgery, but also for the maintenance of previously formed scar tissue. Scar tissue seems to be much more susceptible to vitamin C deficiency than is normal tissue.

Dunphy et al. (1956) conducted an interesting experiment in which guinea pigs were wounded, under general anesthesia, by excision of a 3-cm-diameter disk of skin, connective tissue, and superficial muscles from the interscapular region of the back. The test guinea pigs were placed on a scorbutogenic diet for 20 d before wounding and for 7 d after wounding. Then, starting on the eighth day, some of them were given ascorbic acid (25 mg) daily by intramuscular injection. Control guinea pigs received a diet supplemented with fresh vegetables and oranges.

These workers studied

1. The mucopolysaccharides in the healing wounds, (a) histologically by use of Hale's colloidal iron stain, by toluidine blue for metachromatic staining, and by periodic acid Schiff (PAS) stain and (b) chemically by hydrolysis and analysis for hexosamine
2. The reticulin and reticulin-staining material by silver impregnation
3. The collagen, (a) histologically by van Gieson's stain and by Mallory's connective tissue stain and (b) chemically by hydrolysis and analysis for hydroxyproline

* TAA — total ascorbic acid, reduced and oxidized forms.

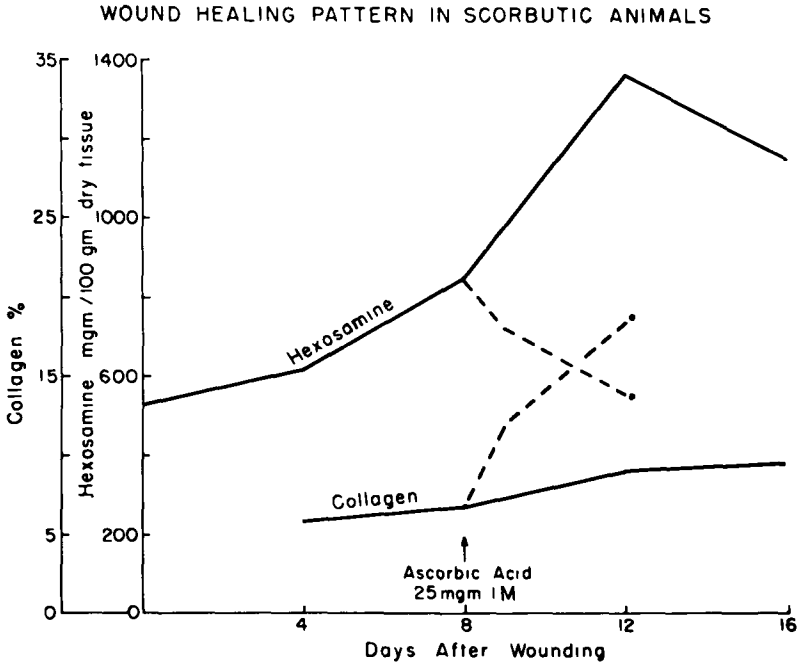

FIGURE 2. The pattern of healing in scorbutic guinea pigs. The dotted lines indicate the alteration which follows the administration of 25 mg of vitamin C daily intramuscularly. (From Dunphy, J. E., Udupa, K. N., and Edwards, L. C. [1956], *Ann. Surg.*, 144, 304. With permission.)

The results of the chemical analyses from that study are shown in Figure 2, where it is evident that the collagen content of the wounds remained low until ascorbic acid was given, when it rapidly increased.

Conversely, the high hexosamine levels in the scorbutic wounds suggested a high mucopolysaccharide content in the scorbutic wounds, which decreased when ascorbic acid was provided. (However, serum glycoproteins also contain hexosamine, so the high hexosamine levels in scorbutic wounds may simply indicate that extravasated serum proteins were not removed until ascorbic acid was administered.) Histological studies using colloidal iron and also PAS-stained sections confirmed that there was no deficiency of acid mucopolysaccharides in scorbutic healing wounds. Undoubtedly, the basic defect of repair in ascorbic acid deficiency is one of collagen synthesis; this was corrected within 24 h following the administration of ascorbic acid.

Haley and Williamson (1957) have made use of the fact the sulfur amino acids are essential for wound healing. They injected 1 mCi of either methionine-^{35}S or cystine-^{35}S, 24 h before surgery and used special equipment to compare the radioactive emission over the wound and over a skin site distant from the wound to study the rate of healing of human wounds without disturbing them. They cited evidence that collagen itself contains essentially no methionine or cystine and suggested that the accumulation of sulfur amino acids at the wound site may be within the fibroblasts themselves.

Reviewing the chemistry of collagen in fibrogenesis and wound healing, Jackson (1958) discussed the confusion arising from the use of such terms as tropocollagen, précollagène, procollagen, collastromin, metacollagen, alkali-extracted collagen, 0.2 and 0.45 *M* NaCl neutral salt-extracted collagen, citrate-extracted collagen, etc. He suggests that there are actually no clear-cut fractions, and that neutral salt-extracted collagen consists of a continuous spectrum of aggregates of collagen molecules of varying size and strength of cross-linkage.

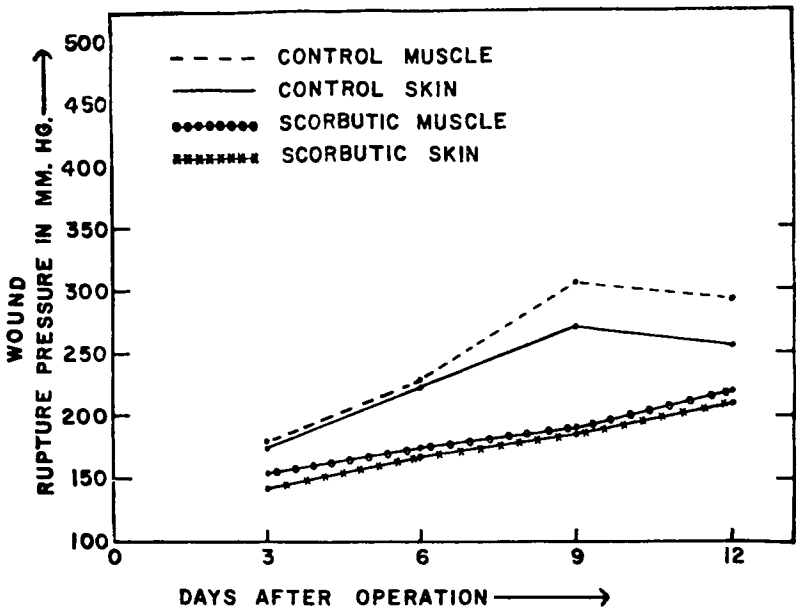

FIGURE 3. Intraabdominal pressure required to rupture the skin and the muscle layers of abdominal wall wounds of anesthetized guinea pigs on normal and scorbutogenic diets on the 3rd, 6th, 9th, and 12th days after surgery. These workers concluded that, "even in the scorbutic animal a certain degree of wound healing occurs, though the level of increment of increase of healing in the scorbutic is considerably lower than in the normal animal on optimal ascorbic acid intake." (From Abt, A. F., von Schuching, S., and Roe, J. H. [1959a], *Bull. Johns Hopkins Hosp* , 104, 163. With permission.)

The fibroblast and its relatives, the osteoblast and the chondroblast, are believed to be responsible for the formation of a soluble form of collagen, which is secreted by the cell and then converted into a meshwork of very fine fibrils of reticulin, which accumulate more and more collagen (of triple helical structure) and coalesce by cross-linkage to form mature collagen in the extracellular space. As discussed in Chapter 2, Volume III, two amino acids unique to collagen, namely, hydroxyproline and hydroxylysine, cannot be incorporated into collagen protein as such. They join the polypeptide chains as proline and lysine and have to be hydroxylated *in situ* after incorporation. This hydroxylation requires the presence of a sufficient concentration of ascorbic acid at the wound site. Gustavson (1956) has shown that the cross-linking of collagen molecules takes place largely by hydrogen bonding between the hydroxyl groups of hydroxyproline and the keto-imide groups of adjacent helices, so clearly there can be no strong cross-linked collagen in the absence of hydroxyproline, which requires ascorbic acid.

Studies by Abt et al. (1959a) showed that the pressure required for the rupture of the abdominal wounds of guinea pigs on scorbutogenic diets was less than that required to rupture similar wounds in guinea pigs on complete diets, at 3, 6, 9, and 12 d after surgery. Nevertheless, even the wounds of the scorbutic animals did show a progressive increase in strength with time after surgery, as seen in Figure 3.

However, Abt et al. (1959b) were able to demonstrate experimentally in guinea pigs that wounds healed for a long time become markedly weaker in scurvy, as the ascorbic acid level of the scar falls. They found that scar tissue normally maintains a higher ascorbic acid (TAA) level than adjacent tissues, even long after a wound is healed, but this advantage is markedly reduced in scurvy (Figure 4). In one man, they found the ascorbic acid (TAA) concentration in the skin of an old scar to be 9.0 mg/100 g, compared with 5.5 mg/100 g

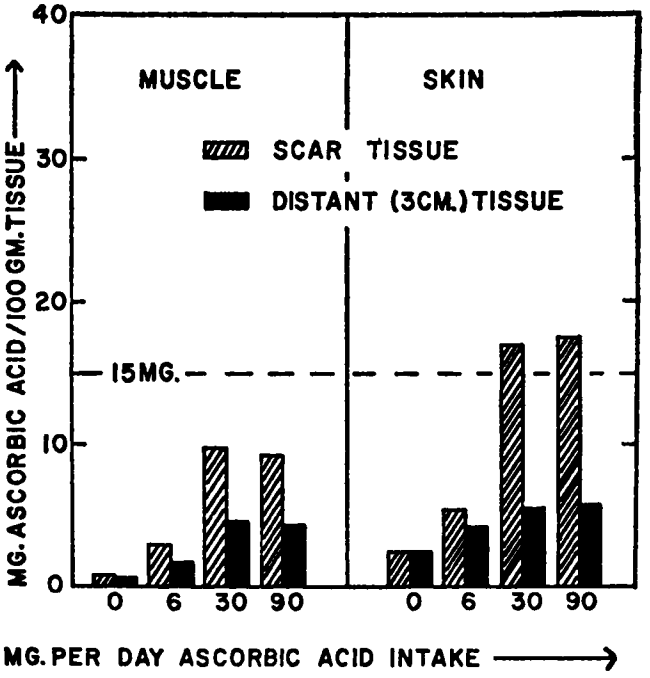

FIGURE 4. Relation of varying levels of dietary intake of ascorbic acid to its concentration in skin and muscle of wound scar tissue and skin and muscle of distant abdominal tissue. Normally, the scar tissue contains more ascorbic acid than surrounding tissues, but this advantage seems to be absent in scurvy. (From Abt, A. F., von Schuching, S., and Roe, J. H. [1959a], *Bull. Johns Hopkins Hosp.*, 104, 163. With permission.)

in abdominal skin 3 cm distant from the scar, even though the wound had been healed for 30 years. They found that the higher levels of ascorbic acid noted in scar tissue are derived in part from recently administered ascorbic acid, as demonstrated by single injections of radioactive ascorbic acid in guinea pigs.

Abt et al. (1960) confirmed their earlier work concerning the increased concentration of ascorbic acid in healing wounds and noted that the central core of the scar 10 d after surgery has an ascorbic acid (TAA) concentration similar to that of the Achilles tendon. Moreover, the ascorbate concentration is also increased in the surrounding tissues and falls off with distance from the wound (Figure 5). In histological studies, they confirmed that the wounds of scorbutic guinea pigs are markedly deficient in collagen, but in chemical studies of collagen (measured as hydroxyproline), they found no difference between the scar skin and other tissues of scorbutic and normal animals.

Crandon et al. (1961) confirmed in humans that the ascorbic acid (TAA) content of the dehisced or granulating wound is considerably higher than that of normal tissues or the blood. In one instance, they recorded the finding of 1.3 mg of ascorbic acid per 100 g in the tissue of a biopsy taken from the dehisced wound of a patient whose plasma ascorbic acid level was zero.

Light and electron microscopic studies of wound healing in guinea pigs by Ross and Benditt (1962) extended and complemented the earlier studies of Wolbach, Bourne, and others. They found that scorbutic wounds clearly differed from the controls in three major aspects. "First there is a marked decrease, although not a total absence, of collagen fibrils, with a large amount of somewhat amorphous dense material within the intercellular space. Second, the great majority of the scorbutic fibroblasts contain irregularly shaped deposits

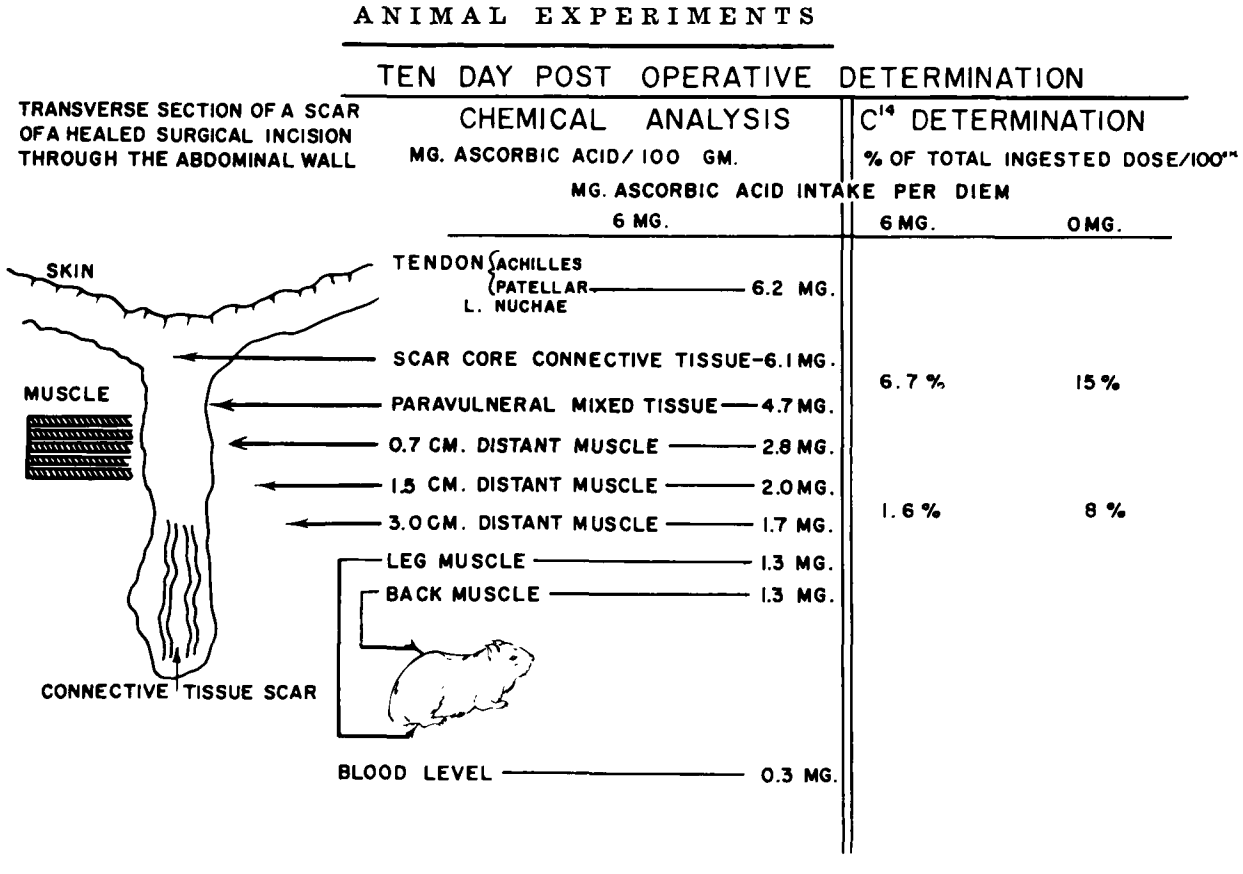

FIGURE 5. Results of analysis of tissues from the abdominal wounds of normal guinea pigs 10 d after surgery. The ascorbic acid (TAA) concentration in the core of the scar was found to be as high as that of the Achilles or patella tendons. The ascorbic acid concentration of surrounding tissues was also elevated and fell off with increasing distance from the wound. (From Abt, A. F., von Schuching, S , and Roe, J. H. [1960], *J. Nutr.*, 70, 427. ©American Institute of Nutrition. With permission.)

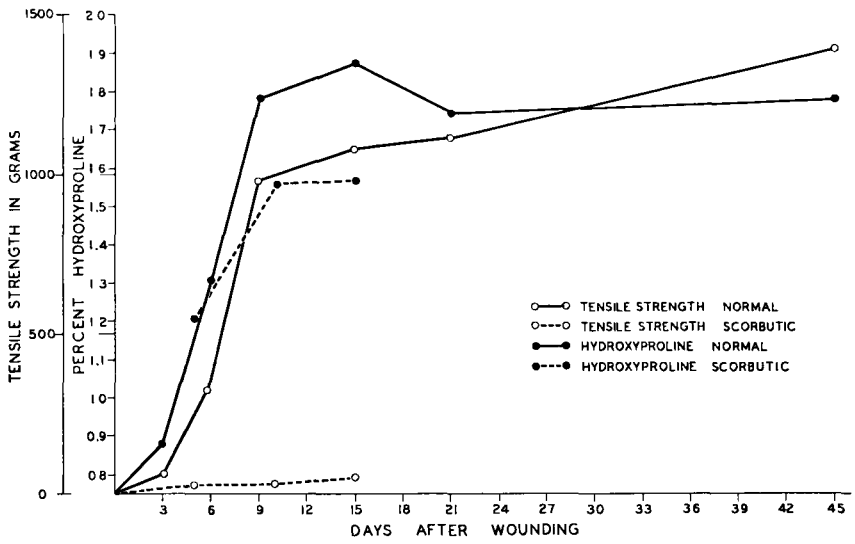

FIGURE 6. Tensile strength and hydroxyproline concentration in healing wounds of normal and scorbutic guinea pigs. (From Adamsons, R. J., Musco, F., and Enquist, I. F. [1964], *Surg. Gynecol. Obstet.*, 119, 323. With permission.)

of lipid which are not obviously membrane-bounded. Third, the endoplasmic reticulum of the scorbutic fibroblasts appears as rounded, often dilated, but rarely interconnected profiles, and this system is not so extensively developed as it is in the fibroblasts of the control wounds." They suggested that the presence of very few individual mature collagen fibrils adjacent to the border of some of the fibroblasts in the scorbutic wounds may be due to the fact that collagen is forming at a very slow rate. Gould (1961) reported his observations that even under the most drastic conditions of ascorbic acid deprivation, some collagen (measured as hydroxyproline) is formed in scorbutic guinea pigs. He has suggested that there are ascorbic acid-dependent and ascorbic acid-independent collagen-forming mechanisms, the independent mechanism being mainly responsible for growth, while the ascorbic acid-dependent process is essential for repair. He suggests that both growth and repair collagen are involved in the healing process, and that the former may serve as a foundation on which the rapidly forming repair collagen accumulates.

Studies of wound healing in scorbutic guinea pigs by Adamsons et al. (1964) showed the expected defect in the tensile strength of abdominal wounds. However, the results of analysis of the scorbutic wound tissues for hydroxyproline showed concentrations almost as high as those found in normal guinea pig wounds at comparable stages (Figure 6). Thus, it would seem that either hydroxyproline is an inadequate measure of collagen or else collagen concentration shows a poor relationship to the tensile strength of wounds. One can conceive of the existence of underhydroxylated collagen or of shorter polypeptide chains containing hydroxyproline to explain the weak tensile strength of precollagen. One can also conceive of otherwise mature collagen chains lacking cross-linkages one to another.

In studies of rats, Sandberg (1964) confirmed that the tensile strength and the hydroxproline content of healing wounds are decreased by the administration of cortisone. Reviewing the literature, he gave reasons for believing that histamine formation is essential as a stimulus to collagen formation in healing wounds and that cortisone inhibits histamine accumulation. This may be so, for Stewart et al. (1953a) observed that cortisone accelerates the reduction of dehycroascorbic acid (DHAA) to ascorbic acid *in vivo* (Figure 1, Chapter 13, Volume I), and Chatterjee et al. (1975a) showed that ascorbic acid accelerates the conversion of histamine to hydantoin-5-acetic acid (Chapter 1, Volume III).

Here we can envision the delicate balance of nature, for any wounding is a form of stress, leading to adrenocorticotropic hormone (ACTH) release, causing ascorbic acid to be released by the adrenals and to become available at the wound site. Presumably the cortisone released by the adrenals is also beneficial in moderate amounts, but too much cortisone can actually prevent healing. In fact, at the time of writing, we had a woman with lupus erythematosus in hospital, whose abdominal wound has just dehisced, most probably because we did not discontinue her cortisone therapy before surgery.

Dunphy (1967), studying the normal healing process in wounds of the large intestine, has observed that the entire wall of the colon, above and below the incision, underwent a rapid and significant loss of old collagen, down to as little as 40% of normal by the third day, but by the fifth day, the synthesis of new collagen resulted in a progressive rise in total collagen. So one must always appreciate that healing is a dynamic process, involving replacement of old collagen by new. Thus, the total collagen content is not as interesting as the rate of formation of new collagen and its progressive gain in strength.

McKibbin and Porter (1967) observed spontaneous fractures and indolent pressure sores in several children with meningomyelocele. On investigation of 25 children with meningomyelocele, they found that 76% of them were vitamin C deficient. The general health and the lassitude of the children improved noticeably following vitamin C administration; "the pressure sores in particularly often showed rapid epithelialization, including one which had previously resisted three attempts at surgical treatment and skin grafting."

Burr and Rajan (1972) studied the leukocyte ascorbic acid concentrations of 91 paraplegics and 41 members of the Hospital Staff at the Stoke Mandeville Spinal Injuries Centre in England. After subdividing the patients and the control subjects into males and females and into smokers and nonsmokers, it was evident that the paraplegics had lower ascorbic acid levels than the controls, possibly due to lack of exercise (Chapter 24, Volume I); 23% of the patients had leukocyte ascorbic acid levels below 15 $\mu g/10^8$ leukocytes. Moreover, the mean leukocyte ascorbic acid level of 33 patients with pressure sores or decubitus ulcers was significantly lower than that of patients without bed sores. Histological study of biopsies taken from the edges of the ulcers, before and after oral administration of vitamin C (500 mg) twice daily for 3 d or placebo, revealed an increased intensity of staining for collagen under the epidermis in all of those who had received ascorbic acid and no such change in those who had received placebo.

Afifi et al. (1975) carried out a "double-blind" crossover study of high-dosage ascorbic acid in the treatment of chronic indolent leg ulcers in eight adults with beta thalassemia. They gave 3 g of ascorbic acid daily for 8 weeks, followed by placebo for 8 weeks, to one group of four patients and reversed the procedure for the other four; there was a highly significant acceleration of healing as a result of the ascorbic acid treatment ($p<0.01$). Indeed, 20 out of 26 chronic leg ulcers, which had been present for at least 2 years before the study, were completely healed at the end of the study and the remaining 6 were reduced to less than half of their original size. These results are most encouraging, but there is always the possibility of the development of ascorbic acid toxicity and diabetes mellitus or cardiomyopathy, due to the release of excessive dehydroascorbate in patients with iron overload, so it would seem preferable to give ascorbic acid with a chelating agent, or a simple chelating fiber, such as *d*-catechin, to remove iron into the lumen of the bowel for excretion as fast as it is released from the liver during ascorbic acid treatment of thalassemics (Chapter 10, Volume I).

Studies of isolated collagen-synthesizing systems have been reviewed by Barnes (1975) who concluded that ascorbic acid participates in the synthesis of collagen hydroxyproline and hydroxylysine, both of which are formed by the hydroxylation of particular prolyl and lysyl residues previously incorporated into peptide linkage during the process of ribosomal collagen protein synthesis. The precise mode of action of the vitamin in these hydroxylations

Table 2
LEUKOCYTE ASCORBIC ACID (TAA) LEVELS (μg/10^8 CELLS) IN PATIENTS STUDIED BEFORE AND 1 MONTH AFTER COMMENCING SUPPLEMENTS

Group A (placebo)		Group B (ascorbic acid)	
Pretrial	After 1 month	Pretrial	After 1 month
40	7	5	56
40	10	14	57
35	13	20	60
30	45	20	60
20	25	20	65
20	15	31	90
16	45	45	75
11	50	21	61
4	22		
Mean 24.0	25.8	22.0	65.5

From Taylor, T. V., Rimmer, S., Day, B., Butcher, J., and Dymock, I. W. (1974), *Lancet*, 2, 544. With permission.

has yet to be elucidated. Many other workers have studied the effects of ascorbic acid deficiency on the hydroxylation of proline and lysine and the roles of these amino acids in the formation of collagen by the fibroblasts (see Chapter 2, Volume III).

Very encouraging findings have been reported in a controlled clinical trial by Taylor et al. (1974), who treated patients with pressure sores by oral administration of ascorbic acid (500 mg) twice daily. The mean leukocyte ascorbic acid (TAA) level of the treated group rose from 22.0 μg/10^8 cells before treatment to 65.5 μg/10^8 cells after 1 month of supplementation (Table 2). The results of treatment were assessed and recorded by ulcer tracings and by serial photography. There was a mean reduction in the size of the ulcers of 84% after 1 month in ten treated patients, whereas the corresponding reduction in the size of the lesions in ten control patients receiving placebo tablets was only 43% (Table 3 and Figure 7). Statistical analysis showed that these finding were highly significant ($p<0.005$); they certainly indicate that ascorbic acid accelerates the healing of bedsores.

Krámer et al. (1979) studied the rate of collagen formation in small polyvinyl alcohol sponges implanted in the subcutaneous tissues of guinea pigs on 0, 5, and 200 mg/d ascorbic acid intakes. Histological examination revealed no normal collagen production in the scorbutic group, but plenty of normal-looking collagen was seen in both the 5- and the 200-mg groups, whose appearances did not differ. However, measurements of hydroxyproline formation in the sponges at 7 d after implantation gave mean values of 0.54, 0.88, and 3.56 μg, respectively; these values did differ significantly from each other ($p<0.05$) in all three groups. At 14 d, the hydroxyproline content of the sponges were 0.62, 37.13, and 54.67 μg, respectively, and the two supplemented groups no longer differed significantly from one another. Thus, we may conclude that high-dosage ascorbic acid accelerated the rate of healing by collagen formation, but did not affect the final collagen content. In all probability, 20 mg/d would have done as well as 200 mg, but even 20 mg/d is a large dose for such a small animal.

Table 3
PERCENTAGE REDUCTION IN AREA OF PRESSURE SORES IN INDIVIDUAL PATIENTS AFTER 1 MONTH

Group A (placebo)	Group B (ascorbic acid)
50	81
54	72
4	87
60	87
22	21
39	90
45	100
14	100
60	100
80	100
Mean 42.8	83.8

Note: $p < 0.005$.

From Taylor, T. V., Rimmer, S., Day, B , Butcher, J., and Dymock, I. W. (1974), *Lancet,* 2, 544. With permission.

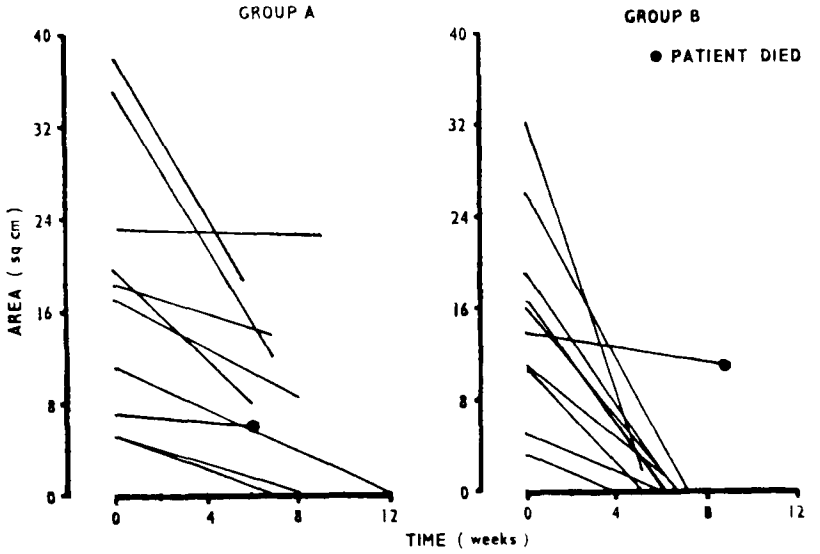

FIGURE 7. Comparison of pressure sore areas before treatment and on completion of trial in placebo-treated patients (group A) and in those receiving ascorbic acid (1 g) daily (group B). (From Taylor, T. V., Rimmer, S., Day, B., Butcher, J., and Dymock, I. W. [1974], *Lancet,* 2, 544. With permission.)

Ringsdorf and Cheraskin (1982) studied the time taken for healing of 3-mm-diameter circular experimental wounds in the gingival tissues of dental students by staining the ulcers with toluidine blue. They reported that 18 d, the healing time on a normal diet, could be reduced to 9 d, by the provision of an ascorbic acid supplement of 250 mg four times a day. These authors also reported great benefits from the administration of high-dosage

ascorbic acid supplements, 500 to 3000 mg daily, for patients recovering from surgery or other injuries, for the healing of decubitus ulcers and for leg ulcers associated with hemolytic anemia. Not only do surgery and trauma decrease blood ascorbic acid levels (Chapter 9, Volume I), so do aging (Chapter 5, Volume I), infection (Chapter 8, Volume I), and hemolysis (Chapter 15, Volume I), so the need for higher doses of ascorbic acid to promote healing in these conditions is quite understandable.

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Chapter 5

BONE CHANGES

I. INTRODUCTION

One of the most basic defects in scurvy is the inability of connective tissue cells to form mature extracellular materials; the fibroblasts, the osteoblasts, the chondroblasts, the odontoblasts, and the cementoblasts are all connective tissue cells which are related to one another and are responsible for the secretion of collagen fibers which are the foundation of fibrous tissue, bone, cartilage, dentin, and tooth cement, respectively. In scurvy, it seems that they continue to secrete a semiliquid protocollagen, but the ability to form mature collagen fibers is lacking, most probably as a result of defective protocollagen proline and lysine hydroxylation (Chapter 2, Volume III). Consequently, the extracellular tissues lack the framework of collagen on which to grow.

II. HUMAN OBSERVATIONS

The consequences are most serious at the time of most rapid bone growth — in infancy; the infant with scurvy tends to lie still on its back in the frog-leg position with its legs flexed at the knees and the hips and its thighs abducted and externally rotated. It avoids movement of the legs because of pain, and this causes a "pseudoparalysis" of the lower limbs. Retarded growth, multiple fractures, and severe pain due to subperiosteal hematomas may lead to a suspicion of child abuse, especially if there are accompanying bruises and ecchymoses. Bruises of the thighs and even fracture of the femur have been recorded as arising from the gentle act of diapering a scorbutic infant. Slipped epiphyses occur both in infants and in older children; osteoporosis and fractures have often been reported in adults with scurvy.

Walter (1748), Chaplain to Lord Anson's expedition around the world from 1740 to 1744, observed many men with scurvy and wrote: "At other times the whole body, but more especially the legs, were subject to ulcers of the worst kind, attended with rotten bones, and such a luxuriancy of fungous flesh, as yielded no remedy." Moreover, as cited at greater length in the context of defective wound healing in Chapter 12, Volume III, he recorded his observation that, "the callous of a broken bone, which had been completely healed for a long time, was found hereby dissolved; and the fracture seemed as if it had never been consolidated."

Smith (1876) described subperiosteal hemorrhages around the shafts of several long bones with separation of the epiphyses in a 2-year-old child, undoubtedly due to scurvy. "The periosteum of the femur was found separated from the bone in its entire extent; it was intensely injected and thickened. The shaft of the femur was found to be completely separated from its articular ends at the epiphysial lines and was surrounded by a maroon-coloured clot from a quarter to half an inch in thickness, which was loosely adherent and lay between the periosteum and the bone." Similar changes were observed in the femur, tibia, and fibula of both legs, but to varying extents.

Swellings of the costochondral junctions in scurvy cause a child's chest to develop an appearance almost identical to that of a "rickety rosary" and are easily mistaken for rickets. Moreover, the gums of infants with scurvy seldom show the sponginess or bleeding which are typical of scurvy in the adult. Indeed, infantile scurvy was labeled "acute rickets" until Barlow (1883), in his classical treatise, pointed out that these cases "approximate more closely to scurvy than to any other disease with which we are acquanted." He reached this conclusion mainly because of the findings of subperiosteal hemorrhages, separation of the

epiphyses, and fracture at post-mortem examination; subsequently his deduction was validated when he found that other infants recovered rapidly with antiscorbutic treatment. Barlow stressed the pain suffered by these infants and their incessant whining, which changed to a scream when they were handled. He described the swelling of their legs and knees, their pseudoparalysis, and the crepitus due to grating of the bone ends sometimes heard on moving a limb. "Extreme fretfulness must be mentioned as a symptom so striking and constant that everybody who has recorded cases has dwelt upon it as something quite remarkable in this affection. In fact this is one of the five constituents, viz. Pallor, wasting, immobility, swelling of limbs and fretfulness, which, with or without swelling of gums, pretty nearly sum up the clinical whole of the so-called acute rickets. This special fretfulness is almost certainly related to affection of the limbs."

Barlow (1883) cited the observation by another physician that, "In a case of scurvy, separation of the ribs from the costal cartilages and fractures of ribs near their anterior extremities occurred, without violence, whilst the patient was in the hospital." Today X-rays assist in the differential diagnosis between rickets and scurvy, but, of course, the two deficiency diseases can coexist. This may have been the situation in the 2-year-old female child described by Sutherland (1894); she had suffered pain in the right thigh for 7 weeks. "On admission the child was found to be fairly well nourished, and had a pale waxy complexion. There were some dirty looking sores along the upper lip, and alae nasi, and also on the buttocks. A large bruise-like mark was present on the right frontal eminence, greenish in colour. The signs of rickets were well-marked. The anterior fontanelle was open, tense and pulsating. The ribs were beaded, the epiphyses of the long bones were enlarged, and the bones of the forearm were curved. On the left upper arm a smooth, firm, tender swelling occupied rather more than the middle third. The swelling felt like periosteal thickening. The right thigh was bent at an angle a little above the centre, and the femur was fractured. The upper fragment was directed forwards and the lower backwards. The lower part of the shaft was much thickened in its whole extent. No crepitus was elicited between the fragments, but there was extreme tenderness over the whole thigh, and the child cried with a shrill, piercing scream when the limb was touched or moved."

The child died 2 d later and was found at autopsy to have subdural and intracranial hemorrhages as well as thrombosis of the basilar artery. "The right femur was broken across almost transversely immediately above the centre. There was no attempt at union of the fragments which were considerably separated. A large amount of soft material was lying round the shaft, under the periosteum, for the most part unorganized, but of a cartilaginous consistence in parts. A similar condition was present in the left humerus, there being no appearance of union between the fragments, and a large quantity of soft material surrounded the broken ends of the shaft."

Aschoff and Koch (1919) made a detailed study of the bone pathology in human scurvy, as seen in soldiers who died of the disease during the World War I. Particular attention was paid to changes in the bone and cartilage at the costochondral junctions and also at the junction of the diaphysis and the epiphysis of the long bones. There was cessation of new bone formation and rarefaction of existing bone, both cortex and spongiosa. Irregularities, absorption, and disappearance of cartilage columns were noted and there was yielding of the bone under strain. A region of fragmentation of bone trabeculae adjacent to the line of junction with cartilage was referred to as the "trümmerfeld zone"; fracture of the bone or separation of the epiphysis was frequently noted to have occurred through that region. Subperiosteal hemorrhages were frequent. Osteoblasts became elongated and disappeared from regions where bone formation would normally occur. The marrow spaces adjacent to the cartilage or the trümmerfeld zone became filled with a loose textured gelatinous connective tissue referred to as the "gerüstmark". Irregular islands of bone formation were seen in the subperiosteal regions and in the gerüstmark.

Hess and Unger (1920) pointed out that, "beading of the ribs, the so-called rachitic rosary, should not be regarded as pathognomonic of rickets. It also occurs very frequently in connection with infantile scurvy and is one of the typical signs of this disorder, developing in the course of the disease, and disappearing rapidly with the recession of the other symptoms when an antiscorbutic foodstuff is given." These authors pointed out that this involvement of the costochondral junctions is the cause of the thoracic tenderness and explains why scorbutic infants scream when they are grasped by the chest.

Giangrasso (1938) reported great benefit from the subcutaneous injection of relatively small doses of vitamin C in all his patients with fractures. Bony callus formation at the fracture site was accelerated, so that the casts could be removed sooner. In this way it was possible to commence active and passive movement of the injured limb at an earlier stage, and this reduced the extent of muscular atrophy. In all probability, equally good results could have been obtained by oral administration of ascorbic acid or vitamin C-rich foods.

One of the most clinically pertinent reports is that of Hyams and Ross (1963) who described a 54-year-old menopausal London housewife with severe osteoporosis and low back pain due to vitamin C deficiency, which had remained undiagnosed for a year until she was admitted to hospital with frank scurvy. The day before admission she had developed very severe lumbar pain, "like a knife in my back;" her husband volunteered the information that the pain had been so severe that she had threatened to put her head in the gas oven. She was also found to have megaloblastic anemia with a hemoglobin level of 7.7. X-rays of the lumbar spine showed osteoporosis with ballooning of the intervertebral disks of L1, 2, 3, and 4 and almost complete collapse of the vertebral body at L1. The thoracic spine also showed osteoporosis with wedging of the mid-thoracic vertebrae. The skull, pelvis, and hands did not show X-ray evidence of osteoporosis. A diagnosis of megaloblastic anemia and osteoporosis due to scurvy was made; she was also found to have histamine-fast achlorhydria. It was decided to treat the patient with ascorbic acid only, since her intake of calcium and vitamin D appeared to have been adequate during the period when the vitamin C content of her diet had been negligible. Her scurvy and her megaloblastic anemia were cured and her osteoporosis was arrested by provision of ascorbic acid, 500 mg daily, and her back pain responded to bed rest on a fracture board. Mobilization commenced after 8 weeks. She was discharged from the hospital after 11 weeks and, adhering to dietary advice, she was gradually able to do more and more of her housework and her shopping, with only a feeling of stiffness in the back, but no pain. It would seem that this patient differed from so many millions of others, only by the the fact that her vitamin C deficiency eventually became severe enough to declare itself as scurvy. Since bone is laid down on a foundation of collagen, it is of relevance to note that Green and Goldberg (1964) observed a very marked increase in collagen produced by cultured human fibroblasts following the addition of ascorbic acid to the culture medium.

Bone fractures, with radiographic appearances resembling scurvy, were observed in the legs of several children with spina bifida associated with meningomyelocele and lower limb paralysis at the King Edward VII Orthopaedic Hospital in Sheffield. Clearly, disuse atrophy can and does lead to osteoporosis, but the X-ray appearance of scurvy including loss of cortical thickness, penciling of the epiphyses, metaphyseal fractures, and subperiosteal hematomas called for further investigation. These findings prompted a study by McKibbin and Porter (1967) of 20 children with meningomyelocele selected at random; 76% were found to be vitamin C unsaturated by a standard loading test; 16 normal children were also studied and all were found to be fully saturated with vitamin C by the same test. Further studies by McKibbin et al. (1968) revealed that the urinary excretion of parahydroxyphenylacetic acid was above the normal range for children, suggesting ascorbate deficiency (Chapter 6, Volume III) in all but 3 of 19 children with meningomyelocele (Figure 1). Seven of these children also had a vitamin C saturation test; all were found to be unsaturated and all had an increased

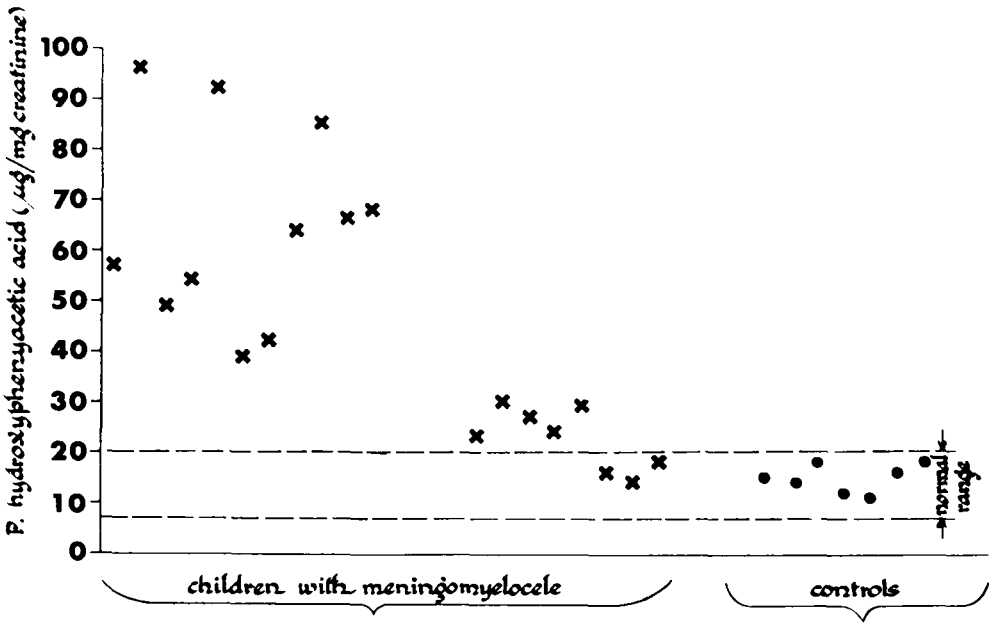


FIGURE 1. Of children with meningomyelocele, 16 out of 19 were found to excrete more parahydroxyphenylacetic acid in the urine than normal controls, and most of them were ascorbic acid deficient. (From McKibbin, B., Toseland, P. A., and Duckworth, T. [1968], *Dev Med. Child Neurol*, 10 (Suppl. 15), 55. With permission.)

p-hydroxyphenylacetic acid excretion level. One cannot help but wonder whether there is not an abnormality of ascorbic acid metabolism in these children and whether this might not have been in some way responsible for their abnormal embryonic development.

Studying the osteoporosis that is so commonly associated with hemosiderosis and scurvy in middle-aged South African Bantu men, Lynch et al. (1967b) observed that the mineral density of the bones was inversely related to the liver iron stores. Moreover, Lynch et al. (1967a) demonstrated an accelerated oxidative catabolism of ascorbic acid in siderotic Bantu; the large deposits of ferric iron undoubtedly contribute to the depletion of tissue ascorbic acid (Chapter 10, Volume I). Subsequent studies by Wapnick et al. (1971) confirmed the earlier finding of an inverse correlation between liver iron levels and bone density in Bantu patients with osteoporosis. Moreover, the low calcium storage in these patients is due to ascorbic acid deficiency, which is mainly due to their hemosiderosis; indeed, their urinary calcium excretion is significantly reduced following administration of ascorbic acid (Figures 2 and 3).

No doubt, many other factors discussed in Volume I of this book, especially smoking and aging also predispose to osteoporosis for precisely the same reason; they decrease tissue ascorbate levels. Indeed, Wilson et al. (1972) in a study of the leukocyte ascorbic acid levels of 159 old people admitted to a geriatric unit in southern England, found that those with low vitamin C levels (under 12 $\mu g/10^8$ leukocytes) included 5 out of 32, or 16% who had sustained fractures within 12 months prior to admission. There was no history of such fractures among the 49 patients with high vitamin C levels (over 25 $\mu g/10^8$ cells). Moreover, as mentioned earlier in the chapter on aging (Chapter 5, Volume I), these authors reported a significantly greater mortality within 4 weeks of admission in those with low vitamin C levels (Table 5, Chapter 5, Volume I).

Clearly, functioning ovaries tend to protect against osteoporosis, as shown by Aitken et al. (1973) and by many others. Likewise, appropriate estrogen replacement therapy is beneficial in estrogen deficiency, but high-dose estrogens can interfere with ascorbic acid metabolism (Chapter 13, Volume I) and could therefore theoretically increase osteoporosis.

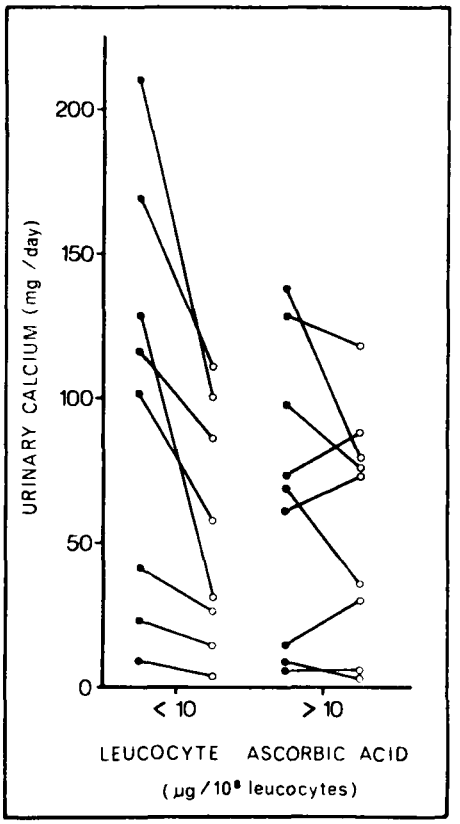


FIGURE 2. The effects of ascorbic acid administration on the urinary excretion of calcium in Bantu hospital patients. The closed circles represent the initial values, and the open circles represent the values after 1 week of therapy. (From Lynch, S. R., Seftel, H. C., Wapnick, A. A., Charlton, R. W., and Bothwell, T. H. [1970], *S. Afr. J. Med. Sci.*, 35, 45. ©1970 Witwatersrand University Press, Johannesburg. With permission.)

A very interesting development is the successful use of ascorbic acid in the treatment of osteogenesis imperfecta. The common form of this hereditary disease is apparently due to a dominant gene with variable expressivity. The disease is characterized by multiple bone fractures and slate-blue sclerotics, probably due to defective collagen formation in the sclera and in the bone matrix. Kurz and Eyring (1974) have reported that daily oral administration of large doses of ascorbic acid (25 to 50 mg/kg/d) to patients with this disease leads to a decreased tendency to bone fracture and allows for greater physical activity. Indeed, one 8-year-old boy who had previously sustained 14 fractures was enabled to play goalkeeper on the school soccer team and now does stunts on the trampoline. Some children have even seemed to show a whitening of the sclera. These authors found no evidence of ascorbic acid deficiency in these children, so the mode of operation of the vitamin supplement is obscure. It was noted that the serum zinc levels of the patients with osteogenesis imperfecta fell somewhat during ascorbic acid treatment, while the serum zinc levels of normal individuals rose, but the implications of these findings are not clear.

Another interesting development, which is difficult to understand, is the successful use of large doses of ascorbic acid for the relief of bone pain, as reported by Cameron and Campbell (1974) in patients with bone metastases and by Basu et al. (1978) in Paget's disease. Using a combination of ascorbic acid, 3 g daily, and calcitonin, 160 MRC units

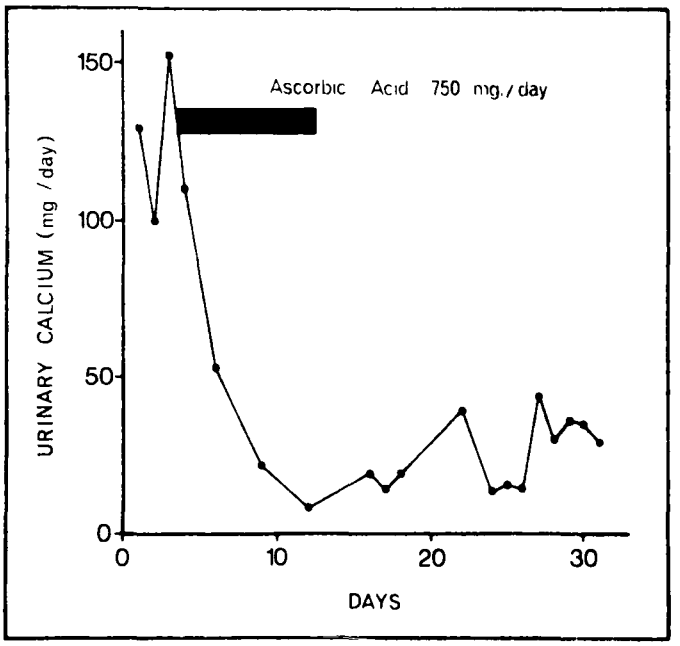


FIGURE 3. The serial changes in urinary calcium excretion induced in one ascorbic acid-depleted subject by 7 d of therapy with ascorbic acid. (From Lynch, S. R., Seftel, H. C., Wapnick, A. A., Charlton, R. W., and Bothwell, T. H. [1970], *S. Afr. J. Med. Sci.*, 35, 45. ©1970 Witwatersrand University Press, Johannesburg. With permission.)

daily, in the treatment of Paget's disease, Smethurst et al. (1981) reported that more patients (62%) claimed marked relief of pain with the combination than with calcitonin alone (36%). Urinary hydroxyproline levels were increased by ascorbic acid and decreased by calcitonin, but were unchanged in patients receiving the combined therapy. While the mechanism of action of ascorbic acid in the relief of bone pain remains unknown, we can conjecture that the vitamin supplement may accelerate remodeling of bone and thus release pressure on trapped nerves.

III. X-RAY FINDINGS

Scurvy affects growing bones, so X-ray changes are seen most frequently in infants and young children and most notably at the ends of the long bones.

Talbot et al. (1913) described the X-ray changes seen in infantile scurvy and gave credit to earlier descriptions by Fraenkel et al. "There is a 'white line' at the end of the diaphysis at the junction of the epiphysis and the diaphysis on the X-ray plate or negative;" it runs transversely across the bone as a narrow or broad shadow, either in a straight or a crooked line. It represents an increased density at the end of the diaphysis, which may persist for as long as 6 months after an apparent cure of the disease. These authors had seen the "white line" at each end of the humerus, ulna, femur, tibia, and fibula. They also demonstrated the X-ray appearance of a large subperiosteal hematoma around the femur in an 8-month-old infant admitted to the Massachusetts General Hospital with scurvy.

Baetjer (1919) described the appearance of a second epiphyseal line about 1/4 to 1/2 in. behind the first, which in reality is a band of localized destruction about $^1/_{16}$ in. thick extending through the entire bone and parallel to the epiphysis. The edges of this band are frequently denser than normal bone and give the appearance of ivory due to deposition of calcium salts.

Baetjer described this band (the trümmer zone) as giving the appearance as if a surgeon had operated and taken out a cross section of the bone.

The early X-ray signs of infantile scurvy described by Pelkan (1925) were as follows:

1. A finely irregular, broadened, well-calcified epiphyseal line;
2. A small spur at the lateral edge of the epiphyseal line. Occasionally dislocation of the entire epiphysis.
3. An area of decreased density (scurvy line) immediately back of the epiphyseal line, which represents lack of calcification of the newly formed spongiosa.
4. A very thin cortex, often merely a narrow white line.
5. Glass-like transparency of the shaft. No trabeculations seen as in normal bone or in rickets.
6. A broad, finely irregular white edge on the epiphyseal center of ossification of the long bones.

Moreover, Pelkan (1925), using a simple triad of X-ray signs of early scurvy (broadened epiphyseal line, edging on the center of ossification of the epiphysis, and especially the ground-glass appearance of the shaft), found it possible to diagnose scurvy in children with questionable signs such as pallor and loss of weight, even before the clinical signs of hemorrhages and pain in the legs had developed. Pelkan also cited four radiological signs of repair in the long bones following scurvy:

1. A double line of ossification across the end of the shaft
2. Duplication of the edges of the epiphyseal center of ossification
3. Calcification of subperiosteal hematomata
4. Realignment of a displaced epiphysis with the shaft of the bone

Schwartz (1927),working at the Milwaukee Children's Hospital, drew special attention to rarified centers of the lower femoral and upper tibial epiphyses and the well-defined ring of greater density, analogous to the trümmerfeld zone, outlining them. He pointed out that, "the fuzzy, indistinct, contourless margin of the rachitic epiphysis is entirely different from the well defined margin of the scorbutic epiphysis." Even 3 and 4 years after the healing of scurvy, Schwartz observed a rarified center and a dense periphery in the epiphyses at the knee, in the capitate and hamate bones of the wrist, and in the os calcis, astragalus, and cuboid in the ankle, giving the appearance of a double ring.

Bromer (1928) noted that the nomenclature, white line, scurvy line, trümmerfeld zone, etc., had been used in an indiscriminate fashion which was very confusing. Winberger called the broadened band of temporary calcification the trümmerfeld zone and the zone of decreased density behind it the white line, while most American writers reverse the application of these terms. Bromer therefore preferred to refer to the dense line at the diaphyseal extremity as the broadened zone of temporary calcification. He called the zone of decreased density next to it the framework marrow. Bromer also stressed the diagnostic importance of the dense edge or ring about the ossification centers of the epiphyses, when combined with the characteristic ground-glass atrophy of their centers.

McLean and McIntosh (1928) made an intensive study of the X-ray findings in infants with scurvy and paid particular attention to the changes seen during the healing phase of the disease. These authors presented 56 X-ray plates of scorbutic infants showing all varieties and all stages of the disease, with emphasis on the differential diagnosis and on the tell-tale residua which sometimes persist for as long as 11 years after treatment. They listed the following changes as being seen at various stages in the diagnosis and treatment of scurvy in 52 infants admitted to hospital, mostly between 8 and 10 months of age and mostly (37 out of 52) with fever exceeding 100°F (37.8°C).

- Broadened zone of preparatory calcification
- Spurs
- Zone of rarefaction
- Center of ossification — intensity of periphery
- Center of ossification — wide peripheral zone
- Center of ossification — rarefaction at center
- Separation of epiphysis (or better, metaphysis, as the diaphysial plate separates with the epiphysis in scurvy)
- Clouding of the trabeculae
- Thinning of cortex
- Hemorrhage into soft parts
- Subperiosteal fracture
- Calcified elevated periosteum
- Transverse calcified lines
- Central scar in the center of ossification
- Thickened shaft
- Curvature of shaft
- Anvil deformity
- Cupped or broadened epiphyses
- Frayed epiphyses

Curvature of shaft does not seem to belong in the list, but otherwise all are findings noted by others in scurvy.

Hartman and Friedman (1931) reported scurvy in a 7-year-old boy whose X-rays showed separation of the left lower femoral epiphysis and absence of the normal trabeculation of the bones, but no evidence of the typical gerüstmark zone or trümmerfeld zone of scurvy. The child presented with fever and localized tenderness; his gums appeared healthy and there were no hemorrhages to be seen in the skin. Traumatic epiphyseal separation was first suspected and it was only when the same lesion developed on the right side that scurvy was recognized. In retrospect, the original X-rays had shown epiphyses with ground-glass centers and accentuated borders. Later films following treatment showed unmistakeable calcification outlining large subperiosteal hematomas around the shafts of both femurs.

Kato (1932) described the X-ray findings in 13 bottle-fed infants with scurvy admitted to hospital in Los Angeles and drew special attention to Wimberger's sign, which is the edging or rimming of the nucleus of ossification in the epiphysis by a comparatively dense, yet thin, white line; its central portion has a pale ground-glass appearance. "This gives a peculiar ring-like shape to the oval, or roughly spherical, center of ossification." It is very evident in infantile scurvy, but Kato pointed out that it may also be seen in rickets, lead poisoning, and phosphorus poisoning, so it is not an absolute sign of scurvy. Moreover, it persists long after the scurvy has been cured. Kato listed ten X-ray signs found more or less constantly in well-developed cases of infantile scurvy as follows:

1. A finely irregular, broadened, intensely calcified zone of preparatory calcification at the epiphyseal end of long bones, the so-called "white line" of Fraenkel.
2. A small spur at the lateral edge of the epiphysis (Pelkan).
3. A zone of rarefaction immediately back of the preparatory calcification, the "scurvy line", the "framework marrow", or the "gerüstmark".
4. A broad, finely irregular edge of dense shadow encircling the nucleus of ossification at the epiphysis, together with rarefaction of the central portion, "Wimberger's sign". This has been shown in the centers of ossification in the carpal and tarsal bones.
5. Separation of the epiphysis.

6. A ground-glass transparency of the shaft, with clouding or obliteration of the trabecular structure which is visible in normal bones.
7. A thinning of the cortical shadow, often represented merely by a narrow white line.
8. Subperiosteal hemorrhage and evidence of hemorrhage into the soft parts.
9. Subperiosteal fractures in the ends of the diaphysis.
10. Enlargement and angulation of the costochondral end of the vertebral junctions of the ribs.

Reviewing each of these signs in turn, Kato concluded that each of them could be mimicked by other disease states with the exception of subperiosteal hemorrhages. He concluded that this is the only sign which is absolutely diagnostic of scurvy, and, of course, it is very late to appear. Nevertheless, Kato considered Pelkan's triad to be valuable in the diagnosis of early scurvy and that a combination of several X-ray signs clearly increased the certainty of diagnosis.

Parks et al. (1935) concentrated on the very earliest signs of scurvy. They made post-mortem comparative X-ray and histological studies of the bones of 532 children dying from any cause between the ages of 2 months and 2 years at the New Haven Hospital in Connecticut and at Johns Hopkins Hospital in Baltimore. In the course of the work, which was originally intended as a baseline study for the evaluation and eradication of rickets, scurvy was discovered in a number of patients in whom the disease had not been suspected during life, and in this way it was learned that scurvy was escaping diagnosis. Of the children, 19 were found on histological examination of the bones to have suffered from scurvy. In only 2 of these 19 children had the diagnosis been made clinically. In 17 the disease was never thought of as a possibility during life. In the pathology department, scurvy was recognized in 5 of the 19 cases, and in 2 additional cases it was suspected. The pathologists, then, in the course of their routine autopsies, did not recognize the disease in 12 cases. The 37 plates accompanying the article by Parks et al. (1935) should be seen to appreciate their findings. Besides early recognition of the dense shadow which lies across the end of the shaft, they observed the very earliest signs of thinning of subjacent cancellous bone at the angles. Thinning of cortical bone led, in some instances, to an appearance as though the angles of the growing bone were actually missing.

Dogramaci (1946) surveyed 241 infants with scurvy admitted to the Infants and Children's Hospitals in Boston in the preceding 10 years and noted that characteristic bone changes were visible in 127 of the 133 infants subjected to X-ray examination. Roughton and Waldron (1953) observed that "subperiosteal hemorrhage is an important feature of infantile scurvy but is seldom seen in the adult."

The X-ray changes of infantile scurvy are discussed in the *Nelson Textbook of Pediatrics* (1975); three distinct changes are described. The earliest changes are usually evident at the knee and consist of rarefication of the bone, which develops a ground-glass appearance with loss of the normal trabecular lines. There is also extreme thinning of the cortex of the bone and the appearance of an irregular white line across the end of the diaphysis. Subsequently, a zone of rarefaction develops under this white line, between it and the diaphysis, and there may even develop a complete linear break across the terminal plate of the diaphysis. More often, the break is incomplete and does not run across the entire shaft, but spurs may appear as lateral prolongations of the white line. Subperiosteal hematomata may be present at this stage, but they may not become clearly visible on X-ray until the healing stage when calcification of the subperiosteal hemorrhages provides a picture suggesting elephant bones containing children's bones within them.

IV. GUINEA PIG STUDIES

Holst and Frölich (1907, 1912) produced scurvy in guinea pigs by feeding them a diet of

oats, barley, rye, or wheat and water. The animals usually died after 28 days on such a diet and they were found on post-mortem examination to have not only multiple hemorrhages and loose teeth, but also a pronounced fragility of the bones and fractures of the bone shafts, "just below the intermediate cartilages," resulting in separation of the epiphyses. They concluded that, "the separations between the epiphyses and the shafts existed *intra vitam*, but that the intact periosteum kept the fractured bones *in situ*." It was observed that, "the periosteum surrounding the upper part of the shaft is, as a rule, thickened both in the outer fibrous and in the inner osteogenetic layer; in both layers there may be seen large extravasations of blood which may communicate through the corticalis with haemorrhages in the marrow." Indeed, the account of the bony changes in scurvy given by these workers was remarkably full and accurate; they even noted that the osteoblasts had come to resemble connective tissue cells. Hart and Lessing (1913) provided a detailed description of scurvy in the rhesus monkey and provided excellent colored illustrations of subperiosteal hemorrhage, completely lifting the periosteum off the bone and surrounding the humerus and the femur.

In her studies of guinea pigs using similar scorbutogenic diets, which may have been also deficient in vitamins A and D and low in protein, Ingier (1915) observed a high percentage of premature births, stillbirths, and maternal deaths. The pregnant animals showed a more advanced state of disease in their bones at an earlier period of defective diet than is the case with nonpregnant animals. On the other hand, both macroscopic and microscopic changes in the fetal skeletons were usually less pronounced than those of the adult animal. Some of the stillborn fetuses showed multiple intrauterine fractures, but the offspring that were born alive were lively and active. They very quickly developed Barlow's disease when fed by their mothers, who were receiving the defective diet.

Chick and Hume (1917) produced scurvy in young guinea pigs on a diet of oats, bran, and water. The animals developed a tenderness of the joints after 17 d and then began to lose weight. Soon they were seen to adopt a characteristic scurvy position; "the sore hind leg is raised in the air while the animal lies down on the other three legs." At post-mortem examination, extensive hemorrhages were found in the muscles of the leg and thigh, especially near the knee joints. "The bones are fragile and easily break off at the junction of the shaft and the epiphysis . . . . . There are marked swellings in the ribs at the bone-cartilage junction which is often found to be fractured." Boycott, in the discussion that followed the paper by Chick and Hume (1917), showed microscopic slides of tissues from scorbutic guinea pigs and concluded that the preparatory changes in the cartilage, before it is turned into bone, are abnormal and occur in some irregular way which obstructs the connective tissue and prevents it from performing its ordinary function. "The result is, that at the growing ends of the bones — and it is particularly well seen at the juncton of the bony to the cartilaginous parts of the ribs — instead of there being an orderly conversion of cartilage to bone, there is disorder. The end of the medullary cavity, instead of being active tissue, forming periosteal bone and being filled with bone marrow, is filled up with a lot of connective tissue which does not go on to the formation of bone."

Chick et al. (1918) found fresh cow's milk to be a poor antiscorbutic and even worse after heating or drying, so they recommended orange juice as an antiscorbutic for children. Studying guinea pigs, they gave a further description of what they called the "scurvy position", undoubtedly indicating pain in the limbs due to hemorrhages under the periosteum, in the muscles, and around the joints. "The animal rests on its side, and the painful leg is held off the ground and may be seen twitching." They also described the "face-ache position" which is reported in Chapter 7 of this volume.

The changes that occur at the interface between bone and cartilage in incipient, acute, and chronic scurvy were clearly illustrated by Tozer in her drawings of the costochondral junctions of guinea pigs (Figure 4), which appeared as an appendix to the work of Delf

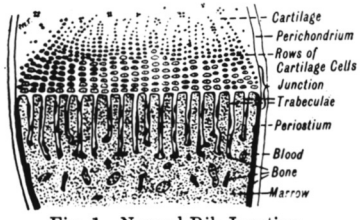


Fig. 1. Normal Rib Junction.

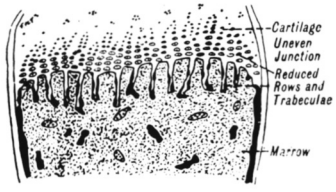


Fig. 2. Nearly Normal Rib Junction, "Incipient Scurvy."

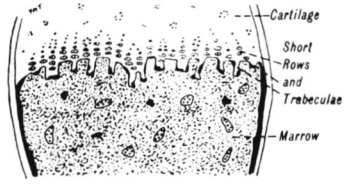


Fig. 3. "Definite Scurvy."

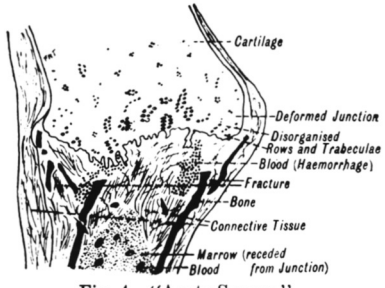


Fig. 4. "Acute Scurvy."

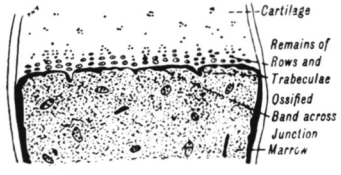


Fig. 5. "Chronic Scurvy" (definite).

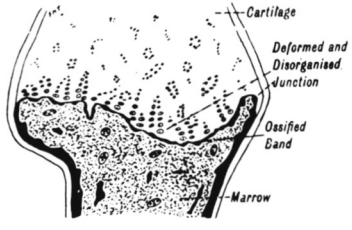


Fig. 6. "Chronic Scurvy" (acute).

FIGURE 4. Drawings by Tozer, who was able to detect even the earliest changes in the appearance of histological sections of the costochondral junctions of guinea pigs with incipient scurvy. (From Delf, E. M. [1918], *Biochem. J.*, 12, 416. ©1918 The Biochemical Society, London. With permission.)

(1918). As noted by Chick et al. (1918), the costochondral junctions become swollen and exhibit a transverse yellow bar — "These appearances correspond with the complete disorganization of the bone-cartilage junction, and with disappearance of the cartilaginous trabeculae and of the rows of cartilaginous growing cells. In many cases the bone is found to be completely fractured and there is great proliferation of connective tissue across the junction." Tozer interpreted the ossified band across the junction in chronic cases as an attempt to strengthen the junction in an abnormal manner.

Randoin (1923) reviewed the work of Tozer on the costochondral junctions of guinea pigs in which young animals (290 to 350 g) were fed on four different diets.

1. A complete diet of oats, bran, autoclaved milk, and orange juice
2. A vitamin C-deficient diet of oats, bran, and autoclaved milk
3. A vitamin A-deficient diet of oats, bran, and orange juice
4. A diet deficient in vitamins A and C, consisting of oats and bran only (This was noted by Randoin as being an unbalanced diet, which was deficient in several essential nutrients.)

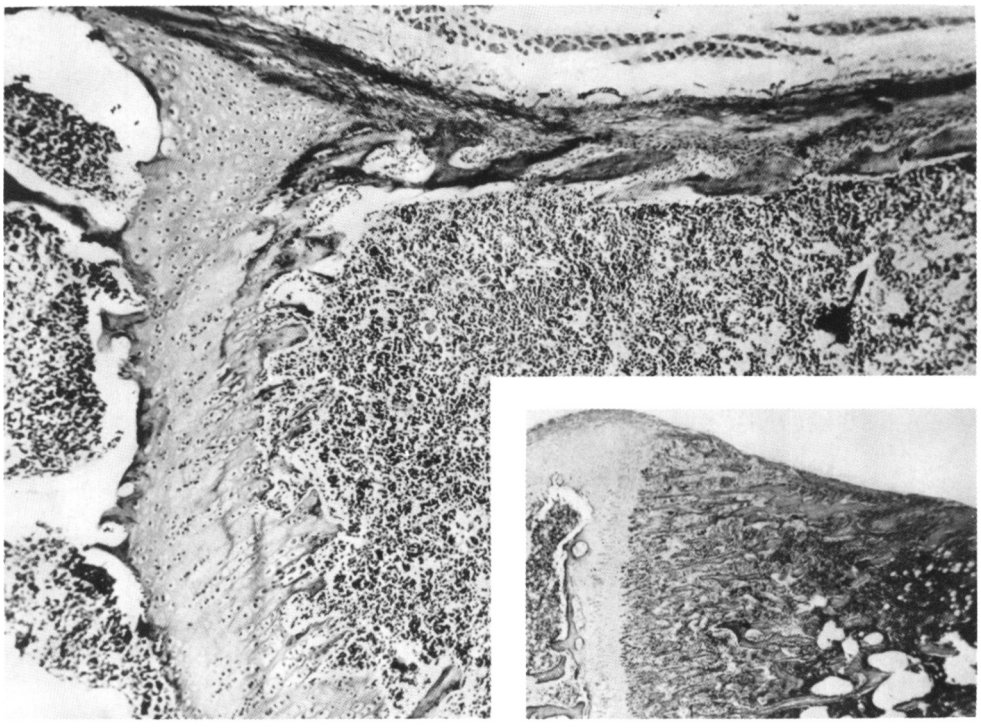
The animals fed on this fourth diet ceased growing immediately, as did those receiving the third diet, also lacking the milk, but when those of the fourth group died around the 25th day, they showed completely irregular cartilage deposition and fractured bones, typical of scurvy, just like those on the second diet.

Summarizing his findings in scorbutic guinea pigs, Höjer (1924) stated, "The enchondral bone formed in the course of scurvy thus differs from the normal bone chiefly in the following points:

1. The osteoblasts more and more assume the appearance of elongated connective tissue cells.
2. The regular form of the bone and its general organization are lacking.
3. The typical bone structure, with bone corpuscles and canaliculi, becomes less and less apparent; instead of that there is a general homogenization of columns and masses.
4. Collagen fibrils are absent."

Wolbach and Howe (1926) also studied the costochondral junctions and the ends of the long bones of guinea pigs with scurvy. They noted that the osteoblasts continued to multiply, but changed their shape, becoming elongated and taking on the appearance of fibroblasts; bone formation ceased and there was resorption of bone deposited on calcified cartilage columns. They concluded that the gerüstmark is a semiliquid intercellular material produced by these altered osteoblasts, lacking something needed for proper bone formation.

Ham and Elliott (1938), at the University of Toronto, studied the effects of protracted moderate scurvy on the bones of young guinea pigs by allowing them 0.5 or 0.75 ml of orange juice a day, while control animals received 2 ml daily. The long bones continued to grow; they were only slightly shorter than those of controls, but they were much lighter. Dried specimens weighed about three fourths of similarly treated control bones. "Furthermore, the bones of the experimental animals were fragile, rough and discoloured, and they frequently exhibited fractures of the shaft near the epiphyseal plate." As noted in Chapter 6 of this volume, many of the joint surfaces were somewhat flattened and not uncommonly invaginated for a very short distance into the epiphysis. That portion of the epiphyseal plate that caps the marrow cavity was for the most part thinner than usual, although in a few areas, it was thicker than normal. The cortex of the shaft was not as dense or as thick as normal. Examples of gerüstmark or framework marrow were not as common as they are in severe experimental scurvy.



B A

FIGURE 5. (A) The normal trabecular bone at the end of the diaphysis, supporting the epiphysis and capping the end of a guinea pig tibia. (B) The lack of normal trabecular bone beneath the epiphysial plate in a guinea pig with moderate protracted scurvy. Normal diet for 1 month after birth, then 0.75 ml of orange juice daily, and no other vitamin C for 50 d, when it died (From Ham, A. W. and Elliott, H. C. [1938], *Am. J. Pathol.*, 14, 323. With permission.)

The disk of trabeculated bone that exists on the diaphyseal side of the epiphyseal plate is normally like a honeycomb, extending across the end of the bone marrow and capping it, but in protracted moderate scurvy, the central portion of this scaffolding was missing (Figure 5A and B). At the periphery, there was still some evidence of cartilage being replaced by bony trabeculae. Thus, growth was able to continue, but at the expense of strength. The bone (and especially this growing region) was markedly weakened and fragile.

The chief manifestation of protracted moderate scurvy in the epiphysis was found to be a diminution in the amount of bone supporting the articular cartilage. Microscopic studies of the epiphyses showed that the articular cartilage lay on a thin shell of calcified cartilage, but was not supported by a scaffolding of cancellous bone, as it should be. Thus, it was often found distorted and fractured, as illustrated in Chapter 6 of this volume which is devoted to joint changes.

These authors suggested that the pathological changes produced by protracted moderate scurvy in adults, after fusion of the epiphyses, would likely be limited to osteoporosis, osteoarthritis, and osteofragility. Of course, these are the very changes that are so common in aged human beings.

Ham and Elliott did not subscribe to the jellation theory of Wolbach and Howe. They observed extensive bony changes in protracted moderate scurvy, but no evidence of a liquid collagen precursor. MacLean et al. (1939), also at the University of Toronto, used pure ascorbic acid and a paired-feeding technique in their experiments and thus were able to

demonstrate that the usual bony lesions seen in scurvy are due to deficiency of ascorbic acid and not to starvation or to lack of any other nutrient. Studying the costochondral junctions, they reported that, "The scorbutic group showed a normal cartilage, but a widened and/or fractured junction of the cartilage and bone. The cartilage side of the zone of ossification appeared normal, but in the region where normally the primitive bone-marrow meets the cartilage layer there was a mass of mesenchymal cells. In some ribs a considerable amount of partially dissolved cartilage columns remained, particularly near the outer edge of the rib."

Studying the complete failure of healing of holes drilled in the femora of scorbutic guinea pigs, Bourne (1942b) remarked that, "all the cells present in the holes appeared to be fibroblasts; there was no sign of osteoblasts." Daily injections of 0.25, 0.5, or 1 mg of ascorbic acid were found to be inadequate for optimal bone repair in guinea pigs on a vitamin C-deficient diet. Injections of 2 mg daily were required to promote full bone-healing activity.

Staining newly formed bone by the use of alizarin dye in the diets of guinea pigs, Bourne (1943) demonstrated that an adequate supply of vitamin C does play a part in the process of laying down bone salt. However, in scurvy, the few bony trabeculae seen were as well calcified as normal; they were simply fewer in number. It was therefore concluded that ascorbic acid is essential for the formation of an adequate phosphatase-impregnated fibrous bone matrix upon which bone salt is immediately deposited.

Gould and Shwachman (1941) studied the serum alkaline phosphatase levels of normal and scorbutic guinea pigs and confirmed their earlier work, showing a marked decrease in the serum level of this enzyme in scurvy. They also found a striking reduction of the phosphatase level of bone (top of tibia) in scurvy, but no change in the levels in the liver, kidneys, adrenals, or intestine. These findings suggested the osteoblasts as the most likely source of serum phosphatase. It was postulated that these cells cease to produce this enzyme when they revert to the appearance of fibroblasts in scurvy.

Follis (1943) conducted a study of the effect of immobilizing a limb on the histological picture of experimental scurvy in guinea pigs. It was found that the classical findings of fractures, trümmerfeld zone, and gerüstmark failed to appear. This was interpreted as indicating that such changes are the result of forces acting on the delicate calcified cartilaginous matrix which fails to ossify properly in scurvy. Likewise, the absence of any pink-staining, fibrin-like material in the metaphyses of immobilized scorbutic long bones suggested that this material derives from rupture of capillaries at the site of small fractures in the trümmerfeld zone.

Perkins and Zilva (1950) observed a pronounced reduction in the alkaline phosphatase activity of the serum, the costochondral junctions, and the epiphyses of young giunea pigs after 12 to 18 d on a scorbutic diet. Follis (1951) applied histochemical procedures to the bones of guinea pigs. In animals dying of scurvy, the hypertrophic cells at the trümmerfeld zone of the costochondral junction gave a positive reaction for alkaline phosphatase, but there was a complete absence of alkaline phosphatase activity in the subjacent gerüstmark — composed of spindle-shaped cells resembling fibroblasts; 24 h after the administration of ascorbic acid (20 mg), a profound change was seen. The "connective tissue cells" of the gerüstmark rounded up, their cytoplasm became more prominent, to resemble osteoblasts, and they exhibited an intensively positive reaction for alkaline phosphatase.

Likewise, in scurvy, the periosteal cells, which normally exhibit a positive reaction, failed to show phosphatase activity. Follis in addition observed that the cytochrome oxidase activity of the gerüstmark cells is reduced in scurvy. This activity promptly returned after the administration of ascorbic acid.

Zorzoli and Nadel (1953), in a biochemical study, confirmed that the alkaline phosphatase activity of the tibia of the scorbutic guinea pig was halved, and also, in a histochemical study, they observed that the black precipitate indicating phosphatase activity at the epi-

Table 1
ASCORBIC ACID AND NONREPARATIVE COLLAGEN SYNTHESIS

Guinea pigs	Specific activity of hydroxyproline[a]		
	Bone	Skin	Liver
Normal (adequate diet for 14 d)	140	86	450
Scorbutic (deficient diet for 14 d)	28	4	560
Recovery (deficient diet for 11 d; then 100 mg of ascorbic acid per day)	150	33	560

Note: At 11 d after beginning the experiment, guinea pigs were injected intraperitoneally with 8 mCi of proline-^{14}C. This was repeated at 12 h intervals until 48 mCi had been injected; 12 h after the last dose, the animals were killed and hydroxyproline was isolated from the collagen fraction of each tissue. The recovery guinea pigs received ascorbic acid only during the period of isotope administration.

[a] cpm/μmol.

From Robertson, W. van B. (1961), *Ann. N.Y. Acad. Sci*, 92, 159. With permission.

physeal plate and at the diaphysis was most notably reduced in scurvy. Friberg and Ringertz (1954) observed a decrease in the uptake of both ^{35}S-labeled sodium sulfate and ^{32}P-labeled orthophosphate by the bones of guinea pigs in scurvy, suggesting a decreased formation of organic sulfomucopolysaccharide as well as mineral deposition in the bones. Pritchard (1956) commented, "It is of interest too that vitamin C deficiency results in regression of osteoblasts to fibroblastic forms with a cessation of their bone building role. Such fibroblast-like cells show no cytoplasmic basophilia, glycogen, alkaline phosphatase, or cytochrome oxidase. The administration of vitamin C, however, causes a reversion to the typical osteoblastic form, with a return of normal histochemical characteristics as well as normal function."

Manoussos and Milhaud (1958) found that experimental ascorbic acid deficiency caused a profound reduction in the uptake of calcium by the bones of guinea pigs. After 20 to 22 d of ascorbic acid deficiency, the rate of uptake of ^{45}Ca from intravenously injected $CaCl_2$ fell from 9.2 or 9.3 to 2.2 or 3.3 mg/h. Studies of the incorporation of proline-^{14}C into hydroxyproline-^{14}C in the tissues of guinea pigs were used as an index of collagen synthesis by Robertson (1961); it was found that guinea pigs deprived of ascorbic acid for 11 d, and not yet showing any signs of scurvy, nevertheless showed a marked impairment of collagen synthesis, both in skin and bone (Table 1). Similar studies of noncollagen protein synthesis in skin, bone, liver, and serum showed no decrease following 11 d of ascorbic acid deprivation.

Nakamura et al. (1965) confirmed that the alkaline phosphatase activities of the jaw bone and the femoral cortex of guinea pigs were strikingly decreased after 3 weeks on a scorbutic diet, but the acid phosphatase activities of these bony tissues were found to be significantly increased in scurvy. Thornton (1968) observed a consistent reduction in the uptake of radioactive calcium and phosphorus by the tibial bones of guinea pigs after 14 d of vitamin C depletion. Moreover, part of the calcium and part of the phosphorus taken up by the bone in vitamin C deficiency were found to be more soluble than usual in aqueous media at pH 7.4. These minerals were apparently less firmly incorporated as part of the bone structure in vitamin C deficiency.

Table 2
EFFECT OF IRON OVERLOAD ON HEPATIC ASCORBIC ACID CONCENTRATION AND ON BONE METABOLISM IN GUINEA PIGS

Mean Values and SD for Nine Animals in Group 1, Nine in Group 2, and Five in Group 3[a]

Measurement	Group 1 (controls)	Group 2 (iron overloaded)	Group 3 (iron overloaded + ascorbic acid supplement)
Final weight (g)	723 (84.7)	725 (66.8)	754 (22.2)
Liver iron concentration (μg/g of wet weight)	308 (156)	5111 (1534.8)	3645 (1059.7)
Liver ascorbic acid concentration (mg/100 g)	29.4 (3.1)	0.7 (0.98)	27.4 (7.5)
Bone mineral density (mg/cm^3)	731 (28.7)	655 (36.8)	691 (34.4)
Percentage bone-formation surface	1.5 (1.5)	0.09 (0.01)	1.9 (3.0)
Percentage bone-resorption surface	1.3 (0.6)	21.7 (14.2)	3.5 (3.9)

Note: Iron overload created in guinea pigs by injection of iron-dextran caused a profound reduction of the liver ascorbic acid level and also osteoporosis in Group 2, even though the animals continued to receive the same amount of ascorbic acid in their diet as did the control animals of Group 1. Group 3 received the iron overload and extra ascorbic acid, which protected them from osteoporosis.

[a] Student *t* test was used for the analysis of the significance of differences. Only the following differences were significant: liver iron concentration, 1 vs. 2 ($p < 0.001$), 1 vs. 3 ($p < 0.001$); liver ascorbic acid concentration, 1 vs. 2 ($p < 0.001$), 2 vs. 3 ($p < 0.001$); bone mineral density, 1 vs. 2 ($p < 0.001$); bone formation surface, 1 vs. 2 ($p < 0.02$); bone resorption surface, 1 vs. 2 ($p < 0.001$), 2 vs. 3 ($p < 0.001$).

From Wapnick, A. A., Lynch, S. R., Seftel, H. C., Charlton, R. W., Bothwell, T. H., and Jowsey, J. (1971), *Br. J. Nutr.*, 25, 367. Cambridge University Press. With permission.

Wapnick et al. (1971) demonstrated that intramuscular iron injections caused ascorbic acid depletion and decreased bone density in guinea pigs. Three groups of guinea pigs all received a standard ascorbic acid-free diet together with ascorbic acid-supplemented drinking water (140 mg/l). Group 1, the control group, received intramuscular injections of dextran, while the second and third groups received injections of iron-dextran, containing 50 mg of iron, three times a week, for 6 weeks. The third group, in addition, received ascorbic acid, 40 mg by intramuscular injection, three times a week. After 6 weeks of iron loading, the iron injections were discontinued, but the ascorbic acid injections for Group 3 were continued. All the animals were maintained on the diet with ascorbic acid in the drinking water for a further 20 weeks. The results in Table 2 show that the hepatic ascorbic acid level in the iron-dextran-treated animals of Group 2 was reduced to less than 3% of that in the other two groups ($p < 0.001$) which were not significantly different from one another. The mean liver-iron stores in the two groups which had received iron-dextran injections were found to be ten times as great as in the control group, but were not significantly different from one another. In the animals given iron-dextran only, bone mineral density was significantly lower than in the control group ($p < 0.001$), percentage bone-formation surface was uniformly and significantly reduced ($p < 0.02$), and percentage bone-resorption surface was significantly increased ($p < 0.001$). In the animals given ascorbic acid injections as well as iron-dextran, however, none of these values was significantly different from the control group. Wapnick et al. commented, "It was noteworthy that osteoporosis and ascorbic acid deficiency developed in spite of the fact that the dietary intake of ascorbic acid was the same as that in the control group. The bone lesions and the vitamin deficiency must, therefore, be ascribed to the siderosis in the test animals, since this was the only variable."

Kassouny et al. (1985) reported that neither megadose ascorbic acid (100 mg/100 g of body weight per day) nor low dietary magnesium intake (600 ppm) for 6 weeks affected the calcium concentration in the bones of growing giunea pigs. Likewise, Tsao et al. (1985) observed that the feeding of high-dose ascorbic acid (25 mg/100 g of body weight per day) for 20 months did not affect the body weight, the total calcium, nor the ionic calcium levels in the blood plasma. Clearly, ascorbic acid deficiency causes profound and serious changes in guinea pigs, while high-dose ascorbic acid does not seem to be harmful to their skeletal development.

V. STUDIES OF OTHER ANIMALS

Hanke (1935) studied the healing of saw cuts in thc leg bones of guinea pigs and rabbits. While dietary ascorbic acid played an indispensable role in guinea pigs, both for survival and for healing of the bones, it was also found to be beneficial even in rabbits which are able to make vitamin C from simple sugars. It was observed that regeneration of bone was complete in rabbits on a vitamin C-deficient diet, but the rate of regeneration was much retarded. Moreover, Giangrasso (1939) observed accelerated callus formation following experimental bone fractures in rabbits if they were given extra ascorbic acid in their diet.

Bourne (1942a,b) found that extra vitamin C given to rats did not accelerate the rate of healing of 1-mm bore holes in their bones, but he did observe that subcutaneous injections of calcium ascorbate accelerated the regeneration of bone, even in rats which synthesize ascorbic acid. No such effect was observed with ascorbic acid alone or with calcium gulono-galactogluconate. It was therefore suggested that the effect was most probably due to the greater ionization and better absorption of calcium when administered as calcium ascorbate. He cited the writings of several other workers who had observed improved healing of fractures in guinea pigs and in rabbits given supplementary ascorbic acid. This is not surprising, as guinea pigs cannot synthesize ascorbic acid, and rabbits with ascorbate-responsive histaminemia, have been observed by the writer, suggesting that they do not always synthesize enough ascorbic acid.

Studies of the turnover times of collagen in various tissues of young rats by Gerber et al. (1960) revealed a wide range — 300 d for kidney, over 110 d for tendon, 25 to 150 d for skin, 50 d for muscle, 30 d for liver, and 20 d for intestine. Bone was found to contain more than one kind of collagen, one with a turnover time of 40 d and one which has a turnover time of 4 d. This rapid-turnover collagen component in bone may well explain why bone formation is so profoundly affected in human and in guinea pig scurvy.

Birge and Peck (1966) observed that ascorbic acid caused a sixfold increase in the rate of collagen synthesis by isolated rat bone cells after they had been cultured for a week and had presumably become ascorbic acid deficient. Likewise, Reynolds (1967) observed ascorbic acid stimulation of collagen synthesis by chick limb bone rudiments in tissue culture. Moreover, Ramp and Thornton (1968) observed that ascorbic acid increased the oxygen consumption and decreased lactic acid production by embryonic chick tibias in tissue culture. In other words, ascorbic acid facilitated aerobic metabolism by the bone cells.

While it is well known and accepted that ascorbic acid plays an important role in bone formation, the work of Thornton and Omdahl (1969), Thornton (1970), and of Ramp and Thornton (1971a,b) has demonstrated that it is also involved in bone destruction. Studying Leghorn chicks, Thornton and Omdahl showed that intraperitoneal injection of ascorbic acid (vs. saline) caused the mobilization of previously deposited skeletal ^{45}Ca and also a significant increase of plasma acid phosphatase activity, while plasma total Ca was decreased. It is perhaps surprising to find that ascorbic acid had such an effect on chicks which supposedly make their own ascorbic acid, but the implications are clear. Ascorbic acid is essential for bone growth and bone growth involves remodeling, so bone destruction by osteoclastic activity is an essential part of bone growth.

Using tetracycline hydrochloride and alizarin red S as fluorescent markers, Messer (1971) studied the rates of bone and dentin formation in *Macaca irus* monkeys on normal and on scorbutogenic diets. Bone formation was found to have ceased completely after 8 weeks of vitamin C deficiency, at a time when dentin formation had been reduced by only 30%. After 16 weeks of vitamin C deficiency, dentin formation had also ceased.

Studies of cultured neonatal mouse calvaria by Golub (1973) showed that ascorbic acid increased the synthesis and decreased the degradation of tritiated hydroxyproline-labeled bone collagen. The data were also interpreted as indicating that ascorbic acid increased the cross-linking and the fiber stability of the collagen in the newly synthesized bone matrix, presumably as a result of increased hydroxylation of lysine.

Machlin et al. (1976) have shown that the pathological changes produced in monkeys (*M. mulata*) made scorbutic by feeding ascorbate 2-sulfate instead of ascorbic acid were virtually identical to those seen in human beings and in guinea pigs with scurvy: "Joint capsules were distended by excess synovial fluid. The epiphyses of long bones were easily separated from their diaphyses by digital pressure, leaving a ragged bloody end. The periosteum was elevated from the cortex of long bones in the region of the epiphyseal plate by red-brown tissue resembling granulation tissue, and was readily stripped from the diaphysis. Haemorrhages were present on the pleural surfaces of the costochondral junctions; these junctions were easily broken. The symphysis pubis was soft and easily cut with a dissecting knife."

VI. CONCLUSIONS

The bone pathology of acute scurvy was described in detail by Wolbach (1937) and by Dalldorf (1938), but the changes observed in protracted moderate scurvy, as produced in guinea pigs by Ham and Elliott (1938), are much more relevant to the human condition than is total ascorbate deficiency. Not only did these workers observe osteoporosis, profound changes at the epiphyseal plates, slipped epiphyses, and the fractures which are so well documented in acute scurvy, they also observed a marked diminution in the amount of epiphyseal bone, resulting in loss of support for the articular cartilage. This proceeded to such an extent in some places as to cause collapse of the thin shell of articular cartilage at the knee, leading to changes typical of osteoarthritis (Chapter 6 of this volume).

The findings of Wapnick et al. (1971) are of especial importance, for they demonstrate that the excessive iron storage of hemosiderosis can cause osteoporosis and subclinical scurvy, even in animals receiving an otherwise adequate ascorbic acid intake. Such findings certainly illuminate the message of this book; vitamin C deficiency may be due to many factors other than a dietary deficiency of ascorbic acid. The question of heavy metal storage should always be considered in osteoporosis. In addition to ascorbic acid, foods containing chelating fiber, such as the bioflavonoids or catechins (i.e., D-catechin), may be needed to remove excesses of heavy metals from the body before the ascorbic acid metabolism can be rectified.

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Chapter 6

JOINT LESIONS

I. INTRODUCTION

Although Smith (1897) seems to have been the first to record the appearance of subcutaneous extravasations of blood, and death within a few weeks, in guinea pigs fed a diet of dry cereals, without any grass, clover, or succulent vegetables, it was Holst and Frölich (1907), who were the first to observe the similarity between this disease and the scurvy which occurs in human beings. The symptoms and signs are very similar in every way, except that swollen painful joints are more evident and bleeding gums are less evident in the guinea pig disease.

II. ASCORBIC ACID DEFICIENCY IN ANIMALS

Jackson and Moore (1916) produced extensive joint lesions in guinea pigs within 11 to 19 d by feeding various cow's milk diets, which were evidently deficient in vitamin C. The wrist joints became painful to pressure and palpable swelling followed in the course of 24 to 48 h.

> Occasionally swelling was found with the first signs of tenderness. The joints continued to enlarge until in some instances they were two to three times their normal size. After two to three days the knee joints underwent similar changes while occasionally the ankle, elbow and shoulder joints became affected in the order named. While the swelling at the wrist joint was at first observed more closely confined to the ends of the ulna and the radius, that at the knee joint was about the heads of the tibia and fibula.
>
> An enlargement of the costochondral junctions resembling a rachitic rosary often was palpable a week after the initial symptoms, and it developed with little noticeable swelling of the surrounding tissues.

Cohen and Mendel (1918), working at Yale university, confirmed that scurvy can be consistently reproduced in guinea pigs. Diets consisting of only dry cereal grains, like oats or barley, produced the disease in about 15 d and produced death in about 24 d, while germinated oats or barley prevented its occurrence, They described the disease in guinea pigs as follows.

> In the guinea pig this is a disease the onset of which is usually characterized by a tenderness of the joints; and in our experience, the wrists, ankles and knees become involved in the order named, though there are many exceptions. There follows in a day or so, a gradual swelling of the affected joints, often to twice or three times the original diameter of the bone. Sometimes, in the younger animals, a joint will fracture spontaneously — the wrist being most susceptible in this regard. The older animals develop a difficulty in using their hind legs, which seem to become stiffened or paralyzed. In order to relieve the pain in the affected member, the animals will lie on their side or back and assume the scurvy position described by Chick and Hume (1917). If curative measures are instituted early enough, the swellings disappear entirely; if not, these knobs harden into exostoses that can be felt through the skin. The disease appears also to affect the junction of the ribs with the cartilages, and in advanced cases we have been able to palpate these costochondral enlargements described by Jackson and Moore (1916). The animals winced when these were touched.
>
> Accompanying the joint enlargement, one notices a marked hypersensitiveness that is succeeded by a dullness or lethargy. The symptoms described may appear while the animals eat well and gain weight steadily. Then follows a loss in appetite with a resulting decline in weight to death.

Studying guinea pigs on a diet of rolled oats and fat-free milk, with the allowance of a very small piece of carrot or a small leaf of lettuce every other day or every third day, Howe (1921) was able to keep guinea pigs alive in a chronic scorbutic state. He increased the

amount of green food or gave orange juice whenever the animals showed difficulty in the use of their legs or difficulty in eating. "The usual joint affections occur to a marked degree; and when this condition has been maintained for a long time, and the animal is then restored to a normal diet, it is found that the legs have become fixed in an abnormal position. This seems to us to be more like rheumatism or arthritis deformans than many experimental conditions that have been called such."

Howe (1923) fed guinea pigs and monkeys a scorbutic diet of soy beans, rolled oats, dried milk powder, calcium carbonate, and sodium chloride, with enough butter and yeast to prevent other vitamin deficiencies. However, he allowed a little orange juice from time to time, so as to keep the animals alive in a state of chronic scurvy. The guinea pigs showed lameness on the 15th day and soon there developed a deep hemorrhagic condition of the muscles around the joints and there developed calcifications on the femur and the tibia. "These calcific deposits appear as loose nodules, nodules attached to the bones, or large plates of calcification occur along the shaft of the bone. The ends of the bone become enlarged and show lipping or may be surrounded by a large amount of pathological calcification which alters the form of the bone. Joint movement is very limited and in some cases ankylosis occurs." The monkeys show the same joint trouble as the guinea pig. "They move about with the greatest difficulty, the joints enlarge to three or four times their natural size and under X-ray examinations it may be seen that extensive bone changes have occurred."

Rinehart (1935) made extensive studies of the joints of guinea pigs with chronic scurvy produced by feeding them inadequate amounts of vitamin C. He described the arthropathy of subacute or chronic scurvy as follows.

Synovial Proliferation and Pannus Formation. A proliferative reaction of the synovial membrane is an almost constant finding. Usually associated with the synovial proliferation and frequently merging with the proliferating cells is a hyaline fibrinoid material. No sharp distinctions can be drawn betwen cells clearly recognizable as synovial and less differentiated connective tissue cells, which intermingled with the fibrinous material, extend from joint recesses as long tongue-like processes into the joint cavity and over the articular surfaces.

Subsynovial and Peritendinous Lesions. The connective tissue beneath the synovial membrane and that about the tendons inserting around the joint present analagous changes. Here again, small extravasations of blood, streaks of fibrin and a reactive hyperplasia of connective tissue cells contribute further to the swelling deformity and limitation of movement of the joint.

Articular cartilage. In addition to diffuse thinning, a retrogressive change, apparently a de-differentiation of areas of the articular cartilage, is not uncommonly seen. In places the surface of the articular cartilage is replaced by undifferentiated and at times vascularized connective tissue. The pannus described is, in some instances, adherent to the articular surfaces . . .

Rarefaction of Bone. Thinning of bone trabeculae is characteristic of chronic vitamin C deficiency. This rarefaction involves apparently the entire skeleton but is most prominent at the bone ends.

Muscle. Some degree of muscle atrophy and degeneration is also a characteristic effect of prolonged vitamin C deficiency. An interstitial edema of the muscle is frequently seen to contribute to the periarticular swelling. In some of the more severe deficient states, hemorrhagic stippling of the muscle may occur. Some degree of muscle atrophy or degeneration is almost regularly found. Occasionally a widespread muscle degeneration dominates the pathologic picture. It would appear that this occasional severe myopathy is an effect of capillary hemorrhage and consequent cellular anoxemia.

Periarticular Reactions — Subcutaneous Nodules. The reactions in the capsular tissues are, perhaps, of greatest interest. The early sanguinous edema has been noted. Somewhat later, the capsular connective tissue undergoes a marked proliferative reaction. Commonly, streaks of hyalinized fibrin lie in intimate association with the reactive connective tissue. The tissue, on section, often shows a striking edema. Somewhat later, a gradual diminution of the periarticular thickening may occur, coincident with the shrinkage of the connective tissue . . . A most interesting observation is the not infrequent development of discrete circumscribed fibrous tissue nodules beneath the skin

about the joints. Sometimes they are movable beneath the skin and at other times are found more or less attached to an underlying bony prominence . . Microscopic sections of the subcutaneous nodules show an edematous, cellular, fibrous tissue, usually associated with irregular strands of brilliantly eosinophilic hyalinized fibrin.

. . . the experimentally produced subcutaneous nodules resemble most closely the pathologic picture of the subcutaneous nodules of rheumatic fever and the earlier nodules of rheumatoid arthritis, excellently described and illustrated by Dawson.

Rinehart described all these changes as occurring in chronic scurvy in the absence of any infection, but he found that certain infections such as pneumonia or lymphadenitis accelerate and intensify the arthritic process. He concluded that subacute or chronic vitamin C deficiency in the guinea pig produces an arthropathy with manifold similarities to rheumatoid arthritis. These include synovial proliferation, intraarticular pannus formation, periarticular fibrous tissue overgrowth, bone atrophy, and subcutaneous nodules.

Schultz (1936) also described the joint changes in guinea pigs with mild and with moderate scurvy, but considered them to be unaffected by the presence or absence of systemic infection. "In the neighbourhood of the joints there was severe and extensive myositis which corresponded to the type noted in the intercostal muscles. Slight thickening and proliferation of the synovial lining was inconstantly present in the animals with mild scurvy but occurred rather regularly in animals with moderate scurvy. In the latter, small fibrin clots enmeshing a few lymphocytes and endothelial cells were occasionally seen free in the joint spaces or adherent to synovial or cartilaginous surfaces; and in the joint recesses similar masses in which some hyalinoid change had apparently taken place sometimes fused with or replaced the synovia."

Mouriquand and Dauvergne (1938a) produced exuberant periarticular osteophytic lesions in guinea pigs by feeding them a vitamin C-deficient diet with a small supplement (3 ml) of orange juice. Subsequently, by feeding the same diet with a meager supplement of ascorbic acid, 0.5 mg daily, they reported that they were able to produce, with perfect regularity, the clinical syndrome of "chronic rheumatism". This chronic vitamin C deficiency developed in the following way: edema and scorbutic hemorrhages were maximal towards about the 35th day, but subsequently diminished progressively and tended to disappear, while a syndrome of pseudoankylosis and muscular retraction developed, rendering the rear end of the animal rigid. Towards the 45th day, X-rays showed intense periosteal thickening associated with epiphyseal and diaphyseal decalcification; on reaching this stage, the lesions could not be reversed by any amount of ascorbic acid. Thus, these workers were able to produce a chronic scurvy clinically characterized by a veritable pseudoparaplegia of the hind limbs due to periarticular changes and muscular retraction, similar to the condition so frequently seen in elderly chronic sick men and women.

Moriquand and Dauvergne (1938b) produced subacute and chronic scurvy in guinea pigs by feeding a scorbutogenic diet plus $^1/_4$, $^1/_2$, or 1 mg of ascorbic acid daily and reported that they developed enormous swellings of the femoral regions and sometimes of the elbows. Some developed what they called the "pseudoankylosing rheumatic syndrome", when they lived long enough, and this was not reversible by feeding vitamin C.

Ham and Elliott (1938), at the University of Toronto, created a protracted form of moderate scurvy in young guinea pigs by feeding them a scorbutogenic diet supplemented by 0.5 or 0.75 ml of orange juice a day. "The chief manifestation of scurvy in the epiphysis was found to be a diminution in the amount of bone supporting the articular cartilage [Figure 1].

"The weakened shell of cartilage and bone was often found distorted and fractured [Figure 2]. Broken fragments of articular cartilage were found in masses of fibrous connective tissue which originated from the capsule . . .

"In some places where the cartilage had given way, the surface was covered with a layer or several layers of large cells with eccentrically located nuclei and basophilic cytoplasm.

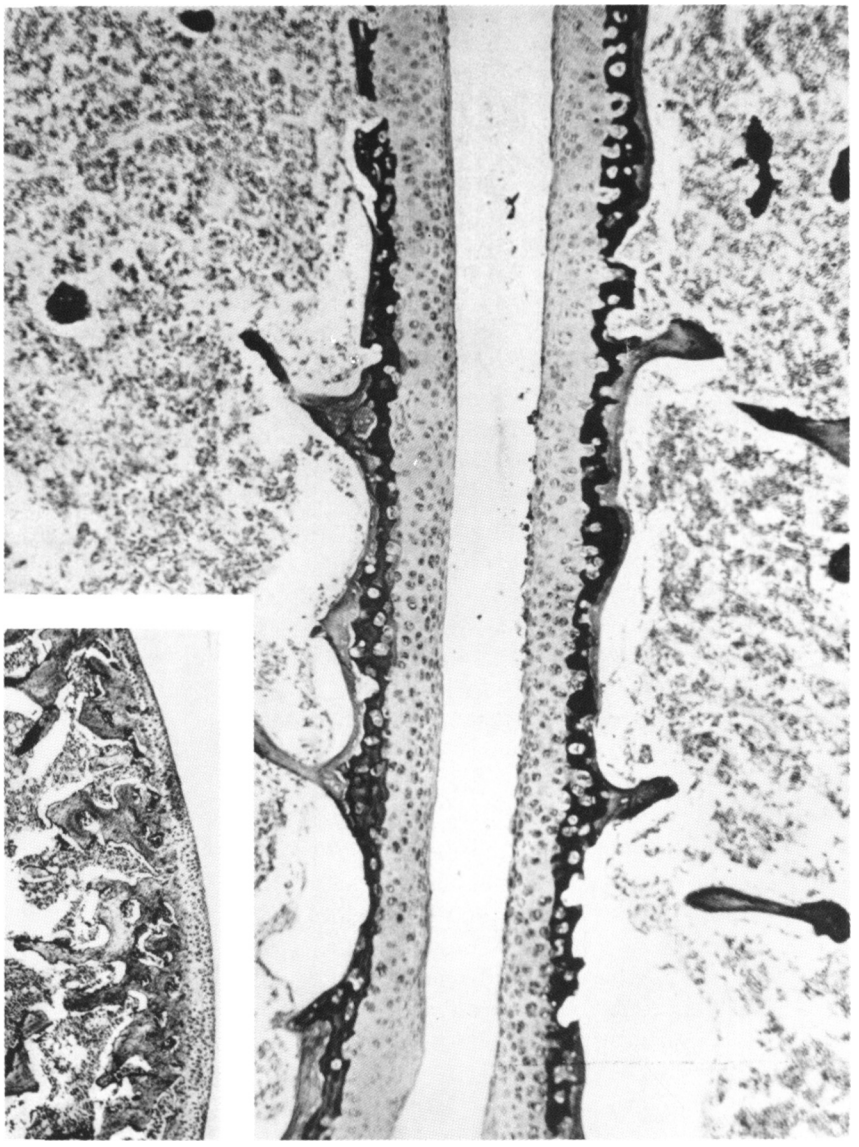

FIGURE 1. (Minor, lower left) Articular cartilage of the knee joint of a normal guinea pig at 5 months of age. (Major) Knee joint of a guinea pig that had received a normal diet for 1 month and thereafter had received 0.5 ml of orange juice daily as a sole source of vitamin C for 120 d, when it died. The scorbutic joint shows a band of intensely staining calcified cartilage between the uncalcified articular cartilage and the supporting bone. The subjacent epiphyseal bone is very thin, and in places there is no bone at all supporting the cartilage. (From Ham, A. W. and Elliot, H. C. [1938], *Am. J. Pathol.*, 14, 323. With permission.)

These cells resembled osteoblasts but were not associated with bone matrix. The tips of the menisci in knee joints were often fibrinous in character; Figure 2 shows this, as well as some fibrinous exudate lying beneath the tip of the meniscus. The connective tissue capsule of the joint, as well as the muscles associated with the joint, also showed lesions."

Ham and Elliott commented on the similarity between these lesions and those characteristic of hypertrophic osteoarthritis in humans. They pointed out that the term hypertrophic referred to the presence of osteophytes and might give a false impression of bone growth; in reality

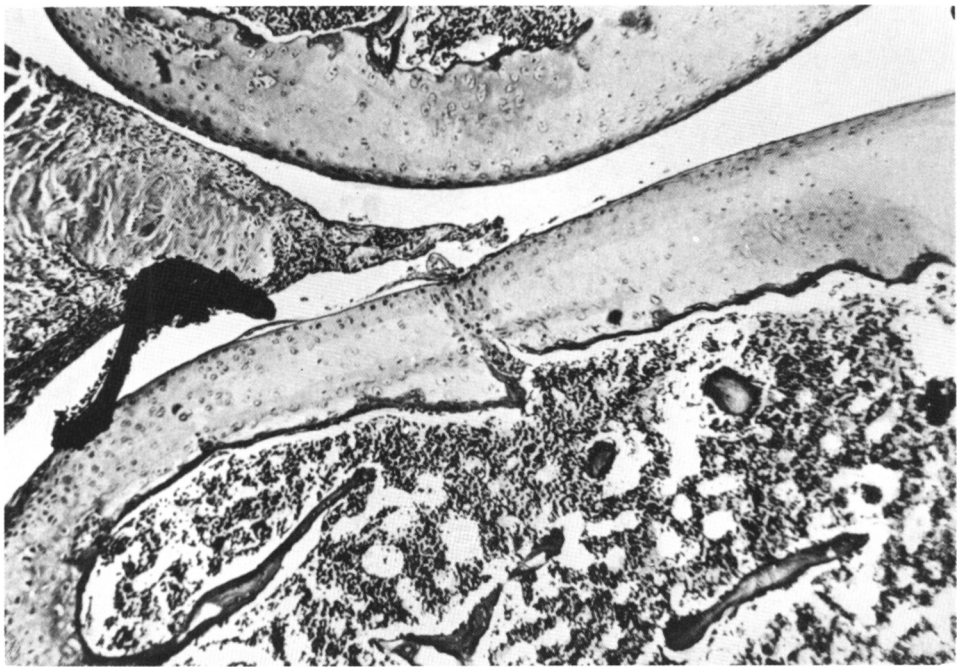

FIGURE 2 Section of the knee joint of a guinea pig that received a normal diet for 1 month after birth and thereafter received 0.5 ml of orange juice daily for 83 d, when it died. The articular cartilage rests on almost no bony support and in one location has given away (center of figure). There is some fibrinous exudate directly above the fracture, and the adjacent tip of the meniscus shows an inflammatory cell reaction A few hyperchromatic nuclei at the site of fracture probably indicate a feeble attempt at repair. (From Ham, A. W. and Elliot, H. C. [1938], *Am. J. Pathol* , 14, 323. With permission.)

there is far less bone in the skeleton than usual. The bones are light and fragile. These authors pointed out that in the adult, when growth is over, the effects of scurvy would become apparent in sites where continual replacement of tissue occurs to compensate for wear and tear. They suggested that certain features of osteoarthritis (the poorly maintained articular cartilages, the generalized diminution of the amount of bone in the skeleton and the osteophytes) would be not unlikely effects of a long continued moderate vitamin C deficiency in the adult.

Mouriquand et al. (1940) confirmed that, by prolonged partial avitaminosis C, they could create a chronic ankylosing type of rheumatism with both epiphyseal and metaphyseal bone decalcification in guinea pigs. The simple periosteal thickening type of lesion was certainly the most frequent, but in some cases there were accompanying bony proliferations of the osteophytic type. Radiologically, the changes began by ordinary periosteal thickening, which progressively hypertrophied and took on an osteophytic appearance after 100 or more days. Once formed, these osteophytic lesions were found to be resistant to ascorbic acid even in large doses. Wolbach and Bessey (1942) reported that they, in collaboration with P. E. Boyle, had found that in prolonged periods on greatly reduced ascorbic intakes (0.3 to 0.5 mg daily) the epiphyseal cartilages of guinea pigs become defective, apparently due to loss of firmness of the matrix.

Meyer (1943/1944), as a result of histological studies of guinea pigs, reported that the fibers of cartilage tissue, which seem to consist of chondroitin sulfate, are not formed in scurvy and suggested that the weakness of connective tissue in scurvy depends on this defect. Follis (1943) reported that the leg of a guinea pig on the side to which a plaster cast had been applied did not develop the trümmerfeld zone (zone of rubble), the gerüstmark (scaffold

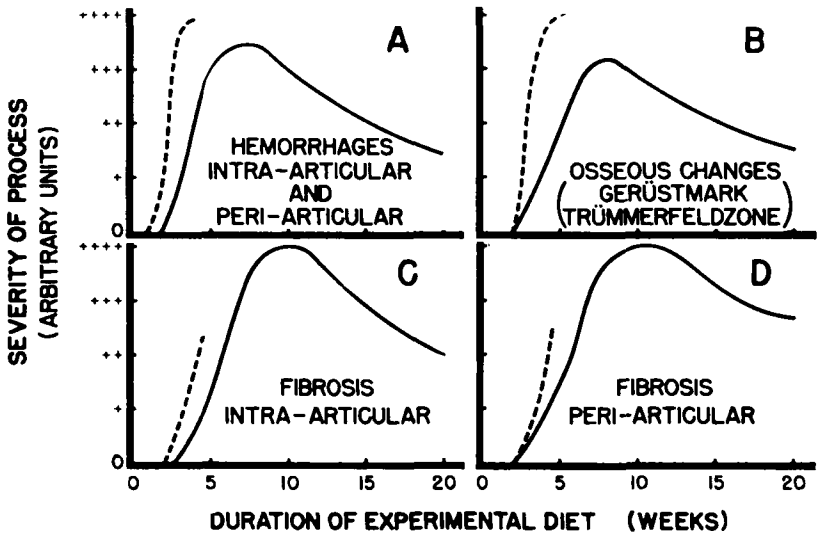

FIGURE 3. A schematic representation of the changes occurring in the joints of scorbutic guinea pigs. Acute scurvy is represented by a broken line. The guinea pigs with acute scurvy died before intraarticular and periarticular fibrosis had reached full expression. (From Pirani, C. L., Bly, C. G., and Sutherland, K. [1950], *Arch. Pathol.*, 49, 710. ©1950 American Medical Association. With permission.)

marrow), nor the fractures at the epiphysiodiaphysial junction, which are typical of scurvy. However, Pirani et al. (1950) found the intraarticular changes to be at least as severe in the immobilized as in the "free" knee joints of scorbutic animals.

Pirani et al. (1950) created chronic low-grade scurvy in 20 guinea pigs by feeding them a scorbutogenic diet supplemented with 0.2 mg of ascorbic acid daily. They studied the progression of the pathological changes in the joints of these animals at frequent intervals for 19 weeks and compared them with two other groups of animals receiving a normal and an acute scurvy diet. The animals with acute scurvy developed evidence of joint involvement at the end of the second or third week, while these changes were manifest a week or two later in the partially scorbutic group. However, the acute scurvy animals were dead a week or two later, while those with chronic scurvy went on to develop more severe and extensive joint lesions (Figure 3).

Clinically, the first evidence of arthropathy was usually at the knee and consisted of enlargement of the ends of the bones forming the joint, associated with subcutaneous hemorrhages and increasing tenderness. The joints became swollen and the animals had progressively more and more difficulty in walking. Healthy guinea pigs usually maintain a position of semiflexion of the knees and elbows, but in scorbutic animals, the flexion, especially of the knees, became more acute with progress and chronicity of the disease. "Passive extension became progressively more difficult until there was complete immobilizaton of the joint in a position of sharp flexion." Similar changes took place in the carpal and tarsal joints of the wrists and ankles, causing a variable degree of fixation and ankylosis.

The vertebral column became gradually more rigid with either disappearance or exaggeration of the normal curvatures. As a result, the scorbutic animal assumed a hyperflexed, hunched, rigid appearance, with voluntary movement reduced to an absolute minimum.

The degree of ankylosis in general eventually became more severe than that noted in terminal acute scurvy (3 to 4 weeks), but after having reached a maximum at about the 12th week it remained stationary. In a few animals a mild "paradoxical" improvement was noted in the condition of the joints after the 13th and 14th weeks. Hemorrhagic manifestations about the joints, which reached a maximum between the 6th and 9th week, diminished

in intensity, as a rule, and in many cases ceased entirely after the 12th week. Subcutaneous nodules measuring up to 2 to 4 mm. in diameter, at first semifirm in consistency and markedly hyperemic, later firmer and less congested, were observed in several animals.

. . . Intra-articular hemorrhages were almost always seen in sections taken from animals that died or were killed during the hemorrhagic phase of the disease, i.e., in the 4th week in acute scurvy and between the 6th and 9th week in chronic scurvy [Figure 4].

Part of the hemorrhage was quickly reabsorbed, and fibrin was deposited in the joint space, where it persisted and slowly underwent organization (Figure 5). "The fibroblasts which were responsible for this process originated either from the subsynovial connective tissue or, more probably, from modified synovial lining cells."

True synovial hyperplasia, with the formation of villi, was observed in a few cases at a more advanced stage (Figure 6). Pannus formation was seen within the joints in chronic scurvy and slight regressive changes were sometimes noted within the cartilage. No typical fractures were observed in the articular cartilage, but occasionally this structure showed a depressed area. "Evidence of excessive and irregular cartilage formation was found not uncommonly at each end of the epiphyseal line, but it was rare and less obvious in the articular cartilage. In most instances the articular cartilage showed only minor changes in spite of the considerable amount of fibrous tissue within the knee joint. In one animal complete fibrous ankylosis was associated with partial destruction of the articular cartilage [Figure 7].

"Considerable fibrosis was observed in the periarticular areas, especially in chronic scurvy . . . In the advanced lesions it was continuous with the proliferating connective tissue of the synovial pads and the joint cavity and especially with the actively proliferating subperiosteal layer of connective tissue along the diaphysis."

Most of the intraarticular lesions appeared to be reversible by treatment with ascorbic acid, but the periarticular fibrosis appeared to be for the most part irreversible and was responsible for persistent functional impairment. Many similarities between scorbutic arthropathy and rheumatoid arthritis were noted; the authors noted that there were also differences. There is a striking absence of inflammatory cells in scorbutic arthropathy. "The fibroblastic proliferation of scurvy, therefore, does not seem to have the destructive properties which, as a rule, are observed in infectious processes and in rheumatoid arthritis. It is probably for this reason that in chronic scurvy with few exceptons the articular cartilage shows mild lesions of a regressive rather than a destructive nature. Despite these differences," these observers wrote, "the possibility that a partial ascorbic acid deficiency may be a predisposing or a contributing factor in certain forms of human arthritis cannot be ruled out."

Herrick et al. (1952) reported that guinea pigs from which vitamin C was withheld developed the usual conditions of scurvy with painful joints and degenerating bones and testes. Death occurred from 12 to 19 d after the animals had been placed on the diet. Histological preparations of ribs and heel joints of all animals were made and studied. Little normal bone tissue remained. Other guinea pigs that received no vitamin C, but were given 5 mg of cortisone daily, lived from 17 to 45 d on the scorbutic diet and developed no painful joints. Animals from this cortisone group that were killed at a time similar to the time of death of the no-cortisone group appeared normal in every respect. It seems that cortisone has a beneficial effect and this may be as a result of maintaining a high ascorbate-dehydroascorbate ratio and a low oxidation-reduction potential, which is especially helpful when ascorbate levels are low. However, even this system fails when no ascorbate remains (Figure 5, Chapter 8, Volume III).

The histological changes in and around the temporomandibular joints of vitamin C-deficient guinea pigs were described by Levy and Gorlin (1953). In addition to the usual bony and cartilaginous changes, there were small hemorrhages in the muscle bundles adjacent to

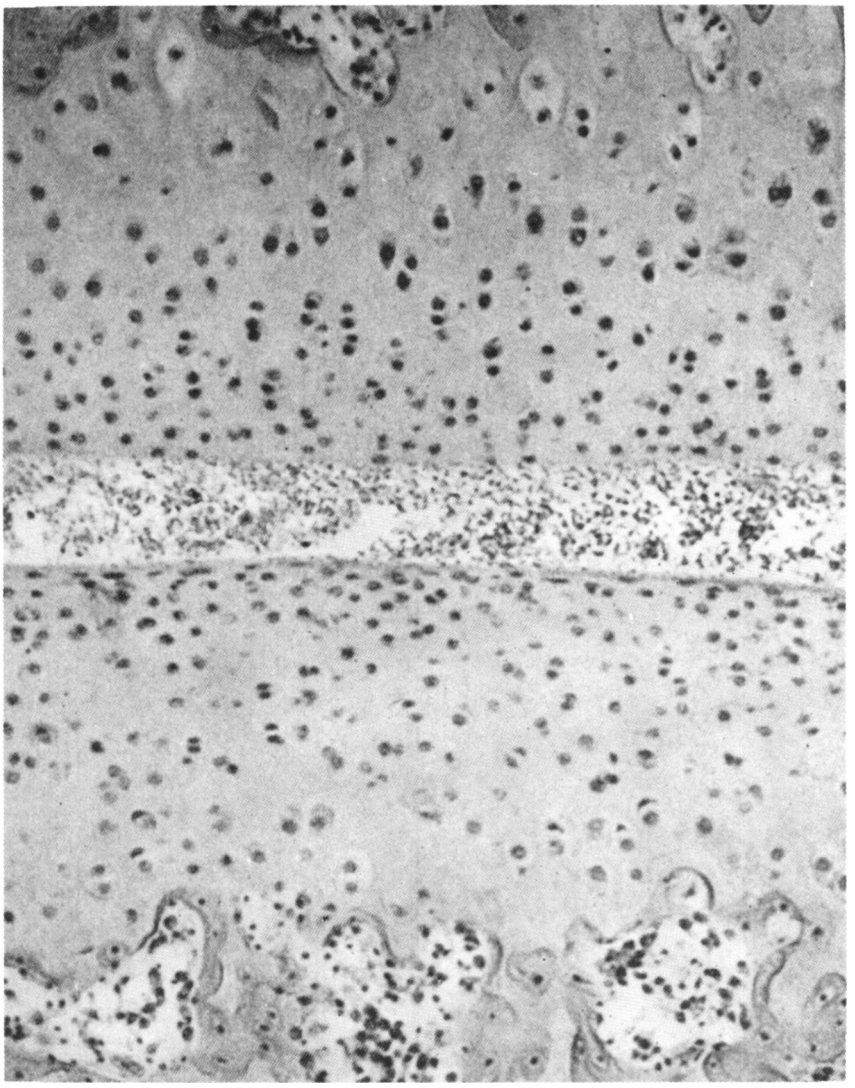

FIGURE 4. Recent hemorrhage in the knee joint of a guinea pig totally deficient in ascorbic acid. (From Pirani, C. L., Bly, C. G., and Sutherland, K. [1950], *Arch. Pathol.*, 49, 710 ©1950 American Medical Association. With permission.)

the joint; amorphous acidophilic material was seen within the joint on both sides of the meniscus and after 4 weeks, there was found hemorrhage within the joint.

Koefoed and Robertson (1966) observed a marked depression of glycosaminoglycan synthesis in the cartilaginous tissues of scorbutic guinea pigs, but concluded that this effect was due to inanition rather than a deficiency of ascorbic acid. Friederici et al. (1966) studied the synovial membrane of the joints of guinea pigs with scurvy by electron microscopy and reported pronounced changes in the endothelial cells and in the pericytes of the capillaries, as well as widening of some of the intercellular junctions.

Schwartz (1980) found that the inclusion of 0.0 mg, 250 mg, or 1.0 g of ascorbic acid in the diets of each of 3 groups of 16 mature beagles caused a dose-dependent increase in their serum protein levels from 5.69 to 6.25 and 6.32 g/dl, respectively, and an increase in the total weight of cartilage in their knee joints from 108.47 to 126.28 and 148.16 mg.

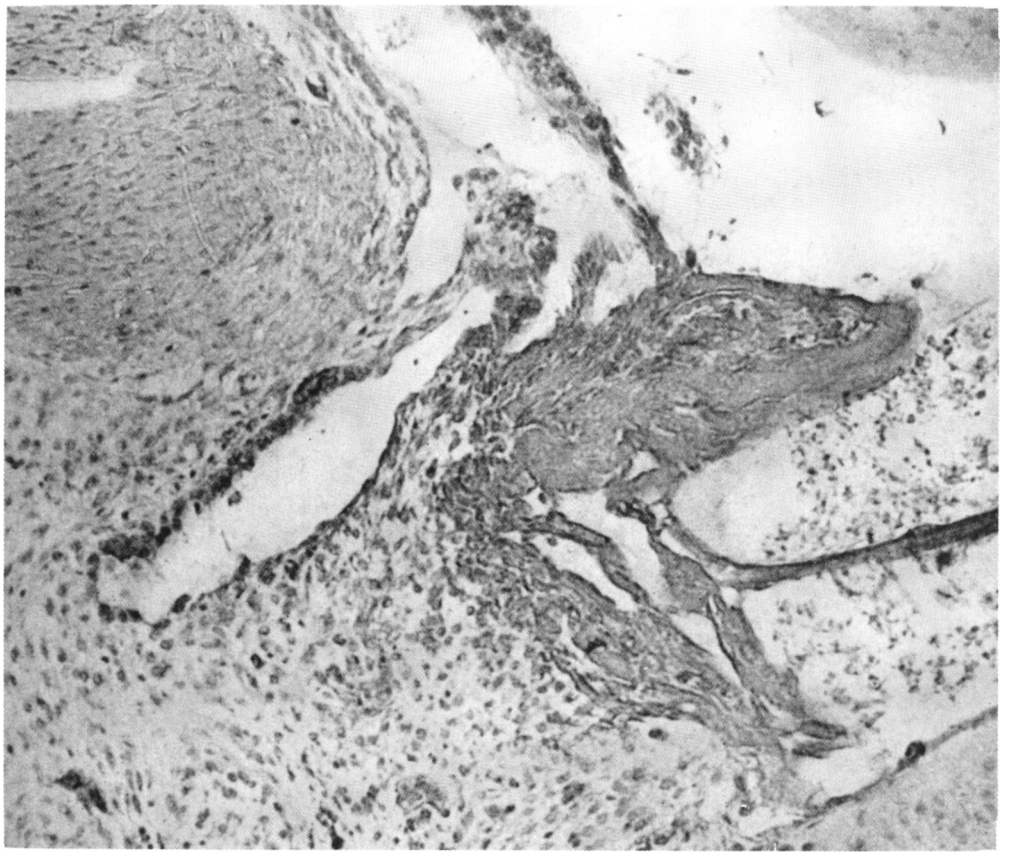

FIGURE 5 Organization of a recent hemorrhage in the knee joint of a guinea pig totally deficient in ascorbic acid. Note the degenerating blood corpuscles, the masses and strands of fibrin, and the proliferating fibroblasts. (From Pirani, C. L , Bly, C B , and Sutherland, K. [1950], *Arch. Pathol.*, 49, 710. ©1950 American Medical Association With permission.)

These results are remarkable in animals which can and do synthesize ascorbic acid, but they are consistent with the findings of the writer, who has observed ascorbate-responsive histaminemia in dogs. Moreover, these findings are consistent with the observation that some dogs develop osteoarthritis of the spine which is responsive to treatment with ascorbic acid.

The value of supplementary ascorbic acid in retarding the development of osteoarthritis was clearly demonstrated by the work of Schwartz et al. (1981). These workers initiated osteoarthritic changes in the right hind knee joints of guinea pigs by transecting the anterior cruciate ligament and the major portion of the medial collateral ligaments or by transecting these ligaments and also removing the anterior portion of the medial meniscus under general anaesthesia. Regardless of the procedure used, the animals receiving the more modest ascorbic acid intake (2.4 mg/d) always developed more severe pathology than those receiving a high dose of ascorbic acid (150 mg/d). The mean plasma ascorbic acid level of guinea pigs with the lower ascorbic acid intake was 0.25 mg/100 ml, while that of the supplemented animals was 1.27 mg/100 ml; the respective adrenal ascorbic acid levels were 14.4 and 151 mg/100 ml. The animals with the lower ascorbic acid intake were certainly not scorbutic, but as the disease in their damaged knees progressed, they showed pitting, ulceration, and eburnation of the joint surfaces much more extensively than did the supplemented group (Table 1).

Sulfated proteoglycans are major constituents of the articular cartilage, which becomes

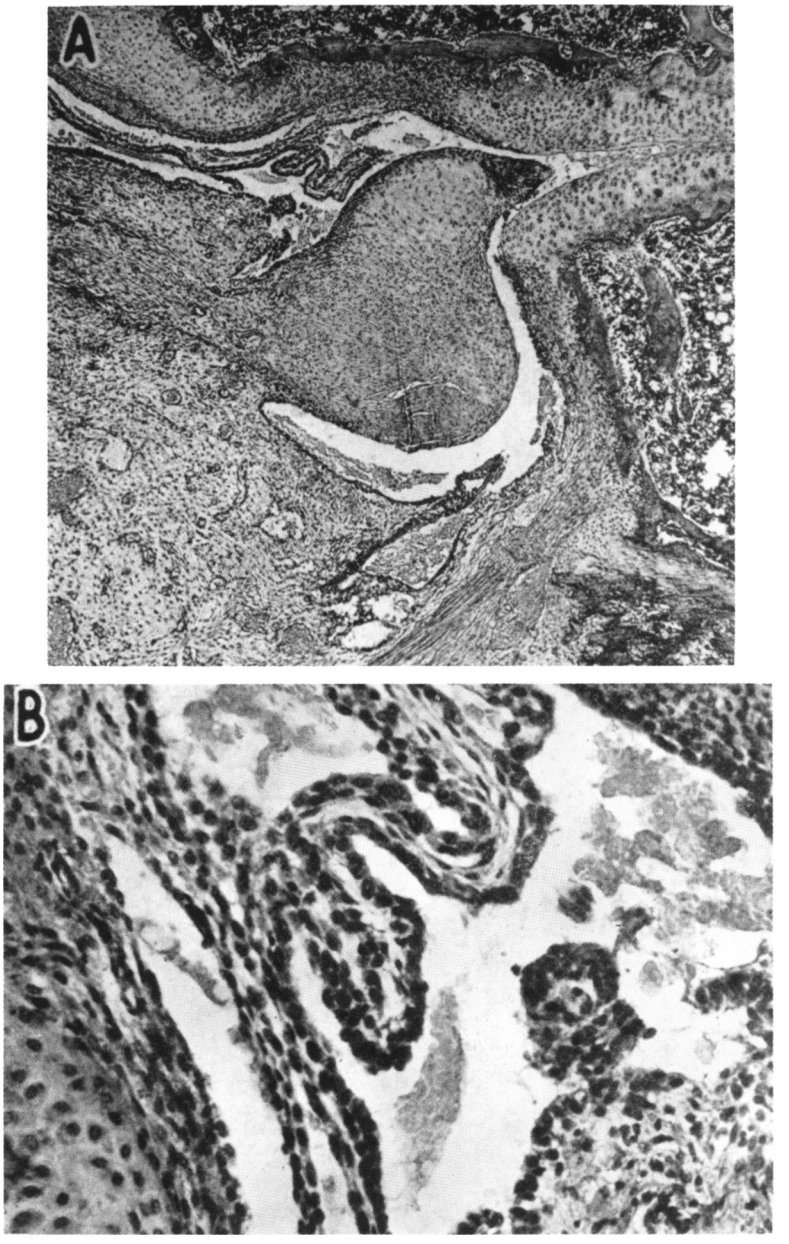

FIGURE 6. (A) Flexion recess in the knee joint of a guinea pig in a subacute stage of scurvy, showing true synovial proliferation and large vascular spaces in the young fibrous connective tissue underneath the synovia. (B) Detail of A, illustrating "true" synovial proliferation. Note the regular lining of synovial cells on newly formed villi (From Pirani, C. L., Bly, C. G., and Sutherland, K. [1950], *Arch. Pathol.*, 49, 710. ©1950 American Medical Association. With permission.)

eroded in osteoarthritis, but Schwartz et al. (1981), in a continuation of their experiments, observed an increased synthesis and metabolic turnover of proteoclycans in arthritic articular cartilages of guinea pigs, both in the supplemented and in the lower ascorbate groups. This undoubtedly represents continuing repair and replacement of cartilage in the face of erosion. It is of interest that proteoglycan biosynthesis was greater in the ascorbate-supplemented

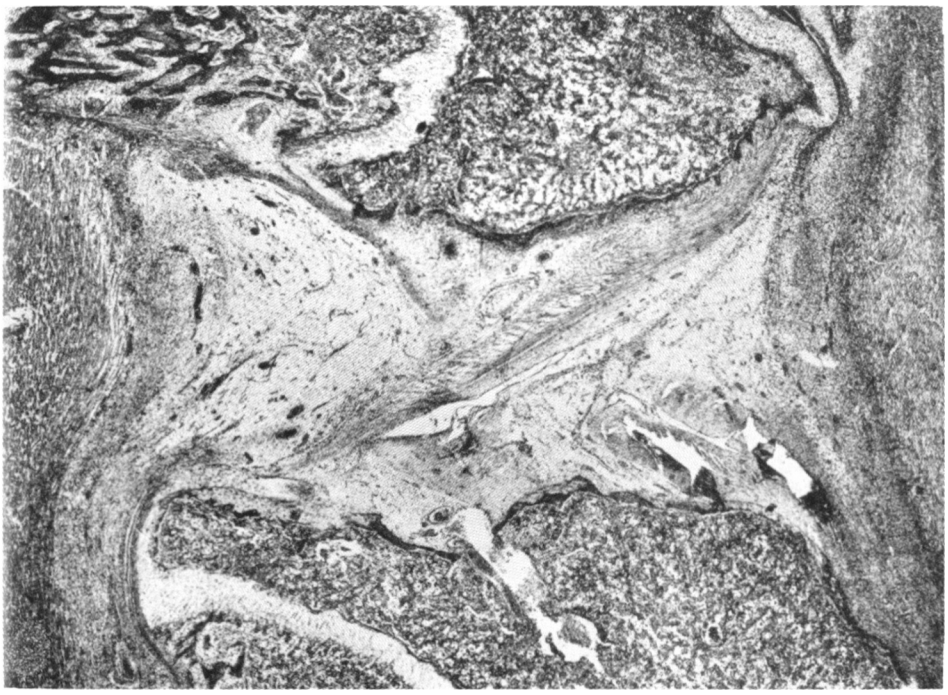

FIGURE 7 Knee joint of a guinea pig in chronic scurvy; there is fibrous ankylosis with partial destruction of the articular cartilage. The entire joint cavity is filled by young proliferating connective tissue. Fibrosis of the periarticular tissue is severe on both the anterior and the posterior aspect of the knee and is continuous with the thick subperiosteal layer of fibrous tissue. (From Pirani, C. L , Bly, C. G , and Sutherland, K. [1950], *Arch Pathol* , 49, 710 ©1950 American Medical Association With permission.)

cartilages, both normal and arthritic. At each site examined, the specific activity was 30 to 60% higher in cartilage from animals receiving high dietary levels of ascorbic acid (Table 2).

Leveille and Schwartz (1982) reported that the acid phosphatase activity was significantly greater in cartilage from the knee joints of guinea pigs on a low but nonscorbutic intake of ascorbic acid (2.4 mg/d) than in guinea pigs receiving a high ascorbic acid intake (150 mg/d). They did not find any significant difference between the cartilage arylsulfatase A or B activities of thse two groups of animals.

III. EXPERIMENTAL HUMAN SCURVY

None of the four subjects in the first scurvy study reported by Hodges et al. (1969) developed arthralgia, but pain and swelling of the joints were complaints by four of the five men who participated in the second study reported by Hodges et al. (1971). "The onset of joint pains began between the 67th and 96th days. Joint effusions appeared between the 68th and 103rd days, by which time the total body ascorbate pool size was below 110 mg and the plasma ascorbic acid level was less than 0.16 mg/100 ml. Joint pain occurred in the knees of three of the five men, in the ankles of two, and in the elbows, wrists and shoulders of one man each. Effusions in both knees occurred in three men, one of whom also had swelling of both of his ankles."

Hood et al. (1970) reported joint effusions in two volunteers who were placed on an ascorbic acid-free diet for 97 d and then on a low-ascorbic acid diet (4 mg daily) for another 116 d. Arthralgia was also recorded in one of three other volunteers who only took the

Table 1
EFFECT OF ASCORBIC ACID ON DEGENERATIVE CHANGES IN KNEE JOINTS OF GUINEA PIGS SUBJECTED TO SURGICALLY INDUCED OSTEOARTHRITIS

Surgical procedure	Anatomic site	Macroscopic changes[a] (% of animals)							
		Surface fibrillation		Osteophyte formation		Structural alterations		Eburnation	
		S[b]	M[b]	S	M	S	M	S	M
A	Medial femoral condyle	56	100	40	50	11	30	—	—
	Lateral femoral condyle	56	80	—	—	—	30	—	—
	Medial tibial plateau	67	90	22	40	—	40	11	50
	Lateral tibial plateau	11	20	—	20	—	—	—	—
B	Medial femoral condyle	83	100	100	83	22	100	—	30
	Lateral femoral condyle	67	50	—	—	—	33	—	10
	Medial tibial plateau	100	100	100	83	33	83	33	66
	Lateral tibial plateau	33	67	17	33	17	—	—	—

Note: Guinea pigs maintained on high levels of vitamin C developed less severe osteoarthritis on the average than those maintained on minimal but nonscorbutic levels of the vitamin.

[a] Operated joints of animals killed 21 (procedure A) and 14 weeks (procedure B) postsurgery were evaluated by three investigators.

[b] S, supplemented animals maintained on 150 mg of ascorbic acid per day; M, minimal animals maintained on 2.4 mg of ascorbic acid per day.

From Schwartz, E. R., Oh, W. H., and Leveille, G. R. (1981), *Arthritis Rheum.*, 24, 1345. ©1981 American Rheumatism Association. With permission.

vitamin C-free diet for 97 d. All five men showed the typical signs of scurvy, such as perifollicular hemorrhages and swollen bleeding gums; all also showed some components of Sjögren's syndrome or keratoconjunctivitis sicca, including dryness, redness, stickiness, and mattering of the eyes, intolerance to light, and transient blurred vision relieved by blinking, as well as tender swelling of the salivary glands, dry mouth, dry skin, excessive hair loss, dental decay, and recurrent breakdown of dental restorations. All received the recommended daily allowances of all the other vitamins and all recovered when they were given ascorbic acid. While the arthritis of Sjögren's syndrome is usually associated with other collagen diseases, such as lupus erythematosus, periarteritis nodosa, polymyositis, scleroderma, or rheumatoid arthritis, it is clear that vitamin C deficiency can cause Sjögren's syndrome, as indeed it can cause scleroderma, so ascorbic acid deficiency or an abnormality of ascorbic acid metabolism must be considered in all forms of arthritis.

Hodges (1971) pointed out that bleeding gums are not the most characteristic feature of scurvy, although they are seen as a late manifestation; the hyperkeratotic follicle with a pink halo, the "perifollicular hemorrhage", was found to be almost pathognomonic. However, a wide range of lesions was observed; these included ocular hemorrhages, Sjögren's syndrome, i.e., loss of secretion of salivary and lachrimal glands, with swelling of the parotid and submaxillary glands, loss of dental fillings, loss of hair, dryness and itching of the skin,

Table 2
NEWLY SYNTHESIZED SULFATED PROTEOGLYCANS IN NORMAL AND OSTEOARTHRITIC ARTICULAR CARTILAGE OF GUINEA PIGS

Ascorbic acid (mg/d)[b]	Anatomic site	Specific activity of proteoglycans $^{35}SO_4$-cpm/μg uronic acid[a]		
		Normal	Osteoarthritic (OA)	Ratio (OA to normal)
150	Femoral condyles	176	269	1.53
	Tibial plateau	191	283	1.48
	Patella	160	222	1.39
2.4	Femoral condyles	118	179	1.51
	Tibial plateau	139	206	1.48
	Patella	120	179	1.49

Note: Proteoglycan synthesis in the articular cartilage was greater in the ascorbate-supplemented animals, both in their arthritic and their normal knees.

[a] Averages of two groups of pooled cartilage samples. For animals on 150 mg/d of ascorbic acid, tissue from two animals comprised one group and tissue from three animals comprised the second group. For animals on 2.4 mg/d of ascorbic acid, tissue from three animals comprised Groups 1 and 2. Animals had been subjected to either surgical procedure A, Group 1, or B, Group 2, in the right (OA) joint and to no surgery in the contralateral (normal) joint.

[b] Dietary intake.

From Schwartz, E. R., Oh, W. H., and Leveille, C. R. (1981), *Arthritis Rheum.*, 24, 1345. ©1981 American Rheumatism Association. With permission.

femoral neuropathy, oliguria and edema, hypochondriasis, hysteria, and depression, impaired vascular reactivity, and "scorbutic arthritis", which is clinically similar to rheumatoid arthritis, with pain, swelling, joint effusions, and limitation of movement. All of the above syndromes responded completely to ascorbic acid.

IV. RELEVANT LABORATORY STUDIES

White and Sandson (1963) reported that incubation of the euglobulin fraction of rheumatoid sera with ascorbic acid caused the complete elimination of all detectable rheumatoid factor *in vitro* and that high-dosage ascorbic acid (4 g daily) was capable of lowering the titer of rheumatoid factor in the serum of some patients with rheumatoid arthritis. Lustberg et al. (1969) studied the fate of labeled homogentisic acid in normal and alkaptonuric human subjects and in tyrosine-fed rats with experimental ochronosis. Their work showed that ascorbic acid supplements decreased the binding of homogentisic acid to cartilage and to fibrous tissue in the rats, so this vitamin might possibly be effective in inhibiting the development and/or the progression of the arthritis of ochronosis in patients with alkaptonuria.

Levenson (1969) studied articular cartilage from the 12 d embryonic chick and found that hypertrophic chondrocytes which normally do not divide, and were usually thought to be degenerative, would multiply actively when released from their matrix and cultured *in vitro*. After 4 to 5 d in culture, they formed rosette-like nests of 10 to 12 rectangular cells with dense cytoplasm enclosed by refractile intercellular material, but this disappeared completely by the 7th day. Addition of ascorbic acid to the culture medium from the third to the ninth

day had a profound effect on the chondrocytes. After only 24 h with the added vitamin, the cells appeared more firmly embedded in matrix than did the controls and formed much more metachromatic extracellular material than the controls. In the presence of ascorbic acid, the cartilaginous masses enlarged and were maintained intact for at least 12 d. After 9d, there was a striking contrast between the ascorbate-maintained and the degenerating controls. Likewise, Lavietes (1970), studying factors affecting tissue reconstruction by dissociated 13-d embryonic chick sternal chondrocytes in culture, observed that the addition of ascorbic acid (50 μg/ml) to the culture medium improved the degree of differentiation and the extent of formation of a metachromatic extracellular matrix.

While glycosaminoglycans have been considered the characteristic constituents of cartilage, Layman et al. (1972) observed that approximately half the dry weight of cartilage is collagen. They found that rabbit articular chondrocytes synthesized collagen *in vitro* provided ascorbic acid (50 μg/ml) was present in the medium. The triple-helical structure of collagen formed *in vitro* by whole cartilage was found to consist of alpha 1 chains only. In contrast, the collagen formed by cultured chondrocytes contained both alpha 1 and alpha 2 chains with a varying (alpha 1/alpha 2) ratio, usually 2:1, occasionally as high as 4:1, resembling skin collagen and suggesting either contamination of the chondrocytes to fibrocytes or altered function due to altered environment.

Goetzl (1976) observed that the polymorphonuclear (PMN) leukocytes of four patients with rheumatoid arthritis and one patient with vasculitis showed normal random and chemotactic migration and that ascorbate stimulation increased the migration of their leukocytes by an average of 55 to 95% above baseline (Figures 8 and 9). In contrast, the PMN leukocytes from four patients with untreated systemic lupus erythematosus (SLE) showed defective random migration and depressed chemotactic responses. Normal *in vitro* enhancement of PMN leukocyte random and chemotactic migration by sodium ascorbate was absent in SLE and in Felty's syndrome, but sodium ascorbate gave normal stimulation of hexose monophosphate shunt activity in the PMN leukocytes (Figure 10), so precluding the possibility of a defect in ascorbate transport.

Studying human cartilage cells in tissue culture, Schwartz and Adamy (1977) observed that ascorbic acid stimulated an increase in the production of cellular protein and in alkaline phosphatase activity. However, this vitamin consistently caused a decrease in the activities of arylsulfatases A and B, both in normal and osteoarthritic cartilage (Figure 11). Moreover, sulfated proteoglycan (mucopolysaccharide) synthesis was significantly increased in the presence of ascorbic acid. These findings are particularly interesting because arysulfatases A and B are enzymes capable of breaking down the sulfated macromolecules of cartilage.

Malemud et al. (1978) reported that the effect of adding ascorbic acid to cultures of rabbit articular cartilage was dramatic. "It caused a three-fold increase in cell proliferation, deposition of copious chondroid matrix immediately about the explants, and an increase in the synthesis of sulfated glycosaminoglycans." Moreover, they cited other studies showing ascorbate stimulation of DNA synthesis and selective ascorbate reduction of arylsulfatase B activity of cultured human chondrocytes.

Schwartz (1979) and co-workers, at Tufts University School of Medicine in Boston, studying osteoarthritic human articular cartilage, have observed that the levels of arylsulfatases A and B become elevated at an early stage of the disease. These enzymes undoubtedly contribute to the erosion of the cartilage. It is therefore very pertinent that vitamin C causes a decrease in the levels of both these enzymes in cultured human cartilage. Schwartz (1979) observed that ascorbic acid enhanced the stability of the sulphated proteoglycans of human articular cartilage in cell culture. "Concurrent with an inhibition of arylsulfatase A and B activities, an increase in sulfated proteoglycan biosynthesis per unit of DNA as reflected in ^{35}S sulfate uptake was present in all cartilage specimens examined 6 days after the introduction of vitamin C in the culture fluid. A larger fraction of the newly synthesized sulfated ma-

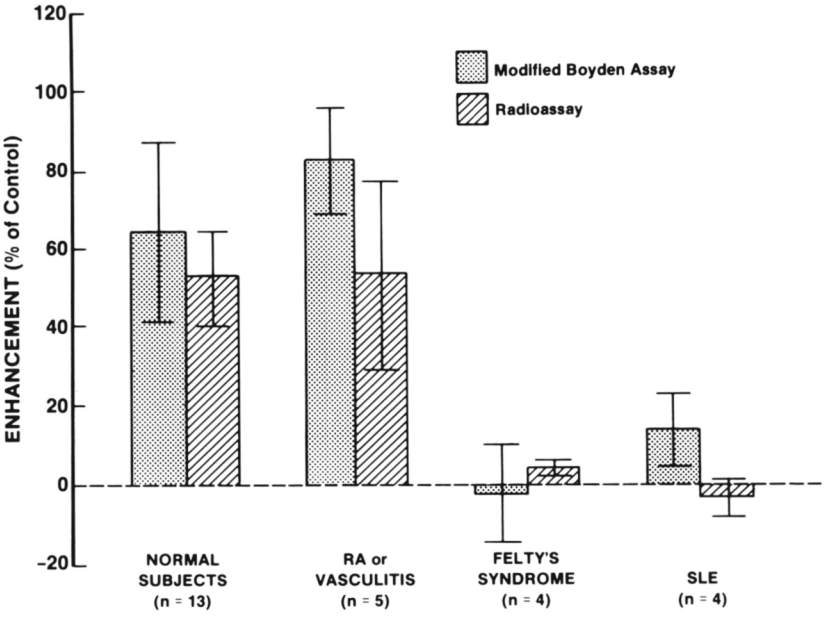

FIGURE 8. Ascorbate enhancement of neutrophil random migration was observed in normal subjects and in patients with rheumatoid arthritis or vasculitis, but was not seen in Felty's syndrome nor in systemic lupus erythematosus. The bars show mean ± 1 SD for each group. An ascorbate dose of 2.5×10^{-3} mol/l was used with a 10-min preincubation interval. (From Goetzl, E. J. [1976], *Ann. Rheum. Dis.*, 35, 510. With permission.)

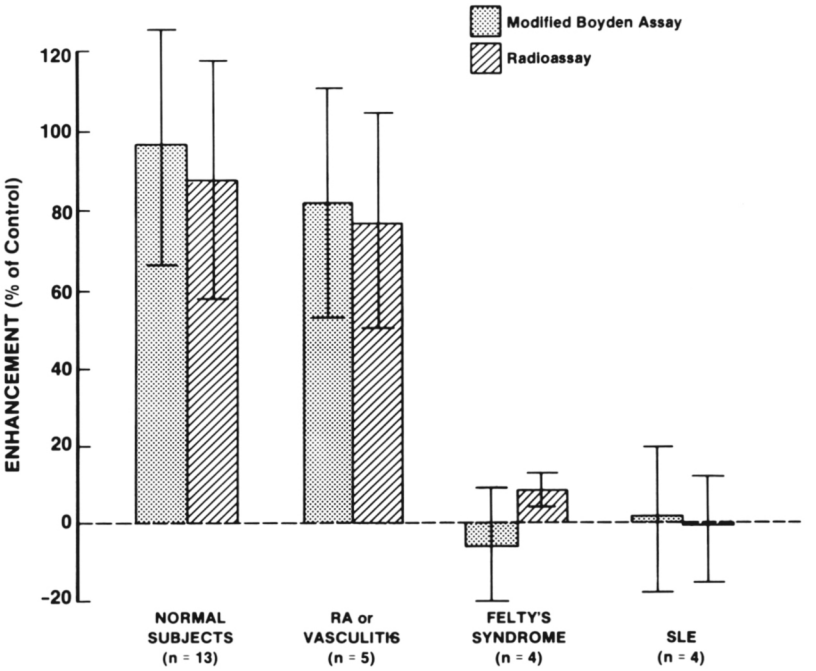

FIGURE 9. Ascorbate enhancement of neutrophil chemotaxis was observed in normal subjects and in patients with rheumatoid arthritis or vasculitis, but did not occur in patients with Felty's syndrome nor in systemic lupus erythematosus. The bars show mean ± 1 SD for each group. An ascorbate dose of 2.5×10^{-5} mol/l was used with a 10-min preincubation interval. (From Goetzl, E. J. [1976], *Ann. Rheum. Dis.*, 35, 510. With permission.)

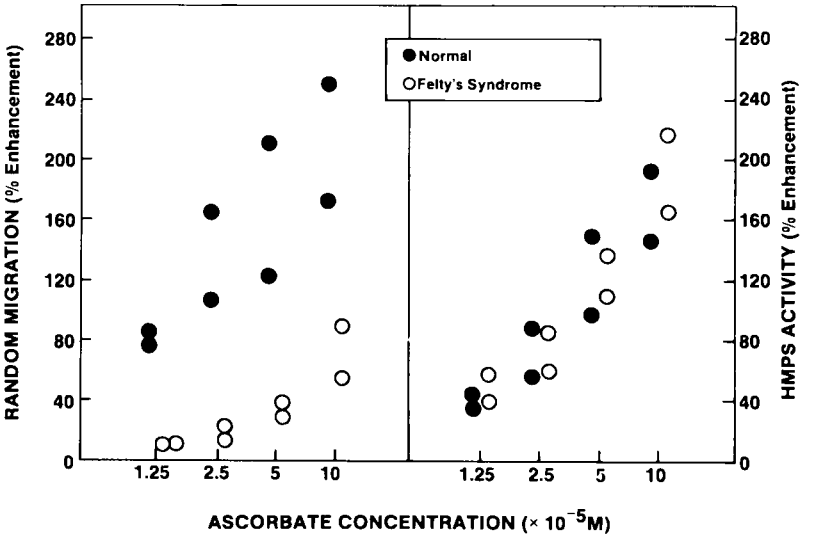

FIGURE 10. Dose response of ascorbate enhancement of neutrophil random migration (left) and of hexose monophosphate shunt (HMPS) activity (right) in normal subjects and in patients with the arthritis of Felty's syndrome. Neutrophil random migration in two patients with Felty's syndrome required an eightfold higher than normal ascorbate level to show any enhancement; however, leucocyte HMPS activity response to ascorbic acid was normal in Felty's syndrome, so there does not seem to be any failure of transportation of ascorbic acid to the leucocytes in that disease. (From Goetzl, E. J. [1976], *Ann. Rheum. Dis.*, 35, 510. With permission.)

cromolecules was incorporated into the matrix of the tissue in samples maintained in the vitamin-supplemented cultures." While ascorbic acid inhibited both arylsulfatase A and B, alpha tocopherol inhibited arylsulfatase A, but had no effect on arylsulfatase B in cartilage slices. Moreover, vitamin C stimulated acid phosphatase activity and vitamin E had a potent inhibitory effect on this enzyme.

Since hemosiderin has been found to accumulate in chondrocytes from the joints of hemophiliacs with the arthropathy that follows hemarthrosis, Choi et al. (1981) studied the effect of 2.5 m*M* $FeSO_4$, and of hemolyzed rabbit serum, on rabbit articular chondrocytes in cell culture; hemoglobin was less effective than Fe^{2+} in inducing siderosis, both morphologically and chemically, but both caused the accumulation of fine cytoplasmic granules containing Fe^{3+} in a proportion of the chondrocytes. Hemosiderin is known to accelerate the catabolism of ascorbic acid (Chapter 10, Volume I), so local ascorbate deficiency or a severe local disturbance of the AA to DHAA ratio within the joint may well be responsible for damage to the chondrocytes. Choi et al. found that sodium ascorbate, 40 μg/ml, caused a proliferation of chondrocytes in monolayer culture, but did not reduce the iron toxicity *in vitro*. Moreover, desferrioxamine, when used to chelate the iron in cell cultures was itself toxic to the chondrocytes, Clearly, studies of nontoxic chelating flavonoids or catechins are indicated, so as to find out whether these natural components of food fiber can remove iron from the joints or whether more powerful chelating agents such as penicillamine need to be administered, along with ascorbic acid, in treatment of the arthritis that develops in association with hemophilia, hemosiderosis, and hemochromatosis.

Krystal et al. (1982) confirmed that sodium L-ascorbate (0.2 m*M*) caused a manyfold stimulation of DNA synthesis by cultured explants of rabbit and also human articular chondrocytes. While articular chondrocytes had traditionally been considered as terminally differentiated and incapable of mitotic division *in situ*, it was confirmed that they can replicate while still surrounded by an extracellular matrix.

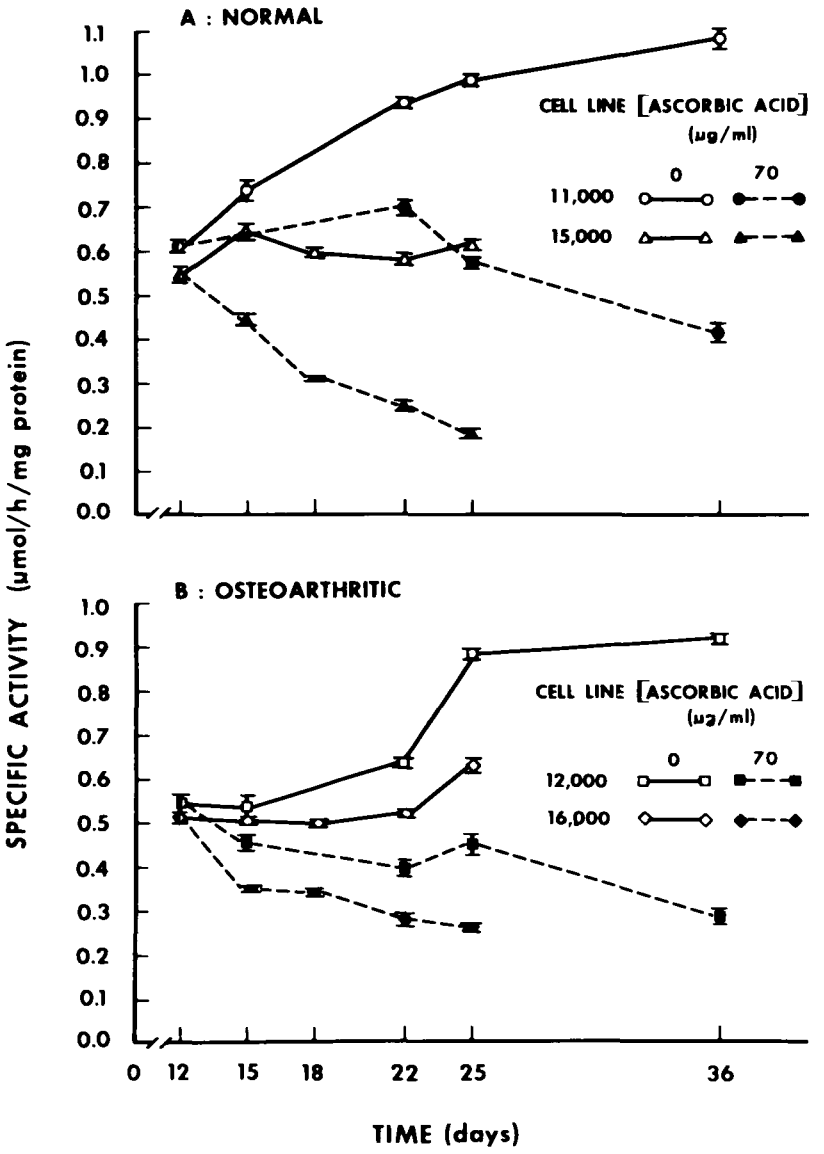

FIGURE 11. Effect of ascorbic acid on the specific activity of arylsulfatase A. (A) In normal human chondrocytes; (B) in chondrocytes from osteoarthritic cartilage. Here it is evident that ascorbic acid inhibited the activity of arylsulfatase A, both in normal and in osteoarthritic chondrocytes. The results obtained for arylsulfatase (B) (not shown) were very similar. (From Schwartz, E. R. and Adamy, L. [1977], *J. Clin. Invest.*, 60, 96. ©American Society for Clinical Investigation. With permission.)

Prins et al. (1982a,b) studied the effects of several growth factors — platelet-derived growth factor, epidermal growth factor, pituitary fibroblast growth factor, heat-activated fetal bovine serum, insulin, and 0.2 mm ascorbate — on (1) DNA synthesis and (2) sulfated proteoglycan synthesis by rabbit articular chondrocytes in monolayer culture and found 0.2 m*M* ascorbate to be unique among the factors tested in stimulating both DNA synthesis and glycosaminoglycan synthesis that was not supressed by higher concentrations of serum.

V. INFANTILE SCURVY

Even though swelling of the joints is not as great in children as it is in guinea pigs or monkeys, the presenting symptoms of 37 infants and young children with scurvy, as reported by Park et al. (1935), make it plainly evident that swollen painful joints may be present and are sometimes the major problem:

— Pain knees flexed 4 weeks.
— Cried when handled 3 weeks.
— Blood in stools 2 weeks; pain 1 week.
— Screamed when knees moved 1 week.
— One foot tender 1 month.
— Cried when legs moved 3 months.
— Legs tender 1 week; bleeding gums.
— Gums bruised 5 weeks. Fretful 3 weeks.
— Arms and legs tender 10 days.
— Cried when touched 4 days.
— Pain, left leg 1 week.
— Disliked movement left leg 2 days.
— Knees drawn up 2 weeks. Fretful.
— Diarrhea; pain in legs 3 weeks.
— Legs drawn up 6 days.
— Pain, right leg 3 days.
— Cried when handled 10 days.
Retrobulbar haemorrhage.
Occult blood in stools.
— Screamed if leg touched 3 days.
— Vomiting, irritable, legs flexed 3 weeks.
Bloody nose and stool. Petechiae on abdomen.
— Pain in legs 3 days.
— Swelling right knee and ankle 2 days.
— Joints tender. Bleeding gums 3 weeks.
— Screamed when touched 2 months.
— Sickly 1 month (Early changes in X-ray).
Then bleeding gums later.
— Pain 2 months.
— Pain in legs 1 week.
— Cried when picked up and arms raised 2 weeks.
Pain in legs later.
— Screamed when legs touched 1 week.
— Pain right leg 5 days.
— Fretful. Screamed 2 weeks.
— Cried when touched or moved 3 weeks.
— Right leg sore 1 week; then left.
— Pain in legs 1 month; later in shoulder.
— Cried when moved 10 days.
— Did not want to be touched 3 days.
— Cried when legs moved 1 month.
— Cried when legs moved 1 week.

The authors cited one mother who said, "Any way you lay him is the way he lays. If you lay him on his side, he makes no effort to move or nothing. It looks like most of his trouble is from the hips down."

VI. ADULT SCURVY AND ARTHRITIS

Freckler (1927) recorded his observations of a 50-year-old school teacher in New South Wales, who presented with a "stiff and sore knee" due to a slight knock on the day before.

He was found to have a large entravasation of blood behind the knee; the inner aspect of both thighs presented a remarkably speckled appearance due to the presence of a small petechial hemorrhage around the base of each hair. His gums were swollen and red and he had been under dental treatment for "trench mouth" for 6 weeks. He said he had not eaten any fruit or vegetables for 7 years as he "hated them like poison," so it would seem that he had survived on what little vitamin C he had obtained from eating meat. A diagnosis of scurvy was made and he was completely cured by proper dietary advice. In conclusion, Freckler speculated on "how many larval forms of scurvy occur in our every day practice, with ill-defined symptoms, anorexia, languor, mild anaemia and even mild stomatitis." A very similar presentation of scurvy was described by Bullowa (1927), who cared for a 55-year-old Irish hotel porter in New York. Two and a half weeks previously he had tripped while at work and, falling, had struck his right knee and thigh. He thought he must have injured his left wrist as well because, since the accident, it had become weak. In a few days, his thigh began to swell and he limped. On examination, he was found to have enormously swollen bleeding gums, a macular hemorrhagic rash over his thighs and forearms, and a left wrist-drop. There was swelling and pitting edema of both legs; the lower portion of the right thigh was tender and much larger than the left. His problems were diagnosed as beriberi causing the wrist-drop and scurvy causing the periosteal and periarticular hemorrhage. A remarkable recovery was accomplished by provision of a proper diet including oranges, meat, and whole meal bread. while he had to be lifted into bed on admission to hospital, he was discharged in good health 12 d later.

Scurvy presented in a very similar manner in a U.S. Navy seaman who was admitted to hospital at Pearl Harbor complaining of bleeding gums, pain in the chest, and pain and swelling of the left knee. Roberts (1927) described how this man had suffered a slight blow on the left knee and it had begun to swell some 3 weeks earlier on board a battleship at sea. He was very weak, with severe anemia (RBC, 1,500,000) on admission to hospital and was found to have the typical bluish discoloration of subcutaneous hemorrhages over both knees. Both knee joints were swollen and painful and the left knee joint space contained fluid which was thought to be blood, although it was not aspirated. He had bleeding gums and many carious teeth. It seems that ever since he was a child he had disliked all fruits and the only vegetable he would eat was an occasional potato. He had probably been in borderline vitamin C deficiency for a long time and his scurvy was finally precipitated by an injection of mercurochrome which he had received as part of his treatment for gonorrhea some 6 weeks previously (see mercury under heavy metals in Chapter 10, Volume I). This sailor made a rapid recovery from the scurvy and the anemia following treatment with citrus juices three times a day.

Jennings and Glazebrook (1938) described two adult Londoners with joint pains as the most prominent presenting symptoms of scurvy. The story of the first of these, a 52-year-old man, who hated fruits and vegetables, is of particular interest:

> In August, 1937, he noticed a sudden pain in the right shoulder, which he ascribed to rheumatism. The shoulder was not swollen or discolored; the pain persisted for about four weeks, and then passed completely away. In November he found on rising one morning that the right knee was swollen. The swelling was followed by pain, which gradually increased in severity over a period of fourteen days and then as gradually passed off. The pain kept him awake at night, but was not increased by movement and did not prevent him from getting about. In December he was admitted to the surgical department at the Postgraduate Medical School for treatment of the condition in the right knee. The knee was found to be grossly swollen, with effusion into the joint and much periarticular bruising and redness. Flexion was possible to a right angle only. It was thought that the condition might be of infective origin, although the Wassermann test, the gonococcal complement-fixation test, and culture of aspirated fluid yielded no positive information. While he was in hospital it was noted that he had anaemia with a colour index below unity and a red count of 3,200,000. Radiographs of the joint showed no recognizable abnormality. With symptomatic measures the swelling and pain in the knee cleared up in three weeks, and he was discharged to the out-patient department for treatment of his anaemia. The anaemia, however, progressed, and by

January, the red count had fallen to 2,240,000 with a colour index of 1.27. Accordingly, he was admitted to the medical department on January 15, 1938, with a provisional diagnosis of pernicious anaemia.

He had a pale yellow complexion, but he was edentulous and his gums looked quite healthy, with no tendency to sponginess or bleeding. The right knee was stiff, full flexion not being possible, and there was infiltration and staining of the periarticular tissues as a result of blood extravasation. Multiple raised petechiae into the hair follicles were present on the extensor aspects of both legs. Investigations revealed a megaloblastic bone marrow and he was found to have no free acid in the gastric juice after histamine. Gastroscopy revealed moderately extensive gastric atrophy with several recent hemorrhages in the mucosa. A diagnosis of pernicious anemia was made and he received standard liver therapy. In fact, it was only when his anemia failed to respond to the liver treatment and when on February 14, 1938, he developed further effusions of blood into the left thigh muscles and around the right ankle and right knee that the diagnosis of scurvy was finally made and he began to receive appropriate and successful treatment. This sad saga serves to show how a patient can seek advice in a most highly respected medical establishment for 6 months, from August until February, without relief, because most of us did not then and do not now recognize the earliest stages of vitamin C deficiency.

Individual case reports are usually considered as being of little value in the medical literature, but they can record great benefit to the individual concerned. In a study of vitamin C and the aging eye at the Hastings State Hospital in Detroit, Bouton (1939) administered ascorbic acid, 350 mg daily, to a group of 12 patients who were selected because of visual disturbances and who were found to be ascorbic acid deficient. "One patient, in particular, a 64-year-old man, suffered severely from pains in all large joints and in the spine, with marked initial stiffness on arising from bed or after sitting in one position for any length of time. After the first week of treatment this patient spontaneously offered the information that the initial stiffness had diminished considerably and that the pains in the joints were definitely less troublesome. Throughout the remainder of the treatment and subsequently he has repeatedly made the statement that he feels 'better in every way than for the past two years.' This feeling of general well-being can probably be ascribed in great part simply to the relief from the constant articular pains."

Roff and Glazebrook (1939) reported their observations of gingivostomatitis associated with ascorbic acid deficiency in many boys from poor homes on enlistment into the Royal Navy. Lassitude and "rheumatic" pain in and around the larger joints were characteristic complaints among those affected in this way; rheumatic fever developed in many of these youths (Chapter 10, Volume III). The authors stated, "It is often impossible to differentiate from the description of the symptoms of the patient a case which will clear up on saturation with vitamin C, from one which will tend to progress to rheumatism and carditis." Rinehart and Greenberg (1942) observed chemical evidence of subclinical scurvy in many patients with rheumatoid arthritis and noted clinical improvement in most of them following ascorbic acid administration as illustrated by the following cases:

- W. K., male, aged 57 — Diagnosis: mild rheumatoid arthritis with slight swelling and stiffness of metacarpophalangeal and interphalangeal joints and soreness in knees and shoulders; initial plasma ascorbic acid, 0.0; curve following test dose of ascorbic acid, very flat; 3-h peak, 0.13 mg/100 ml; deficit, 3g; initial capillary resistance, 90 petechiae at −15 cmHg; elevated to 8 petechiae at −16 cmHg in 6 d; very mild reticulocyte response; 2 months later, general health and arthritis greatly improved
- M. M., male, aged 31 — Diagnosis: rheumatoid arthritis of the spine; initial plasma ascorbic acid level, 0.0 mg/100 ml; curve following test dose of ascorbic acid, very flat; 3-h level, 0.17 mg/100 ml; deficit, 4 g; questionable slight rise in capillary

resistance from four petechiae at −16 cmHg to one petechia at −20 cmHg after 21 d; no reticulocyte studies; improvement in general health, sleeps better, less back pain

- W. H., male, aged 38 — Diagnosis: rheumatoid arthritis of the spine; initial plasma ascorbic acid, 0.0 mg/100 ml; curve following test dose of ascorbic acid (15 mg/kg), medium rise to peak of 0.6 mg/100 ml; tissue deficit, 2 to 3 g; capillary resistance, rose from six petechiae at −15 cmHg to six petechiae at −20 cmHg in 60 d; increased energy and appetite, less pain and increased movement in spine, clear-cut slowing of sedimentation rate
- E. L., female, aged 30 — diagnosis: mild rheumatoid type of arthritis; initial plasma ascorbic acid, 0.28 mg/100 ml; curve, medium rise to 0.68 mg/100 ml; 5-h level higher; no excretion; second curve, 3-h level, 1.28 mg/100 ml; no excretion; deficit 2.5 g; capillary resistance normal; no reticulocyte rise; marked symptomatic improvement in general health and arthritis; diet history good; probable absorptive fault

Glazebrook and Thomson (1942), studying naval cadets, found that supplementary ascorbic acid (200 mg daily) had only a slight effect on the incidence of common colds and tonsillitis, but afforded complete protection against the development of rheumatic fever (Chapter 10, Volume III) which as we know, "licks the joints but bites the heart." There were 16 cases of rheumatic fever and 17 cases of pneumonia among 1100 controls and no case of either disease among 335 youths receiving ascorbic acid supplements.

Israels (1943) reported studies of three men aged 57, 47, and 66 who were admitted to the Manchester Royal Infirmary with classical scurvy and anemia. All three presented with pain, stiffness, swelling, and discoloration of a knee or an ankle or both; only one of the men had sprained his ankle 8 weeks previously. The other two also had large ecchymoses, stiff swollen joints, and pain on movement without knowledge of any injury.

The advanced stage of vitamin C deficiency at the time of diagnosis is well illustrated by the report of Cox et al. (1962) who observed stiff, swollen, painful knees or ankles associated with periarticular extravasation of blood in six out of seven patients treated for scurvy at the General Hospital in Birmingham, England. Nevertheless, in most instances the joint lesions of acute scurvy are recorded as resolving completely following oral administration of ascorbic acid. A few, like the second patient reported by Will and Murdoch (1960), are recorded as having some residual stiffness of the knee following complete resolution of a scorbutic hemarthrosis.

The report by Hyams and Ross (1963), already mentioned because of osteoporosis in Chapter 5 of this volume, devoted to bone lesions, must also be mentioned here in this chapter on joint lesions because ballooning of the intervertebral disks of L1, 2, 3, and 4 was seen on X-ray, in addition to the almost complete collapse of L1 and osteoporosis with wedging of the mid-thoracic vertebrae. This 54-year-old London housewife had suffered severe backache for a year and had consulted physicians at another hospital, as well as an osteopath, seeking relief without avail. Indeed, none of these changes would have been properly diagnosed as being due to vitamin C deficiency if her condition had not progressed to frank scurvy, nor would she have received proper treatment. These observations are particularly pertinent because Basu (1981) has reported lower plasma vitamin C levels in five patients with prolapsed intervertebral disk (mean 0.46 mg/100 ml) than in normal subjects (mean 0.94 mg/100 ml), and Greenwood (1964), of Baylor College of Medicine in Houston, reported the successful use of ascorbic acid supplements in the treatment of patients with sciatica due to herniation of an intervertebral disk. He prescribed 500 mg daily, increasing to 750 or 1000 mg when necessary, for hundreds of patients, and after 5 years of experience, he gained the impression that he was able to avoid surgery and hasten recoovery in most of the patients by this treatment. Moreover, he expressed his belief that the recurrence rate in 300 patients who had undergone surgery was reduced. It certainly seems wise to make sure

that there is no deficiency of ascorbic acid in "disk" patients; for the nucleus pulposus of the intervertebral disk is normally held in placed by a collagenous fascial sheath and collagen is an ascorbic acid-dependent tissue.

Scobie (1969) reviewed the findings in seven men who were admitted to the Wellington Hospital in New Zealand with scurvy between 1960 and 1969. Three of them had effusions into one or both knee joints, but only one had the classical hemorrhagic gingivitis of scurvy. Indeed, only two of the seven were suspected of having scurvy on admission. Nevertheless, clinical improvement often began on hospital diet and bed rest, even when the administration of vitamin C was delayed, possibly because the blood histamine level is reduced by rest and sleep (Chapter 17, Volume I). Thus, there is a real danger that the diagnosis may be missed, and if the dietary advice is not provided, the problem wll surely recur at home. Indeed, one of the men recorded a similar bout of leg trouble 2 years before admission.

Sahud and Cohen (1971) reported that the plasma ascorbic acid level was significantly lower than normal (0.60 vs. 1.03 mg/100 ml) in patients with rheumatoid arthritis ($p < 0.001$); but the level in the platelets did not differ significantly from normal. However, the platelet ascorbic acid content was also found to be significantly reduced (30.9 vs. 68.6 $\mu g/10^8$ cells) in patients with rheumatoid arthritis receiving high-dose aspirin treatment ($p < 0.001$). these authors suggested that ascorbic acid deficiency might contribute to the pathology of rheumatoid disease. They noted that many physicians include supplemental vitamin C in the management of rheumatoid arthritis and pointed out that this becomes especially important in patients receiving high-dose aspirin therapy.

Bevelaqua et al. (1976) observed hemarthrosis due to scurvy in a 56-year-old bartender in Philadelphia. They aspirated 35 ml of blood-stained fluid from his right knee and also obtained a synovial biopsy specimen. Stained sections showed massive fresh hemorrhage into the synovium as well as some hemosiderin in the macrophages of the deep connective tissue, suggestive of older bleeding. There was no evidence of an inflammatory cell infiltrate, but there were increased numbers of unusually large fibroblasts. The synovial lining cells appeared normal, and Congo red stain for amyloid was negative. "Electron micrographs showed many fibroblasts with prominent, dilated rough endoplasmic reticulum, Golgi apparatus, and nucleoli. Occasional vacuoles containing ferritin granules and other dense, unidentified material were also noted. Mature collagen fibers were seen occasionally in the interstitium but were definitely decreased in number. Hemosiderin laden macrophages were found, as were many extravasated erythrocytes and some erythrophagocytosis. Small-vessel endothelial cells were thick, with normal appearing inter-cellular junctions, and were rich in organelles, including endoplasmic reticulum, Golgi apparatus, free ribosomes, dense bodies, and mitochondria. Vascular basement membrane was present but poorly defined, and perivascular collagen fibers were sparse. The pericytes had no identifiable alteration." This patient was a heavy smoker who also abused alcohol and had suffered two cerebrovascular accidents before presenting with scurvy and hemarthrosis. The authors suggested that scurvy may be a more common cause of hemarthrosis than is generally appreciated, The present writer would also suggest that ascorbic acid deficiency may have been largely responsible for the two episodes of cerebral hemorrhage preceding this admission with scurvy (see Chapter 19, Volume III).

Bird (1983) reported the proceedings of a conference on heavy metals and arthritis which was held at Harrogate on April 7, 1983.

- Grennan of Manchester noted that serum levels of copper and ceruloplasmin were significantly higher and serum levels of zinc were significantly lower in patients with osteoarthritis than in control subjects. Copper and ceruloplasmin were still further raised in the rheumatoid group and zinc was lowered in the rheumatoid group, though not more so than in osteoarthritis.

- Ward et al. of Harrow made similar observations as regards copper and zinc and studied these metals in patients with rheumatoid arthritis during treatment with various chemicals; it was argued that even gold compounds might be working through copper metabolism.
- Blake of Birmingham cited experimental evidence that iron may exacerbate rheumatoid arthritis.
- Dixon of Harrogate discussed the use of penicillamine in active rheumatoid arthritis and gave reasons for believing that its benefits result more than its –SH (reducing agent groups) than from its metal-chelating action.

Clearly, the deleterious effects of copper and iron and the beneficial effects of reducing and chelating agents may be due to their effects on ascorbate metabolism. While dietary ascorbic acid deficiency is certainly less common than a disturbance of ascorbate metabolism, and correction of the abnormal metabolism provides the best results, there is little doubt that ascorbic acid deficiency can be a potent predisposing factor in various forms of arthritis. It is therefore unfortunate that Price (1983), a "health educator", should include vitamins among the quack remedies and should imply that all the physicians who give dietary advice or recommend vitamins for arthritic patients are ignorant and uninformed.

VII. CONCLUSIONS

It is very pertinent that not only cortisone, but also penicillamine, a chelating and reducing agent, should prove to be so useful in the treatment of rheumatoid arthritis; they both have the effect of reducing dehydroascorbic acid to ascorbic acid (see Figure 1, Chapter 13, Volume I, and Clemetson and Anderson, 1966). Moreover, penicillamine brings the additional bonus that it removes heavy metals, like copper, from the body.

More emphasis should be placed on maintainng an adequate supply of ascorbic acid during treatment of arthritis. Also, greater use should be made of the chelating fiber of foods, such as the bioflavonoids, catechins, and tannins. Though slower in action than the strong chelating agents, chelating food fiber avoids the possibility of toxic reactions, resulting from the use of penicillamine, which were noted in an editorial in the *British Medical Journal* (1975). Of course, one cannot restore a ruined joint, but one can hope to prevent further deterioration.

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Chapter 7

DENTAL AND PERIODONTAL CHANGES

I. INTRODUCTION

The teeth and the gums are affected by vitamin C deficiency, both in guinea pigs and in human subjects, but the teeth are more severely affected in guinea pigs and the gums seem to be affected more in man. Nevertheless, the teeth do become loose and may fall out both in guinea pigs and in man.

II. ANIMAL STUDIES

The teeth of guinea pigs differ from those of man, notably by their persistent growth. This and the ascorbic acid dependency of the guinea pig make it a very useful animal in which to study the effects of vitamin C depletion on dental growth and development. Holst and Frölich (1907), in their classic description of the experimental production of scurvy in guinea pigs by feeding diets of oats, barley, rye, or wheat and water, noted that the teeth took on a greenish-grey discoloration and the molars became loose in all of 36 animals studied; the incisors have long curved roots and are not easily loosened. These authors also noted the development of a gap between the teeth and the walls of the alveoli and extreme fragility of the maxillary and mandibular bones in scurvy. Horseshoe-shaped holes, the size of millet seeds, developed in the outer surfaces of the mandibular bones opposite the root tips of the molar teeth and mandibular fractures were frequent. Hyperemia and swelling of the gums were apparent in only 18% of the animals, but diffuse subperiosteal hemorrhages at the inside of both rami horizontales of the lower jaw or hemorrhages elsewhere in the body were seen in nearly all the animals. When antiscorbutics were fed, the teeth were firm and shining white.

Hart and Lessing (1913) produced experimental scurvy in rhesus monkeys by feeding them on condensed milk, cooked rice, and dried pig nuts. The first symptom was bleeding of the gums, with later loosening of the teeth, but not much swelling or ulceration of the gums.

Talbot et al. (1913) observed loose teeth and hyperemic or hemorrhagic gums in nearly all of 18 guinea pigs made scorbutic by feeding on oats and water, or rye bread and water, or white bread and water. Jackson and Moore (1916), studying experimental scurvy induced in guinea pigs by feeding various milk diets, noted a dull yellow discoloration of the teeth extending two thirds of the distance from the base to the end and submucous hemorrhages at the base of the incisors. "In sections of the lower jaw hemorrhages were numerous in the bone marrow, periosteum and tooth pulp. The hemorrhages in the periosteum were especially noticeable in the portions covering the alveolar process. There was great dilation of some of the vessels in the pulp of the molar teeth at about their middle with considerable hemorrhage about them." In guinea pigs with scurvy produced by feeding oats and hay, they reported almost complete necrosis of the pulp of the incisor teeth and also more or less necrosis in the pulp of the molars.

In their description of experimental scurvy in the guinea pig, Cohen and Mendel (1918) wrote, "Very rarely there was an appearance of submucous hemorrhage at the base of the lower incisor. Oftener, there was observed a hyperemia or congestion at the same site. Loosening of the teeth was quite a common occurrence in the animals with scurvy. Occasionally during life, the lower incisors were shown to be loose. Sometimes they would break off, though never in normal pigs or in animals with scurvy that was recently developed. At

autopsy the lower molars were nearly always loose, at times to such a degree as to permit the teeth to be removed easily with forceps. The upper molars on the contrary were not so often affected in this manner."

Besides describing the "scurvy position" relating to pain in the legs, Chick et al. (1918) also described the "face-ache position", as being indicative of scurvy in young guinea pigs: "The animal lies curled up with the side of its face pressed on the floor of the cage. This is a frequent attitude in adult guinea pigs when in normal health, but we have never seen a young animal adopt it except when ill with scurvy. It seems to indicate haemorrhage of the jaw, with soreness and looseness of the teeth. The state of the molar teeth and of the whole gums cannot be inspected during life, and it is only possible to judge of the condition by the greater or less capacity for eating, and by the assumption of the face-ache position." These workers observed that, "the jaws and teeth are usually profoundly affected, the teeth become brittle and loose and the jaw is easily fractured on pressure."

Zilva and Wells (1919), also studying guinea pigs, reported that the tooth is one of the first parts, if not the first part, of the body to be affected by deficiency of antiscorbutic material in the diet. The teeth showed all the appearances of senility. In advanced cases, the teeth were loosened by gradual absorption of the cement membrane of the alveolar sockets, which left exposed that portion below the neck.

Howe (1920) pointed out that the dental effects of vitamin C deficiency in guinea pigs more closely simulate pyorrhea than does any condition that has been artificially produced in animals. Moreover, he observed that the microorganisms present in pyorrhea are also present in the normal mouth and do not cause infection until vitamin C deficiency lowers the resistance of the tissues. Howe (1921a) observed that while it was possible to produce lactic acid by fermenting bread with saliva *in vitro,* and this could be shown to affect extracted teeth in a test tube, he had been unable to cause dental caries by the addition of sugars, starches, and microorganisms to the diets of guinea pigs. However, he found that a diet deficient in fat-soluble, water-soluble, and antiscorbutic vitamins caused decalcification of the teeth, absorption of alveolar bone, loosening of the teeth, pyorrhea, and such erosion of the incisors that a probe could be passed down directly into the pulp.

Howe (1921b) also observed that guinea pigs fed a diet of rolled oats and fat-free milk, with a very small piece of carrot or a small leaf of lettuce every other day or every third day, developed loose teeth, bleeding gums, and pyorrhea, with distinct dental cavity formation in some cases. Howe (1923) fed a diet containing adequate supplies of yeast and butter, to prevent other vitamin deficiencies, and observed the development of cavities in the teeth of both guinea pigs and monkeys which were kept alive in a chronic scorbutic state. The teeth showed degeneration of the odontoblasts and the formation of "osteodentin", replacing the pulp far down toward the apex of the tooth, with only a narrow rim of orthodentin surrounding it.

Toverud (1923) studied the mineral content of the teeth of normal and scorbutic guinea pigs. While the percentage of calcium oxide in the ash was virtually unchanged (48.7 to 47.1%), the percentage of magnesium in the ash was more than doubled in scurvy (from 1.7 to 4.0%).

Höjer (1924) found microscopic evidence of incipient scurvy in the teeth of guinea pigs as early as 8 d after starting them on an absolute scurvy diet:

> The first change affects the odontoblast layer. This layer which forms the intermediate link between pulp and dentin, has normally a very regular structure of long, slender, parallel cells with processes into the dentinal tubules. In scurvy this regularity is broken, and at the same time a hyperemia appears in the pulp. The parallelism of the odontoblasts becomes less pronounced. At intervals more rounded, osteoblast-resembling cells are seen in their stead. In these places the corresponding part of the predentin seems to have lost the protoplasm processes from the odontoblasts. It lacks Tomes' canals and seems amorphously calcified. In the dentin, however, which was already calcified at the onset of the scurvy, Tomes' canals seem to remain, and here they are widened. Inside the

calcified dentin, which resembles an amorphous cement, a hard tissue is deposited in the course of the process, stained blue-grey by hematoxylin. In the place of the odontoblasts, at the inner, notched edge of this tissue, osteoblast-resembling cells are seen. Similar cells are also seen further in towards the centre of the pulp and in different places the above-mentioned hard tissue reaches into the pulp in diversiformed strands, surrounded by osteoblast-resembling cells. These strands or columns already from the first show a certain regularity in their arrangement, standing radially in relation to the centre of the pulp. They are early connected by transverse columns and show a distinct bone structure.

If, however, there has been a small amount of antiscorbutic in the food, which has somewhat lengthened life, but not precluded the fatal issue, the bone formation is more vigorous.

If the animals, however, after a time of scorbutic diet, receive a life-preserving daily dose of antiscorbutic substance, the development becomes a different one. . .we see the pulpa bone reorganized to irregular dentin. Slender odontoblast-like cells appear again, even if they are not at once as regularly arranged as in animals which are sound from birth. The bone columns are perforated by Tomes' canals, running irregularly. This irregular dentin possesses at the same time bone canals with cells and blood vessels, and dentin canals, and it may therefore rightly be called osteodentin.

. . .The hard tissue formed in the pulp of the teeth in fully developed scurvy resembles bone, but it lacks the constitutive quality of dentin, Tomes' canals.

Höjer called this pulpa bone as it is formed by cells, which behave like and look like osteoblasts. He reserved the term osteodentin for the irregular dentin, which is formed when the scorbutic pulpa bone is reorganized by odontoblasts which reappear when antiscorbutic foods are provided. Höjer summarized the changes he observed in scorbutic guinea pigs as follows:

1. The gradual change of the odontoblast layer;
2. The amorphous calcification of the predentin;
3. Widening of Tomes' canals in the dentin formed before the onset of scurvy;
4. New formation of bone instead of dentin;
5. Dilatation of vessels and in early stages hyperemia; sometimes hemorrhages in the pulp;
6. Atrophy and resorption of pulpa tissue;
7. In the healing of the scurvy — reorganization of the pulpa bone into irregular dentin, osteodentin, with bone canals and dentinal canals;
8. In a scurvy which is latent all through or very much mitigated. . .forms which are the most common in man — the progress is similar, though not so pronounced and presents pictures, that differ considerably less from the normal.

Höjer and Westin (1925) claimed that it is possible in guinea pigs so to arrange the dosage of vitamin C in the food as to produce "scorbutus mitior latens levior", in which only dental changes occur and there are no other clinical signs of scurvy. Moreover, Höjer (1926) demonstrated that he could use microscopic studies of the teeth of young guinea pigs to estimate the vitamin C content of the diet. In this way, Key and Elphick (1931) were able to determine the vitamin C potency of foods and to express it in terms of orange juice equivalents.

Wolbach and Howe (1926) also observed early changes in the fine structure of the teeth of guinea pigs, beginning at the apical end of the tooth after 7 to 12 d on a scorbutogenic diet. Normally, the odontoblast layer is closely applied beneath the dentin, but these authors recorded the development of a cleft between these layers as the earliest sign of scurvy. At first, there was a partial separation, but later there was complete separation of the odontoblast layer from the dentin. Thus, there was formed a liquid-filled space between the pulp and the dentin which was presumed to be filled with a defective product of the odontoblasts.

Wolbach and Howe observed the change in appearance of the odontoblasts, which came to resemble osteoblasts and the wavelike shape formed by this layer of cells in scurvy, but they did not observe any of the bone formation in the pulp that had been reported by Höjer.

These apparent contradictions were resolved by the work of Fish and Harris (1934, 1935) who studied longitudinal sections of the cheek teeth of guinea pigs instead of the transverse sections used by Höjer and Wolbach and Howe. Fish and Harris pointed out that, "A

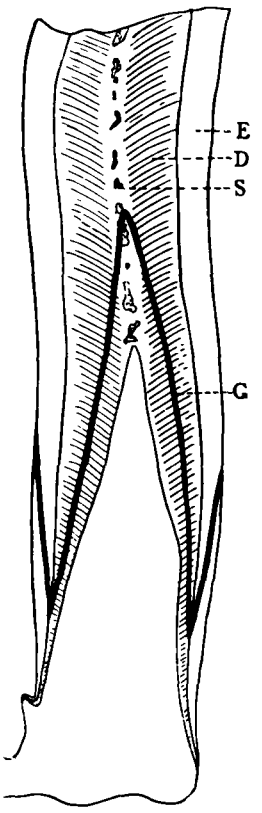

FIGURE 1. Diagram of a longitudinal section of a guinea pig's cheek tooth, relatively shortened for convenience: E, enamel; D, dentin; S, calcific scar tissue or secondary dentin. The line G represents graphically a particular point in time such that the enamel, dentin, and calcific scar tissue below the line were developed after this particular moment, and the hard tissues above the line were developed before. (From Fish, E. W. and Harris, L. J. [1935], *Br. Dent. J.*, 58, 3. With permission.)

transverse section may happen to be taken through a part of the tooth that was formed before the experimental diet was started and, in any case, it is not easy in a transverse section to say how much of the tooth was formed before and how much after the experimental period" (Figure 1). Guinea pig incisor teeth curve in more than one direction and cannot be used for this purpose.

Fish and Harris reported that in every case of scurvy all of the odontoblasts, ameloblasts, cementoblasts, osteoblasts, osteoclasts, and bone corpuscles had undergone degeneration and that all production of new dentine, enamel, cement, and bone had ceased. They pointed out that it is normal for odontoblasts to degenerate and lose their fibrils and to become embedded in secondary dentine at the senile end of the tooth, but this process was seen lower down in the pulp in subscurvy. "As the odontoblasts move up with the persistently growing tooth in the guinea pig, they pass through every phase of their life history. At the growing end of the tooth they may be seen differentiating from the embryonic cells of the papilla. Halfway up they are typical mammalian odontoblasts — oval cells with a pronounced fibril running into a tubule in the dentine. At the older end of the pulp they are seen degenerating." When odontoblasts lose their fibrils, they die and the dentin dies, too. Fish and Harris envisioned calcific scar tissue being laid down over the pulp ends of these tubules, sealing them off from the more vital pulp tissues further down in the tooth.

In scurvy there appears to be simply an acceleration of this process of degeneration. Instead of only the odontoblasts at the senile end of the tooth dying and their particular tubules being sealed up by calcific scar, the younger odontoblasts all the way down the pulp also degenerate and lose their fibrils, so that the primary dentine all down the tooth also dies and is sealed off by a barrier of lime salts.

In subscurvy the connective tissue cells of the pulp and some of the youngest odontoblasts are able to remain alive for a time. They soon begin to degenerate, however, and their fibrils become detached, and a deeply staining deposit of calcium salts is then deposited over the ends of their tubules, effectively sealing them off from the pulp. Lime salts are deposited throughout the pulp, in a well-developed collagen matrix, which encloses islets of the degenerating cells and presents the same appearance as the normal calcific scar tissue at the senile end of the healthy tooth.

In full scurvy these phenomena are modified because the pulp is more severely affected. All the odontoblasts lose their fibrils and die, so that all the primary dentine dies and is sealed off by the deeply staining barrier of lime salts, but the pulp is not able to continue the reaction and lay down the expanded collagen matrix to accommodate the lime salts, as it does in subscurvy, and fill up the pulp chamber.

In subscurvy, the enamel was reported as continuing to form, but in full scurvy it completely failed to do so. "Not only does the enamel fail to form, but the ameloblasts which otherwise should be functional at that moment undergo permanent degeneration from which they never recover. They either disappear altogether or become keratinized, so that even if the animal is cured there will never be any enamel on that section of the tooth which is forming when the disease was at its height.

"The effect of scurvy on the development of cementum conforms strictly to the type of reaction observed in other hard structures. The cementum developed before the onset of the acute stage of the disease remains unaltered, but during the actual attack the cementoblasts undergo degeneration similar to that of the osteoblasts."

In the opinion of Fish and Harris, "The massive calcific deposits in the pulp in subscurvy, with their prolific cell inclusions, are simply calcific scar tissue, the natural result of a hasty attempt to seal off dead primary dentine which became a source of irritation to the pulp when its dentinal fibrils were cast off by the degenerating osteoblasts." They preferred the terms calcific scar tissue, or secondary dentin, to the terms pulp bone or osteodentin used by others. As regards the failure of normal enamel formation observed in scurvy, Fish and Harris agreed with Howe that this could be of significance as regards susceptibility to dental caries, but they concluded that the lesions they had seen in scurvy bore only a superficial resemblance to caries.

Meyer and McCormick (1928) produced scurvy in guinea pigs by feeding them on a diet of heated alfalfa hay, heated rolled barley, and water and found similar changes in animals provided with supplementary wheat germ and yeast or milk. In late scurvy he reported, "shortening of the roots of the teeth by absorption so that the molars can be depressed below the level of the gums." No doubt alveolar bone resorption contributed to the production of this phenomenon.

Randoin and Lecoq (1929) observed very abundant gingival and intestinal hemorrhages and particular friability of the bones and teeth of guinea pigs made scorbutic by the use of a semisynthetic artificial scorbutic diet which included butter fat and yeast to ensure adequacy of A, D, and B vitamins.

Dalldorf and Zall (1930) found retardation of the rate of growth of the incisor teeth in scorbutic guinea pigs to constitute a reliable index of the severity of the disease. This index was later used by Schultz (1936) in his work on the cardiovascular and arthritic lesions in scurvy (Table 1).

Boyle et al. (1936) observed that in vitamin C deficiency dentin deposition stops, but long spicules of dentin project into the apex of the pulp cavity. This confirmed the accepted belief that calcification is not prevented by scurvy.

Boyle (1938) noted that the ameloblasts are of ectodermal origin in contrast to the fibroblasts, osteoblasts, chondroblasts, and odontoblasts, which are all mesodermal cells. One

Table 1
RATES OF GROWTH OF TEETH WITH DIFFERENT DOSAGES OF ORANGE JUICE IN CHRONIC SCURVY

Number of guinea pigs	Period of observation (d)	Weekly supplement of orange juice (cc)[a]	Average daily growth (mm)	Designation
12	16th—20th	0	0.31	Acute scurvy[b]
3	26th—30th	3	0.34	Moderate scurvy[b]
3	41st—45th	3	0.38	Moderate scurvy[b]
9	17th—21st	6	0.43	Mild scurvy[b]
3	26th—30th	9	0.60	Slight scurvy
3	41st—45th	9	0.65	Slight scurvy
4	26th—36th	30	0.76	No scurvy
4	41st—45th	30	0.78	No scurvy
3	—	0[c]	0.85	No scurvy

Note: The rate of growth of the teeth of young guinea pigs may be used as an index of the severity of scurvy.

[a] The basal diet was given without supplement in each instance from the 1st to the 14th day.
[b] Terms employed by Dalldorf and Zall (after Sherman) designating a similar rate of growth of the teeth.
[c] Diet of cabbage, oats, and hay.

From Schultz, M. P. (1936), *Arch. Pathol.*, 21, 472. ©1936 American Medical Association. With permission.

would therefore not expect ascorbic acid deficiency to affect ameloblasts and one would expect enamel formation to continue as normal in scurvy. In accord with this, Boyle found that the normal enamel to dentin thickness ratio of 3:4 is increased to 3:1 or even to 4:1 in guinea pigs with scurvy. However, he observed regional atrophy of the ameloblasts and markedly hypoplastic or irregular enamel formation in areas where there had been hemorrhage in the overlying tissues.

MacLean et al. (1939) used pure ascorbic acid and a paired-feeding technique in their experiments and thus ensured that the changes they observed in scorbutic guinea pigs were due to ascorbic acid deficiency and not to an insufficiency of any other nutrients. They confirmed that in scurvy, "the odontoblasts lose their regularity of formation and form a wavy border or serrated edge. The dentine formed after this time is laid down inside the original dentine but outside the odontoblastic layer, and lacks the tubular appearance of normal dentine."

Boyle et al. (1940) used spaced alizarin dye injections to study the rate of dentin formation by the incisor teeth of guinea pigs on diets containing different amounts of ascorbic acid. Even in normal animals, the rate of dentin deposition, as seen in cross-sections of the teeth, was found to vary almost 100% in different parts of the tooth. Nevertheless, the rate was essentially the same in corresponding parts of the incisor teeth of the same animal or in different animals on the same diet. It was found that the rate of growth in thickness of dentin at a defined site near the formative ends of the teeth was directly related to the ascorbic acid intake of the animals (Figure 2). Wolbach and Bessey (1942), reviewing these studies, concluded that dentin matrix (predentin) formation ceases completely in scurvy and that ascorbic acid is necessary for the formation of a matrix on which new dentin can be formed. Today we might say that ascorbic acid is needed to facilitate the hydroxylation of proline and lysine in protocollagen already secreted by the odontoblasts, so as to form a collagen matrix on which dentin can be laid down in an orderly manner.

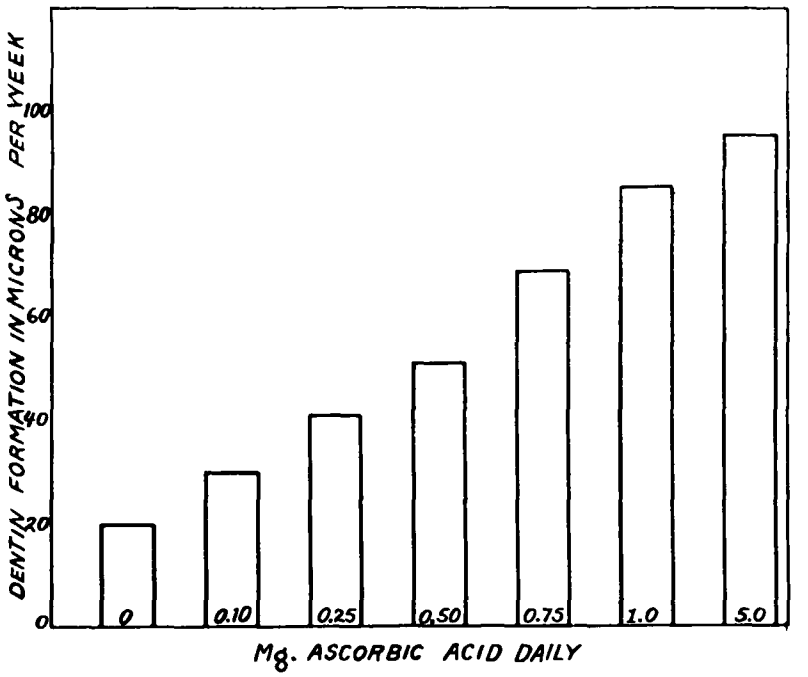

FIGURE 2. The graph shows that the amount of dentin produced during a given time at the formative end of the guinea pig incisor is proportional to the amount of ascorbic acid administered. (From Boyle, P. E., Bessey, O. A., and Howe, P. R. [1940], *Arch. Pathol.*, 30, 90. ©1940 American Medical Association. With permission.)

Kuether et al. (1944) fed guinea pigs on a low ascorbic diet, supplemented with eight different levels of ascorbic acid. They found a close correlation between the dietary intake and the blood and tissue levels of this vitamin. Studying longitudinal sections of the incisor teeth, they found pathological changes in the odontoblastic layer of cells when the whole blood total ascorbic acid (TAA)* level fell below 0.22 mg/100 ml, while hemorrhages in the knee joints did not usually appear until the ascorbate level fell below 0.15 mg/100 ml.

Crampton (1947) devised an elegant method for the biological assay of the vitamin C content of various diets by measuring the length of the odontoblast cells in the incisor teeth of young guinea pigs. The length of mature odontoblasts was found to bear a logarithmic relation to the vitamin C fed, in the range between 0.5 and 2.0 mg of ascorbic acid per guinea pig per day. Guinea pigs fed 0.25 mg daily all developed frank scurvy after 28 d on the diet. At the upper end of the range, there was no increased response with intakes of 4.0 or 8.0 mg daily over that shown for 2.0 mg.

Studies of *Macaca mulatta* (rhesus) monkeys by Chapman and Harris (1941) revealed that susceptibility of the gingival tissues to infection with the fusospirochetal group of organisms is markedly increased by vitamin C deficiency and also by a diet deficient in certain components of the B_2 complex other than riboflavin or nicotinic acid. Boyle (1941) observed that long-term suboptimal ascorbic acid intake in the guinea pig caused weakness and breakage of the collagen-suspending fibers and migration of the teeth, just as in the systemic or diffuse atrophy type of human periodontal disease. Glickman (1948a) studied the histological changes in the periodontal membrane and in the connective tissue core of the interdental papillae of acutely scorbutic guinea pigs and observed generalized periodontoclasia or diffuse alveolar atrophy; but he concluded that gingivitis and pyorrhea do not occur unless there is a complicating local factor.

* TAA — total ascorbic acid, reduced and oxidized forms.

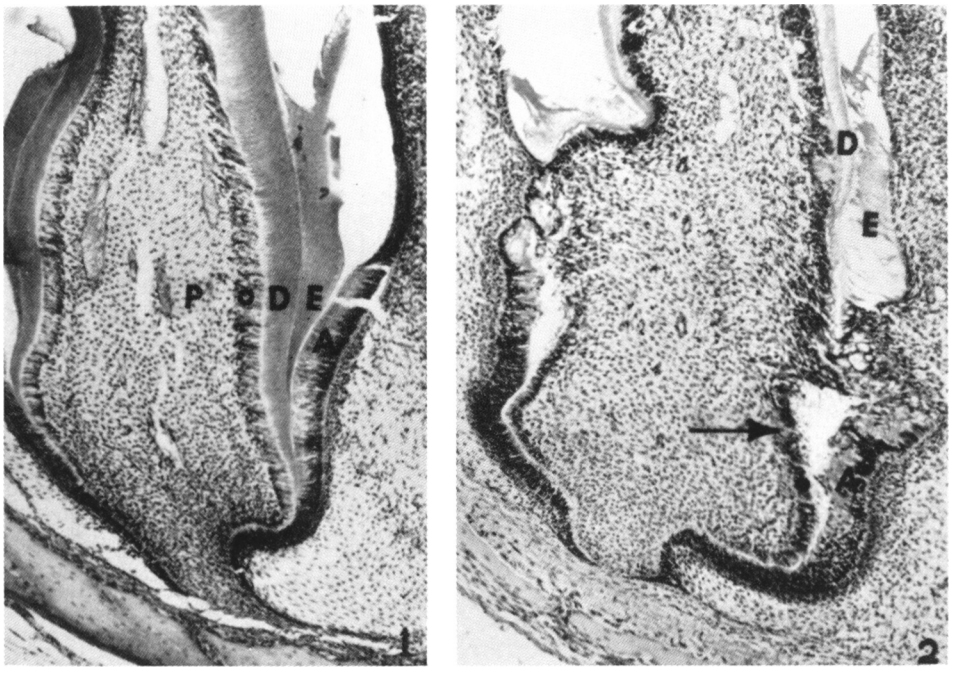

FIGURE 3. Photomicrographs of the growing ends of molar teeth from guinea pigs (1) fed a scorbutogenic diet for 2 weeks and then given ascorbic acid, 1 mg daily, for 1 week and (2) fed a scorbutogenic diet for 3 weeks: P, pulp; O, odontoblasts, E, enamel matrix, A, ameloblasts: D, dentin Note the failure of dentin production in (2); it should extend further apically Profound changes in the odontoblast and ameloblast layers are also very evident in (2) Loss of bone is also evident. (From Fullmer, H. M., Martin, G. R., and Burns, J. J. [1961], *Ann. N.Y. Acad. Sci.*, 92, 286 With permission)

Glickman (1948b) induced local gingival ulceration and periodontal inflammation in normal and scorbutic guinea pigs by the application of a 10% solution of silver nitrate to a small area of the labial gingival sulcus for 30 sec. He found that acute vitamin C deficiency markedly impaired the ability of the periodontal tissues to limit the injurious effect of local irritation. "Whereas a well-formed barrier of collagen fibrils, fibrin, and inflammatory cells separated the artificially induced area of injury from the underlying bone in the control animals, no such barrier occurred in the vitamin C deficient animals."

Lowry (1952) observed that, "the mildest signs which have been detected for ascorbic acid deficiency in the guinea pig are defects in the developing incisors." These appear when the tissue concentrations fall below about 40% of maximum. Scurvy, however, does not develop until the tissues are less than 20% saturated.

Hunt and Paynter (1959) studied guinea pigs fed an ascorbate-free semisynthetic diet supplemented by 0.0, 0.4, or 5.0 mg of ascorbic acid a day. Using a paired-feeding technique, they confirmed that the alveolar bone resorption and the defective formation of collagen fibers, cementum, and dentin were caused by ascorbic acid deficiency and were not due to starvation or to any other dietary deficiency. Guinea pig studies by Fullmer et al. (1961) demonstrated that ascorbic acid deficiency causes marked changes in the ameloblasts or enamel-producing cells at the roots of the teeth where there is no subjacent dentin. Amelogenesis was seen to proceed normally over normal dentin and enamel (Figure 3).

Cabrini and Carranza (1963) observed markedly reduced histochemical evidence of alkaline phosphatase activity in the connective tissues of 7-d-old gingival wounds in vitamin C-deficient guinea pigs. They related this to the lack of a collagen defensive barrier in the

wounds. Likewise, Nakamura et al. (1965), using biochemical techniques, found markedly decreased alkaline phosphatase activity in the gingiva and in alveolar bone of scorbutic guinea pigs, but found the acid phosphatase activity of these tissues to be increased.

Schow (1966) reported notching of the incisor teeth of guinea pigs after 20 d on an ascorbic acid-deficient diet, when their mean whole blood total ascorbic acid (TAA) level had fallen from 1.20 to 0.28 mg/100 ml. Studies of ascorbic acid-deficient guinea pigs by Kanouse (1966) revealed no change in the "oxytalan fibers" in the periodontium. As the collagen fibers in the periodontal membrane decrease in number with the severity of ascorbic acid deficiency, the oxytalan fibers become more evident, but it seems that this is only because they are more readily seen where there are less collagen fibers.

Collins et al. (1967) studied the healing of 2-mm-diameter punch biopsy wounds in the gingiva of groups of guinea pigs receiving 0, 2, and 20 mg of ascorbic acid daily by intraperitoneal injection. The healing times were 16.7, 12, and 8 d, respectively. A dose of 2 mg daily was reported as being adequate to prevent scurvy, but it is evident that larger doses were required for optimal wound healing.

Dreizen et al. (1969) produced scurvy in cotton ear marmosets and observed histological evidence of hemorrhages, edema, and decreased collagen density in the alveolar part of the periodontal ligament. There was some widening of the periodontal ligament space due to osteoclastic activity, but gingival changes were minimal. It was suggested that gingivitis, when present, is due to decreased resistance to local irritants.

Messer (1971) studied the effects of ascorbic acid deficiency on the jaw bone and on the teeth of *Macaca irus* monkeys, using tetracycline and alizarin red as fluorescent chemical markers to delineate the amounts of growth occurring after set intervals of time. It was found that bone formation had ceased completely after 8 weeks of vitamin C deficiency, at a time when dentin formation had decreased by only 30%. All dentin formation had ceased after 16 weeks of ascorbic acid deficiency.

Berkovitz (1974) studied the rates of impeded and unimpeded eruption of incisor teeth in scorbutic and "pair-fed" guinea pigs and observed that from day 11 to day 23 there was a profound reduction in both rates. After 20 d, the unimpeded eruption rate of the scorbutic incisors had decreased from 0.54 to 0.12 mm/d, while those of the control animals fell only from 0.58 to 0.52 mm/d. The fact that the incisor eruption rates of rats had been found to be unaffected by lathyrogenic drugs was used as an argument against defective collagen synthesis alone being the cause of impaired tooth eruption in scurvy. Thaete and Grim (1974) studied the buccal epithelium of guinea pigs by electron microscopy and observed enlarged intercellular spaces in scurvy, "possibly due to decreased production of cementing substances between the cells."

Østergaard and Löe (1975) studied the proline and hydroxyproline contents of the collagen from skin, gingival tissues, and subcutaneous polyvinyl sponges in normal and scorbutic green monkeys *Cercopithecus aethiops*. The hydroxyproline content of the gingiva started to fall within the first 4 weeks, while that of the skin fell later and to a lesser extent, suggesting a higher rate of collagen turnover in the gingival tissues. Synthesis of hydroxyproline in the subcutaneous polyvinyl sponges was markedly reduced at 8 weeks and was almost completely arrested after 12 weeks on the scorbutogenic diet. Not only did ascorbic acid deficiency impair the synthesis of collagen, it was also found to result in the formation of a collagen with an abnormally high proline to hydroxyproline ratio, both in the gingiva and the polyvinyl sponges. The findings of this study are most likely valid, but it is unfortunate that 1 g of $CuSO_4$ was added per kilogram of milk powder in the diets of both test and control animals, so that even the control animals had inflamed gingiva; moreover, the animals were not pair fed.

Veen-Baigent et al. (1975) reported studies of tooth formation in guinea pigs maintained on nine levels of ascorbic intake. Normal tooth structure was observed in animals given 0.5

mg of ascorbic acid daily per 100 g of body weight. At intake levels below 0.5 mg, dentin was deficient and marked buckling of the formative tips of the tooth roots was evident. In these experiments, wound healing and also the percentage hydroxyproline content of regenerated tissue became normal at the same ascorbic acid intake level (0.5 mg/100 g of body weight or 2.0 mg for a 400-g guinea pig).

Machlin et al. (1976) produced scurvy in rhesus monkeys by feeding an ascorbic acid-deficient diet and observed the development of gingivitis, bleeding gums, inflammation of the palate, ulcers on the lips, loose teeth, capillary fragility, and swollen joints. Similar changes were observed in animals on the same diet supplemented with dipotassium L-ascorbate-2-sulfate; those provided with L-ascorbic acid were protected.

Alvares et al. (1979) studied young adult monkeys on a scorbutogenic diet and observed a marked increase in the permeability of the epithelium of the gingival sulcus. This defect preceded and could have contributed to the development of scorbutic gingivitis.

Kramer et al. (1979), of the Boston University School of Dentistry, studied collagen formation in polyvinyl sponge implants placed beneath the skin of guinea pigs after 7 d on diets containing 0- (scorbutic), 5- (normal), or 200-mg (megadose) daily allowances of ascorbic acid. Mature collagen was absent from sponges in the scorbutic animals, 7 and 14 d later; it was replaced by an amorphous intercellular material. Collagen formation in the sponges of the normal and the megadose groups were similar in histological appearance, 7 and 14 d later, but the collagen (hydroxyproline) content of the megadose group was significantly greater after 7 d ($p < 0.05$), suggesting that megadose ascorbic acid may be useful in accelerating the rate of wound healing following dental extraction or other trauma.

Aleo (1981) reviewed the relationship between diabetes mellitus and periodontal disease and suggested that

1. The microangiopathy of diabetes mellitus may be due to local ascorbic acid deficiency. Thickening of the capillary basement membrane may be present prior to the appearance of any detectable abnormality in glucose metabolism according to Siperstein (1971).
2. Hyperglycemia decreases the availability of ascorbate in the tissues by impairing the transport of this vitamin, as shown into red cells by Mann and Newton (1975).
3. Endotoxin-induced histamine sensitivity is increased by ascorbic acid deficiency (Subramanian et al., 1973).
4. Ascorbic acid deficiency weakens the mucosal barrier to endotoxin, as suggested by Alfano et al. (1975) and Alfano (1978).

Aleo (1981) concluded that, "The recent finding by this laboratory that ascorbic acid under specific conditions is able to modify the toxicity of endotoxin, opens a new approach to the potential management of cementum-bound endotoxin in periodontal disease." M'Barek and Brocheriou (1981) observed delayed healing of gingival wounds in vitamin C-deficient guinea pigs, even at a stage before they showed any significant weight loss.

Alvares et al. (1981), working at the Regional Primate Center of the University of Washington in Seattle, studied chronic subclinical ascorbic acid deficiency in young adult *Macaca fascicularis* monkeys. After 23 months, there was no evidence of spontaneous gingivitis or periodontitis in any of the six experimental and four pair-fed control monkeys. However, by the simple expedient of tying a silk ligature at the cemento-enamel junction of the left first molar tooth of each animal at 23 weeks, local plaque-associated periodontitis was induced; 2 weeks later, gingival scores and pocket depth measurements on the ligatured side were significantly greater in the ascorbate-deficient animals than in the controls. These workers therefore suggested that ascorbic acid deficiency does not cause gingivitis, but does reduce resistance to gingivitis and periodontitis, perhaps by impairing leukocyte chemotaxis and phagocytic activity.

Sodek et al. (1982), at the University of Toronto, studied the effect of ascorbic acid on the synthesis of collagen and noncollagenous protein by bone of the alveolar process and the periodontal ligament in organ cultures of adult mouse periodontium. Collagen synthesized in the presence of less than 10 μg/ml ascorbic acid was found to be highly underhydroxylated (proline to hydroxyproline, 2.3 to 3.1:1) in both tissues. When the ascorbic acid levels were between 25 and 100 μg/ml, the synthesis of collagenous proteins was selectively stimulated and hydroxylation significantly improved (proline to hydroxyproline, 1.72 to 1.89:1).

Studies of collagen synthesis and mineral uptake by cultured molar tooth germs from newborn hamsters have been reported by Bronckers (1983). Total protein and collagen synthesis were equally retarded in vitamin C-deficient explants, and the collagen that was produced was found to be underhydroxylated. This resulted in the formation of an abnormal predentin matrix, which seemed to be the cause of a failure of the ameloblasts to differentiate. The uptakes of ^{45}Ca and $^{32}PO_4$ were also retarded, and the molar Ca to PO_4 uptake ratio was reduced in ascorbic acid deficiency.

III. HUMAN OBSERVATIONS

Accounts of a disease afflicting the Crusaders on their journeys to the Holy Land include descriptions of a black discoloration of the legs, associated with gangrene of the gums, grievous pain, and many deaths. Those who survived were cured with the arrival of spring. There is little doubt that they had scurvy. Likewise, many accounts tell of scurvy among sailors on long sea voyages; swollen bleeding gums and loss of teeth were always prominent signs of this disease.

Kahle, writing in the *New Orleans Medical and Surgical Journal* (1909/1910), reported his notes on the development of scurvy in an infant weaned on a commercial malted milk preparation. It had been quite well and standing with support at 8 months of age, but slowly became unable even to sit without support and then lost the use of its legs completely. It lay on its back with legs everted and immobile and screamed when it was approached. Examination revealed a pale child with enlargement of the lower end of the radius and ulna and beading of the junctions of the ribs and the cartilages, associated with extreme tenderness. The mouth contained 8 teeth; the gums were much swollen and spongy, of a purplish hue, and bled easily. The mother was advised to give 3 or 4 teaspoonsful of orange juice daily; this led to a rapid recovery.

Chick and Hume (1917) suggested that, "the spongy and septic conditions of the gums characteristic of scurvy is probably partly, if not wholly, due to bacterial infection. The defenses against invasion are well known to be weaker in the mouth than in many other parts of the body, and in scurvy are further reduced owing to the abnormal condition of the blood system." However, it is now known that the abnormalities of the gums, teeth, and alveolar bone in scurvy involve far more than just a reduced resistance to infection. There is complete cessation of function by the osteoblasts, odontoblasts, cementoblasts, and fibroblasts in scurvy, so that no new bone, dentin, cement, or collagen can be formed when vitamin C is lacking. Moreover, capillary fragility is profoundly affected in scurvy and is largely responsible for the swelling and bleeding of the gums.

O'Shea (1918) warned against the use of mercury to treat syphilis in patients with scurvy, for he knew that mercury was injurious in scurvy. The current relevance of this fact is discussed Section IV, Conclusions, at the end of this chapter. Randoin (1923) reported that acute scurvy had become extremely rare in peacetime, but she noted that prescorbutic states, characterized by weakness, with slight pains in the legs, bleeding gums, and intestinal troubles, were quite frequent among the inhabitants of large towns because many people either could not or did not get anough fresh fruits and vegetables.

Öhnell (1928), in Sweden, recording his observations in 22 patients he had treated for scurvy over the years, made the following observation:

The gingivitis had manifested itself as an intensively reddened swelling of the gums, localized to the nearest surroundings of the teeth. In particular has one been struck by the intensively swollen interdental papillae. In only a small number of cases has there been any more marked bleedings within the swollen areas. The intensity of the gingitivis has not proceeded hand in hand with gravity of the condition in other respects. In the case most severe of all, for example, a man aged 61, with general condition exceedingly impaired and with marked bleedings — the lower limbs were almost entirely discoloured from haemorrhages — there was only a mild gingivitis present. In another case, just mentioned as an example of rapidly occurring changes, the general condition was exceedingly good; no subcutaneous haemorrhages were observed but the gingivitis was exceedingly well marked. This is probably connected, at least to some extent, with the different oral hygiene observed in the respective areas.

Moreover, Öhnell (1928), in collaboration with Westin, found "typical scorbutic changes in the teeth" not only in cases of frank scurvy, but also in cases of suspected latent scurvy.

One case — a chronic dysentery, with marked intestinal fermentative dyspepsia and consequent deficiency in C-vitamins — shows beautiful denticles and root-denticles, verified not only radiologically but also microscopically in an extracted tooth, in spite of complete absence of gingivitis or haemorrhages. In four other cases there were no other symptoms of manifest scurvy than gingivitis. These cases also show fully convincing changes in the teeth . . .it would seem, therefore, as if these investigations have given us a previously unknown possibility to diagnose latent scurvy, a condition which I fear is exceedingly common in our country.

The dental changes consist in porosity of the dentine as well as new formation of pathologically built up dentine zones along the inner surface of the dentine bordering to the pulp.

The highly differentiated dentine cells, odontoblasts, are successively destroyed and replaced by other cells of less differentiated types (osteoblasts and fibroblasts).

In connection with this the normal structure of the pulp undergoes changes and becomes the seat of severe atrophy and degeneration.

The degenerative areas in the pulp undergo calcification. The walls of the veins are most severely attacked and phleboliths occur in the pulp. Thereby exceedingly large areas in the pulpar tissue may become calcified, giving rise to so-called denticles of peculiar structure, partly lying free in the tissue, partly being attached to the wall. The former are common in the cavity of the pulp, i.e., the crown — the latter in the root-canal.

Öhnell gained the impression that pathological new formations in the tooth occur to a considerably greater extent in the mitigated forms of scurvy than in absolute scurvy, where degenerative phenomena predominate:

It is of particular interest that Prof. Westin is able to demonstrate not only a series of cases of healed Möller-Barlow's disease but also scurvy in different stages of healing where it has been established with certainty that under vitamin C treatment the changes in the teeth are capable of being healed, in full accord with the conditions present in experimental scurvy in guinea pigs. The changes are therefore reversible. This is of decisive import for proving the scorbutic nature of the changes.

Unfortunately, however, to prove the existence of these changes it is necessary to extract the tooth and examine serial sections under the microscope.

Luckily, however, we have in radiological examination a means by which certain intimations are afforded us for a right estimation of the changes. The denticles appear on the x-ray negative as light spots in the dark pulpar shadow and the new formation is evidenced by closure of the dental canals.

All these examined cases have proved to be typical denticular cases with crown-denticles as well as root-denticles.

On account of certain röntgenological factors the root denticles are on the whole more reliable signs. Even crown denticles, however, may be established with a faintly greater amount of certainty, provided the röntgen technique has been up to the mark. It would seem likely, however, that a still further development will take place along this line.

It must not be concluded from this, however, that the mere establishment of denticles is sufficient for diagnosing latent or manifest scurvy, for according to odontological literature denticles may also arise as a result of certain external irritative factors, such as erosions or fillings.

But should there exist a generalized denticular formation in all the teeth independently of the above mentioned factors, it must be deemed of great importance and render the presence of scurvy suspected.

Hanke (1929), in collaboration with 17 dental surgeons of the Chicago Dental Research Club, conducted dietary inquiries and careful examinations of the mouths of 114 men, women, and children. They classified their observations into four groups: no dental caries now, caries, caries and pyorrhea, and gum inflammation, but no caries. The study included

blood calcium and phosphorus determinations and there was special interest in the possibility of an association between vitamin D deficiency and dental caries, The results (Table 2), however, showed no association between caries and a dietary history suggestive of vitamin D deficiency. Instead, both dental caries and alveolar inflammation showed a definite relationship to vitamin C deficiency in this nutritional study. Hanke stated, "From this entirely unprejudiced survey we have been forced, rather contrary to our expectations, to the conclusion that if diet is a factor in dental disorders, vitamin C is probably the most important constituent."

In view of these findings, Hanke (1930) and colleagues began to advise large quantities of vitamin C-rich foods, especially orange and lemon juice, lettuce, and tomatoes for their patients following dental surgery. They observed a markedly beneficial effect in curing gingival disease and in arresting the progress of dental caries. "The amount of vitamin required appears to be very much greater than we have heretofore suspected and it appears not to be the same for all people;" for adults they usually prescribed a pint of orange and lemon juice a day, as well as one or two eggs a day and any desired amount of fresh fruit and vegetables. The increased needs of two subjects were so great that their gingival tissue could be healed and made firm in 2 weeks on this diet, but their gums became spongy and hemorrhagic again within 2 weeks when the citrus fruit was withheld. They treated over 500 patients in this way and observed excellent results in those who cooperated, even in some whose teeth were heavily coated with plaque and who went without scaling. Of course, the best results were obtained by a combination of dental surgery and diet combined, but the improvement in general health that resulted from the dietary supplement was remarkable. "The patients have almost invariably observed an increased alertness and an increased ability to work or play without fatigue. . . . The resistance to infection appears also, in many cases, to have been materially increased."

Hanke was of the opinion that the development of dental caries was arrested in these patients, but Hess (1932) and others found no geographic or epidemiological evidence to support the idea that dental caries might be due to ascorbic acid deficiency.

Hanke et al. (1933) found orange and lemon juice to be so effective in the treatment of gingivitis in children that they advised it for all their patients. They left open the question as to whether it was vitamin C and/or some other ingredient in orange juice that was so beneficial.

Boyle et al. (1937a,b), at Harvard University, drew attention to all of the similarities between diffuse alveolar bone atrophy of pyorrhea alveolaris, as seen so commonly in human beings, and the changes produced by chronic vitamin C deficiency in guinea pigs. "Thus, in both acute and chronic ascorbic acid deficiency, generalized rarefaction of the bone occurs. This rarefaction, a result of the inability of the osteoblastic cells to form bone matrix, is accompanied by a similar failure of the fibroblastic cells to form collagen fibers. The loosening and wandering of the teeth is an expression of the weakness of the bone and collagen fiber suspending apparatus." They did not dispute the effects of trauma, infection, food impaction, and calculus formation, but these and Vincent's infection were thought to be more closely related to local pyorrhea.

There is plenty of evidence that bleeding gums and periodontal disease may be due to vitamin C deficiency. Kramer (1937) investigated 34 cases of gingivitis and stomatitis among German army personnel and found a vitamin C deficit ranging from 2000 to 2500 mg. Treatment with ascorbic acid cured all but one of them. Kramer was impressed by the improvement in general health that followed saturation with vitamin C. Not only did the gums become firmer and the hemorrhages cease, but the lassitude, anorexia, and rheumatic pains of these patients also cleared up.

Roff and Glazebrook (1939) also reported great success from the use of ascorbic acid in the treatment of Royal Navy cadets who were found to have gingivostomatitis on enlistment.

Table 2
THE RELATION OF DIETARY DEFICIENCIES TO CARIES AND TO OTHER DENTAL DISORDERS[a]

	No dental disorders				Caries				Caries and gum inflammation				Gum inflammation and pyorrhea; no caries			
	Group I dietary deficiency				Group II dietary deficiency				Group III dietary deficiency				Group IV dietary deficiency			
Age range (years)	None	C and D	C	D	None	C and D	C	D	None	C and D	C	D	None	C and D	C	D
0—10	2	1				2	4			1						
11—20	4		2	1		9	16			2	4			1	2	
21—30	1			1		6	3			2	3			1	5	
31—40			1			3	3			3	3			3	3	
41—50	2						1			3	3			2	4	
51 up	2										1		1	3		
Totals	11	1	3	2	None	20	27	None	None	11	14	None	1	10	14	None

Note: Of 17 patients without dental caries (Group I), 4 had a vitamin C-deficient diet. None of 47 patients with dental caries (Group II) had an adequate vitamin C intake. Likewise, none of the patients in Group III and only one in Group IV had an adequate vitamin C intake.

[a] In no case were any of the dental disorders associated with a pure D deficiency; 55 cases, i.e., 50% of them, were deficient only in C.

From Hanke, M. T. (1929), *J. Am. Dent. Assoc.*, 16, 2263.

All the cadets received full dental care and scaling to remove any calculus and they were instructed in dental hygiene; 300 boys who were treated with ascorbic acid, 200 mg daily for 3 weeks, showed a reduction of gingivostomatitis from 17.6 to 4.9%, while in 300 boys who acted as controls, and did not receive the vitamin supplement, the incidence changed from 16.3 to 12.6% on the communal diet alone. However, Stamm et al. (1944) reported no benefit from the use of ascrobic acid, 200 mg daily for 7 d, followed by 100 mg daily for 14 d, in the treatment of bleeding gums in Royal Air Force personnel. Perhaps the dose and the duration of treatment were inadequate. Certainly, the writer would recommend ascorbic acid, 200 mg, with rutin, D-catechin, or citrus bioflavonoids, 200 mg three times a day for 2 months, before acknowledging failure, for many people have abnormalities of ascorbic acid metabolism and do not respond to ascorbic acid alone. Moreover, bioflavonoids often take 2 months to act (Chapter 11, Volume III).

Crandon et al. (1940) reported their findings in a normal, active adult (Crandon) who placed himself on a vitamin C-free diet supplemented by all other known vitamins for a period of 6 months. His plasma ascorbic acid level fell to 0 after 41 d and his white cell-platelet layer reached 0 after 82 d on the diet; but the perifollicular hemorrhages of scurvy did not appear until 161 d. "During the first five months of the diet no changes were grossly apparent in the teeth or gums. A competent dentist pronounced the gums to be normal in appearance at the end of this time." At the end of 6 months, when clinical scurvy, as manifested by perifollicular hemorrhages over the legs, had been present for 3 weeks, examination of the gums revealed that they were slightly more boggy on pressure than usual, but no other gross changes could be seen. A biopsy specimen of the gingivum at this time was absolutely normal. "Of interest is the fact that although the gross findings were negative, X-ray films of the teeth taken at this time showed occasional interruptions of the lamina dura."

Data concerning the gingival and periodontal conditions and the plasma ascorbic acid (AA)* levels of 1396 patients in Chicago were presented by Burrill (1942). It was found that the blood plasma vitamin C levels tended to be lower in patients with gingivitis (mode 0.20 to 0.40 mg/100 ml) than in those who were free from gingivitis (mode 0.40 to 0.60 mg/100 ml). Dietary histories revealed that the incidence of periodontal disease among people over 20 years of age who ate citrus fruits (43.5%) was lower than in those who did not (49.9%). While these differences may represent a benefit from the higher intake of citrus fruits, the difference was small, and the author noted that these findings could simply mean that a patient who neglects his mouth will also be likely to neglect his diet.

Stuhl (1943), working in England during the winter of 1941/1942, reported seeing many soldiers with gingivitis and stomatitis, associated with severe degrees of ascorbic acid deficiency. He reported that both local antiseptic treatment and systemic vitamin C supplementation were necessary to provide an effective cure. Many of these men had other symptoms including listlessness, lethargy, mental apathy, irritability, lessened endurance, and lack of energy, all of which were improved by treatment with vitamin C.

Studying military personnel with ulcerative and necrotic gingivitis at a crowded camp site in time of war, Kent (1943) obtained careful dietary histories and made bacteriological studies to differentiate between scurvy and Vincent's infection or "trench mouth" due to spirochetal and fusiform bacilli. Finding that three quarters of those with classical trench mouth also showed evidence of hypovitaminosis C, he concluded that overcrowding and cross-infection from the use of shared dining utensils were contributory factors, but that ascorbic acid deficiency due to overcooking of vegetables had caused a decreased resistance to infection. Moreover, he suggested that Vincent's infection may interfere with vitamin C absorption (or metabolism) and so set up a vicious circle, furthering the deficiency. It was suggested that many of the cases recorded as scurvy in former centuries were in fact cases

* AA — ascorbic acid, reduced form.

Table 3
INCIDENCE OF GINGIVAL BLEEDING, TENDERNESS, REDNESS, AND SWELLING AT INITIAL (AFTER LOCAL TREATMENT) AND FINAL EXAMINATIONS

		Group 1		Group 2		Group 3		Group 4	
Symptom	**Examination**	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Bleeding	Initial	0	0.0	5	16.7	5	16.1	3	11.5
	Final	11	33.3	12	40 0	9	29.0	5	19.2
	Difference	11	**33.3**	7	**23.3**	4	**12.9**	2	**7.7**
Tenderness	Initial	3	9.1	2	6.7	1	3.2	1	3.8
	Final	25	75.8	15	50.0	13	41.9	6	23 0
	Difference	22	**66.7**	13	**43.3**	12	**38.7**	5	**19.2**
Redness	Initial	2	6.1	3	10.0	1	3.2	1	3.8
	Final	21	63.6	14	46.7	8	25.8	3	11 5
	Difference	19	**57.5**	11	**36.7**	7	**22.6**	2	**6.7**
Swelling	Initial	14	42.4	10	33.3	13	41.9	11	42.3
	Final	22	66.7	14	46.7	13	41.9	8	30.8
	Difference	8	**24.3**	4	**13.4**	0	**0.0**	−3	**−11.5**

Note: Detailed study of the recurrence of gingivitis in 150 men and women of the Royal Canadian Air Force who had received local treatment and were then placed on four different diets for 8 months. Group 1, food containing 10 mg of ascorbic acid daily; Group 2, food containing 25 mg of ascorbic acid daily; Group 3, food containing 10 mg of ascorbic acid plus 70-mg ascorbic acid tablet daily; Group 4, usual station ration providing approximately 75 mg of ascorbic acid daily.

From Linghorne, W. J., McIntosh, W. G., Tice, J. W., Tisdall, F. F., McCreary, J. F., Drake, T. G. H., Greaves, A. V., and Johnstone, W. M. (1946), *Can. Med. Assoc. J.*, 54, 106. With permission.

of Vincent's infection, and that many of the men diagnosed as having trench mouth in World War I also had scurvy.

Farmer (1944) reported no alveolar nor dental changes even on X-ray examination in five young men, aged 20 to 30 years, who were maintained for 7 months on a very low vitamin C diet, nor in five young men kept on a diet that was relatively deficient in vitamins C and B complex for 7 months. The plasma ascorbic acid levels fell to zero after 70 d and the white cell-platelet ascorbic acid level had reached zero in all these subjects in the latter part of the fifth month on the diet, but no dental or periodontal pathology was detected.

It was suggested that the oral conditions frequently ascribed to an inadequate intake of vitamin C may in reality be due to preexisting caries or to improper oral hygiene. Another way of interpreting these same observations would be that ascorbic acid deficiency increases susceptibility to gingitivis, but does not directly cause it. Roth (1955) emphasized the need for prolonged administration of ascorbic acid, 500 mg daily, as well as scaling and gum massage in the treatment of gingivitis. He listed the other vitamin supplements used in the treatment of diseases of the mouth, tongue, and lips at the New York University College of Dentistry and noted that vitamin A had also been found useful in the treatment of gingivitis. Linghorne et al. (1946), in an initial pilot study involving 120 Royal Canadian Air Force personnel with gingivitis, found that high doses of ascorbic acid (375 mg daily), niacin alone (225 mg daily), ascorbic acid with other vitamins, or placebo tablets did not affect the number showing improvement in 5 months. However, in a subsequent experiment, these workers studied the effects of four different ascorbic acid intakes on the recurrence of gingivitis following local treatment to remove, as far as possible, all clinical evidence of gingivitis; 8 months of carefully controlled observations on 150 volunteers revealed a significantly greater incidence of recurrent gingivitis in men and women on diets containing 10 or 25 mg of ascorbic acid than in others receiving 75 or 80 mg/d ($p < 0.01$) (Table 3).

The gingival bleeding recurrence rates were 33.3% in Group 1 (diet containing 10 mg/d), 23.3% in Group 2 (diet containing 25 mg/d), 12.9% in Group 3 (diet containing 10 mg plus 70-mg supplement), and only 7.7% in Group 4 patients who received a diet containing 75 mg of vitamin C a day. Daily analysis of the diets revealed mean daily ascorbic acid intakes of 7.9 mg in Group 1, 22.3 mg in Group 2, 77.9 mg in Group 3, and 78.3 mg in Group 4. So the better results in Group 4 do suggest the presence of a beneficial factor in vitamin C-containing foods besides vitamin C itself. Plasma ascorbic acid levels averaged 0.2 mg/100 ml in Group 1, 0.25 mg/100 ml in Group 2, and 0.7 to 1.0 mg/100 ml in Groups 3 and 4. The mean leukocyte ascorbic acid levels were 11.9, 13.2, 25.4, and 24.3 mg/100 g in Groups 1, 2, 3, and 4, respectively. It was suggested that vitamin C deficiency may well be the reason that so many people in Newfoundland had lost all their teeth by the age of 20.

Walsh (1947) drew attention to the gingivitis of pregnancy and suggested that not only defective oral hygiene, but also hormonal changes and vitamin C deficiency were factors in the development of this condition. Krebs (1953) observed typical scorbutic changes in the gums of most of the volunteers on an ascorbic acid-deficient diet in the "Sheffield experiment". "The earliest signs were tiny haemorrhages in the tips of the interdental papillae, and swelling, seen first after 26 weeks of deprivation. About 10 weeks later, nine of the ten deprived volunteers had developed abnormalities of the gums, gross in two cases, less advanced but definite in five other cases, and slight in the remaining two cases."

While experimental ascorbic acid deficiency does not always cause the typical alveolar changes of scurvy, it is comforting to know that ascorbic acid still cures these changes when they occur. Indeed, Roughton and Waldron (1953) reported a typical case treated in the Veterans Hospital at Jefferson Barracks, MO. "A patient was admitted with subcutaneous hemorrhages on one leg, gingival enlargement and a 1 by 2 cm ulcer on the palate. A dietary survey revealed grossly inadequate intakes of vitamins C, A and B complex. A clinical diagnosis of scurvy was made; the plasma ascorbic acid level was 0.14 mg/100 ml; the patient was given 2 grams of ascorbic acid daily, with iron, multivitamin tablets and a high protein diet and the response was dramatic. Within two weeks the gingival tissues were normal and the ulcer had completely healed." Similarly, Harris and Hutchinson (1955) observed perifollicular hemorrhages of the skin of the face and swollen, ecchymotic bleeding gums in a 39-year-old man who was living on a very poor diet of white bread and cake in Sydney, Australia. His plasma ascorbic acid level was recorded as 0.26 mg/100 ml, which is certainly not usual in scurvy, but treatment with ascorbic acid, 700 mg daily for 3 d, and then 200 mg daily, resulted in complete healing of the gums in 14 d.

A delayed effect of ascorbic acid supplementation was observed by Cohen (1955), treating a group of mentally retarded boys aged 11 to 15 years. Administration of ascorbic acid, 500 mg daily, produced no evident improvement in the very poor condition of their gingival tissues in the first 4 weeks, but beginning in the fifth week, the inflammation and the swelling decreased, and after 90 d all were markedly improved. No local treatment was undertaken during the study.

In the Vanderbilt cooperative study of maternal and infant nutrition, Martin et al. (1957) studied the dietary histories and serum ascorbic acid levels of 2129 consecutively encountered pregnant women in Nashville, TN. "Analysis of findings relative to the health of the mother and baby revealed only five categories which may possibly be associated with ascorbic acid nutriture: hematologic findings, gingivitis, premature separation of the placenta, premature birth and puerperal fever . . . For groups with consistent levels of serum ascorbic acid, the percentages with gingival abnormalities were 21, 17 and 8 for the low, medium and high concentrations in the serum, respectively, for parity two and under. These differences are significant ($p < 0.002$). A significant relationship between gingival findings and vitamin C intake appeared only when groups with consistently low, medium and high vitamin C intakes

were compared on the basis of the number having gum findings on two or more physical examinations. Among women of parity two or less with consistently low vitamin C intakes, 19 per cent had gum findings on at least two examinations. Among the consistently medium and high intake groups the corresponding percentages were 14 and 9, respectively. Thus, it seems that vitamin C played a role in causing the gingival lesions, but it was not of major importance."

The use of a combination of ascorbic acid and citrus bioflavonoids (C.V.P.®) in the treatment of gingival hemorrhage was evaluated by Roth et al. (1957), working in New York City; they obtained encouraging results. Subsequently, Carvel and Halperin (1961), working at Loyola University School of Dentistry in New Orleans, observed complete arrest of gingival bleeding and visible regression of gingival lesions in all of 13 men and women taking citrus bioflavonoids, 200 mg, and ascorbic acid, 200 mg (Duo C.V.P.®) three times a day. It is worthy of note that these were not all poorly nourished individuals. The patients included college teachers, college and high school students, and a lawyer. Usually the bleeding ceased in 2 weeks, but some patients required prolonged treatment and their bleeding tended to recur when treatment was discontinued. None of seven patients receiving placebo capsules showed any improvement. Clearly, these were not cases of scurvy, but were most probably instances of a local or a general disturbance of ascorbic acid metabolism which s slowly rectified by treatment with bioflavonoids.

Tillman (1961), noting that the gingival changes of classical scurvy are seen only in patients with poor oral hygiene, reported such findings in a 44-year-old man with many loose carious teeth and a foul mouth due to ulceromembranous gingivitis. Many abnormalities including anemia (Hb, 9.0 g/100 ml), distension of the abdomen, edema and folliculitis of the legs, as well as the gingivitis, were all cured by treatment which included bed rest, vitamin C, a nutritious diet, and intramuscular injections of folic acid and iron. No doubt the carious teeth remained loose due to alveolar bone recession and some may have needed removal at a later date. Parfitt and Hand (1963) found poor gingival health and low plasma ascorbic acid (AA) levels (mean, 0.265 mg/100 ml) among acutely and chronically ill mental patients. Supervised tooth brushing caused a definite improvement in the gingival condition of these patients, but administration of ascorbic acid, 500 mg daily for 6 weeks, to 20 patients with severe gingivitis produced no evident improvement in their gingival condition.

Barros and Witkop (1963) found periodontal disease to be very prevalent in Chile. It was seen mostly in people with poor dental hygiene, but no significant correlation was found between the periodontal disease and any specific nutritional deficiency. A carefully controlled double-blind study of 100 men with gingivitis by Keller (1963) showed conclusively that scaling to remove calculus was a more effective form of treatment for periodontal disease than vitamin C supplementation when assessment was made 21 d later. However, among those patients who had concomitant ascorbic acid deficiency, the dietary supplement, which provided 450 mg of ascorbic acid as well as other vitamins, did cause a marked improvement in the results following scaling. Some patients receiving placebo capsules containing methylcellulose showed an increase in their plasma ascorbate (TAA) levels during the study, and some receiving the vitamin supplement showed no change or even a decrease in plasma ascorbate. Those whose ascorbate levels rose during the study showed the best results as regards healing of their gingivitis following scaling.

Glickman and Dines (1963) studied the blood and gingival tissue ascorbic acid levels of 15 patients with gingivitis before and after administration of ascorbic acid, 250 mg daily for 14 d. They observed a significant increase in the blood ascorbic acid level, from 1.0 to 1.3 mg/100 ml, but no significant change in the mean gingival ascorbic acid level of 1.54 mg/100 ml. Normally one expects tissue ascorbate levels to be many times greater than blood levels, so the fact that they were not suggests an abnormality of transport, storage, or utilization of ascorbic acid at the gingival level, such as one might find as a result of

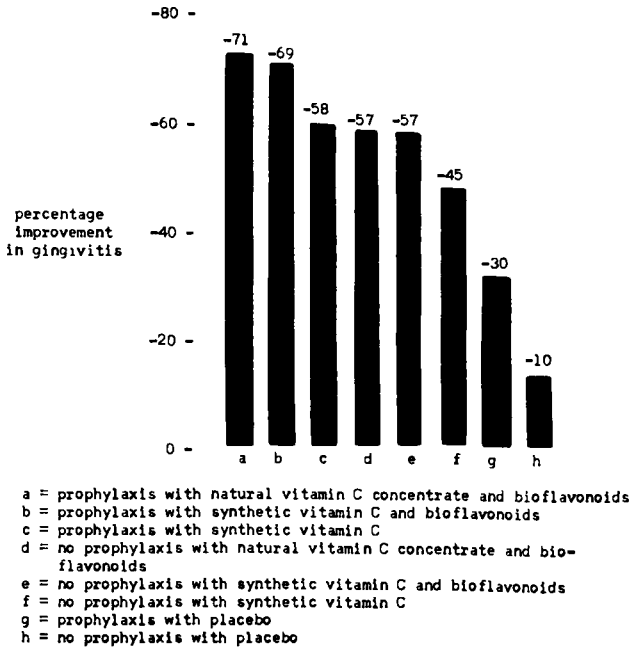

FIGURE 4. The percentage of improvement in "gingival scores" 3 weeks after "prophylaxis" (removal of calculus from all the teeth on one side of the mouth) and administration of one of the following dietary supplements three times a day for 3 weeks (25 subjects per group): No. 1, 100 mg of lactose per capsule; No. 2, 100 mg of synthetic vitamin C; No. 3, 100 mg of synthetic vitamin C plus 100 mg of citrus bioflavonoids; No. 4, 100 mg of natural vitamin C concentrate plus 100 mg of citrus bioflavonoids. Both "prophylaxis" and ascorbic acid were of significant benefit, but scaling plus ascorbic acids and bioflavonoids gave the best results.

diabetes mellitus, dilantin, mercury, iron, or copper toxicity, or sepsis. Chelating flavonoids aid tissue storage of ascorbic acid, so this may explain the better results obtained when bioflavonoids are combined with ascorbic acid therapy.

El-Ashiry et al. (1964), in a careful scrutiny of the teeth and gums of 102 subjects at the University of Alabama, found a closer correlation between calculus and gingivitis ($r = +0.568$, $p < 0.001$) than between fasting plasma ascorbic acid levels and gingivitis ($r = -0.218$, $p < 0.005$). However, in a double-blind study comparing local treatment by removal of calculus and systemic treatment by dietary supplementation, vitamin C (100 mg), and citrus bioflavonoids (100 mg), three times a day for 3 weeks, they found the combination of vitamin C and bioflavonoids to be more effective in the treatment of gingivitis than vitamin C alone or removal of calculus alone (Figure 4). The benefits to be obtained from the combined use of vitamin C and bioflavonoids in the treatment of hemorrhagic gingivitis were confirmed by Caprioglio et al. (1967) and by other speakers from Rome, Turin, Milan, Bologna, Pavia, Modena, Stresa, Verona, and Gorizia, at a symposium on bioflavonoids, which was held at Stresa on Lago Maggiore in April of 1966.

An extensive study of the dental and periodontal health of 21,000 people in 8 widely dispersed countries, reported by Russell (1963), showed no correlation with ascorbic acid intake. Dental caries tended to be lower in areas where there was a low intake of sugar and an optimal intake of fluoride; populations with high scores for periodontal disease tended to be deficient in vitamin A, but faulty dental hygiene and aging seemed to be more important factors. Russell et al. (1965) found strong associations between oral hygiene and periodontal

disease in South Vietnam, but no meaningful correlation with any specific dietary factor. Coven (1965), studying the periodontal health of children, observed greater benefit from the use of a multivitamin supplement containing 75 mg of ascorbic acid than from ascorbic acid alone or from vitamin B complex alone.

Solomon et al. (1968) observed that the prevalence of periodontal disease is significantly greater in smokers than in nonsmokers of similar age, both in men and in women. The periodontal condition of cigarette smokers, as a group, is as poor as that of nonsmokers who are 15 years older. There is no evidence that the increased prevalence of periodontal disease in smokers is due to ascorbic acid deficiency, but it may well be, for blood ascorbic acid levels are low in smokers (Chapter 4, Volume I), decline with increasing age (Chapter 5, Volume I), and tend to be lower in men than in women (Chapter 6, Volume I), while the prevalence of periodontal disease is inversely related to these parameters.

Some writers, like El-Ashiry et al. (1964) and Cheraskin (1975), have reported that ascorbic acid is markedly beneficial in the treatment of periodontal disease, while others have reported little if any benefit. Clearly gingivitis is a multifactorial disease and ascorbic acid supplements are most helpful in those instances where ascorbic deficiency has played an important role in the development of the disease.

O'Leary et al. (1969) found no significant difference in the tooth mobility of four subjects who received 300 mg of ascorbic acid daily for 12 weeks and four subjects who received placebo capsules. However, the initial periodontal status of these subjects ranged from normal to advanced disease. Moreover, there is no evidence that they were ascorbic acid deficient at the beginning of the experiment.

Mukherjee (1969) compared the rate of dissolution of calculus, dentin, and enamel by various anticalculus agents and found that a commercial product — ascoxal — at pH 4.5 to 5.0 had a much greater effect on calculus than on dentin or enamel *in vitro*. However, the composition of this product was given as ascorbic acid, 33 g; sodium percarbonate, 23 g; cupric sulfate, anhydrous, 65 g; others, 100 g. Some part of this product will inevitably penetrate the gingiva and some may be swallowed; this could lead to the accumulation of copper in the gingiva and in the liver, so it would most likely have a deleterious long-term effect on both local and systemic ascorbic acid metabolism. The present writer would certainly avoid any copper-containing product (see Chapter 10, Volume I).

Hood et al. (1970) observed the development of dental caries in one of five volunteers after 84 d of complete ascorbic acid deprivation and after a further 36 d on an inadequate ascorbic acid intake (2.5 mg/d), when his plasma ascorbic acid (TAA) level was 0.24 mg/100 ml. Not only did he develop dental caries, he also had difficulty in retaining new dental fillings; the fillings had to be replaced twice before ultimate retention, which did not occur until after full replenishment with ascorbic acid.

Studying periodontal disease in western Nigeria, Enwonwu and Edozien (1970) found it to be much more prevalent and much more severe at a younger age among Yoruba villagers of low socioeconomic groups than among Yoruba people of higher educational and economic level on the faculty of the University of Ibadan. Indeed, one study of the Yoruba people showed that 82.5% of those aged 25 years or over showed extensive pocket formation, with destruction of the supporting structures of the teeth. Many factors, including malnutrition in infancy, parasitic disease, and poor dental hygiene each may have played a role in decreasing the resistance of the villagers, but a notable fact emerging from the study of Enwonwu and Edozien (1970) was that 71% of the villagers had serum total ascorbic acid (TAA) levels below 0.2 mg/100 ml (43% below 0.1 mg/100 ml), while only 15% of the higher socioeconomic group had ascorbic acid levels below 0.2 mg/100 ml. The authors interpreted the serum ascorbic acid levels as being indicative of the vitamin C content of the diets, but it is very likely that the low protein content of the diet of the villagers contributed to their ascorbic acid deficiency (Chapter 12, Volume I), as there was a high incidence of kwashiorkor in the villages.

Buzina et al. (1973) observed marked seasonal variations in the prevalence of both angular stomatitis and bleeding gums in the school children of four villages in Croatia, as the diet was particularly short of ascorbic acid, riboflavin, vitamin A, and animal protein in the winter, spring, and early summer. Supplementation with riboflavin, 3 mg daily for 8 months, reduced the prevalence of angular stomatitis from 17.2 to 3.4%, while in nonsupplemented groups, the prevalence rose to 29% in the same period. In one school, the addition of 70 mg of ascorbic acid daily to the hot soup of each child's school lunch failed to prevent an increase in the prevalence of gingival bleeding from 13.3% in April to 27.7% in early June. Nevertheless, following the availability of fresh fruits and vegetables in summer and early autumn, there was a reduction in the prevalence of gingival bleeding in October to between 1.7 and 9.4% in all four schools. No doubt most of the ascorbic acid supplement was lost in the hot soup, because this vitamin is unstable when heated; in all probability, much better results would have been achieved by supplying oranges or vitamin C with bioflavonoids. Moreover, the low protein content of the winter diet may have been a contributory factor, causing a disturbance of ascorbate metabolism in these children (Chapter 12, Volume I).

Shannon (1973) studied 341 individuals at a Veterans Administration Hospital in Houston, TX, and found a highly significant negative correlation between the whole blood total ascorbic acid levels and the extent of periodontal disease ($p < 0.01$) in these subjects. Stambaugh et al. (1973), at the University of Washington, Seattle, drew attention to the relationship between ascorbic acid deficiency and dilantin hyperplasia of the gums. They observed marked ascorbic acid deficiency, with a plasma total ascorbic acid level of 0.08 mg/100 ml, in a 54-year-old man receiving diphenyl hydantoin and phenobarbitone treatment with an inadequate diet. The gingival tissues were enlarged and fibrotic, with the exception of the marginal gingivum which was soft, erythematous, and bled easily. X-rays revealed moderate to advanced periodontal disease. Other related findings included hemiplegia due to cerebral hemorrhage, extreme irritability, guaic positive stools, and mild chronic joint pains. His appetite, attitude, and gingival disease were all improved within a week of starting treatment with ascorbic acid, 1000 mg daily. The adverse effects of phenytoin (diphenyl hydantoin) on ascorbate and on folate metabolism have been noted in Chapter 24, Volume I.

Pressman et al. (1974), working at the National University of Cordoba, found only 4 out of 27 patients with gingivitis to have subnormal plasma ascorbic acid levels and concluded that vitamin C deficiency was not a major cause of bleeding gums in their patients. In contrast, Cowan (1976), in Dublin, conducted a double-blind study and found that prolonged high-dosage ascorbic acid, following scaling and polishing of the teeth, caused a definite improvement in the regularity and definition of the periodontal membrane, as seen in X-ray films in 30 out of 37 test and in only 9 out of 32 control subjects. The usual dose of ascorbic acid was 1 g daily for 1 to 3 months.

Cheraskin and Ringsdorf (1977) wrote an article entitled "Dentists Need More Vitamin C". They analyzed the results of a dietary questionnaire completed by over a thousand dentists and their wives and found that 12.5% of them had diets providing less than the minimum daily requirement (MDR) of 60 mg/d, set by the Food and Nutrition Board of the National Academy of Sciences (U.S.) in 1968, and that 6.8% had diets that did not provide the revised MDR of 45 mg/d set in 1973. Moreover, they pointed out that smoking, the use of aspirin, or the taking of oral contraceptives would increase the percentage with vitamin C deficiency. They concluded, "We, as dentists, will not sell this type of prevention to patients unless we practice it ourselves in our families."

Falconer (1979) reported a case of frank scurvy with swollen, ulcerated, and purplish gingiva in a Pakistani immigrant at St. Luke's Hospital in Bradford, England, and found that his diet of curry and chappatis, twice a day, had been providing about 3 mg of vitamin C daily. Woolfe et al. (1980), reviewing the relationship between ascorbic acid deficiency and human periodontal disease, found the literature to be conflicting. In summary, they

concluded, "It is apparent that while ascorbic acid deficiency may be a factor which results in deleterious effects on the periodontium, ascorbic acid supplementation has little or no effect on gingival health, the course of periodontal disease or tooth mobility. The combination of oral hygiene and initial therapy procedures together with ascorbic acid supplementation has been shown by some workers to result in improved gingival health." Unfortunately, they drew no distinction between the use of ascorbic acid alone and treatment with ascorbic acid and bioflavonoids which seems to be much more effective.

Hutt (1981) studied the effect of ascorbic acid, 500 mg twice daily for 2 weeks, before third molar tooth extraction. There was no case of delayed healing among 80 patients who received the preoperative vitamin C, but there were 3 cases of abnormal healing among 70 patients who did not receive the vitamin supplement. These data suggest a possible benefit for the few who may have been vitamin depleted, but the numbers are too small to allow any meaningful conclusions.

Mallek and Nakamoto (1981) discussed the extra folic acid needs of patients with gingival hyperplasia due to dilantin anticonvulsant therapy. They pointed out that these patients often have megaloblastic anemia, especially in association with pregnancy, lactation, birth control pills, poverty, febrile illness, alcoholism, or following gastrectomy. However, they did not mention ascorbic acid deficiency in these patients, which can cause a disturbance of folate metabolism (Chapter 4, Volume III) and was observed by Stambaugh et al. (1973) in association with dilantin hyperplasia.

Dannenberg (1982) and Giunta (1983) have observed that the practice of chewing ascorbic acid tablets, instead of swallowing them, can cause such acidity in the mouth as to dissolve much of the occlusal enamel and cause deep cupping of the dentin on the molars. Chewable ascorbic acid tablets for children should therefore be discouraged.

Aurer-Koželj et al. (1982), in Zagreb, observed marked improvement in the ultrastructure of the epithelium and the connective tissue in the gingival lamina propria of patients with progressive periodontitis when their plasma ascorbic acid level was raised from a mean of 0.28 mg/100 ml to 1.31 mg/100 ml. The dietary ascorbic acid intake of these patients had been estimated as being between 20 and 35 mg/d. They were studied before and after 6 weeks of supplementation with 70 mg of ascorbic acid daily.

Ringsdorf and Cheraskin (1982) compared the rates of healing of 3-mm circular gingival wounds in dental students on normal diets, with and without 1000 mg of daily ascorbic acid supplement. These authors observed a markedly increased rate of healing when the students were receiving the ascorbate supplement, but the number of subjects was not sufficient to prove it. There have been many studies in which a possible relationship between periodontal disease and dietary ascorbic acid intake has been investigated. One of the largest included data from dental examination of 8609 individuals aged 25 to 74 in the continental U.S. during the National Health and Nutrition Examination Survey (NHANES), as reported by Ismail et al. (1983). Only a weak association was found between ascorbic acid intake, as calculated from the diet of the previous day, and periodontal health; and even this association could have been due to other factors such as age, sex, social class, or smoking habit, etc. It seems to the writer that we need to know to what extent the plasma ascorbate and the gingival tissue ascorbate levels vary independently of the dietary vitamin intake. This raises the question of local ascorbate metabolism, which may be affected by sepsis and by heavy metal contamination of the tissues.

Shaw et al. (1983), studying the effects of astringents in miniature swine, observed severe changes in the gingival connective tissue following the application of a cotton cord soaked in ferric sulfate to the gingival sulcus for 10 min. Most of the severe changes subsided within 2 weeks, but clearly iron destroys ascorbic acid (Chapter 10, Volume I) and is likely to have an even greater and possibly a longer lasting effect in species that do not synthesize ascorbic acid.

Arensen et al. (1982) have produced preliminary histochemical evidence of increased copper levels in the subepithelial gingiva of patients with periodontitis. Moreover, Knuuttila et al. (1983) have found a high copper content (48 μg/g) in human subgingival calculus. Clearly, a local accumulation of copper could be a cause of local ascorbate depletion (Chapter 10, Volume I).

Mercury is found in the marginal gingivum 1 to 7 d after an amalgam tooth restoration and can cause a moderate gingivitis as noted in a review of mercury toxicity by Bauer and First (1982). This, too, could lead to local ascorbate deficiency, as mercury causes significant destruction of ascorbic acid (Chapter 10, Volume I). Indeed, Bauer and First note that small amounts of mercury ions tend to inhibit or dimish synthesis of collagen by fibroblasts, as one would expect in ascorbate-deficient tissue. The gums recover as the amalgam becomes overlaid with an oxidized layer of tin, but may become inflamed again if more mercury is released during the corrosion process. Abraham et al. (1984) have shown a significantly higher mean blood mercury level in young adults with mercury amalgam dental fillings (0.7 ng/ml) than in control subjects with no amalgam restorations (0.3 ng/ml; $p < 0.01$). Moreover, both the blood and the postchewing mouth air mercury levels showed highly significant positive relationships to the mean occlusal surface area of the posterior amalgams ($p < 0.001$). While the possible health hazards resulting from the increased blood mercury levels associated with dental amalgams have not yet been determined, they do constitute a cause for great concern. These blood mercury levels are similar to those reported by Kuntz et al. (1982) in a group of 57 prenatal patients (0.79 ng/ml) in whom a significant positive correlation was found between a history of stillbirth ($p < 0.05$) and mercury levels in maternal and cord blood. These results are just as one might expect (Chapter 14, Volume III) if mercury does indeed destroy ascorbic acid (Chapter 10, Volume I). A further cause of great concern is the finding that the prenatal blood mercury levels of these women showed a positive correlation with earlier deliveries of malformed infants ($p < 0.05$). Clearly this question needs to be explored in depth and if these findings are confirmed, there will be a need for removal of the cause, which will certainly keep the dentists busy, and also a need for a study of the capacity of natural chelating food fiber such as D-catechin to remove heavy metals from the body. Ascorbic acid supplements will also be needed to balance any increased rate of loss of this vitamin.

Nakamoto et al. (1984) discussed the role of ascorbic acid as an antihistamine in the prevention and treatment of gingivitis. Ascorbic acid would probably be more effective in the treatment of gingivitis if it were not for the adverse general effects of factors such as pregnancy, smoking, and dilantin, which deplete the body stores of ascorbic acid, and the local effects of diabetes mellitus, bacterial endotoxin, and mercury amalgam, which mitigate against the entry of ascorbic acid in the tissues.

IV. CONCLUSIONS

The gingivitis of frank scurvy is rarely seen nowadays, but disturbances of ascorbic acid metabolism are relatively more common. We see pregnancy gingivitis, diabetic gingivitis, alcoholic gingivitis, senile gingivitis, dilantin gingivitis, and mercury gingivitis. These conditions have different causes, but one may note that they all seem to involve local or general ascorbic acid deficiency. Likewise, gingivitis is common in smokers and in protein-deficient subjects who are also ascorbate deficient.

Barlow (1883) pointed out that sponginess of the gums is not an essential finding in scurvy, being rare in infants before the eruption of the teeth and in elderly people who have lost all their teeth. Moreover, referring to the use of mercury in the treatment of syphilis, he stated, "The difficulty of inducing sponginess of the gums in young infants by the administration of mercury is well known." Clearly, Barlow saw an analogy between scurvy

and mercurial hyperplasia of the gums as early as 1883. It is therefore appropriate that there should be renewed interest in the toxicity of mercury by Utt (1984), MacDonald (1984), and an Editorial in the *California Dental Association Journal* 100 years later, and that we should begin to understand the reason for this connection. The present writer suggests that heavy metal chelating agents like D-catechin, antioxidants like alpha tocopherol, and ascorbic acid supplements will all find a useful place in the treatment of certain forms of gingivitis. Certainly, catechin-coated ascorbic tablets would seem to be desirable; these should be swallowed whole and not chewed, Moreover, the use of copper, mercury, and iron compounds as mordant, styptic, astringent, or restorative agents should be reduced to a minimum. Sometimes ascorbic acid supplements, or ascorbic acid and chelating flavonoids, will relieve the gingivitis, but often the primary cause must also be treated in order to rectify an abnormality of ascorbic acid metabolism.

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Chapter 8

ATHEROSCLEROSIS

Aschoff (1924), in his analysis of the nature of atherosclerosis, was the first to postulate that the earliest morphological lesion is a disturbance of the intercellular ground substance of the arterial intima. Observing a deposit of stainable lipid along the internal elastic membrane of the artery, he supposed that the mechanical stress imposed upon the artery caused a change in the properties of the intimal ground substance, rendering it more permeable to plasma lipids.

Mettier et al. (1930) suggested that arteriosclerosis might enhance the development of scurvy, particularly in elderly men, because they found cardiovascular damage, chiefly arteriosclerotic in type, to be present to a greater or lesser extent in all of eight patients with scurvy seen by them in Boston, MA. However, the relationship seems to be the other way around, for it is now known that chronic subclinical ascorbic acid deficiency causes hypercholesterolemia (Chapter 5, Volume III) and endothelial damage, as well as changes in the mucopolysaccharides and the collagen in the walls of the blood vessels of guinea pigs. Subendothelial hemorrhages undoubtedly appear more frequently in hypovitaminosis C, especially in areas subjected to mechanical stress; subendothelial cholesterol deposition may then develop as an aberrant form of wound healing in the affected regions of the arteries.

Menten and King (1935) observed the production of diffuse hyperplastic vascular lesions in guinea pigs maintained on diets containing insufficient vitamin C and also given injections of sublethal amounts of diphtheria toxin. Such animals also developed hydropic degeneration of the beta cells of the islets of Langerhans, associated with hyperglycemia and a low tolerance to sugar. These authors expressed the opinion that a combination of dietary vitamin C deficiency and infection might be relevant to the origin of diffuse hyperplastic arteriosclerosis in humans. Boyd (1938) reported observing yellow streaks of atheroma in the intima of the aortas of children dying after infectious fevers, which are known to deplete ascorbate stores (Chapter 8, Volume I).

MacKay (1941) described coronary artery hemorrhage in association with mild atheromatous changes as the cause of death in a 62-year-old woman. Certainly, coronary hemorrhage is rare and it is much more usual to find thrombosis in association with atheroma of these arteries. However, we must remember that blood coagulation is the normal mechanism for the arrest of hemorrhage, so subendothelial hemorrhages may actually be the initiating cause of the formation of a thrombus. Mjasnikova (1947) provided evidence that ascorbic acid deficiency promotes the development of atherosclerosis in experimental animals. Trimmer and Lundy (1948) studied 556 private patients. They observed that 42% of all patients, 59% of patients with heart diseae of mixed etiology, and 70% of patients with coronary thrombosis had plasma ascorbic acid levels of 0.5 mg/100 ml or less. Davis and Oester (1952) reported that vitamin C had an inhibitory effect on the development of aortic arteriosclerosis in rabbits. Becker et al. (1953) reported their early studies of the relationship between ascorbic acid and cholesterol metabolism. That subject has stimulated much research which has added to our understanding of the etiology of atherosclerosis during the last 30 years, as described in Chapter 5, Volume III.

An important study was reported by Willis (1953); working at the Montreal General Hospital in Canada, he noted that atherosclerosis begins at points of stress or damage to the endothelium of the large arteries and chose experimental scurvy to initiate damage to the intercellular ground substance of the intima of the aorta in guinea pigs. "A total of 145 guinea pigs was divided, with equal distribution of the sexes, into the following groups: (1) Chronic scorbutic diet. (2) Chronic scorbutic diet with oral ascorbic acid. (3) Chronic

scorbutic diet with cholesterol and oral ascorbic acid. (4) Chronic scorbutic diet with cholesterol and intra-peritoneal ascorbic acid. (5) Acute scorbutic diet. (6) Acute scorbutic diet with oral ascorbic acid. (7) Acute scorbutic diet with cholesterol. (8) Acute scorbutic diet with oral ascorbic acid and cholesterol." The guinea pigs were killed at regular intervals over a period from 12 to 41 d in each of the groups. Blood samples were obtained for cholesterol analysis; the aorta and spleen of each animal were removed, fixed, frozen, and stained with Scharlach R for lipid studies. Both acute and chronic scurvy were effective in producing lesions of the arterial intima indistinguishable from the lesions seen in early human atherosclerosis. The lesions developed within 15 d; they occurred at normal plasma cholesterol levels and were not accompanied by lipid deposits in the spleen. Cholesterol feeding resulted in similar lesions with approximately the same rapidity, but with hypercholesterolemia and with lipid deposits in the spleen (Table 1).

A combination of ascorbic acid and rutin was used in the treatment of atherosclerosis by Gale and Thewlis (1953), who reported favorable results on the basis of symptomatic changes, but it was not a controlled study. Willis et al. (1954) studied atherosclerosis of the femoral and popliteal arteries of men varying in age from 55 to 77 years, by the use of femoral angiography. Treating ten patients with ascorbic acid, 500 mg three times a day, and using six patients as untreated controls, they observed the progression or regression of atheromatous plaques over periods ranging from 2 to 6 months. The numbers were too small to make any definite conclusions, but interpretation of the X-rays by an unbiased observer revealed that six of the ten treated patients improved, three became worse, and one was unchanged, while none of the six untreated patients improved, three deteriorated, and three were unchanged.

Willis and Fishman (1955) studied the ascorbic acid (AA)* content of human arterial wall tissues obtained within a few hours of death and found severe degrees of arterial ascorbic acid deficiency to be common, even in otherwise well-nourished individuals. Moreover, they found that that ascorbic acid depletion was often localized to certain segments of arteries, especially to regions which are subject to mechanical stress, where atherosclerosis is most common. Adjacent segments, where mechanical stress is less, tended to have a higher ascorbic acid content, and atherosclerosis there was rare. Willis and Fishman stated, "A good example of the localization of atherosclerosis by mechanical stress occurs in the carotid artery. Because of the dilation associated with the carotid sinus and bifurcation of the common carotid artery, this site is highly susceptible to atherosclerosis. The immediately adjacent internal carotid artery is only rarely involved by atherosclerosis. Our results suggest that the local point of excess mechanical stress in the carotid sinus is associated with a relative depletion of ascorbic acid in most instances as compared with the adjacent internal carotid artery."

One can readily understand that subendothelial petechial hemorrhages will occur most readily in ascorbic acid-deficient individuals and most commonly in areas subjected to stress. The process may be locally self-accelerating because hemolysis around the subendothelial hemorrhages will destroy ascorbic acid locally and thus make the situation worse. Fortunately, Willis and Fishman have provided preliminary evidence that the arterial ascorbic acid level can be increased by ascorbic acid supplementation.

Bolker et al. (1956) observed an increase in the serum hexosamine level of guinea pigs with scurvy. This elaborated on similar findings of increased serum glycoprotein levels in scorbutic guinea pigs by Pirani and Catchpole (1951) and was considered analagous to the observation by Berkman et al. (1953) of increased serum glucosamine and total protein-bound polysaccharides in diabetic patients with degenerative vascular disease and in non-diabetic patients with chronic glomerulonephritis. Bolker et al. suggested that it implied depolymerization of the mucopolysaccharides of the ground substance in scurvy and in

* AA — ascorbic acid, reduced form.

Table 1
THE RESULTS OBTAINED IN EACH OF EIGHT DIETARY GROUPS

Diet	No. of animals	Plasma cholesterol range (mg%)	Average plasma cholesterol (mg%)	Fat staining of spleen	No. of animals with atherosclerosis	No. of animals without atherosclerosis	Average degree[a] of atherosclerosis in those having it
Chronic scorbutic	20	23—120	80.5	0	9	11	2.5+
Chronic scorbutic with oral ascorbic acid	22	26—106	53.5	0	0	22	0
Chronic scorbutic with oral ascorbic acid and cholesterol	18	41—270	162.0	3+	16	2	2.5+
Chrome scorbutic with i.p. ascorbic acid and cholesterol	18	85—301	179.4	2+	7	11	1 6+
Acute scorbutic	32	17—80	42.0	0[b]	19	13	2+
Acute scorbutic with oral ascorbic acid	16	23—77	40.0	0	0	16	0
Acute scorbutic with cholesterol	11	Not done	Not done	3+	11	0	2.6+
Acute scorbutic with oral ascorbic acid and cholesterol	8	Not done	Not done	3+	4	4	2+

Note: Atherosclerosis was produced both by an acute and by a chronic scorbutic diet. The combination of scurvy and cholesterol feeding was the most potent method of producing atherosclerosis, being effective in all the animals in which it was tried. None of the 38 control animals developed atherosclerosis.

[a] Average obtained by dividing total of plus signs by the number of animals having lesions.

[b] One animal in this group was found to have 1+ fat staining of the spleen. It is omitted in the representation of the figure as it was a solitary finding.

From Willis, G. C. (1953), *Can. Med. Assoc. J.*, 69, 17. With permission.

degenerative vascular disease. Pirani and Catchpole suggested that these glycoproteins may lead to the development of amyloid disease.

Willis (1957) studied the reversibility of atherosclerosis in guinea pigs by first depriving them of ascorbic acid for 21 to 30 d, and then rescuing half of them by ascorbic acid supplementation for 1 to 27 d. Early atheromatous lesions stained less intensely with Scharlach R after only a few days of ascorbic acid therapy and soon lost their diffuse lipid deposit completely. Advanced atherosclerotic plaques, on the other hand, were much more resistant. The lipid of the advanced lesions showed diffusion to smaller droplets, but did not disappear during the 27 d of study.

Atherosclerosis has been observed in the young as well as in the old; Moon (1957), studying the coronary arteries of 105 individuals, found evidence of early atherosclerotic changes even in infants and children. "The association of lesions of the internal elastic membrane with deposition of mucopolysaccharide and subendothelial fibroblastic activity was a constant finding in the proximal segments of the coronary arteries of infants and children; this association was noted also in previous studies of arteriosclerosis in adults." Thus, it is evident that we must start young if we are to prevent the development of atherosclerosis.

The work of Banerjee and Ghosh (1947), Sarkar and Banerjee (1957), and Banerjee et al. (1958) had already demonstrated decreased glucose tolerance in scorbutic guinea pigs and monkeys. Now Banerjee and Singh (1958) and others observed that the total body cholesterol content was significantly increased in scorbutic guinea pigs. If fact, chronic ascorbic acid insufficiency is associated with profound disturbances of both carbohydrate and lipid metabolism, as discussed in Chapters 3 and 5, Volume III. Thus, chronic vitamin C deficiency may well explain the known association between atherosclerosis and decreased carbohydrate tolerance which was discussed in an Editorial in *Nutrition Reviews* (1967).

Kawishwar et al. (1963) found that the defects of carbohydrate and lipid metabolism observed in scorbutic guinea pigs could be reversed either by insulin treatment or by supplementation with ascorbic acid. Simonson and Keys (1961) reviewed the Russian literature on atherosclerosis and noted the many enthusiastic reports of success from the use of ascorbic acid, 1000 mg daily, in the treatment of coronary artery disease by lowering blood cholesterol levels. However, Simonson and Keys were unable to confirm the cholesterol-lowering effect in experiments at a Minnesota State Hospital when they gave ascorbic acid, 1000 mg daily, in alternating periods of 3 to 4 weeks. It is now clear that the treatment should have been continued for much longer than 3 or 4 weeks.

Studies by Myasnikov (1958), McConnell and Sokoloff (1963), Zaitsev et al. (1964), and by Sokoloff et al. (1966) provided evidence that ascorbic acid supplements afford considerable protection against cholesterol atheromatosis in rabbits. Moreover, Sokoloff et al. reported that ascorbic acid therapy (1.5 to 3.0 g daily) was very effective in lowering elevated tryglyceride levels and increasing blood lipoprotein lipase levels in patients with cardiovascular disease. It was less effective in lowering the cholesterol levels. Mason (1963) reported post-mortem examination of 180 professional airmen (mean age, 27.3 years) who had died accidental deaths. He observed macroscopic atherosclerotic disease in one or more coronary arteries in 40% of them.

Studying the endothelial cells of the aorta of the scorbutic guinea pig, Gore et al. (1965a) observed that, "The actual junction space as revealed by electron microscopy is widened, and there is also depletion of subendothelial collagen fibers." They concluded that, "since subendothelial collagen is sparse and inconstant in the capillary channels from which scorbutic bleeding originates, endothelial cell disjunction must be the essential structural basis for the occurrence of hemorrhage in scurvy." It is particularly interesting that they drew attention to the similarity between the widening of the junction space in scurvy and that induced by histamine, for there was no knowledge of the histaminemia of ascorbic acid

deficiency when they were writing. Gore et al. (1965b) observed a significant decrease in the hydroxyproline ($p < 0.05$) and chondroitin sulfate ($p < 0.05$) contents and a significant increase in the hyaluronic acid ($p < 0.05$) content of the aortas of guinea pigs on a scorbutogenic diet. However, the fatty deposits observed by Willis (1958) were not seen either in this or in the previous study involving a total of 105 scorbutic animals.

Bailey and Butler (1966) found that rabbits fed a 1% cholesterol diet were protected from the development of atherosclerotic aortic plaques by the administration of cortisone, but not by supplemental ascorbic acid. This effect of cortisone is interesting, as Stewart et al. have shown that cortisone accelerates the reduction of dehydroascorbic acid (DHAA) to ascorbic acid *in vivo* (Chapter 13, Volume I).

Sokoloff et al. (1966) reported that in atherosclerosis and related disorders the concentration of DHAA in the blood and tissues is about three times higher than in healthy persons of the same age group. The reason for this prooxidant effect is not known, but it could be due to an increased copper concentration in the aorta and other tissues of affected individuals. Henzel (1968) reported that he and co-workers had performed copper assays on specimens obtained from nine patients requiring resection of a diseased abdominal aorta for either occlusive or aneurysmal disease. The average copper concentration in specimens from patients with atherosclerosis but no aneurysm was 26.3 ppm. For patients with atherosclerosis and aneurysms, it was 21.2 ppm, whereas for those with aortic aneurysms without visible evidence of atheroslerosis, it was 14.2 ppm. Of course, it is true that vascular disease has been produced in swine by feeding a copper-deficient diet, but that is a quite different disease, being due to an elastic defect in the arterial wall.

Sokoloff et al. (1967) reported extensive studies of the effects of ascorbic acid supplements on the blood cholesterol, lipoprotein lipase (LPL), and triglyceride levels of animals and man; 60 rabbits on a normal diet were compared with 60 rabbits fed increasing doses of cholesterol and 60 rabbits receiving ascorbic acid (150 mg/kg of body weight per day) in addition to the same amount of cholesterol. After 8 months, their cholesterol levels were 88.5, 1234, and 308 mg/100 ml, their lipoprotein lipase levels were 0.576, 0.189, and 0.45 units, and their triglyceride levels were 26.2, 195, and 89 mg/100 ml, respectively, showing a very marked benefit from ascorbic acid supplementation on all three scores. Moreover, the rabbits fed ascorbic acid with cholesterol showed fewer pathological changes in the vascular system than those fed cholesterol alone. The coronary arteries of the cholesterol-fed group were severely affected and showed marked luminal narrowing, while the coronary arteries of the ascorbate and cholesterol group showed only a very slight narrowing of the lumen. Likewise, the aortas of the cholesterol-fed group showed extensive lipid plaques, while the ascorbic acid and cholesterol group had aortas which were much less severely and less extensively affected. Studies of rats gave almost identical results. Studies of the effects of ascorbic acid supplements, 1.5 to 3 g daily, on the cholesterol, lipoprotein lipase, and triglyceride levels of men and women of different ages, over periods ranging from 5 to 24 months, gave less consistent results than the animal studies, but, "a group of 18 cardiac patients, 58 to 72 years of age, gave the most markedly beneficial response. The patients were given ascorbic acid, 2.0 to 3.0 g, for 15 to 24 months. The total cholesterol decreased from 320 to 245 mg/100 ml, average, the LPL activity was almost tripled, from 0.12 to 0.36 unit, and triglycerides were reduced from 209.4 to 102.3 mg/100 ml. This group of cardiac patients, ten with one coronary occlusion and eight with two occlusions, were given ascorbic acid therapy from 15 to 60 days after the last attack . . . " Little effect was seen in the first 2 months of treatment, but the LPL began to rise and the triglycerides began to fall in the third month of treatment. "The total cholesterol response to ascorbic acid was not uniform. Only in cases with original levels above 280 mg/100 ml was there a definite reduction of it." Sokoloff used the term "clearing factor" for LPL or lipoprotein lipase and noted that it appears in the blood almost immediately after the injection of heparin. He

described it as an essential enzyme in the capillary wall, which hydrolyzes the triglycerides of the lipid globules or chylomicrons in the blood after a fatty meal, releasing fatty acids which combine with plasma albumin to form a soluble molecule. He suggested that fats may be as important as cholesterol in the development of atheroma, so he saw the consistent elevation of LPL activity following high-dose ascorbic acid treatment as a very positive gain.

Ginter et al. (1969) observed that longer exposure (for 80 to 82 d) to a low-ascorbic acid diet (0.5 mg/d) was more effective than short exposure (for 10 to 20 d) to an ascorbate-free diet in causing cholesterol accumulation in the tissues of guinea pigs on a diet containing 0.3% cholesterol. All of the guinea pigs on this cholesterol-containing diet developed diffuse fatty infiltration of the liver. Cholesterol deposition in the wall of the aorta was significantly more marked in the low-ascorbate group than in those with a higher intake of ascorbic acid. Ginter et al. (1969) made similar observations of an increased incidence of atheroma not only in the aorta, but also in the coronary arteries of guinea pigs on a low-ascorbate, 0.3% cholesterol diet.

Ginter et al. (1970) studied 50 people over 40 years of age, living in a rural area of Czechoslovakia, who were known to have a seasonal deficit of vitamin C; 24 of these people took ascorbic acid, 300 mg daily for 47 d, while 18 served as controls. Blood analyses before and at the end of the study revealed a significant reduction in the mean serum cholesterol level of the test group from 255 to 238 mg/100 ml, while the respective control values were 251 and 263 mg/100 ml. A review of the literature on ascorbic acid and atherosclerosis by Schaffer (1970) led him to conclude that deficiency of ascorbic acid may well be a contributing factor in the development of coronary, aortic, and cerebral arteriosclerosis.

Kishikawa et al. (1971), in an electron microscopic study of the aortic endothelium of scorbutic guinea pigs, observed an increased incidence of nuclear swelling and vacuolization, separation of endothelial junctions, and reduction of cytoplasmic organelles. In chemical studies of the aorta in scurvy, they found an increase of total mucopolysaccharides, essentially increased hyaluronic acid, a decrease in chrondroitin sulfate B, and a decrease of collagen measured as hydroxyproline. Beta glucuronidase, which participates in the degradation of mucopolysaccharides, was depressed in the aorta and in the serum. Triglycerides, cholesterol esters, and beta lipoproteins were moderately increased in the serum of scorbutic guinea pigs and markedly so in scorbutic guinea pigs on a diet containing 5% coconut oil. Ginter et al. (1971) provided evidence that the catabolism of cholesterol to bile acids is significantly decreased in guinea pigs with chronic ascorbic acid deficiency, as discussed in Chapter 5, Volume III.

The low and falling leukocyte ascorbic acid levels recorded within 6 h after coronary infarction — mean 15.4 $\mu g/10^8$ cells (normal, 16 to 36 $\mu g/10^8$ cells) — and the somewhat increased ascorbic acid content of the cardiac muscle 33 h after coronary deaths — 4.79 mg/100 g (vs. 3.57 mg/100 g in noncoronary deaths) — as reported by Hume et al. (1972) are believed to reflect a transfer of ascorbic acid from the leukocytes to the injured heart muscle, but unfortunately these data do not allow us to know the ascorbic acid content of the diseased coronary vessels before the infarction. Patients with cardiovascular disease do seem to have a poor vitamin C status, as reported by Kishikawa et al. (1971), or a deficiency of vitamin C, bioflavonoids, and vitamin B_6, as reported by Samsonov et al. (1972). Moreover, Knox (1973) carried out a correlation analysis between the standardized mortality rates for ischemic heart disease and cerebrovascular disease in different regions of England and dietary intakes of a number of nutrients. Vitamin C intakes showed a strong negative correlation, i.e., mortality from cerebrovascular disease was high in regions with low vitamin C intakes and vice versa, as shown in Table 1 of Chapter 20, Volume III.

Ginter (1974) reviewed the extensive studies by his group at the Institute of Human

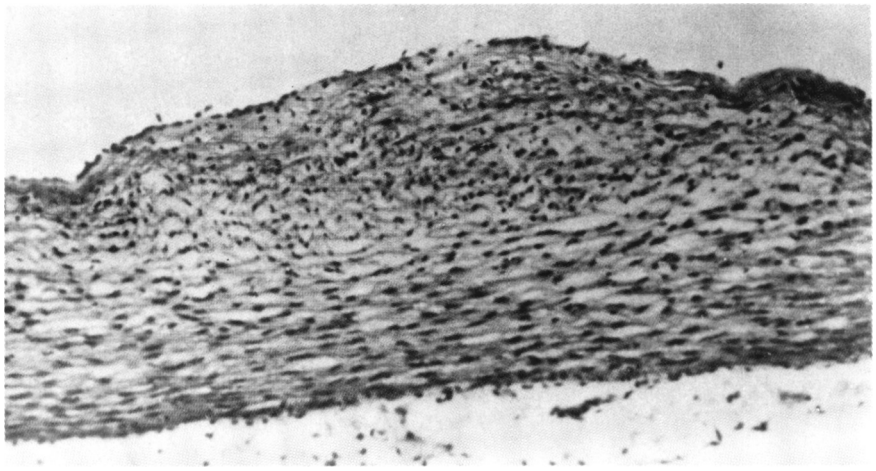

FIGURE 1. A section of the aorta from a guinea pig that had been on a marginal vitamin C-deficient diet for 104 d. The large intimal plaque appears well formed and consists mainly of musculofibrotic tissue. (Hematoxylin and eosin.) (From Sulkin, N. M. and Sulkin, D. F. [1975], *Ann. N Y. Acad. Sci.*, 258, 317. With permission.)

Nutrition Research in Bratislava, Czechoslovakia, and concluded that latent ascorbic acid deficiency belongs in the list of risk factors which predispose to atherosclerosis; he emphasized the importance of preventing hypovitaminosis C. Krumdieck and Butterworth (1974) reviewed the evidence concerning vitamin C deficiency and atherosclerosis. They expressed the belief that atherosclerosis, at least prior to the development of scarring, fibrosis, and calcification, must be a reversible disorder. They assume that injury and repair of the arterial wall are processes that go on continuously during life and that disease will ensue only when the rate of the former predominates. Atherosclerosis should therefore not be considered as an inescapable consequence of aging.

Sulkin and Sulkin (1975) studied the effects of chronic borderline ascorbic acid deficiency in guinea pigs by feeding no ascorbic acid for 14 d and then 0.5 mg per guinea pig per day. Microscopic examination of the aortas of these animals after 100 to 150 d demonstrated large intimal plaques (Figure 1) which appeared to be of the fibromuscular type, as demonstrated by van Gieson-Verloff and by Mallory stain (Figure 2). This proliferation of collagen in the aortic wall is of particular interest, as one might have expected the opposite in vitamin C deficiency, but collagen proliferation was also seen by these workers in the extracellular spaces of the autonomic ganglia following chronic ascorbic acid deficiency. Another observation of note was the presence of marked endothelial proliferation in many sections of the aortic epithelium (Figure 3). This was someties accompanied by intimal thickening and sometimes without such thickening. Other changes included a consistent high degree of metachromasia in the ground substance in the walls of the aortas from the experimental animals and, in some instances, the presence of a fibrous, amorphous material of varying thickness underlying the endothelium (Figure 4). Lipid studies of that material had not been performed at the time of writing, but the cholesterol contents of the livers of these chronically scorbutic animals were reported to be markedly increased. These findings point to a direct link between vitamin C deficiency and arteriosclerosis. We do not yet know all the factors which determine whether guinea pigs with chronic marginal ascorbic acid deficiency will develop this form of musculosclerotic arteriosclerosis, or amyloid degeneration, or atheroma of the aorta. Human autopsy findings show that all three forms of arterial disease often coexist in the same segment of artery. This may well be due to the

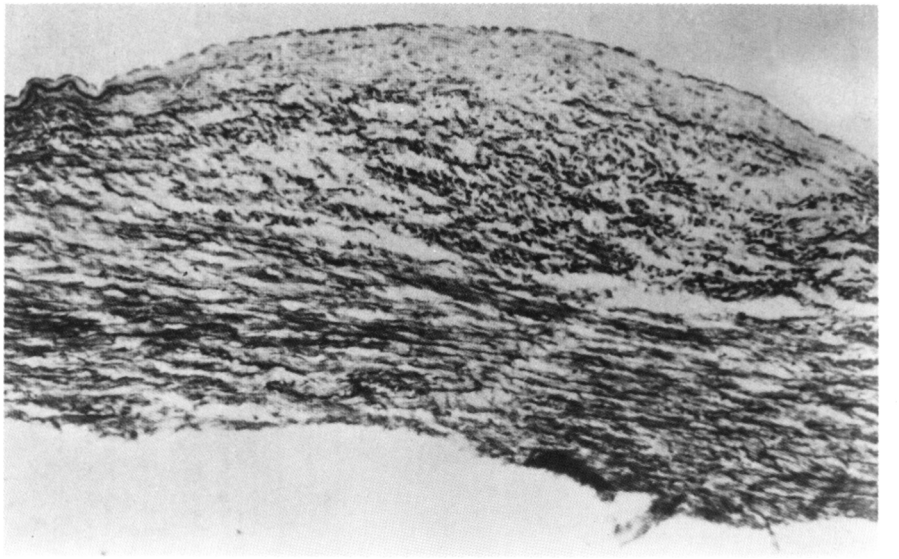

FIGURE 2. A section of an aortic arteriosclerotic plaque from a guinea pig that had been on a marginal vitamin C-deficient diet for a period of 109 d stained with van Gieson-Verhoff stain to demonstrate the fibrotic nature of the lesion. (From Sulkin, N. M. and Sulkin, D F. [1975], *Ann. N Y. Acad. Sci.*, 258, 317 With permission.)

fact that they all arise from the same underlying problem, namely, ascorbic acid deficiency. The ascorbate deficiency may be systemic or local; moreover, it may be due to dietary deficiency or to any of the other factors listed in Volume I.

Reviewing the role of ascorbic acid in the regulation of cholesterol metabolism and in the pathogenesis of atherosclerosis, Turley et al. (1976) stated that, "Chronic dietary inadequacy of vitamin C may influence the pathogenesis of atherosclerosis, as it affects not only plasma cholesterol and triglyceride concentrations but also the integrity of the vascular wall." They pointed out that dietary inadequacy of ascorbic acid seems to be involved in the regulation of cholesterol in several ways. Not only did they review reasons for believing (1) that ascorbate deficiency decreases the conversion of cholesterol to bile acids, they also suggested (2) that indirectly ascorbate depletion decreases cholesterol absorption as a result of the reduced availability of bile acids, monoglycerides, and fatty acids; they also reviewed evidence (3) that cholesterol synthesis may be decreased in vitamin C deficiency. Changes in the rate of cholesterol synthesis would certainly affect serum cholesterol levels more in animals on low-cholesterol diets, so this does seem to fit with several experimental observations. Ascorbic acid seems to increase low serum cholesterol levels and usually has the opposite effect on high cholesterol levels.

Verlangieri and Bakos (1976) observed that the feeding of a high-cholesterol diet (0.5% cholesterol) to rabbits caused a reduction in the concentration of chondroitin sulfates, especially chondroitin sulfate A in the aorta, and that intravenous injections of ascorbic acid, 100 mg/kg of body weight, reversed this reduction. They suggested that this mucopolysaccharide depletion of the aortic wall may be the initiator of atherosclerosis in rabbits. In further studies, Verlangieri et al. (1977) demonstrated that L-ascorbic acid and also L-ascorbic acid-2-sulfate had a protective effect against, and significantly reduced, the intimal thickening that occurs in the arch of the aorta of cholesterol-fed rabbits.

Hanck and Weiser (1977) reported that their experiments, both in guinea pigs and in human subjects, showed a definite action of ascorbic acid on blood and tissue lipid levels. "When there was a deficiency of ascorbic acid, plasma and tissue lipid levels tended to be

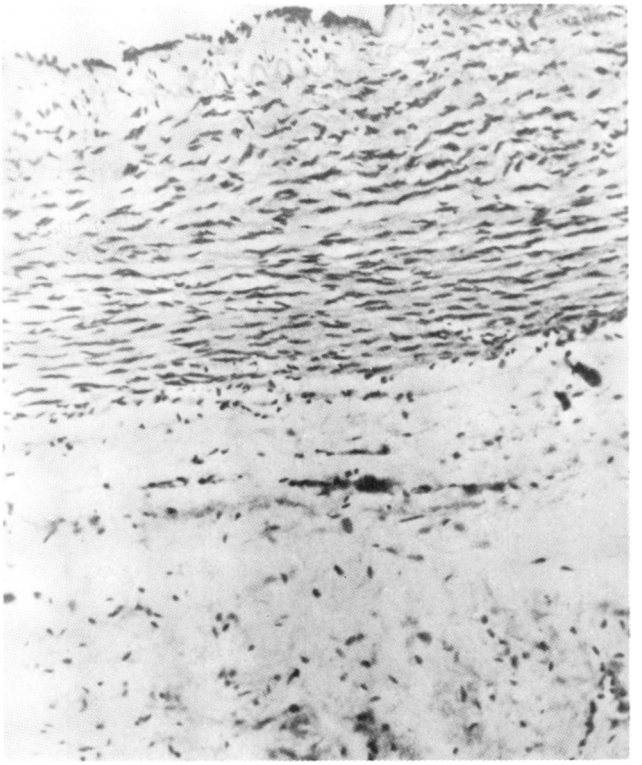

FIGURE 3. A section from an aorta of a guinea pig that had been on a marginal vitamin C-deficient diet for 110 d. This section is characterized by endothelial proliferation and intimal thickening. (From Sulkin, N. M. and Sulkin, D. F. [1975], *Ann. N.Y. Acad. Sci.*, 258, 317. With permission.)

raised without dietary intake of cholesterol. When ascorbic acid intake was normal, lipid levels were elevated by dietary intake of cholesterol. In both cases the elevated cholesterol levels would be reduced by continuous intake of supplementary ascorbic acid for a prolonged period." After 8 weeks, the cholesterol level of a group of guinea pigs receiving only 50 ppm of ascorbic acid in their diet rose by over 40% compared with pair-fed and *ad libitum*-fed groups receiving the same diet with 2000 ppm of ascorbic acid. After 9 weeks, the difference in the plasma cholesterol level of the group on the low-ascorbate diet had risen by about 65% compared with the control groups. Moreover, the cholesterol concentration in the aortas of the animals on the low-ascorbate diet was measured after 8 weeks and was 75 mg/100 g of wet tissue compared with 46 mg in pair-fed control animals and 60 mg/100 g in *ad libitum*-fed controls. These same workers, studying ten healthy human volunteers aged 25 to 45, who had normal initial plasma ascorbate levels (1.03 mg/100 ml), found that a dietary ascorbic acid supplement of 4 g daily for 3 weeks caused a 10% reduction in their plasma cholesterol levels.

Ginter (1978) has observed extensive atheromatous changes in the coronary arteries of cholesterol-fed guinea pigs with latent ascorbic acid deficiency. Almost complete occlusion of one branch of such a coronary artery after 202 d of chronic marginal ascorbic acid deficiency is shown in Figure 5. There are good reasons for believing that such observations are pertinent to human beings, for several studies have shown that vegetarians have a much lower incidence of coronary heart disease than omnivorous people, and Sacks et al. (1975)

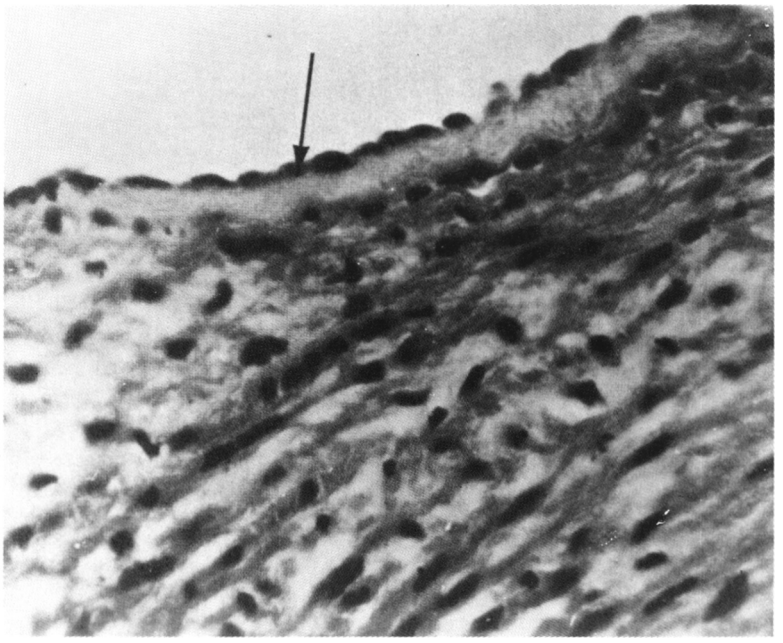

FIGURE 4 A section from the aorta of a guinea pig that had been on a marginal vitamin C-deficient diet for 105 d Note the thick band of amorphous substance underlying the endothelium (From Sulkin, N. M. and Sulkin, D. F. [1975], *Ann. N.Y Acad Sci.*, 258, 317. With permission.)

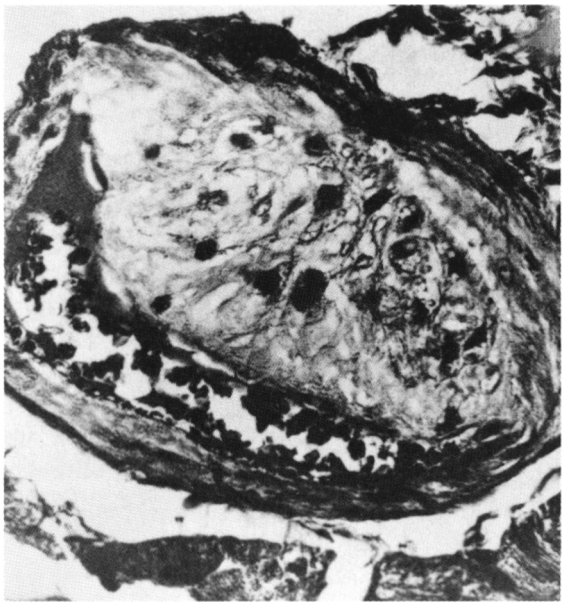

FIGURE 5. Endothelial atheroma infiltrated by histiocytes and almost completely occluding a branch of the coronary artery of a guinea pig after 202 d on a marginal vitamin C-deficient cholesterol-loaded diet. (From Ginter, E. and Bobek, P. [1981], *Vitamin C: Ascorbic Acid*, Counsell, J. N. and Hornig, D. H., Eds., Applied Science, London, chap. 17. With permission.)

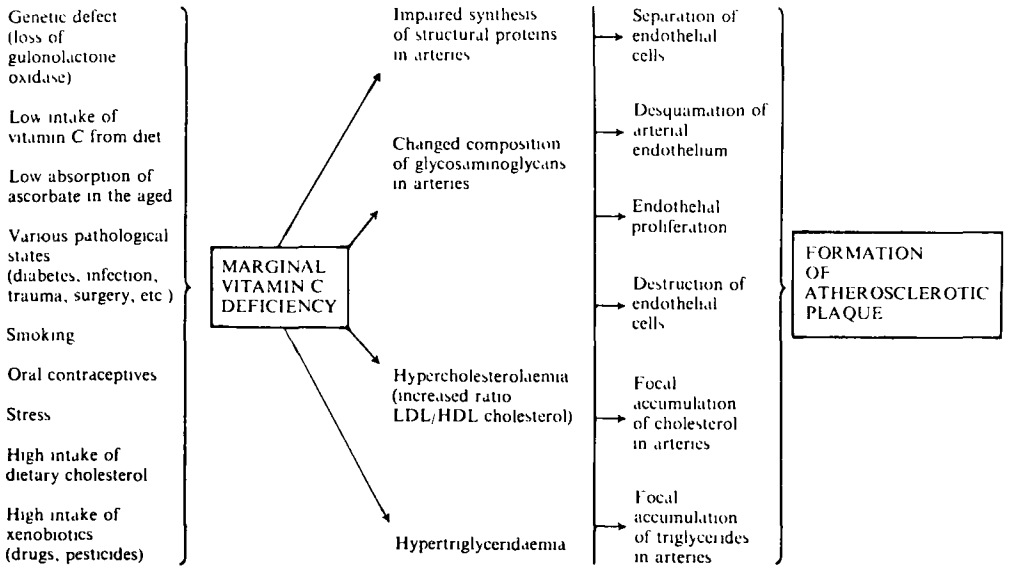

FIGURE 6. In this scheme, the authors summarize the evidence that latent vitamin C deficiency predisposes to atherosclerosis in four ways by (1) increasing the blood cholesterol level, (2) increasing the blood triglycerides, (3) altering the composition of the mucopolysaccharides of the vessel wall, and (4) impairing the synthesis of vessel wall proteins. (From Ginter, E. and Bobek, P. [1981], *Vitamin C: Ascorbic Acid*, Counsell, J. N. and Hornig, D. H., Eds., Applied Science, London, chap. 17. With permission.)

have shown that vegetarians have much lower plasma cholesterol and triglyceride levels than control subjects eating the usual American diet. Clearly, the high ascorbic acid and the low cholesterol of the vegetarian diet are both beneficial in this regard.

Ginter (1978) and Ginter and Bobek (1981) have written extensive reviews of the evidence relating hypovitaminosis C to atherogenesis. They point out that subclinical vitamin C deficiency predisposes to atherosclerosis, not only by increasing the blood cholesterol and triglyceride levels, but also by interfering with the integrity of the mucopolysaccharides and the collagen of the arterial wall (Figure 6). Ascorbic acid deficiency also damages the endothelium of the aorta, as shown by Gore (1965a) and Fujinami (1980). This may be the most serious initiator of atheroma, as the lipids accumulate just beneath the damaged endothelium. Lopez et al. (1978) have studied the effects of ascorbic acid supplements on platelet aggregation in human subjects. They reported an increase in the lag time of collagen-induced platelet aggregation, but no change in the lag time of epinephrine- or ATP-induced platelet aggregation.

Studying 63 patients with angiographically proven arteriosclerosis obliterans of the lower limbs, Heine and Norden (1979) reported the results of treatment with ascorbic acid (1 g daily) for an average of 16.2 months (3 to 53 months). They found a significant decrease in the overall mean cholesterol level of these patients from 330 to 293 mg/100 ml, but found no significant change in the overall serum triglycerides. Grouping these patients according to the hyperlipoproteinemia classification of Frederickson, they found that the best results had been obtained in patients with type IIa lipoproteinemia, who showed a significant reduction in both serum cholesterol and triglycerides. There was also a significant fall in the cholesterol level in group IIb, but the reduction of the triglyceride level was not significant. In type IV, the mean cholesterol level fell significantly, but the triglycerides showed a nonsignificant rise. In type V, there was no significant change in either the cholesterol or the triglyceride levels. However, since 62% of the patients belonged to type IIa or IIb and 31.5% belonged to type IV, the overall cholesterol-reducing effect was encouraging. Un-

Table 2
DISTRIBUTION OF LEUCOCYTE ASCORBATE BETWEEN PATIENTS WITH NORMAL AND ABNORMAL CORONARY ARTERIES

Group	Low leucocyte ascorbate (<21 μg per 10^8 cells)	Normal leucocyte ascorbate (>21 μg per 10^8 cells)
Normal coronary arteries		
Total group	51.0% (25)	49.0% (24)
Smokers	54.2% (13)	45.8% (11)
Nonsmokers	48.0% (12)	52.0% (13)
Abnormal coronary arteries		
Total group	91.1% (92)	8.9% (9)
Smokers	89.4% (42)	10.6% (5)
Nonsmokers	92.6% (50)	7.4% (4)

Note: Figures in parentheses give the numbers of patients in the subgroup. Analysis of the data provided by Ramirez and Flowers (1980) concerning the leukocyte ascorbic acid levels of patients with and without coronary artery disease.

From Ginter, E. and Bobek, P. (1981), *Vitamin C: Ascorbic Acid*, Counsell, J. N. and Hornig, D. H., Eds., Applied Science, London, chap. 17. With permission.

fortunately, there was no difference regarding the incidence of hemorrhagic or thromboembolic complications in the groups having or not being given vitamin C; it would seem that their disease was too far advanced for reversal.

Studies by Manku et al. (1979) have revealed that ascorbic acid in the concentration range from 1 to 10 mg/100 ml caused a dose-dependent and highly significant enhancement of the conversion of dihomogammalinolenic acid (DGLA) to prostaglandin (PG) E1 and to thromboxane (TX) B1 by human blood platelets, but ascorbic acid in this physiological range had no effect on the conversion of arachidonic acid to PGE2 and TXB2. Vitamin C can therefore selectively enhance the formation of cyclooxygenase-generated products from DGLA (Series 1) without changing those of arachidonic acid (Series 2). This may be of great importance as regards the beneficial effects of ascorbic acid on the vasculature, as Series 1 products of DGLA metabolism inhibit platelet aggregation, while Series 2 products of arachidonic acid metabolism promote platelet aggregation.

Horrobin et al. (1979) point out that the essential fatty acid, arachidonic acid, is usually in plentiful supply, while DGLA is an essential fatty acid which is found only in small amounts in the body and may often be wanting. They therefore stress the importance of adequate supplies of essential fatty acids in the diet and suggest that giving ascorbic acid supplements without an adequate supply of DGLA may be ineffective. Ramirez and Flowers (1980) compared the ascorbic acid status of patients with and without coronary artery disease, as demonstrated by cardiac catheterization and coronary cinearteriography. The mean leukocyte ascorbic acid level in patients with coronary atherosclerosis (13.49 μg/10^8 cells) was significantly lower than that found in people with normal coronary arteries (22.83 μg/10^8 cells; $p < 0.001$). Indeed, 90% of those with coronary heart disease were found to have low leukocyte ascorbic acid levels (<21 μg/10^8 cells), as shown in Table 2.

Verlangieri and Sestito (1981) have demonstrated that increasing quantities of insulin cause an increased transport of radioactive ascorbic acid into bovine heart endothelial cells. Moreover, studies of human cell preparations by Bigley et al. (1982) and by Moser and Weber (1984) have demonstrated that hyperglycemia impairs the cellular uptake of ascorbic acid. So it now seems probable that the microangiopathy of diabetes and the atherosclerosis, which is so common in diabetes, may both be initiated by endothelial cellular ascorbic acid

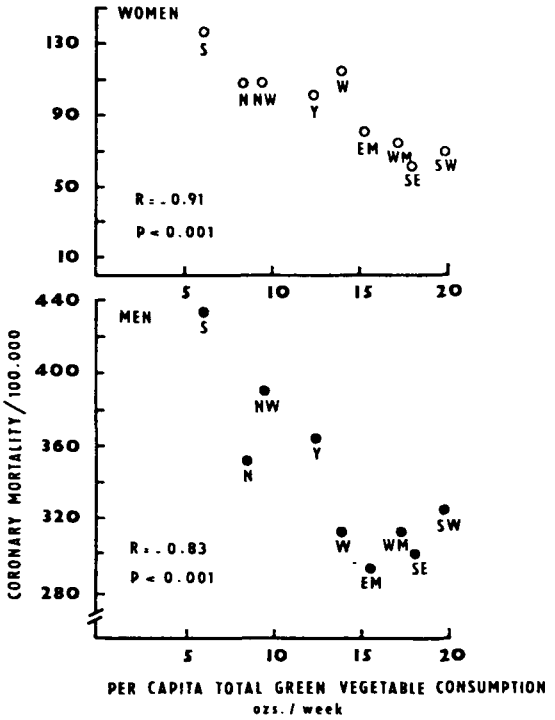

FIGURE 7. Male and female ischemic heart disease mortality plotted against total fresh green vegetable consumption in nine regions of England, Wales, and Scotland. Key: S, Scotland; N, northern region; NW, northwestern region; Y, Yorkshire and Humberside; W, Wales; WM, west midlands; EM, east midlands; SW, southwestern region; SE, southeastern region. (From Armstrong, B. K., Mann, J. I., Adelstein, A. M., and Eskin, F. [1975], *J. Chronic Dis.*, 28, 455. ©1975 Pergamon Press, Ltd. With permission.)

deficiency. Sadava et al. (1982) confirmed that ascorbic acid has a protective effect against cholesterol-induced aortic changes in rabbits.

Epidemiological studies reported by Armstrong et al. (1975), Phillips et al. (1978), Stamler (1979), Ginter and Bobek (1981), and Hanck (1982), including data from different countries and different regions of the world, have all reported significant negative correlations between the intake of fresh fruits and vegetables and the incidence of coronary heart attacks. Armstrong et al. (1975) reported a highly significant negative correlation between the total consumption of fresh green vegetables and mortality from ischemic heart disease in men and women in nine regions of England, Scotland, and Wales (Figure 7).

Studying members of the "Seventh-Day Adventist" religious denomination in California, Phillips et al. (1978) found that the risk of fatal coronary heart disease among nonvegetarian men aged 35 to 64 was three times greater than in vegetarians of comparable age ($p < 0.01$). This differential was much smaller for older men and for women. Ginter and Bobek (1981) have constructed a graph showing a significant negative correlation between the consumption of fruits and nonstarchy vegetables and coronary heart disease by analysis of World Health Organization data for 20 countries, assembled by Stamler (1979) (Figure 8).

Hanck (1982) has reported that a high rate of heart attacks in Scotland is associated with a low intake of ascorbic acid and unsaturated fatty acids, while in northern Italy, there is a low incidence of heart attacks associated with a higher intake of ascorbic acid and unsaturated

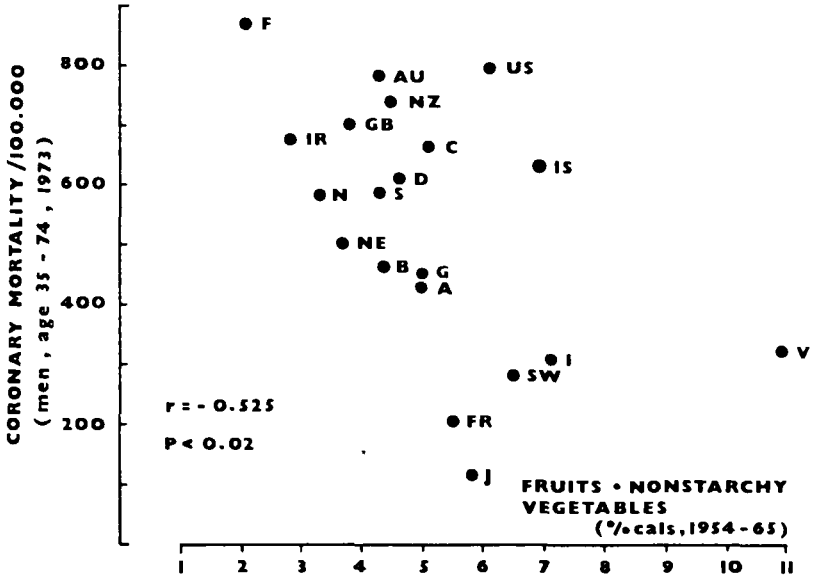

FIGURE 8. Significant negative correlation between consumption of fruits and nonstarchy vegetables and coronary mortality. Key: F, Finland; AU, Australia; GB, Great Britain; US, United States; NZ, New Zealand; IR, Republic of Ireland; C, Canada; IS, Israel; D, Denmark; S, Sweden; N, Norway; NE, Netherlands; B, Belgium; G, German Federal Republic; A, Austria; V, Venezuela; I, Italy; SW, Switzerland; FR, France; J, Japan. The graph was constructed on the basis of World Health Organization data provided by Stamler (1979). (From Ginter, E. and Bobek, P. [1981], *Vitamin C: Ascorbic Acid*, Counsell, J. N. and Hornig, D. H., Eds., Applied Science, London, chap. 17. With permission.)

fatty acids (olive oil). Pauling (1982) reported work he has conducted in association with Emstrom, in California, where several hundred people, aged 65 years or older, receiving an average of 1800 mg of ascorbic acid daily, have been found to have an age-standardized death rate around 45% of that for California as a whole.

Sinclair (1979) has pointed out that, "Eskimos when on their traditional diet, have the highest fat intake in the World and no dietary fibre, but even relatively long-lived Eskimos have almost none of our non-infective 'Western' diseases (ischaemic heart disease and other thrombotic disorders, cancer, diabetes mellitus, dental caries, multiple sclerosis, intestinal disorders, varicose veins)." This certainly seems to be a paradox. Sinclair attributes the apparent immunity of the Eskimos from atheroma to their high intake of essential fatty acids from fish and seal meat. This suggestion is certainly consistent with the DGLA-deficiency theory of Horrobin. Another explanation suggested by the writer is that the absence of heavy metals from the snowy water supply of the Eskimos makes dietary chelating fiber unnecessary; they obtain vitamin C by eating uncooked fish and meat; they do not waste this vitamin by the accumulation of heavy metals from their water supply. As mentioned in the section of this book devoted to heavy metals and water supplies, there is overwhelming evidence that deaths from cardiovascular disease are more common in soft-water areas than in hard-water areas of the U.S., the U.K., Japan, and other countries. The first morning water drawn from copper pipes, in soft-water areas, often contains as much as 2 ppm of copper, while hard water coats the pipes with "fur" and thus reduces the amount of metal dissolving in the water.

Clearly, the most successful use of ascorbic acid supplements will be in areas where there is a seasonal dietary deficit of vitamin C, as observed by Ginter et al. (1970). When tissue ascorbic acid deficiency is due to an abnormality of ascorbic acid metabolism, the treatment

will not be so simple. Heavy metals may need to be removed from the body by the use of insoluble chelating fiber, as indeed is the case with the Bantu, who have ascorbic acid deficiency due to hemosiderosis. Tea catechins have been shown to reduce iron absorption in the Bantu. The writer recommends that ascorbic acid should always be given with a chelating antioxidant such as (+)-catechin.

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Chapter 9

MENTAL DEPRESSION

I. OBSERVATIONS IN HUMANS

It is interesting to note that Woodall in his book *The Surgeon's Mate*, published in 1639, recognized that depression was one of the earliest signs of scurvy. He advised ship's surgeons to look for men who had failed to appear in the mess and to search the cabins night and morning. The psychological effects of scurvy were vividly described by the Reverend Richard Walter, Chaplain on board the *Centurion*, in his account of Lord Anson's voyage around the world from 1740 to 1744, published in 1748. He states, "This disease is likewise attended with a strange dejection of the spirits, and with shiverings, tremblings and with a disposition to be seized with the most dreadful terrors on the slightest accident. Indeed, it was most remarkable, in all our reiterated experience of this malady that whatever discouraged our people, or at any time damped their hopes, never failed to add vigor to the distemper; for it usually killed those who were in the last stages of it, and confined those to their hammocks who were before capable of some kind of duty."

Discussing the onset of the disease in 22 patients with scurvy who had come under his care at the Sabbatsberg Hospital in Stockholm, Öhnell (1928) stated, "The symptoms associated with latent scurvy, fatigue, mental depression, drowsiness, feeling of oppression, rheumatic pains, dry skin, keratosis, and perhaps anaemia are so little characteristic as to make a definite diagnosis impossible, even should a previous diet poor in C-vitamines and perhaps endemic conditions lead the suspicion in the right direction. A method of diagnosing latent, sporadic scurvy, particularly in our country with a fare during a great part of the year deficient in C-vitamines would be of exceedingly great service." Öhnell also stated that, "Neurotic conditions have been of exceedingly frequent occurrence, obvious psychosis in two further cases and suspicious psychosis in one case."

The sad dejection resulting from scurvy is very well illustrated in the description by Göthlin (1931) of a child he observed during a nutritional survey conducted under the auspices of the University of Uppsala in 1930. "Of all the Uppland school children examined, the most deficient in vitamin C was a 12-year-old girl from a woodland parish. She had the typical gums of scurvy, spongy, swollen and hyperemic at the edges, and her breath was foul. Some of her teeth were slightly loose. In the capillary strength test, she exhibited no less than eight capillary hemorrhages with as low a pressure as 35 mm Hg. I shall never forget how this little girl with the dull, tired, resigned expression of face, through five weeks' intensive treatment with orange juice awakened, so to speak, how her movements became livelier and her eyes grew bright, and how her looks showed what pleasure she got out of life. She told her teacher herself that she was much better through eating oranges. It was also observed that the foulness of her breath disappeared, although no change was made or even suggested in her highly primitive mouth hygiene during the orange juice treatment." In April and May, 18% of the children in Uppland were found to be vitamin C deficient; their nutrition was markedly improved in summer, as soon as the cows were put out to fresh pasture, and when the new potatoes and the raw berries and fruits became available.

Wood (1935) gave a full account of the medical history and physical findings relating to a 53-year-old woman with scurvy, but his description of her mental attitude is most pertinent here. "She complained of pains in various parts of her body, a rash on her legs and weakness. She was depressed, irritable and negativistic, so that a complete history was difficult to elicit, and investigations had to be limited." She received a 600-mg test dose of ascorbic acid followed by 80 mg daily, but she discharged herself from hospital before obtaining full

benefit from the treatment. "It was not possible to estimate the reticulocyte response owing to the patient's objections."

Bersot (1936) reported decreased urinary excretion of vitamin C in patients with certain mental disorders. The results of subjecting ten young men, aged 20 to 30 years, to a slowly induced vitamin C deficiency were reported by Farmer (1944). They all ate overcooked food from a cafeteria, such as an individual might ordinarily obtain by poor selection. It provided 0 to 5 or 10 mg of ascorbic acid a day. The diets of five of them were also designed to be deficient in vitamin B complex. Two control subjects received daily supplements of B complex and 75 mg of ascorbic acid, which was later increased to 150 mg. The plasma ascorbic acid levels of the test subjects fell to 0 after an average of 70 d, and their white cell platelet ascorbic acid levels reached 0 toward the end of the fifth month. Subjects in both depletion groups showed a measurable decrease in work output on the bicycle ergometer, that of the controls remaining constant. All of the vitamin C-deficient subjects complained of very severe fatigue during the last 2 months of depletion, even though they showed no gum changes or other clinical signs of scurvy, except some hyperkeratotic papules around the hair follicles of their legs. Their "choice-reaction-time" increased after the third to fifth month on the deficient diet. Individuals making the greatest number of errors in choice reactions or whose reaction time showed the greatest variation appeared to show the greatest debilitating effects of vitamin C deficiency. The latter effect was associated with loss of interest or motivation. Characteristics such as aggressiveness, submissiveness, etc. became exaggerated during vitamin C depletion, but there was no measurable effect on the threshold of perception, the coordination of motion on a "pursuit meter", or the critical-fusion frequency of visual flicker.

Wade et al. (1946) reported on four patients with adult scurvy. One of them was a 35-year-old male imbecile who was refusing all food when admitted to hospital. He screamed constantly and had to be restrained in order to carry out any sort of an examination or procedure. He was given 500 mg of ascorbic acid intravenously, followed by 100 mg three times daily by mouth. There was prompt cessation of the hemorrhagic manifestations and disappearance of the edema. The patient improved mentally and began to eat regular ward diet several days later.

Cutforth (1958) reported 11 cases of adult scurvy seen in the years 1951 to 1957 in 2 London teaching hospitals. He stated that more than half of these patients on admission to hospital were depressed, resentful, and rather uncooperative. After a few days of ascorbic acid treatment, however, this state disappeared and the patients became normal and cheerful.

The work of Kubala and Katz (1960), of Texas Woman's University, showed a significant correlation between plasma ascorbic acid levels and performance in a range of "intelligence quotient" tests by school children. They also observed a significant increase in the test performance following dietary supplementation with oranges, even among groups where nutritional deficiencies would not be expected. It was therefore concluded that alertness or sharpness of mind is influenced by nutritional state and that some component of oranges has a decidedly beneficial effect.

Punekar (1961) found the mean blood ascorbic acid (TAA)* level of five patients with depression to be lower (0.49 mg/100 ml) than in normal individuals (0.87 mg/100 ml), but provided no evidence concerning the cerebrospinal fluid (CSF) ascorbate levels. Using two standard psychiatric evaluation systems, Minnesota multiphasic personality inventory (MMPI) and Wittenborn psychiatric rating scales (WPRS) in a double-blind controlled study, Milner (1963) observed a highly significant improvement in the depression scores of chronic psychiatric patients following treatment with ascorbic acid, 1 g daily for 3 weeks.

Shafar (1965) reported three adult patients with frank scurvy due to exclusion of all

* TAA — total ascorbic acid, reduced and oxidized forms.

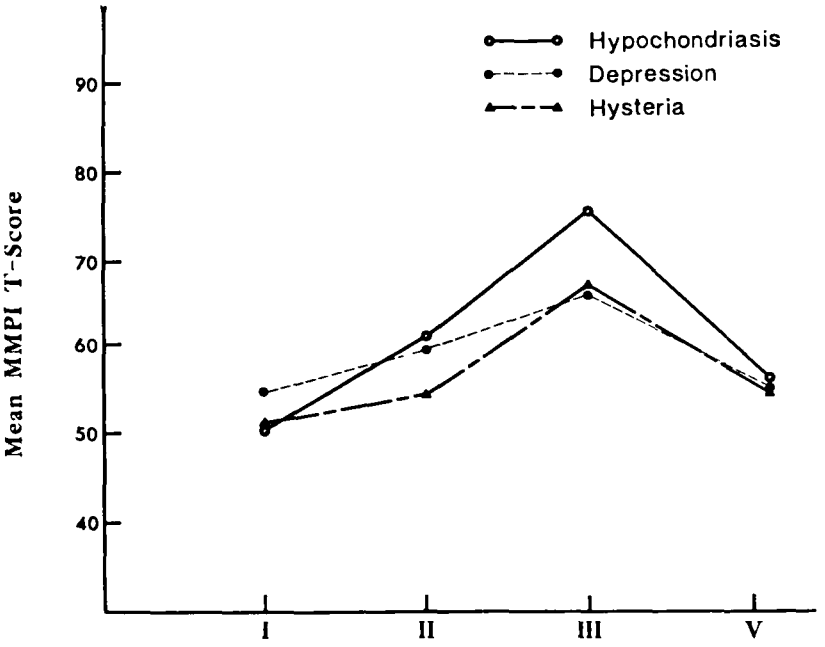
vegetables and fruit from their diets on medical advice; two had received this advice for duodenal ulcers and one for ulcerative colitis. One patient after treatment of his scurvy said, "he had not felt as well for years." One complained of tiredness and listlessness and had received psychiatric treatment for a chronic anxiety state. The other, described below, had attempted suicide and had been diagnosed as having a depressive state. "The patient, a male aged 72, was admitted with severe asthenia, which latterly had confined him to bed. Examination revealed the obvious features of scurvy over the lower limbs. Anaemia was pronounced (haemoglobin 40 per cent, red blood cells 1.4 m per c. cm) and the bone marrow was of hyperplastic megaloblastic type. Treatment with vitamin C and folic acid produced a rapid response, and after four weeks the scorbutic manifestations had resolved and the haemoglobin was 80 per cent. Six years previously the patient had been admitted to a surgical unit where a diagnosis of duodenal ulcer had been established. He improved with conservative measures but was instructed by the attending doctor to refrain from vegetables and fruit. Several months before the present admission he had attempted suicide, and a depressive state was diagnosed. The depressive features cleared with the restoration to normal of his vitamin status. Barium-meal examination revealed a chronic duodenal ulcer."

Zara and Vinci (1967) reported very encouraging results from the use of vitamin C and bioflavonoids as adjuvants in the treatment of patients with senile depression. Pauling (1968) has suggested the possibility of "a localized cerebral deficiency disease" and has postulated, "that some human beings have a sort of cerebral scurvy, without any other manifestations," which may be the cause of depression or even schizophrenic psychosis. Studies of seven women with scurvy who presented at the dermatology clinic of the Sheffield Royal Infirmary were reported by Walker (1968). All seven patients presented with swelling, pain, and discoloration of the legs. The most striking feature was a curious "woody" feel of the calves of the legs. One further striking clinical feature noted in all of these patients was, "their severe depressive state at the time of admission. This cleared within a few days of starting vitamin C therapy." Initially Walker stated that she attributed this change in mood to the relief of pain, but in one case, "the depression was cured long before the pain in her ulcerated legs settled."

The clinical manifestations of scurvy include fatigue, petechial hemorrhages, follicular hyperkeratosis, aching limbs, swollen bleeding gums, and effusions into the joints. However, studies of five human volunteers by Kinsman and Hood (1971) during 15 weeks of vitamin C depletion have shown by psychometric testing that personality changes, notably hypochondriasis and depression, precede the usual clinical manifestations of scurvy (Figures 1 and 2). There was a significant deterioration in mood and as tested by the MMPI ($p < 0.05$) when the mean whole blood ascorbic acid levels were low (0.44 mg/100 ml) compared with when the blood levels were high (mean, 1.65 mg/100 ml). These authors also observed a decline in psychomotor performance on the 5-min digit symbol substitution test (DSST) ($p < 0.05$) associated with reduced arousal and motivation, but the psychomotor changes did not occur until there were obvious signs of scurvy.

Hankes et al. (1974), studying hemosiderosis and scurvy in the Bantu, suggested that abnormalities in tryptophan and serotonin metabolism may be responsible for the depression which is associated with scurvy. Roger Lewin (1974) cited the findings of Smola who, while studying the effect of an ascorbic acid supplement (100 mg daily) on the respiratory diseases of Czechoslovakian miners, observed a raised mental alertness in the test group. Lewin believes this could have been due to an increase in the level of cyclic AMP in the brain. Sherry Lewin (1976), noting that ascorbic acid is necessary for the first stage of the conversion of tryptophan to serotonin, has elaborated on the idea that an impairment in the synthesis of this neurotransmitter is a possible cause of the mental depression which is associated with ascorbic acid deficiency.

Ridge et al. (1976) conducted a study of plasma and cerebrospinal fluid (CSF) ascorbic



	Depletion Phase			Post Loading Phase
Test Session	I	II	III	V
Day of Study	23	72	107	240
Mean Body Pool Ascorbic Acid (mg)	1054.60 (σ=655 20)	219.20 (σ=117.20)	75 75 (σ=43 24)	1500 (σ=0)

FIGURE 1. Changes in mean Minnesota multiphasic personality inventory (MMPI) T-scores of five volunteers at three test sessions during ascorbic acid depletion and one after resaturation, showing an inverse relationship between the body ascorbic acid pool and the T-score value for three personality scales. (From Kinsman, R. A. and Hood, J. [1971], *Am. J. Clin. Nutr.*, 24, 455. ©American Society for Clinical Nutrition. With permission.)

acid levels in epileptic patients, with and without treatment. They found the CSF ascorbic acid levels to be higher than the plasma levels, indicating an active transfer of ascorbic acid, as shown in Figure 3, where it may be seen that a plasma ascorbic acid level greater than 0.8 mg/100 ml is required to ensure saturation of the transfer process. This plasma level is believed to require a dietary ascorbic acid intake of at least 70 mg/d. Of course, any impairment of the blood/brain transfer mechanism or increase in the rate of destruction of ascorbic acid in the brain could cause a marked increase in this requirement. Indeed, one wonders whether the high brain copper levels in Wilson's disease might not cause an increased rate of destruction of ascorbic acid. This could be studied by obtaining data similar to those in Figure 3 for patients with Wilson's disease.

Mashek et al. (1977) reported that supplementation with vitamin C (100 to 150 mg/d) reduced the number of industrial accidents and increased mental alertness and efficiency. They also found that the psychomotor performance of a group of 16- to 18-year-old subjects

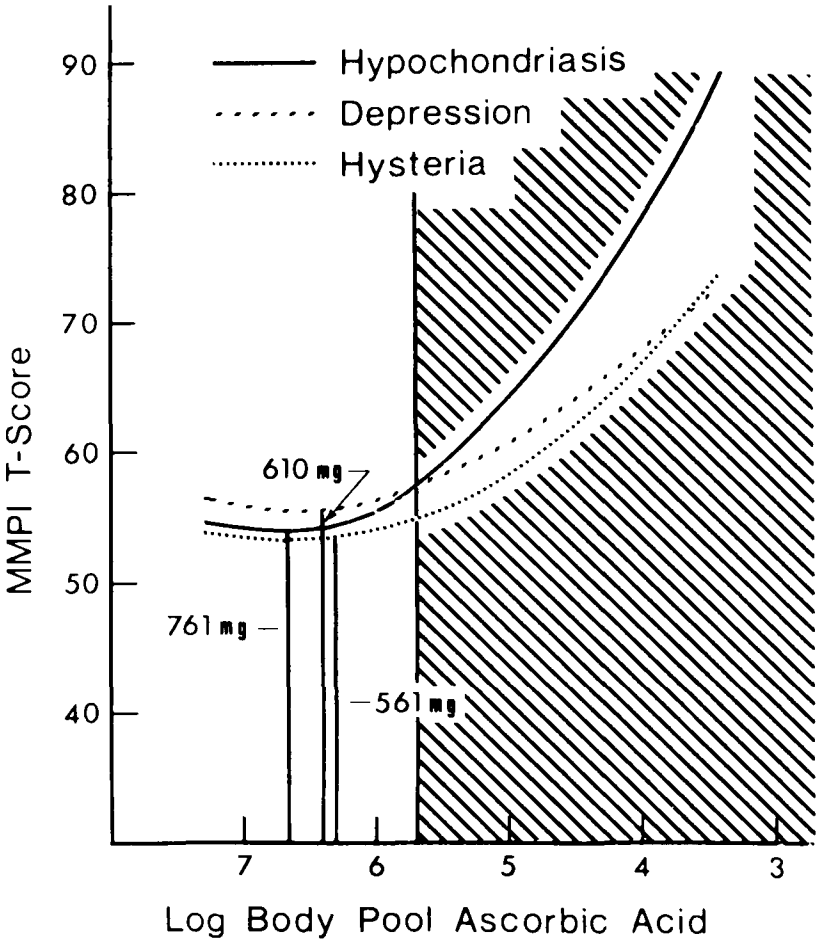


FIGURE 2. Quadratic regression lines relating MMPI T-scores for three MMPI scales to log body pool of ascorbic acid, showing points of inflection (vertical lines drawn from each regression line) at which the scale scores show initial elevation. The shaded area represents the level of body pool ascorbic acid at which obvious clinical signs of scurvy were present (log equivalent of 300 mg of body pool ascorbic acid). (From Kinsman, R. A. and Hood, J. [1971], *Am. J. Clin. Nutr.*, 24, 455. ©American Society for Clinical Nutrition. With permission.)

was improved after 1000 mg/d of vitamin C for 2 weeks. Night shift workers were found to have improved psychotechnical test results when their blood ascorbic acid level was maintained above 0.5 mg/100 ml.

On the other hand, Adam (1981), conducting a double-blind cross-over study of ascorbic acid supplementation (160 mg daily) on 20 Edinburgh University student volunteers, found no change in psychomotor performance during periods of high (1.63 mg/100 ml) and periods of low (0.62 mg/100 ml) plasma ascorbic acid levels. However, 0.62 mg/100 ml is not a very low plasma ascorbic acid level, so the results are as one might expect.

When treating patients with vitamin C and bioflavonoids for regular ovulatory menorrhagia or hypermenorrhea associated with capillary fragility, in Saskatchewan in winter, the present writer was struck by the expressions of well being volunteered by the patients when they returned after 2 months of treatment. They had not complained of feeling unwell before treatment, but often volunteered the information that they felt so much better after it.

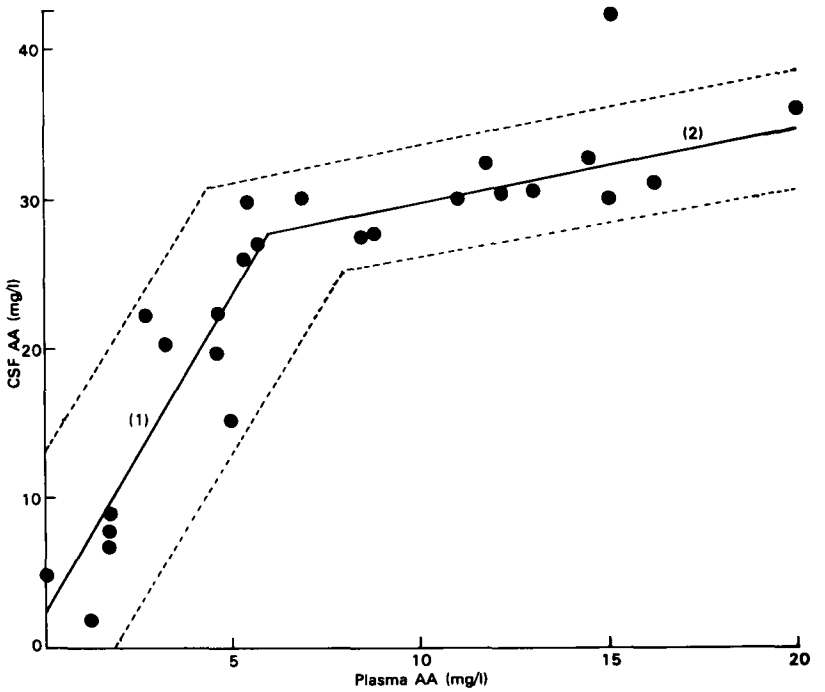


FIGURE 3. Relationship of cerebrospinal fluid (CSF) ascorbic acid (AA) level to plasma AA level in 25 subjects being treated for epilepsy. (From Ridge, B. D., Fairhurst, E., Chadwick, D., and Reynolds, E. H [1976], *Proc. Nutr. Soc.*, 35, 57A. With permission.)

II. OBSERVATIONS IN MONKEYS

Studying a Formosan long-tail female monkey *(Macacus cylopsis swinhoi)* on a vitamin C-deficient diet consisting mainly of bread and autoclaved milk, Miura and Okabe (1933) gave a close description of the animal's behavior. After 12 weeks on the diet, the animal showed decreased vigor and mild congestion and swelling of the gums; 10 d later it developed bloody diarrhea and the affected gums grew worse, both hemorrhages and swellings extending to the inner side of the upper molars on both sides. So completely depressed was the animal that it sat most of the time crouched at one corner or another of the cage. All joints appeared more or less weak. While mentally alert, the animal assumed a peculiar position the whole day long; both hind legs were straightened up at the knee joints and were thrown up together; both forelimbs grasped the iron bars on the front door of the cage; the upper half of the body was bent forward as if to place its whole weight on the forelimbs, rather than to rest upon the hips, while the face was buried between the limbs with eyes mostly staring in front. It is not possible to know whether the depression preceded the manifestations of scurvy, but the description leaves no doubt that the animal became completely dejected and inert, nor is it possible to know to what extent the inertia was due to pain. The finding by Enwonwu and Okolie (1983) of increased brain histamine levels in infant monkeys *(M. nemestrina)* on a low-ascorbic acid diet (Table 7, Chapter 12, Volume I) and the observation of a significant increase in the phenylalanine to tyrosine ratio in the brains of ascorbate-deficient animals provide plenty of room for speculation as to the cause of mental depression and apathy in ascorbic acid deficiency.

III. OBSERVATIONS IN GUINEA PIGS

Randoin (1923) cited her colleague, Lopez Lomba, as describing four stages in the behavior

of guinea pigs on a scorbutogenic diet. During the first 6 d, the animals remain perfectly well and behave normally; during days 6 to 14 (period A), they show excitation and polyuria; from day 15 to day 18 (period B), they show depression; then during days 19 to 30 (period C), the depression deepens to torpor before they die. During the stage of excitation, the animal turns in its cage and reacts quickly when one tries to catch it. Lopez Lomba was particularly struck by the depression which follows the state of excitation. Clearly these authors are describing a state of agitated depression, giving way to melancholy, followed by withdrawal, immobility, and death.

Adlard et al. (1974) studied the learning ability of guinea pigs on low- and high-ascorbic acid diets. Although the forebrain ascorbate levels of the low- and high-ascorbate groups were 0.30 and 1.11 μmol/100 g, there was no significant difference in their performance in T-maze tests.

IV. *IN VITRO* FINDINGS

The finding of Moffatt et al. (1972), that 10^{-4} *M* ascorbic acid has an inhibitory action, like that of the phenothiazine tranquilizers on the activity of cyclic 3′,5′-nucleotide phosphodiesterase activity, *in vitro* is certainly food for thought as regards the antidepressive activity of this vitamin.

V. CONCLUSIONS

The marked decrease in norepinephrine and increase in dopamine levels observed by Deana et al. (1975) in the brains of ascorbic acid-deficient guinea pigs (Table 1, Chapter 6, Volume III) suggest abnormal tyrosine metabolism as a possible cause of the mental depression of ascorbic acid deficiency. Dixit (1979) has suggested that one might find a decreased quantity of 3-methoxy-4-hydroxyphenyl glycol, the breakdown product of norepinephrine, in the urines of patients with depression due to scurvy. However, the precise state of catecholamine metabolism in scurvy is still uncertain. Cerebral histamine intoxication is another possible cause of the depression that is so characteristic of ascorbic acid deficiency.

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Chapter 10

AMYLOID

Studies of chronic scurvy in guinea pigs by Pirani and Bly (1949) and by Pirani et al. (1949) revealed definite deposits of amyloid-like material in certain organs. A total of 30 Guinea pigs were divided into 3 groups and all were fed a scorbutogenic diet. Control animals received 2 mg of ascorbic acid daily, those in the chronic scurvy group received 0.2 mg daily, and the acute scurvy group received no ascorbic acid. Amyloid-like material was found only in the guinea pigs with chronic scurvy and only in those animals which were sacrificed 8 weeks or more after the beginning of the experiment. Seven of the eight animals in the latter group showed distinct deposits of amyloid-like material. "In these cases the spleen was severely affected, the liver moderately, and the adrenal cortex only minimally. No other organ appeared to be involved."

"Microscopically, this material appeared in the spleen in the form of a thick band in the peripheral portions of the Malpighian corpuscles. In the liver it appeared in moderate amounts between the hepatic cords and the wall of the sinusoids with resulting compression and atrophy of the liver cells. In the adrenal cortex small amounts were noted in close apposition with and at times completely surrounding the walls of the capillaries." The results of tests and special stains were those usually observed both in human amyloidosis and experimental amyloidosis in animals.

Chemical analyses reported by Pirani and Catchpole (1951) revealed significant increases in the serum glycoprotein levels of guinea pigs, both in acute and in chronic scurvy. This raised the question as to whether such polysaccharide-proteins are involved in the genesis of amyloid. In any event, the finding of high serum glycoprotein levels in scurvy was consistent with the theory that scurvy involves depolymerization of mucopolysaccharides of the connective tissue ground substance, as suggested by the histochemical observations of Gersh and Catchpole (1949).

Lautsch and Gagné (1951) repeated the work of Pirani and Bly (1949) and observed definite deposits of amyloid in the spleens of four out of six guinea pigs with chronic scurvy, but found no sign of amyloid in other organs when the animals were killed after 8 weeks on the diet. The amyloid formed wide bands around the Malpighian bodies of the spleen and appeared to encroach more and more the lymphoid tissue, in some places entirely replacing it. In the two animals where no positive amyloid could be demonstrated, "there occurred a halo-like area around the Malpighian bodies, where the reticulo-endothelial cells appeared swollen and the intercellular substance was more abundant." It was felt that this represented a preamyloid stage.

Amyloidosis is a disease involving the extracellular deposition of a microscopically homogeneous fibrillary protein in various tissues, leading eventually to death when this material replaces the cells in vital organs such as the heart and the liver. It is largely responsible for senile degeneration of the brain; it also affects the pancreas in 90% of diabetics, as reported by Ravid et al. (1967). The name "amyloid" was introduced by Virchow because, like amylum or starch, this substance becomes walnut brown when treated with iodine. There are many varieties of amyloid disease, including at least two hereditary or familial types, and also several kinds of localized amyloid tumors. Amyloid disease also occurs in association with several lymphoproliferative disorders including Hodgkin's disease and multiple myelomatosis. Secondary amyloidosis also develops as a result of a variety of chronic disease states, including tuberculosis, osteomyelitis, leprosy, rheumatoid arthritis, pyelonephritis, and any form of chronic suppuration. It usually affects the liver, spleen, lymph nodes, kidneys, and adrenals, but can involve other organs. The precise chemical nature of amyloid

is not certain, although it is said to be a glycoprotein in which a mucopolysaccharide is attached to a globulin. Amyloid stains red with methyl violet; Congo red stains it pink and gives an apple-green birefringence under polarized light. The most specific method for identification of amyloid is electron microscopy, by which the typical fibrillary structure of amyloid tissue may be seen.

Primary amyloidosis develops as a progressive degenerative change in all of us with advancing age and tends to occur earlier or more markedly in men than in women. It affects the brain, the heart, the aorta, the tongue, the gastrointestinal tract, the nerve roots, the skin, the periarticular tissues, and other organs to varying extents. One should distinguish between this slowly progressing amyloid degeneration of advancing senility, which is diagnosed only by histological studies after death, and the much more rapidly progressing amyloid disease which makes itself evident by clinical manifestations such as macroglossia, hepatomegaly, proteinuria, bilateral carpal tunnel syndrome, muscle pain, bone pain, cardiac failure, or spontaneous rupture of the spleen. In these, the diagnosis may be proven by bone marrow, muscle, lymph node, or rectal biopsy; purpura is present in 40% of these clinically evident forms of amyloid disease; they have a mean survival time of only 13 months, according to Barth et al. (1969).

Amyloidosis has been produced experimentally in animals by many different agents and in many different ways, including the injection of live or killed bacteria or by *Eschericia coli* endotoxin (Barth et al., 1968) and also by repeated injections of a foreign protein such as casein. Many authorities believe that amyloid results from an autoimmune process or an antigen-antibody reaction, but the variety of procedures used to produce amyloid make it difficult to develop any unifying hypothesis concerning its pathogenesis. Its occurrence in old dogs as well as its production in hamsters, mice, cats, and horses make it difficult to believe that ascorbic acid deficiency could be an essential feature in its formation, as these species are all capable of synthesizing ascorbic acid. Of course it is true that there is evidence that they do not always make enough ascorbic acid under certain circumstances, for we have demonstrated a marked reduction in the blood histamine levels of dogs and rabbits following oral administration of ascorbic acid.

Teilum (1952) has observed that amyloidosis usually develops following an accumulation of plasma cells, fibroblasts, and reticuloendothelial cells. It occurs in reticuloendothelial dyscrasias and neoplasias and following chronic antigenic stimulation of the reticuloendothelial system. Teilum (1954) has also observed that nitrogen mustard, a toxic radiomimetic chloromethyl amine, caused the rapid development of extensive amyloid degeneration in the spleens of mice that had been pretreated by repeated injections of sodium caseinate. The amyloid degeneration developed within a few days following nitrogen mustard administration in mice that had not yet developed amyloid following several weeks of sodium caseinate injections.

Suggestions that amyloid might be derived from immunoglobulins arose from the high incidence of anomalous immunoglobulins in the blood and urine of patients with primary amyloidosis, as reported by Osserman (1959) and Cathcart et al. (1972), and from the demonstration of immunoglobulins in amyloid deposits by immunofluorescent studies. However, Teilum (1964) reported the development of amyloid disease in a patient with agammaglobulinemia; so one is inclined to agree with the view expressed by Barth et al. (1969) that serum proteins probably adsorb onto amyloid after its formation and are not specifically related to its pathogenesis.

It is true that the amyloid fibril protein from a patient with primary amyloidosis, from one with secondary amyloidosis, and from a patient with myeloma were studied in a sequential amino acid analyzer by Glenner et al. (1971a) and were reported as being homologous to fragments of immunoglobulin. Furthermore, Glenner et al. (1971b) have created amyloid-like "antiparallel β-pleated sheet fibrils" *in vitro* by proteolytic digestion of Bence-

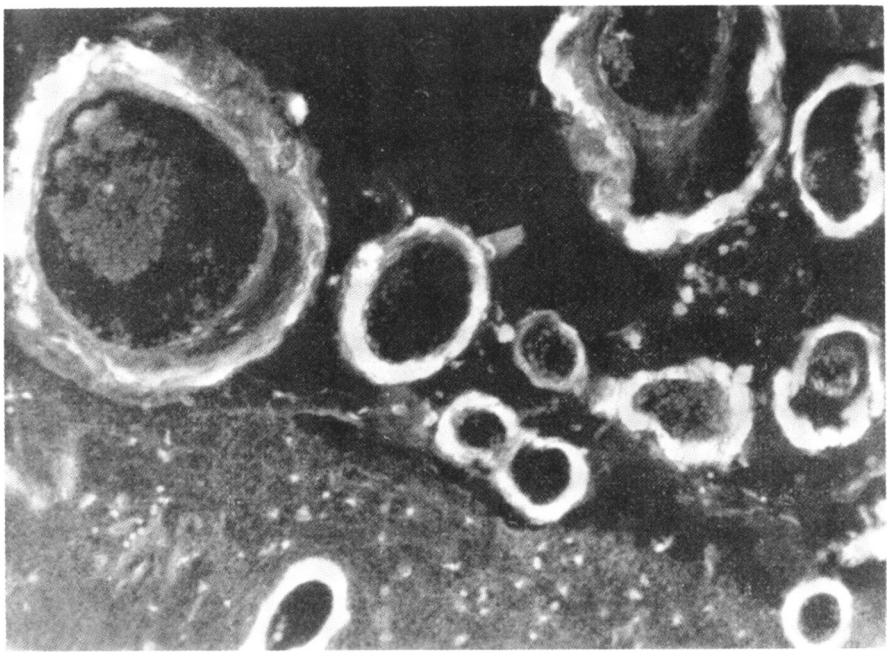

FIGURE 1 Intense meningocortical amyloidosis in the insular cortex of a 69-year-old woman (clinical diagnosis: chronic brain syndrome, senile brain disease, psychotic reaction) The emaciated patient died of pneumonia. (From Schwartz, P [1967], *Psychosomatics*, 7, 12. With permission.)

Jones protein. However, one should not be surprised to find immunoglobulins among the debris of dead reticuloendothelial cells, no matter how they were killed. Speculation that amyloid degeneration might be due to some prooxidant toxin, like dehydroascorbic acid (DHAA), affecting the reticuloendothelial cells, might explain why amyloid occurs in ascorbic acid-deficient guinea pigs, and this might explain its occurrence following chronic infections where AA* levels are low and DHAA predominates in the tissues, but speculation is all it is.

It would be interesting to know whether amyloid could be produced by intravenous injection of DHAA or by repeated injections of histamine, such as might be released by dying mast cells, plasma cells, or eosinophils. Goodman and Gilman (1941), writing about histamine, stated, "An amount, which would be toxic if it were liberated, is present in a bound and inactive form in the tissues of all mammals." Schwartz (1967), the senior pathologist at a large state mental institution in Pennsylvania, reported being able to detect amyloid in a previously unprecedented frequency in the organs of aged persons by special treatment of histological sections. He used thioflavin-S, a fluorochrome which causes a characteristic fluorescence by even minimal amounts of amyloid when viewed in UV light. Schwartz stated, "In the aged, infiltration of many organs and tissues, particularly of the brain, the cardiovascular system and of the pancreatic islets by a morbid substance, amyloid, occurs independently of additioal changes and even more frequently than hardening and atheromatous lesions of the arteries."

As a result of post-mortem studies on 400 cases of presenile and senile deterioration of the brain, Schwartz and colleagues created a basis for the recognition and definition of pathological changes characterizing these disorders. They found meningeal and cortical vascular amyloidosis (Figure 1), as well as amyloid deposits in the form of "senile plaques",

* AA — ascorbic acid, reduced form.

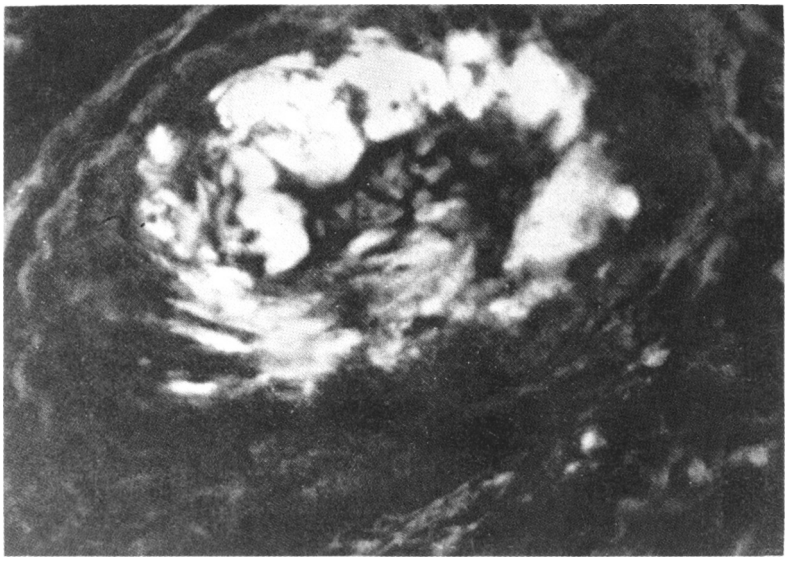

FIGURE 2. Intensely fluorescent multinodular massive amyloid degeneration of a pericardial coronary branch (89-year-old male). In the same case, a diffuse interstitial fibrillary amyloidosis of the auricles and of the atria was also present The aorta displayed extensive amyloid deposits. (From Schwartz, P. [1967], *Psychosomatics*, 7, 12. With permission.)

and amyloid deposits in cortical neurons (so-called Alzheimer cells) to be the most significant cerebral manifestations of common senile dementia and Alzheimer's disease. These changes were associated with destruction of nervous substance and extensive disappearance of functional elements.

In the heart, Schwartz observed amyloid deposits within the spaces between muscle fibers, very frequently producing diffuse, fibrillary, interstitial amyloidosis; more rarely there was multifocal, massive, interstitial amyloidosis; diffuse, massive, interstitial amyloid infiltration also occurred. These types of amyloid infiltration eventually destroy the heart muscle fibers. He also noted a peculiar form of amyloid degeneration of middle-sized and small branches of the coronary arteries (Figure 2): a multinodular, massive accumulation of amyloid thickened the vascular walls and eventually occluded the vessels (multinodular, massive, stenosing amyloidosis of coronary arteries). The aorta was one of the organs most frequently affected: amyloid deposits were found to penetrate its media, the intima, and sometimes also the adventitia. Amyloid deposits with and without sclerotic and atheromatous changes were also observed in the large and middle-sized aortic branches.

Amyloid degeneration of pancreatic islets was another very common and typical finding in the aged (Figure 3). Generally speaking, amyloid degeneration of pancreatic islets was particularly accentuated in cases of senile diabetes. However, in quite a few observations, intense insular amyloid of the pancreas prevailed, although diabetes was not recognized clinically. He stated, "Our findings indicate that there is a connection between senile amyloidosis and senile diabetes, which seem to be the two most frequent metabolic disorders in human pathology. According to our pathoanatomical findings, amyloidosis occurs more often than diabetes."

"Cerebral, cardiovascular and pancreatic insular amyloidosis are quite often associated manifestations of severe senile degeneration. In certain instances amyloid degeneration of the brain, heart, aorta and pancreatic islets displays the same high intensity. In other observations, cerebral, or perhaps cardiovascular, amyloidosis predominates. We also know of cases in which pancreatic insular amyloid was prevalent. Thus we have the impression

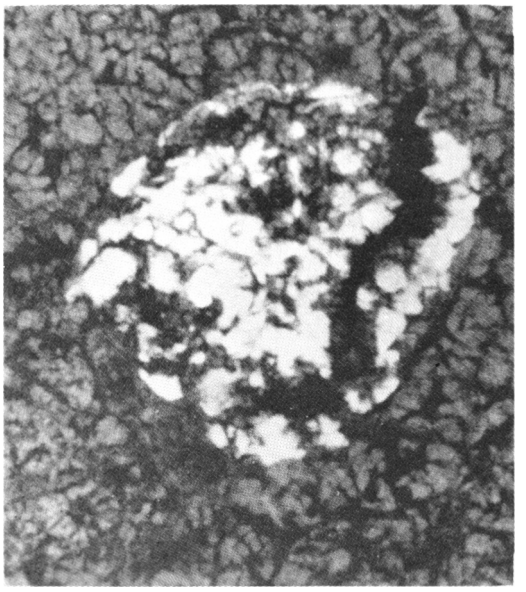

FIGURE 3. Massive amyloid deposits in an enlarged islet of Langerhans of the pancreas (clinical diagnosis: chronic brain syndrome, cerebral arteriosclerosis, diabetes mellitus, 68-year-old woman). (From Schwartz, P. [1967], *Psychosomatics,* 7, 12. With permission.)

that cerebral, cardiovascular, and insular amyloid degeneration do not always begin coincidentally. However, in due time if the patient survives, brain, cardiovascular system, and pancreatic islands will be involved more and more. At any rate, the simultaneous presence of cerebral, cardiovascular and pancreatic insular amyloid degeneration can be regarded as a characteristic triad of senile degeneration. Amyloidosis is the most common morphologically recognizable significant disease in human pathology; it seems that no one, living long enough, escapes it."

Scheinberg (1977) reviewed the evidence concerning the nature of two major protein constituents of amyloid fibrils: one known as AL, apparently derived from the terminal segment of a light chain of an immunoglobulin, the other known as AA, being a new protein that bore no relationship to any known serum protein. Scheinberg (1977) suggested that highly activated macrophages occupy a central position as mediators of the immunological responses of T and B lymphocytes, which play a key role in the pathogenesis of amyloid disease.

Evidence that immunoglobulins are not of primary importance in the pathogenesis of amyloid disease has been provided by Powers et al. (1981), using the immunoenzyme-bridge technique. With this immunoperoxidase procedure, they found that cerebral amyloid plaques from patients dying with Alzheimer's presenile dementia, senile dementia, or "normal" aging were more reactive with antibodies to neurofilament than with antibodies to plasma protein or intracellular endogenous proteins. These same workers observed that cerebral arterial amyloid was most reactive with plasma protein antibodies. It seems that there are several different amyloids and that the structure of each is related to its environment and to the dying tissues from which it has developed.

The facts that amyloid disease can be caused by ascorbic acid deficiency, that chronic infection causes ascorbic acid deficiency and predisposes to amyloidosis, and that aging leads to ascorbic acid deficiency and causes amyloidosis make one wonder what part ascorbic

acid deficiency plays in human amyloidosis. Schwartz, in a lecture delivered at the New York Academy of Sciences on October 9, 1967, I believe, suggested that diabetes mellitus, atherosclerosis, and amyloidosis, which so commonly affect patients over the age of 55, may all have a common etiology; it is therefore interesting to note that they are all accelerated by ascorbic acid deficiency and by factors that tend to decrease ascorbic acid levels.

A thought for future research involves the nature of Reye's syndrome. Some children following a febrile illness, especially when treated with aspirin, develop hepatic failure and cerebral degeneration. Since aspirin has been found to decrease the ascorbic acid content of both the brain and the liver and since infections are also known to decrease ascorbic acid stores, it becomes pertinent to ask whether the thioflavin-S fluorescent technique has been used to study the brains of children dying of Reye's syndrome. If, indeed, these children do show evidence of amyloid degeneration of the brain, we might have an important clue toward the prevention of this tragic disease. Regression of amyloidosis has been noted by some authors after cure of the primary underlying disease, so there is definitely hope that this disease process may be arrested. However, it goes without saying that dead cerebral cells cannot be replaced.

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Chapter 11

VENOUS THROMBOSIS

Studying the arterioles, capillaries, and venules in the webb of the foot of the frog, Wharton-Jones (1850) found that any type of injury to the small blood vessels was followed by the adherence of a gray granulous substance (platelets) to the inner lining of the injured vessel. These platelets subsequently coalesce to form platelet thrombi, and fibrin is deposited around them. Hayem (1871) reported his findings on post-mortem examination of four people who died of scurvy during the seige of Paris. Hemorrhage was the most notable feature, but some veins were seen to be partly occluded by red cells surrounded by fibrin. However, this author did not think that the blood had been coagulated during life.

Virchow (1886) outlined three variables operating in the pathogenesis of thrombosis: coagulability of the blood, impedence to the flow of blood, and changes in the vessel wall. Thrombosis of a large vein was observed by Jackson and Moore (1916) in only one of their scorbutic guinea pigs, but in human studies, Aschoff and Koch (1919) observed thrombosis of large veins at autopsy in 33% of people who had died of scurvy during World War I. Hess (1920) also observed that thrombosis formation was common in scurvy.

Ralli and Sherry (1941) recorded thrombophlebitis as a complication of scurvy in a 47-year-old diabetic woman who had piled up gums, large purpuric spots, petechiae of the arms, legs, and face, and an ascorbic acid deficit of 6.7 g. Vilter et al. (1946) described their findings in 19 patients who were seriously ill with scurvy on admission to the Cincinnati General Hospital. They noted that "thromboses of saphenous, femoral, or cephalic veins often were associated with the larger ecchymoses." In one man, "there were extensive ecchymoses over the left popliteal and left antecubical areas, extending up and down the left leg and left arm. Phlebothrombosis was present in both areas, and considerable collateral venous circulation was observed over the left shoulder. There was fluid in the left knee. Small areas of purpura and perifollicular hemorrhages were noted over the arms and legs." In another scorbutic man, "there was a massive ecchymosis associated with phlebothrombosis behind the right knee." Yet another scurvy patient presented with the complaint of pain and weakness in the knees; there were confluent ecchymoses in the popliteal regions associated with phlebothrombosis.

Lamy et al. (1946) reported pleural effusions, hemosiderosis, and adrenal hypertrophy, as well as venous and arterial thrombosis, in young adults who died of malnutrition following release from German prison camps at the end of World War II. Samuels and Webster (1952), working at McGill University in Montreal, studied thrombosis in the veins of dogs. These workers noted that "the endothelium of the veins is extremely delicate, and seemingly minute trauma to the vein wall will lead to disruption of the lining and subsequent thrombosis." They observed that platelets adhere along the lines of interendothelial cement when a vessel is injured.

Crandon et al. (1958) demonstrated an increased need for ascorbic acid following major surgery. One example they cited was that of a malnourished 52-year-old man who underwent total gastrectomy and splenectomy for carcinoma of the stomach. "He successfully weathered his operation without vitamin supplements. Postoperatively, he received only 100 mg of ascorbic acid a day. With this supplement, the plasma ascorbic acid was kept just above the level that we consider to mark borderline deficiency [Table 1] and the buffy coat (leucocyte and platelet) value hovered around borderline levels." He developed thrombophlebitis on the fifth postoperative day. On the 13th postoperative day, a bilateral femoral vein ligation became necessary because of extension of the thrombosis. Shortly thereafter

Table 1
MAINTENANCE STUDIES IN A SERIOUSLY DEPLETED PATIENT UNDERGOING TOTAL GASTRECTOMY AND SPLENECTOMY

Condition of patient	Period of assay	Plasma ascorbic acid (mg/100 ml)	Buffy coat ascorbic acid (mg/100 g)	Remarks
No hypotension during operation — 8 h in duration; 4 blood transfusions given	Immediately before operation	0.12	3.1	Thoracoabdominal approach
	1st h of operation	0.15	3.7	Total gastrectomy with splenectomy and enterostomy
	3rd h of operation	0.10	7.5	
	Immediately after operation	0.10	9 3	
	1st postoperative d	0.13	6.2	100 mg of ascorbic acid intramuscularly per day
	3rd postoperative d	0.26	7.5	Wangensteen suction
Phlebitis developed; temperature 102°F and pulse 100; chlortetracycline intramuscularly begun	5th postoperative d	0.27	9.0	Eosinophil count dropped to 6/mm^3
	7th postoperative d	0.30	9.3	Wangensteen suction discontinued
	10th postoperative d	0.25	7.9	Soft-solid diet free of vitamin C begun
Draining sinus developed in wound; culture showed no growth	13th postoperative d	0.28	11.1	
	18th postoperative d	0.20	4.4	
Bilateral femoral-vein ligation	25th postoperative d	0.23	7.2	

Note: Data concerning a 52-year-old man who underwent a total gastrectomy and splenectomy for carcinoma of the stomach. He had suffered epigastric discomfort for 7 months and had been on a diet containing no fruit or vegetables. Following surgery, he received vitamin C injections, 100 mg daily for 10 d, until he was able to take solid food. Nevertheless, his ascorbate status remained inadequate and he developed venous thrombosis on the 5th day following surgery; bilateral femoral vein ligation became necessary on the 13th day; he also developed a large incisional hernia due to failure of wound healing.

From Crandon, J. H., Landau, B., Mikal, S., Balmanno, J., Jefferson, M., and Mahoney, N (1958), *N. Engl. J. Med.*, 258, 105. With permission.

the plasma and buffy coat ascorbic acid (TAA)* levels fell even further. Although this patient had no cough or distension postoperatively, a large incisional hernia developed which was subsequently considered as having been due to deficiency of ascorbic acid. It would seem likely that the bilateral femoral vein thrombosis was also precipitated by ascorbate deficiency. We can envisage very small subendothelial hemorrhages from the capillary vasa vasorum, beneath the intimal lining of the femoral veins, leading to endothelial damage, platelet aggregation, and thrombosis.

Gore et al. (1965) made a detailed study of the aortic endothelium in scorbutic guinea pigs and observed that the actual junction space between the endothelial cells, as revealed by electron microscopy, is widened and there is also depletion of subendothelial collagen fibers. They concluded that, "endothelial cell disjunction must be the essential structural basis for the occurrence of haemorrhage in scurvy." They compared these changes to the

* TAA — total ascorbic acid, reduced and oxidized forms.

effects of histamine or inflammation. This is interesting, for we are now aware of the histaminemia of scurvy. The cell junctions were considered either to consist of acid mucopolysaccharides or a substance that has affinity for them. We may conjectuve that there is depolymerization of the mucopolysaccharides in scurvy. It is highly probable that a study of the endothelium of the large veins in scurvy would reveal a similar picture, for endothelium is the same tissue which extends along the inside of all the arteries, arterioles, capillaries, venules, and veins, as well as the endocardium.

Spittle (1973) conducted a double-blind study of the incidence of deep-vein thrombosis by the ^{125}I-fibrinogen scanning technique, in patients receiving ascorbic acid (1g daily) or placebo, both before and for 2 weeks after major surgery, and also in patients with serious medical disorders. She found that vitamin C had a powerful protective effect, reducing the incidence of detectable thrombosis from 20 out of 33 (or 60%) in the placebo group to 10 out of 30 (or 33%) in those receiving ascorbic acid supplements. Subsequently, Spittle (1974) reported that with routine administration of ascorbic acid, 500 mg daily, to all surgical patients, she still saw a very occasional case of clinically evident deep-vein thrombosis, but since increasing the dose to 1 g daily, before and after surgery, she had seen no further cases. "This suggests that in the elderly, 1 gram daily is needed to provide protection against thrombotic disease." Spittle reported that vitamin C is now routinely administered to all surgical and orthopedic patients at Pinderfields Hospital in Wakefield, Yorkshire, and deep-vein thrombosis has disappeared from the wards.

Taylor et al. (1979), however, reported no significant reduction in the incidence of deep-vein thrombosis as a result of giving ascorbic acid (1 g daily) for 7 d before surgery. Postoperative thrombosis occurred in 9 out of 20 (or 45%) of those who received ascorbic acid before surgery, and in 13 out of 23 (or 57%) of those who received placebo. "Thrombus scores" were calculated by multiplying the number of elevated points on a limb by the number of days the point was elevated; the thrombus scores were 3.5 for the placebo and 2.0 for the ascorbic group. The leukocyte ascorbic acid levels of both groups of patients fell following surgery, and this fall was significantly greater on days 6 and 9 in those who developed thrombosis after surgery ($p < 0.001$), as shown in Figure 1. The authors suggested that the significantly lower leukocyte ascorbate levels of those with postoperative thrombosis resulted from adhesion of leukocytes to the vein wall, but the low ascorbate levels could equally well have been a potent contributory cause of the thrombosis. It seems unfortunate that these workers did not continue ascorbic acid supplements after surgery, as did Spittle, and also that they did not study a greater number of patients.

The common types of venous thrombosis, which can occur at any time or, more commonly, following surgery and during or after pregnancy, affect the legs and include the self-evident superficial thrombophlebitis affecting the long saphenous veins and the less evident but more dangerous phlebothrombosis involving the deep veins in the soleus muscles of the calves. Either can extend to occlude the entire femoral vein and present a serious threat of embolism and death. Pelvic vein thrombosis also presents a diagnostic problem and a serious hazard following certain forms of pelvic surgery. Certainly, stasis of blood due to inactivity plays a role, so early ambulation following childbirth or following surgery is very important in reducing the incidence of deep vein thrombosis, but such thromboses do still occur and ascorbic acid deficiency has not been abolished. Moreover, abnormalities of ascorbic acid metabolism due to surgery, etc. may play a more important role than does a simple dietary deficiency of the vitamin.

Clemetson (1968) suggested that low ascorbic acid levels might predispose to endothelial damage and thromboembolism. Moreover, Clemetson (1979) pointed out that nine factors predisposing to cerebral thrombosis, coronary thrombosis, or thrombosis in the deep veins of the calf all have one thing in common: estrogen administration, pregnancy, aging, smoking, infection, trauma, surgery, soft water, and winter season are all associated with a

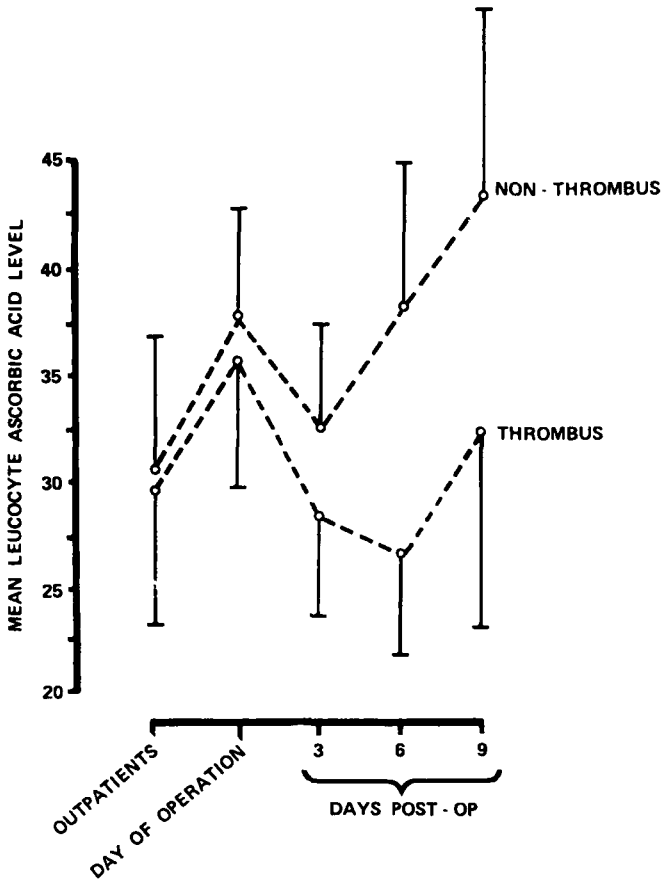

FIGURE 1. Postoperative patterns of leukocyte ascorbic acid (TAA) levels in thrombus and nonthrombus patients (95% confidence limits for each mean are shown). The ascorbate levels of the patients with evidence of deep-vein thrombosis were significantly lower than those of nonthrombosis patients on postoperative days 6 and 9 (p <0.,001). (From Taylor, T. V., Raftery, A. T., Elder, J. B., Loveday, C., Dymock, I. W., Gibbs, A. C. C., Jeacock, J., Lucas, S. B., and Pell, M. A. [1979], *Br. J. Surg.*, 66, 583. ©Butterworth & Co., Ltd. With permission.)

tendency to decreased plasma ascorbic acid levels. Since we now know that ascorbic acid deficiency is associated with elevated blood histamine levels and that the stress of long hours without sleep leads to histaminemia, we can, if we include stress, count ten factors which tend to elevate blood histamine levels and which predispose to thrombosis.

Much research and many theories have been devoted to the cause of deep-vein thrombosis and embolism, especially as regards the increased risk of these occurrences and other forms of cardiovascular disease in women taking the old high-dose combined estrogen-progestagen birth control pills. Enhanced blood coagulability, increased blood viscosity, reduced blood cell filtrability, enhanced platelet adhesiveness and aggregation, a rise in clotting factors VII and X, an enhancement of fibrinolytic activity, a fall in antithrombin III activity, and a low content of plasminogen activator in the walls of the veins were all discussed by Girdwood (1976) when he reviewed this subject, but no definite conclusions were reached.

Some authors, like Crowell et al. (1971), have reported a slight reduction in the coagulation time of the blood in women "on the pill", as measured by the partial thromboplastin time (PTT). However, it does not necessarily matter if the PTT is reduced, just so long as the

blood does not start to clot. The question is, "What causes the coagulation mechanism to be initiated?" and not, "How long does it take the blood to clot?"

So, we must look for changes in the endothelium of the blood vessels, as first suggested by Quick (1963). We are particularly interested in knowing what deleterious effects estrogens may have on the endothelium. Also we need to know whether other factors which increase the risk of death from thromboembolism could act by damaging the endothelium. We know that pregnancy and labor predispose to deep vein thrombosis and embolism (Nixon, 1955); venous thrombosis is also well recognized as occurring after infection, trauma, or surgery. Moreover, Lawrence et al. (1977) have reported that the incidence of deep-vein thrombosis is higher in the cold half of the year. Schroeder (1966) has reported that deaths from all forms of cardiac and vascular disease are higher in soft water areas than in hard water areas. Evidence that estrogen administration, pregnancy, aging, smoking, infection, trauma, surgery, soft water, and winter season are all associated with disturbances of ascorbic acid metabolism is provided in the several chapters of Volume I. Whether the association between the resultant ascorbate depletion and venous thrombosis is causal or coincidental remains to be demonstrated, but the evidence is certainly suggestive.

Horrobin (1973) recorded the development of deep-vein thrombosis in the calf on the morning after taking 3 g of vitamin C for a febrile illness the night before; he wondered if the ascorbic acid might not have precipitated the thrombosis. This is, of course, possible, as a high ratio of reduced to oxidized ascorbic acid in the blood plasma seems to be more important than the absolute amount of the vitamin. The level of dehydroascorbic acid (DHAA) is known to rise in some people under certain circumstances following the ingestion of ascorbic acid. Moreover, we have evidence of a paradoxical effect of high-dose ascorbic acid on carbohydrate metabolism, as described by Chatterjee et al. (1975), both in humans and in guinea pigs on high-cereal diets, and we have evidence of a similar paradoxical effect of high-dose ascorbic acid on bile acid and cholesterol metabolism, as described by Holloway and Rivers (1981). It would, therefore, seem wiser to recommend modest doses of ascorbic acid such as 200 mg three times a day, taken with D-catechin as an indirect chelating antioxidant, rather than megadose vitamin C, especially in areas where heavy metal catalysts such as iron or copper are prevalent in the drinking water.

In all probability, high concentrations of ascorbic acid in the endothelial cells lining the blood vessels and lower levels in the blood plasma contribute to the transmembrane potential across the endothelial surface. It may be that a plasma ascorbate level exceeding the endothelial ascorbate level, as may occur soon after consumption of a high dose of ascorbic acid, may be counterproductive in this scheme of things.

It had been customary to think of the surface charge or zeta potential (due to fixed surface radicals on the cells) as being responsible for repelling cells one from another. However, Weiss (1972) has shown that substances like ouabain, which reduce the transmembrane potential of cells by altering the assymetric distribution of K^+ and Na^+ inside and outside the cell, enhance cell adhesion. Weiss points out that this is achieved without any alteration of the zeta potential. Clearly, differences in the oxidation-reduction states (or E_h) of substances inside and outside the cells, as well as quantitative differences in the concentrations of substances such as ascorbic acid inside and outside the cells, will contribute to the transmembrane potential. Undoubtedly, streaming potentials created by blood flow and also zeta potential or surface charge play important roles in maintaining the integrity of the endothelium, but it seems quite likely that a positive injury potential due to hemorrhages beneath the endothelium will attract negatively charged platelets.

Sawyer et al. (1953, 1954) have shown that platelets are attracted to the positive pole when an electric potential difference is placed across the outside of a blood vessel. Moreover, they have demonstrated that injury to the vessel wall sets up a positive injury potential which attracts platelets and leads to thrombus formation. Born and Payling Wright (1967) reported

that platelets from scorbutic guinea pigs had a diminished capacity for adhering to glass, and Wilson et al. (1967) made similar observations in two patients with scurvy. However, Harrison and Honour (1967), studying guinea pigs with scurvy, observed that the hemostatic plugs produced by injury are firm and have their integrity unimpaired.

Sarji et al. (1979) observed that the ascorbic acid levels in washed platelets from insulin-dependent diabetics were significantly lower than those from normal subjects (25.5 vs. 45.2 $\mu g/10^{10}$ platelets; $p < 0.001$). This is presumably due to impaired ascorbic acid transport, similar to that observed by Chen et al. (1983), into the monocytes of diabetic subjects (Chapter 2, Volume II). Sarji et al. suggested that this low platelet ascorbate level might account for the hyperaggregability of platelets from diabetics. They found that a dietary supplement of ascorbic acid, 500 mg four times a day for 8 d, caused a significant inhibition of platelet aggregation in normal subjects, but had no such effect in diabetics. While this work does suggest that ascorbic acid supplements may decrease platelet aggregation in normal subjects, there does not seem to be any solid evidence that ascorbic acid deficiency or scurvy cause an increase of platelet aggregation.

Johnson et al. (1981) carried out detailed studies of platelet function in one patient with scurvy and in five volunteers who were fed an otherwise complete ascorbic acid-deficient diet for 96 d. Despite the fact that the volunteers had plasma ascorbic acid levels (mean, 0.12 mg/dl) and leukocyte ascorbic acid levels (mean 8.1 $\mu g/10^8$ cells) in the range found in scurvy, no consistent abnormality of platelet function developed. Platelet number, glass bead column retention, aggregation in response to ADP, collagen, epinephrine, sodium arachidonate, and ristocetin, serotonin release, bleeding time, and platelet retention during bleeding were all normal in the patient with scurvy. Platelet retention during bleeding decreased somewhat in all five volunteers, but none became abnormal. The bleeding time remained normal in all five subjects. Slight thrombocytopenia developed in one subject, but otherwise the platelet number and all platelet functions were normal in the ascorbic acid deficient volunteers. It was concluded that the platelet changes sometimes reported in scurvy may be due to a coexistent folic acid deficiency and that the hemorrhages of scurvy are not a consequence of any defect in platelet function.

Findlay (1921), Shattuck (1928), Bronte-Stewart (1953), Cutforth (1958), and Ginter et al. (1968) have all observed that the blood coagulation time is normal in hypovitaminosis C and in scurvy. It would seem that the hemorrhages of scurvy are due to endothelial damage and capillary fragility. They do not seem to be attributable to a failure of the blood coagulation mechanism, nor to failure of platelet function.

The role of ascorbic acid deficiency in venous thrombosis and embolism is a fascinating subject for further research.

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Chapter 12

DECREASED RESISTANCE TO INFECTION

I. THE VICIOUS CYCLE

Hess (1917), in a paper on the pathogenesis of infantile scurvy, emphasized the fact that a lack of the antiscorbutic factor which leads to scurvy at the same time predisposes to infections. In 1932 he observed that this susceptibility to infections had been confirmed by Ludwig Meyer and many others; Hess stated, "It exists before the scorbutic signs are manifest, in the stage which is better termed latent scurvy."

Höjer (1924) observed that guinea pigs fed a diet deficient in vitamin C readily contract infections. Of 189 animals with scurvy, 60 suffered from acute infections, mainly of the upper respiratory tract, and from penumonia, septicemia, and enterocolitis. Animals with latent scurvy showed about twice as many infections leading to death as those on a full diet. Of 72 animals fed an insufficient antiscorbutic diet, 47% showed signs of infection as compared with 20% of the controls. Of 62 animals fed an absolutely scorbutic diet, 29% showed signs of actue infections. However, the life span was twice as long in guinea pigs fed a relatively deficient antiscorbutic diet as in those fed an absolutely scorbutic diet.

Lawrynowicz (1931) reported observations of two vitamin C-deficient guinea pigs which led him to assert that scurvy can provoke infection from a bacterial source which, during normal nutrition, had lain dormant within the body of the animal. One was an infection with bacillus diphtheriae and the other was an infection with bacillus paratyphoid C, which developed in vitamin C-deficient guinea pigs. Analysis of these cases established that the guinea pig from which the diphtheria bacillus was obtained had been used — 2 months previously — to determine the virulence of sources of diphtheria by "crude tests". The guinea pig remained active a month later and was then used to produce scurvy; as a result of this experiment, the guinea pig died on the 37th day of the deficient diet. The anatomical changes consisted of enlargement of the spleen and enlargement and hyperemia of the adrenals. Bacteriological study showed the presence of the diphtheria bacillus in the spleen. The origin of the diphtheria bacillus could only be explained by introduction of diphtheria bacilli of low virulence at the time of the "crude tests" and that when the experiment was finished, these remained in the body without the ability to cause disease. It must have been scurvy which created the conditions in which pathological changes began to develop in the host. This was interpreted as strong evidence that scurvy caused an increase in the virulence of the microorganism. Paratyphoid C was likewise obtained from the spleen and from the blood of a guinea pig which died on the 41st day of a scorbutogenic diet. This animal had not been used in any previous experiment, and there was no known source of extrinsic infection, so it was believed that an inoffensive source within the animal had become virulent and multiplied as a result of the scurvy. Lawrynowicz suggested that this increase of virulence of organisms in the scorbutic milieu is the likely manner in which paratyphoid arose during the Russian famine of 1921 and the way in which many other epidemics may have arisen.

There is an extensive literature concerning vitamin C and resistance to tuberculosis, and some of it is contradictory and confusing. However, the work of McConkey and Smith (1933) seems to have been very direct. These authors fed tuberculous human sputum to 72 guinea pigs daily, for periods ranging from 6 weeks to 4 months. Of 37 that were maintained on a low ascorbic acid diet, 26 acquired intestinal tuberculosis. Of the other 35 animals, on a diet that was supplemented by an adequate amount of vitamin C, only 2 showed tuberculous ulcers in the intestines.

Perla and Marmorston (1937) pointed out that patients with scurvy who die generally

present at autopsy evidences of severe infection: tuberculosis, pneumonia, otitis media, furunculosis, and pyuria are reported as having been common in children dying of scurvy. Indeed, these authors report, "The frequent association of scurvy and spontaneous infection led to the erroneous conclusion that scurvy was due to an infection, and, as late as the war (World War I), organisms were isolated from the tissues of scorbutic animals, that were held responsible in part for the changes observed in scurvy." Perla and Marmorston concluded, "The latent scorbutic state is often converted into frank scurvy by infections, and under such conditions hemorrhagic phenomena are frequent."

Banerjee (1943) wrote a review of many studies implicating ascorbic acid deficiency as a cause of decreased resistance to infection. Moreover, infection depletes ascorbic acid stores, as discussed in Chapter 8, Volume I. So ascorbic acid deficiency and infection compound each other, as in a vicious cycle. This undoubtedly accounts for the fulminating infections which have so often accompanied human scurvy.

Martin et al. (1957), in the Vanderbilt cooperative study of maternal and infant nutrition, in Nashville, TN, found no relationship between puerperal fever and vitamin C intake by dietary history, but did find the incidence of puerperal fever to be significantly lower in those women whose serum vitamin C levels had been highest during pregnancy. Clearly, the serum levels of vitamin C are controlled by many factors besides dietary intake of the vitamin.

II. INCREASED SUSCEPTIBILITY TO BACTERIAL TOXINS

Work on guinea pigs by Harde (1934), Harde and Phillipe (1934), King and Menten (1935), Jungeblut and Zwemer (1935), Kligler (1936), and others showed that ascorbic acid has a direct detoxifying effect on diphtheria toxin, both *in vitro* and *in vivo*. Moreover, in their studies, King and Menten observed that there is a wide zone of vitamin C deficiency, without the appearance of scurvy, where physiological processes are subnormal and the animal is more sensitive to injury from bacterial toxin.

Recent studies by Aleo and Padh (1985), cited in Chapter 8, Volume I, have shown that the endotoxin of *Escherichia coli* inhibits the uptake of ascorbic acid by mouse fibroblasts. If endotoxin is found to have a similar effect on ascorbic acid uptake by other cells, then it will be quite evident why *E. coli* endotoxin leads to septic shock and why this condition can be so rapidly fatal. Indeed, intravenous multivitamin and ascorbic acid supplementation, as well as high-dosage cortisone and antibiotic therapy, must be started early to save the life of anyone with endotoxic shock.

III. DECREASED LOCALIZATION OF BACTERIAL INFECTION

Studying guinea pigs inoculated with tubercle bacilli, Höjer (1924) noted that, "the strength of the connective tissue reaction after a certain time is in a direct ratio to the dose of the antiscorbutic given." Studying human subjects with tuberculosis at the Väsby Sanatorium in Uppland, Sweden, he noted that the death rate was highest during the months of March, April, and May, before the cows were put out to grass and when the diet in the north was most deficient in antiscorbutic properties. In a carefully controlled study, he compared the progress of 22 patients given an orange a day and 21 patients given a special pastry during those 3 months; the orange-supplemented group fared much better than the controls. Among the 21 control patients, 11 progressed according to expectation, 2 progressed better, and 8 were worse. Among the 22 orange-supplemented patients, 6 progressed according to expectation, while 16 progressed more favorably than might have been expected.

Meyer and Meyer (1944) studied experimental staphylococcal abscesses in vitamin C-deficient guinea pigs. Not only did they find that the vitamin C-deficient animals were more

susceptible to infection, there was a more widespread inflammatory reaction, a greater diffusion of the organisms, and less localization of the infection. This may be largely due to the defective intercellular ground substance which is so characteristic of scurvy.

IV. INCREASED CAPILLARY PERMEABILITY TO VIRUSES

Sokoloff (1955) suggested that the increased capillary protein leakage associated with capillary fragility is associated with increased leakage of virus particles into the tissues. He gave vitamin C and citrus bioflavonoids, 200 to 300 mg of each, every 3 or 4 h, to five patients with virus A influenza and was impressed by the rapidity of their recovery. Likewise, Biskind and Martin (1955) found vitamin C and citrus bioflavonoids, 300 mg of each by mouth, 4 times a day, to be of inestimable value in the treatment of 69 patients with acute respiratory infections including rhinitis, pharyngitis, influenza, and tonsillitis. These authors attributed the beneficial effects to restoration of the normally impaired capillary permeability and fragility associated with these infections, but they were unable to obtain equally good results with vitamin C alone.

V. INCREASED SUSCEPTIBILITY TO PLASMODIAL INFECTION

Studying a virulent form of rodent malaria, Bourke et al. (1980) demonstrated that the administration of ascorbic acid is effective in decreasing the number of parasites in the blood and in extending the mean survival time of *P. berghei*-infected mice.

VI. INCREASED SUSCEPTIBILITY TO FUNGAL INFECTION

Rogers et al. (1983), working at Oregon State University, inoculated guinea pigs with *Candida albicans* by intracardiac injection, while they were receiving an ascorbic acid-free diet supplemented with 0.5, 20, or 100 mg/d of sodium ascorbate. The growth of *Candida* in the kidneys of the animals receiving 0.5 mg of ascorbate a day was significantly higher than in either the 20- or the 100-mg dose groups ($p < 0.02$ and $p < 0.01$, respectively) on day 3 after inoculation. It was concluded that ascorbic acid deficiency renders guinea pigs more susceptible to *Candida* during the first 3 d of infection.

VII. IMPAIRED LEUKOCYTE METABOLISM

McCall et al. (1971) reported that 0.01 *M* ascorbate inhibits the H_2O_2-myeloperoxidase-halide reactions which have been implicated in the bactericidal activity of leukocytes. Nevertheless, they found that the clearance of *Staphylococcus aureus* and *E. coli* in intact human leukocytes was normal in the presence of this concentration of ascorbic acid; they therefore concluded that some other mechanism must be active. There are three well-defined metabolic phenomena which normally accompany the ingestion of a bacterium by a neutrophil, namely, increased oxygen consumption, increased production of hydrogen peroxide, and increased hexose monophosphate shunt (HMS) activity. *In vitro* studies by Cooper et al. (1971) have shown that very low concentrations of ascorbic acid (0.0001 *M*) cause a definite increase in the HMS activity of human leukocytes, as shown in Figure 1. Cooper et al. (1972) have observed that adrenal corticosteroids decrease leukocyte HMS activity, but much less so when they have been stimulated by ascorbic acid. De Chatelet et al. (1972) observed that dehydroascorbic acid (DHAA) also caused a dramatic increase in the resting HMS activity. A sequence of reactions involving DHAA, reduced glutathione, and reduced nicotinamide adenine dinucleotide phosphate (NADPH) was postulated to explain this stimulation.

Bigley and Stankova (1974) carried out experiments in which human leukocytes were

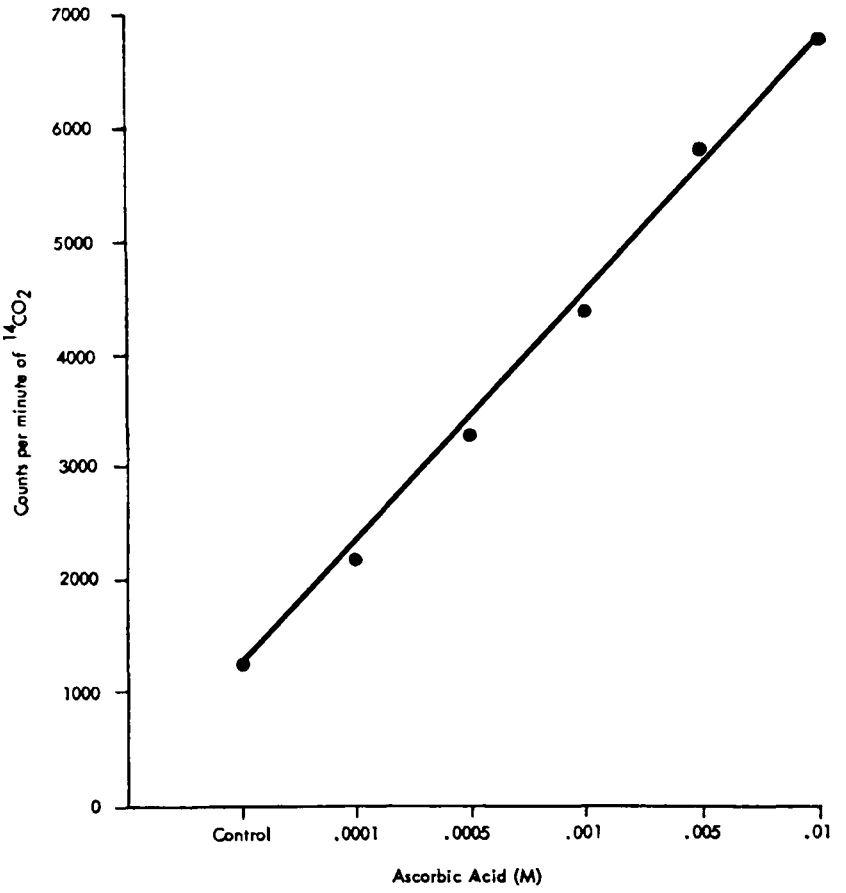

FIGURE 1. Effects of increased concentrations of ascorbic acid on the hexose monophosphate shunt activity of human neutrophils. (From Cooper, M. R., McCall, C. E., and De Chatelet, L. R. [1971], *Infect. Immun.*, 3, 851. With permission.)

incubated aerobically or anaerobically in the presence of DHAA. They found that the HMS activity of the leukocytes was directly proportional to the rate of uptake of DHAA and its reduction to AA* within the cells, but was not related to the concentration of AA already in the leukocytes. When the cells were incubated in the presence of AA, they found that HMS activity was not stimulated under anaerobic conditions and was stimulated only slowly under aerobic conditions. It seems that HMS activity of leukocytes, so closely related to phagocytosis, is dependent on the conversion of DHAA to AA within the leukocytes.

Shilotri (1977a), using *E. coli,* observed a decrease in phagocytosis-induced glycolytic and HMS activities and decreased bactericidal activity by leukocytes from vitamin C-deficient guinea pigs (vs. pair-fed controls). Further work by Shilotri (1977b) suggested that this impairment of phagocytosis-induced HMS activity of ascorbic acid-deficient guinea pig leukocytes, when challenged with *E. coli,* may be due to a lowered capacity of the cells to stimulate the enzyme NADPH-oxidase; this in turn would explain the impaired bactericidal activity. Shilotri found no change in glutathione reductase or myeloperoxidase in vitamin C-deficient guinea pigs.

* AA — ascorbic acid, reduced form.

VIII. INCREASED PLASMA CORTISOL LEVELS

Plasma corticosteroid levels are markedly increased in scorbutic guinea pigs (Chapter 8, Volume III), so the question arises as to what role cortisone plays in the impairment of the immune response in scurvy. This will be difficult to ascertain; one approach would be to maintain adrenalectomized guinea pigs by twice daily injection of gluco- and mineralocorticoids. One could then study the response to infection of these animals on high and low ascorbic acid diets.

A very interesting study was reported by Chretien and Garagusi (1973), showing that ascorbic acid was able to correct the abnormal leukocyte metabolism of six patients receiving long-term corticosteroid therapy. Normal polymorphonuclear (PMN) leukocytes reduce the colorless dye nitroblue tetrazolium (NBT) after phagocytosis to black formazam deposits which appear within the cells and serve as a histochemical marker for the enzymatic activity which normally follows phagocytosis. This burst of metabolic activity includes increased oxygen consumption, stimulation of HMS activity, oxidation of NADH, and hydrogen peroxide production and is necessary for the killing of bacteria. However, corticosteroids interfere with this metabolic activity and impair NBT reduction. Phagocytosis and NBT reduction can be stimulated by latex particles; Chretien and Garagusi (1973) demonstrated that this latex-stimulated function was impaired in leukocytes from patients receiving steroid therapy, but was completely restored to normal following the oral administration of 2 g of ascorbic acid. They suggested that all patients receiving steroids for any length of time should be given ascorbic acid supplements. They also suggested that ascorbic acid may prove to be valuable in the treatment of chronic granulomatous disease of childhood, a condition characterized by early death from infection, suppressed NBT reduction by the leukocytes, and deficiency of NADH oxidase, etc.

IX. ALTERED GENERAL METABOLISM

Hepatic enzyme glycolytic activities have been found to be depressed in ascorbic acid-deficient guinea pigs by Ganguli and Banerjee (1961). Rose and Warms (1966) have shown that the availability of NAD is a rate-limiting factor in glycolysis by human red blood cells. Wood and Beutler (1973) have reported that addition of high-dosage ascorbic acid to stored human blood results in a reduction of the lactate to pyruvate ratio of the blood, reflecting a change in ratio of NAD to NADH, due to oxidation of NADH to NAD, presumably by DHAA. Thus, it would seem that there may be a reduction in the rate of formation of NAD from NADH in ascorbic acid-deficient blood: that could cause a reduction in the rate of glycolysis and HMS activity.

X. DECREASED LEUKOCYTE MIGRATION AND CHEMOTAXIS

Bourne (1948) observed that vitamin C deficiency impairs leukocyte migration. Chemotaxis appears to play a role in the direction of ascorbate transport by leukocytes. Leukocytes are known to migrate toward wounded or infected areas. Thus, Hume et al. (1972) observed that leukocytes move toward an infarcted area of myocardium and deposit ascorbate there. This movement may be partly chemotactic and partly electrostatic. Lewin (1976) believes it may be triggered by the release of histamine from local mast cells. Preliminary experiments reported by Lewin indicate that leukocytes migrate preferentially toward areas of higher histamine concentrations.

Studies by Goetzl et al. (1974) showed that exposure of human leukocytes to glutathione or to ascorbic acid at neutral pH increased both random migration and chemotactic responsiveness to diverse stimuli, but did not affect phagocytic acitivity under the conditions of

their experiment. Sandler et al. (1975) have shown that ascorbic acid enhances the chemotactic responsiveness of human monocytes and PMN leukocytes, so it would seem likely that leukocyte chemotaxis may be impaired in scurvy.

Ganguly et al. (1976) observed that there were 25% fewer macrophages in the peritoneal exudates of ascorbic acid-deficient guinea pigs and that the macrophages showed a significant reduction in their ability to migrate. However, they observed no difference between the phagocytic abilities of normal and ascorbic acid-deficient guinea pig macrophages for *S. aureus;* the cells had been washed several times and kept cold until they were used. Anderson et al. (1980) observed enhanced neutrophil motility to a chemotactic stimulus of endotoxin-activated autologous serum in normal adult volunteers after ingestion of 2 and 3 g of ascorbate daily. Moreover, Anderson (1981) wrote an extensive review of his own works and those of others concerning the effects of ascorbic acid on neutrophil motility and bactericidal activity, not only in patients with Chediak-Higashi syndrome, but also in chronic granulomatous disease, in hyperimmunoglobulinemia E, in bronchial asthma, in patients with recurrent and chronic bacterial infections, and also in normal individuals. There are many reports of increased PMN leukocyte motility and chemotaxis following ascorbic acid treatment and many of the patients showed definite clinical improvement. Anderson pointed out that the *in vitro* effects of ascorbic acid on human neutrophils and lymphocytes are seen only in the presence of autologous serum.

Pecoraro and Chen (1987) report that normal human mononuclear leukocytes (MNL) (lymphocytes and monocytes) have a total ascorbic acid concentration 43 times that of blood plasma and that the polymorphonuclear leukocyte (PMN) level is 17 times that of plasma. However, these leukocyte ascorbic acid levels were found to be markedly reduced by the administration of glucose, resulting in a significant reduction of chemotaxis, both by MNL and PMN leukocytes.

XI. STUDIES RELATING TO CHEDIAK-HIGASHI SYNDROME

Chediak-Higashi syndrome (CHS) is a rare autosomal recessive disease characterized by partial ocular and cutaneous albinism, frequent pyogenic infections, neutropenia, and characteristic giant lysosomal PMN leukocyte granules. Boxer et al. (1976) described an 11-month-old girl with CHS. The motility and bactericidal functions of her PMN leukocytes were impaired and there was a markedly increased level of intracellular cyclic adenosine monophosphate (cAMP) in these cells. After 2 months of treatment with ascorbic acid, 200 mg daily, the leukocytes acquired normal migratory responsiveness, postphagocytic degranulation, and antimicrobial activity. Moreover, the intracellular cAMP levels were considerably decreased following the ascorbic acid treatment.

Vanderbilt et al. (1977a, b) reported that ascorbic acid enhances microtubule assembly and confirmed an improvement in PMN function in the blood of patients with CHS. Kraut (1977a, b) also found that ascorbic acid improved the functional defect of PMN function in a patient with CHS. These workers reported that ascorbic acid stimulated the HMS activity (fivefold) and formate oxidation (fourfold) in resting granulocytes without changing the level of reduced glutathione (GSH) or generating chemiluminescence. During phagocytosis, on the other hand, they found that ascorbic acid appears to act as a scavenger for oxygen radicals, descreasing the fall in GSH and the normal rise in chemiluminescence and formate.

Gallin et al. (1979) observed no improvement in the neutrophil motility or antimicrobial activity in two adult brothers with CHS following treatment with ascorbic acid, 6 g daily, for 8 months, but did find that CHS mice treated with ascorbic acid showed improved leukocyte migratory and bactericidal activities and increased resistance to infection by *C. albicans*.

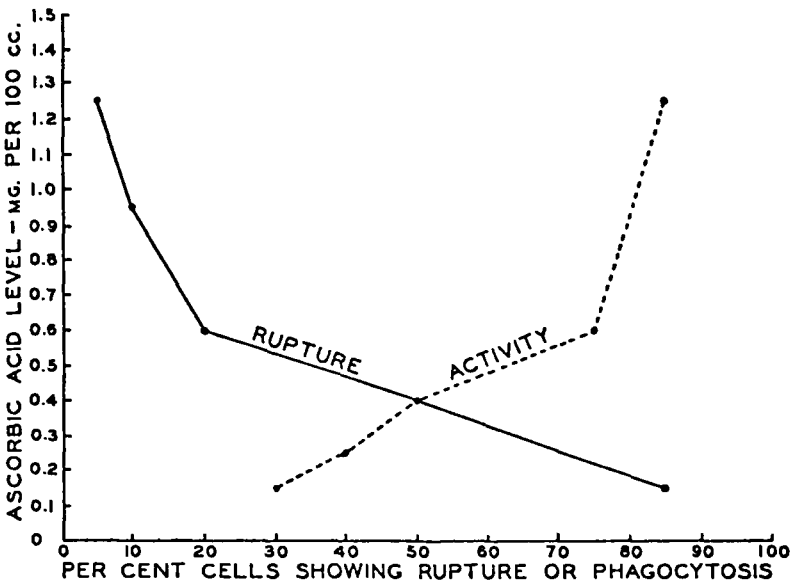

FIGURE 2. The percentage of polymorphonuclear leukocytes found to contain bacteria, after 15-min incubation with a suspension of killed beta hemolytic streptococci, is plotted against the ascorbic acid concentration of the peritoneal exudate from which the leukocytes were obtained. The number of leukocytes found to be ruptured is also shown for each ascorbate concentration. (From Nungester, W. J. and Ames, A. M. [1948], *J. Infect. Dis.*, 83, 50. ©1948 University of Chicago Press. With permission.)

XII. IMPAIRED PHAGOCYTIC ACTIVITY

A pronounced reduction in the phagocytic power of the leukocytes with respect to *E. coli* and *Mycobacterium tuberculosis* was reported in scorbutic guinea pigs by Lawrynowicz (1931). Studies by Cottingham and Mills (1943) revealed a marked reduction in the phagocytic activity by vitamin C-deficient guinea pig leukocytes. Nungester and Ames (1948) also observed a marked reduction of phagocytic activity for β-hemolytic streptococci by PMN leukocytes from ascorbic acid-deficient guinea pigs. Moreover, they noted a tendency for leukocytes from scorbutic guinea pigs to undergo spontaneous rupture. In fact, there was a positive correlation between the serum ascorbic acid level and phagocytosis and also a negative correlation with leukocyte rupture, over the physiological ascorbic acid range from 0.1 to 1.2 mg/100 ml, as shown in Figure 2.

Merchant (1950) observed a significant decrease in the activities of leukocytes from peritoneal exudates of guinea pigs which were ascorbic acid deficient. Cell diameter, pseudopod formation, motility, and phagocytic activity were all significantly greater when the ascorbic acid concentration of the peritoneal exudate was greater than 0.3 mg/100 ml. Marcus et al. (1953) found supplementary ascorbic acid to enhance the phagocytic ability of leukocytes in the mouse peritoneal cavity. This is particularly interesting, as mice make their own ascorbic acid. Even so, they seem to have benefited from a supplement of ascorbic acid in this series of experiments.

Bigley and Stankova (1974) observed that human neutrophil leukocytes have an impressive capacity for reducing dehydroascorbate and thus regenerating their content of reduced ascorbate. They have postulated that this property may function to preserve leukocyte integrity by inactivating free radicals and oxidants produced during phagocytosis. Chatterjee et al. (1975b) studied the effects of guinea pig leukocytes on *Bacillus subtilis* and observed a decreased phagocytic index in scorbutogenic diet-fed guinea pigs.

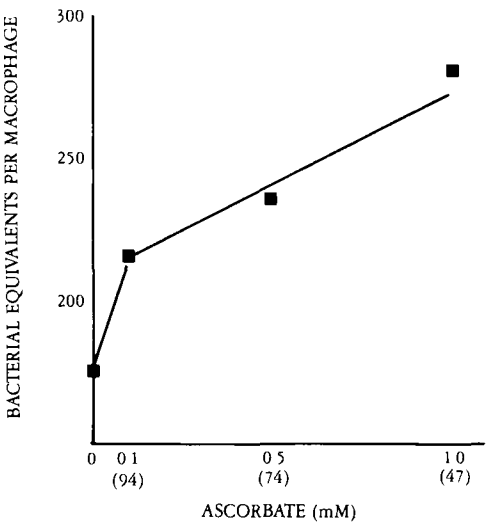

FIGURE 3. Phagocytic activity of mouse peritoneal macrophages after incubation with media supplemented with ascorbic acid. After 20 h of incubation, media containing fresh ascorbate were added and after 1 h, their ability to phagocytose heat-killed radiolabeled *Pseudomonas aeruginosa* was measured. The results are the means of three cultures in bacterial equivalents accumulated after incubation for 2 h. Ascorbic acid caused a dose-dependent increase in the phagocytic activity of the macrophages. (From Thomas, W R. and Holt, P. G. [1978], *Clin. Exp. Immunol.*, 32, 370. With permission.)

Thomas and Holt (1978) studied mouse peritoneal macrophages which had been incubated with increasing concentrations of ascorbic acid for 20 hr. Ascorbic acid caused a dose-dependent increase in their phagocytic activity, as shown in Figure 3. Bigley et al. (1982) observed that DHAA and 2-deoxy-D-glucose enter leukocytes by the same pathway. Then Chen et al. (1983) reported that high blood sugar levels cause human monocyte ascorbic acid deficiency *in vivo* (Figure 5, Chapter 2 of this volume) and that diabetics have significantly lower mononuclear leukocyte ascorbic acid levels than normal individuals. Moreover, Moser and Weber (1984) have found that glucose inhibits the uptake of ascorbic acid (1-^{14}C) by cultured human granulocytes. This may well explain the decreased resistance to infection by patients with diabetes mellitus. It seems that the granulocytes take up D-glucose and L-ascorbic acid by the same insulin-dependent active transport system, which can become saturated with sugar to the exclusion of ascorbic acid. Moser and Weber report that this transport system is inhibited by KCN and by phlorizin and is temperature sensitive. It is quite specific, as it is not inhibited by fructose, only by galactose at higher concentrations.

XIII. ALTERED BACTERICIDAL ACTIVITY

Miller (1969) suggested that hydrogen peroxide and ascorbic acid constitute a bactericidal system in phagocytic cells, which facilitates the digestion of bacteria by lysozyme. The antibacterial action appeared to depend on free radicals liberated during the oxidation of ascorbic acid by hydrogen peroxide. Model systems containing ascorbate, hydrogen peroxide, and copper or cobalt have been studied by Drath and Karnovsky (1974) and have been shown to possess marked bactericidal activity. Divalent copper in concentrations as low as

5×10^{-6} *M* still showed antimicrobial activity when present in the complete model system, whereas cobalt was active only at concentrations above 10^{-4} *M*.

Smith et al. (1975) agreed that exposure of human granulocytes to ascorbic acid *in vitro* resulted in stimulation of the HMS and augmentation of random migration and chemotaxis, but reported that neither the bactericidal nor phagocytic activity of these cells was altered by increasing ascorbic acid concentrations. Stankova et al. (1975) reported no difference between the hydrogen peroxide production or the bactericidal activity of leukocytes from guinea pigs on an ascorbic acid-deficient diet and others on a massive ascorbic acid intake (1 mg/g of body weight per day), when challenged with *S. aureus*.

Shilotri (1977a, b) observed not only decreased phagocytic activity, but also decreased bactericidal activity against *E. coli* by leukocytes from ascorbic acid-deficient guinea pigs. However, data presented by Shilotri and Bhat (1977) indicated that daily supplements of ascorbic acid (200 mg daily) did not affect the bactericidal activity of human leukocytes. Moreover, these authors reported that daily intakes of 2 g of ascorbic acid for 15 d significantly impaired bacterial killing by leukocytes. This effect was reversible, as evidenced by return to normal activity following withdrawal of the vitamin supplement.

The reason for this anomalous effect of high-dosage ascorbic acid on human leukocyte activity is not known. It could be that ascorbate-free radical or monodehydroascorbic acid produced in plasma during the oxidation of AA to DHAA can damage the leukocytes instead of the bacteria. Chatterjee et al. (1975a) have shown that high-dose ascorbic acid, both in guinea pigs and in human subjects on high-cereal diets, causes a marked increase in the DHAA content of the blood. They have shown that this accumulation of DHAA in the blood can be prevented by a high-protein diet. There are also reasons for believing that this oxidation of ascorbic acid can be prevented by the provision of enough dietary fiber to chelate heavy metal catalysts in the lumen of the bowel. If so, this is further reason for believing that a supplement of 200 mg of ascorbic acid coated with D-catechin may be preferable to 2 g of ascorbic acid alone (see Chapter 11, Volume I).

The work of Kraut et al. (1980) "suggests that ascorbic acid can play a dual metabolic role in human polymorphonuclear leukocytes. Ascorbic acid can, not only generate H_2O_2, but it may also scavenge oxygen metabolites. This reflects its ability to undergo cyclic oxidation-reduction and to act either as an oxidizing or a reducing agent." If so, we can readily understand that too much or too little ascorbic acid could swing the redox potential to one extreme or the other and this could slow or arrest the oxidation-reduction cycle.

XIV. DECREASED HUMORAL IMMUNE RESPONSE

Much of the early work on the effects of vitamin C deficiency on natural and acquired immunity was reviewed by Perla and Marmorston (1937) and Bourne (1949). Although Bourne found the work on vitamin C and complement to be contradictory, he did conclude that vitamin C plays an important role in antibody production and increases the inactivation of toxins, not only in guinea pigs, but also in rabbits.

Long (1950) observed a reduced secondary immune response to diphtheria toxoid in guinea pigs which had received their primary inoculum on day 35 of a low-ascorbic acid diet, which did not produce scurvy. In contrast, Kumar and Axelrod (1969) found no alteration in the primary or secondary antibody response to challenges with the same antigen in severely scorbutic guinea pigs. The reasons for this apparent paradox may simply be the ascorbate status of the animals at the time of the primary challenge. Kumar and Axelrod gave the first inoculation after only 1 week on a scorbutogenic diet when testing for primary immunity and 1 week before starting on the diet when testing for secondary immunity. The high mortality rate after 3 or 4 weeks in guinea pigs fed on this diet (which was completely devoid of ascorbic acid) precluded the primary administration of antigen at a later stage in these experiments.

Prinz et al. (1977) carried out a study on 45 human volunteers between the ages of 18 and 21 years; 25 were given 1 g of ascorbic acid a day for 75 d and 20 received no supplement. Estimations, carried out before and after the period of supplementation, showed significant increases in serum immunoglobulins A and M (IgA and IgM) and C-3 complement levels. Cortisol and transcortin levels were not affected. Serum IgG increased slightly.

Studies reported by Vallance (1977) of work he conducted during the British Antarctic Survey in 1972 showed direct relationships between plasma ascorbic acid (TAA)* levels and serum IgG and IgM levels. Those between IgG and leukocyte ascorbic acid ($p < 0.05$) and plasma ascorbic acid ($p < 0.01$) and between IgM and leukocyte ascorbic acid ($p < 0.01$) were statistically significant, while that between IgM and plasma ascorbate was not. IgG (but not IgM) significantly increased ($p < 0.02$) in four subjects who received a dietary supplement of ascorbic acid, 1 g daily, for a week. The specific immunoglobulin (IgG) levels were affected more than those of the nonspecific immunoglobulin (IgM), suggesting that ascorbic acid could improve response to infection rather than having a prophylactic effect against infection.

XV. DECREASED PRODUCTION OF INTERFERON

Isaacs and Lindenmann (1957) reported the discovery of interferon, a protein produced by cells infected by a virus, which has the property of increasing the resistance of neighboring cells to virus attack. Pauling (1970) proposed the hypothesis that ascorbic acid may provide some protection against virus diseases by playing a part in the synthesis and activity of interferon. This theory has since been substantiated by the work of Siegel (1975), Geber et al. (1975), Dahl and Degre (1976), and Siegel and Morton (1977). Dahl, using human tissue cultures, found that addition of ascorbic acid led to increased production of interferon when stimulated by several viruses.

XVI. DECREASE IN THYMIC HUMORAL FACTORS

Dieter and Breitenbach (1968) reported a marked decrease in the ascorbic acid content of the thymus of cockerels during age involution, accompanied by a threefold rise in the ratio of oxidized to reduced ascorbic acid. These findings suggested an important role for ascorbic acid in the development and maintenance of lymphoid tissue. Dieter (1969) studied the effects of thymus extracts from groups of guinea pigs on high and low ascorbic acid intakes. He used the restoration of lymphoid organ weights and HMS enzyme activities in X-irradiated rats as indicators of "thymic humoral factor" effects. The thymic humoral factors from ascorbic acid-supplemented animals restored the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconic dehydrogenase in the thymus, lymph nodes, and spleen. Conversely, thymus extracts from ascorbic acid-deficient animals resulted in a further weight loss in the lymph nodes and spleen. Thus, it would seem that vitamin C is necessary for the normal production or optimal activity of thymic humoral factors in the guinea pig.

While the concentration of total ascorbic acid in the thymus of the guinea pigs receiving the low-ascorbic acid diet had decreased only 40% below normal, the ratio of oxidized to reduced ascorbic acid had increased fivefold (Table 1). This and subsequent studies by Dieter (1971) have led to the conclusion that the oxidation state of the ascorbic acid in the thymus may be of particular importance for the production of "thymic humoral factor".

XVII. DECREASED CELL-MEDIATED IMMUNITY

Siegel and Morton (1977a, b) reported that ascorbic acid-supplemented mice showed no

* TAA — total ascorbic acid, reduced and oxidized forms.

Table 1
COMPARISON OF THE TISSUE CONCENTRATIONS OF ASCORBIC ACID IN ORGANS OF GUINEA PIGS MAINTAINED ON HIGH AND LOW ASCORBIC ACID INTAKES[a]

Supplement in drinking water	Thymus			Spleen		
	AA	DHA	TAA	AA	DHA	TAA
Ascorbic acid, 1%	50.6 ± 5.3	16.8 ± 2.1	67.4 ± 5.5	34.9 ± 1.0	22.5 ± 7.7	57.3 ± 11.4
Ascorbic acid, 0.1%	9.9 ± 2.8[b]	16.1 ± 2.6	26.1 ± 3.8[b]	16.1 ± 1.5[b]	15.0 ± 1.6	31.3 ± 3.0[b]

Note: Values represent the means ± SEM of four animals per group.

[a] Data from Dieter (1980).
[b] Indicates means are significantly different for those in the high ascorbate group at $p < 0.05$; Student t test.

change in humoral antibody response, but these workers did observe an increased cell-mediated immune response in vitamin C-treated mice. Hahn and O'Connor (1978) reported that sodium ascorbate in concentrations ranging from 10^{-7} to 10^{-4} *M* stimulated the mitogenic responsiveness of cultured human T lymphocytes, but inhibited responsiveness in concentrations ranging from 10^{-4} to 10^{-3} *M* and was toxic to the lymphocytes in a concentration of 10^{-2} *M*.

Fraser et al. (1978) observed that lymphocytes from scorbutic immunized guinea pigs showed an impaired thymidine incorporation response to certain mitogens, and that similarly treated guinea pigs receiving large doses of sodium ascorbate (250 mg daily) showed a much greater level of incorporation of tritiated thymidine, indicative of greater lymphocyte mitotic activity. This led on to the important finding by Anthony et al. (1979) that T cell-mediated immunity is markedly impaired in scorbutic guinea pigs; *in vitro* ^{51}Cr release from labeled chicken erythrocyte target cells incubated with lymphoid cells from spleens of ascorbic acid-deficient guinea pigs was significantly less than with spleens of pair-fed or *ad libitum*-fed control guinea pigs.

Delafuente and Panush (1980) cited their earlier studies showing that ascorbic acid enhanced pokeweed mitogen-stimulated DNA and protein synthesis by human lymphocytes. They also described experiments showing that a physiological concentration of ascorbic acid (1 mg/100 ml) caused a significant increase in the concanavalin A-stimulated DNA synthesis by human peripheral blood lymphocytes cultured *in vitro*. However, it would seem that it was not a change in the cells, but a change in their environment that caused these effects, for the provision of 10 g/d of ascorbic acid for a week to 16 volunteers had no effect on their leukocyte ascorbic acid levels or the responses to stimulation by their washed lymphocytes *in vitro*.

The concentration of ascorbic acid seems to be critical in the stimulation of lymphocyte transformation, but the results of studies by different workers show a wide variation of effects at the same concentration, as noted by Anderson (1981a) in his review of the literature on this subject. Anderson reported that, "stimulation of lymphocyte transformation to mitogens in normal human volunteers, following the intravenous injection of 1 g of vitamin C, was associated with a serum-related increase in lymphocyte reactivity to mitogens. This stimulation of proliferation was also associated with a serum-dependent-stimulation of neutrophil motility and inhibition of the myeloperoxidase/hydrogen peroxide/halide (MPO/H_2O_2/halide) system." He concluded that available evidence suggests that vitamin C is a stimulant

of leukocyte functions, especially PMN leukocyte motility and antimicrobial activity, as well as lymphocyte transformation, and that it is safe to use in pharmacological dosage. Further studies by Anderson (1981b) were confirmatory. In contrast, Kay et al. (1982) reported absolutely no change in the T lymphocyte responsiveness or other T cell characteristics in five human volunteers after 9 weeks on a scorbutogenic diet when their blood plasma ascorbate (0.09 to 0.15 mg/100 ml) and their overall leukocyte ascorbic acid levels (6.2 to 10 $\mu g/10^8$ cells) had reached borderline scorbutic levels.

This apparent paradox is most likely explained by the work of Evans et al. (1982), who observed that mononuclear cells, including small and large lymphocytes, as well as monocytes, appear to maintain a high-ascorbic acid content (3800 mol/l) or 80 times that of normal blood plasma, even when the plasma, red cells, platelets, and granulocytes are depleted and the overall "buffy layer" (leukocyte and platelet) ascorbic acid level has become markedly reduced. Kay et al. were studying purified T lymphocytes, while Anderson (1981a, b) studied a serum-related effect of ascorbic acid on lymphocyte functions. Panush et al. (1983) reported changes in the cyclic nucleotide levels of washed human peripheral blood mononuclear cells as a result of exposure to 10^{-3} *M* ascorbate *in vitro,* but the physiological significance of changes induced by such a high extracellular concentration of ascorbate is questionable.

Eftychis and Anderson (1983) reported that the activation of both suppressor activity and oxidative metabolism in mononuclear leukocytes (MNL) was almost totally inhibited by 10^{-3} *M* ascorbate or by the same concentration of cysteine *in vitro.* It was suggested that ascorbate and cysteine prevent the inhibition of nonspecific suppressor activity in MNL by an antioxidant mechanism, but of course it is difficult to know the physiological significance of such high extracellular ascorbic acid concentrations (normally ranging from a low of 10^{-5} *M* to a high of 10^{-4} *M*).

Their earlier finding of increased T cell-mediated immunity and the enhancement of interferon production by dietary ascorbic acid supplementation in mice led Siegel and Morton (1983) to investigate the effect of ascorbic acid on the "natural killer" (NK) cells. Since interferon has been noted to increase the activity of NK cells, it was expected that the inclusion of ascorbic acid (250 mg/100 ml) in the drinking water of mice would increase the activity of the killer lymphocytes. However, Siegel and Morton (1983) found that supplementary ascorbic acid did not influence NK cell activity. This paradox was later explained by Siegel and Morton (1984) when they observed that mouse bone marrow-derived macrophages secrete increased quantities of prostaglandin E_2 (PGE_2) as well as interferon in response to ascorbic acid supplementation. Suppression of NK cell activity by PGE_2 apparently counteracts the stimulation of NK cell activity by interferon.

XVIII. DELAYED SKIN GRAFT REJECTION

Kalden and Guthy (1972) have shown that vitamin C is necessary for normal skin graft rejection. Skin allografts had a mean survival time of 6.8 d in guinea pigs on a normal diet, but the mean survival time of such grafts was 16.8 d in guinea pigs which had been vitamin C-deficient at the time of receiving the transplant. They believe that ascorbic acid deficiency causes a defect in the metabolism of the small "immunologically competent lymphocytes".

XIX. LOSS OF PRIMARY IMMMUNE RESPONSE

While Anthony et al. (1979) reported no change in the humoral immune response of vitamin C-deficient guinea pigs, Thurman and Goldstein (1980) found a severe loss of the primary immune response (to sheep red cells), that is to say, failure of response to a new antigenic challenge during vitamin C deficiency.

Guinea pigs placed on diets deficient in ascorbic acid rapidly lost their capability to respond to primary and secondary antigen challenges *in vivo;* also their spleen cells became unresponsive to mitogens *in vitro.* The recovery of immune reactivity following re-introduction of ascorbic acid to the guinea pigs' diet was found to be greatly dependent upon the amount of ascorbic acid given. Memory type immune responses returned at much lower ascorbic acid doses than did primary type responses. This suggests that primary immunological reactivity to new antigens requires higher ascorbic acid levels than are needed for secondary reactions to antigens. This study points to the dependence of immune responsivity on appropriate dietary intake of ascorbic acid. It suggests that immunological memory is not affected by the ascorbic acid deficient state, but rather that effector mechanisms for the initiation and expression of immunity are altered, thereby depressing the capacity to respond immunologically. It also indicates that re-initiation of adequate amounts of ascorbic acid into the diet of ascorbic acid-deficient animals can restore immune responses to normal. Inadequate amounts of dietary ascorbic acid lead to a dichotomy in the immune response in which memory responses are normal but reactivity to new antigens is depressed.

These findings were confirmed by the work of Prinz et al. (1980) who studied the humoral antibody response to a T-dependent cellular antigen. They fed one group of guinea pigs an ascorbic acid-free diet for 2 weeks and then provided them with 9 g of cabbage a day to keep them alive in a vitamin C-deficient state. These animals, and an ascorbic acid-supplemented group, were challenged by injection of sheep red cells 2 weeks after the onset of the diet. The primary antibody response of the ascorbate-supplemented animals was found to be significantly greater than that of the controls in two respects. The peak antibody titer was both higher and occurred earlier in the test animals. No significant difference in the antibody response to a second injection of antibody was manifest.

XX. PERIPHERAL STASIS

Findlay (1921) observed a considerable discrepancy between the red cell counts obtained from heart blood and from the capillaries of scorbutic guinea pigs. The erythrocyte counts of peripheral blood from the ears of guinea pigs with acute scurvy were much higher than those of blood obtained from the heart, while similar studies of control guinea pigs showed very little difference (Table 2). He interpreted this as indicating severe peripheral blood stasis due to endothelial damage and stated that, "stagnation of blood in the capillaries appears to be one of the factors leading to deficient oxygenation of the tissues and thus to death." In all probability, endothelial damage of such degree as to cause so much transudation of tissue fluid is a late phenomenon, but the resultant capillary dilation, congestion, stasis, and tissue edema would undoubtedly contribute to a decreased resistance to infection.

XXI. IMPAIRED LEUKOCYTOSIS

Hryniewicz and Lawrynowicz (1927) reported a reduction in the mean white blood cell count of 22 guinea pigs from 11,345 to 8887 per cubic millimeter in experimental scurvy, due to a reduction in the number of mononuclear cells and a moderate increase in the number of PMN leukocytes, as shown in Table 3. On the other hand, Maxia (1930), also studying scorbutic guinea pigs, observed a shift to the left in the Arneth index and a relative increase in the number of lymphocytes, which decreased before death.

Perla and Marmorston (1937) reported that the leukoblastic reaction to infection was feeble in guinea pigs infected with pneumococci. Bartley et al. (1953) observed a progressive fall in the leukocyte counts of 5 men on a vitamin C-deficient diet, from a mean of 8050 at the beginning of the experiment to 5300 per cubic millimeter at the time when signs of vitamin C deficiency became manifest, but found no alteration in their differential white cell counts. Kalden and Guthy (1972), studying guinea pigs, on the other hand, observed no difference between the total or the differential white cell counts of scorbutic and normal animals.

Fraser et al. (1978), studying immunized guinea pigs, observed that the percentage of the total lymphocytes belonging to the T variety decreased progressively, and the percentage

Table 2

Guinea pig	Red counts (in millions)		Hemoglobin	
	Heart	Capillary	Heart	Capillary
A. Acute Scurvy				
1	3.4	4.6	82	101
2	3.7	4.8	—	—
3	4.2	5.1	—	—
4	3.9	4.9	84	98
5	3.7	5.0	92	99
6	4.0	4.8	78	96
7	4.1	4.6	80	100
8	4.9	5.1	—	—
9	4.2	4.8	81	89
10	4.1	4.9	84	98
11	3.9	5.0	77	95
12	3.7	4.7	80	94
B. Controls				
25	4.8	4.9	98	98
26	5.0	5.1	96	98
27	4.7	5.0	99	101
28	4.8	4.8	95	96
29	4.6	4.9	98	99
30	4.8	5.2	97	99

Table 3

	Nombre des leucoytes	Cellules mononucléaires		Cellules polynuclearies	
		%	Nombre absolu.	%	Nombre absolu.
Cobayes normaux.	11.345	66	7.487	33	3.743
Cobayes scorbutiques	8.887	43	3.820	54	4.798

Note: In this table, "cellules mononucléaires" refers to the small and large lymphocytes, the monocytes, and the "globules de Kurlow", while "cellules polynucléaires" refers to neutrophil, eosinophil, and basophil polymorphonuclear leukocytes. It was a marked reduction in the number and percentage of small lymphocytes that accounted for the reduction of the leukocyte count in the scorbutic guinea pigs.

From Hryniewicz, M. and Lawrynowicz, A. (1927), *J. Physiol. Pathol. Gen.*, 25, 674. With permission.

belonging to the B variety increased, during a 4-week period of vitamin C deficiency, as shown in Table 4. Studies by Vallance (1979), during the British Antarctic Survey in the years 1972 and 1973, revealed a highly significant inverse relationship between the leukocyte ascorbic acid (TAA) level and the leukocyte count ($p < 0.0001$), even though none of the men showed any evidence of infection. This relationship was less evident among subjects whose blood was at or near saturation with ascorbic acid, so it is presumably a dilutional effect caused by leukocytes sharing available ascorbic acid.

Table 4
EFFECT OF Na-ASCORBATE ON WHITE BLOOD CELL COUNTS AND ON THE NUMBER OF B AND T LYMPHOCYTES IN PLASMA OF IMMUNIZED GUINEA PIGS[a]

Na-ascorbate/ d (mg)	Day	n	White blood cells/mm³ (× 10³)	Percentage of total lymphocytes (mean ± SEM)	
				B lymphocytes	T lymphocytes
0	0	5	3.64 ± 0.49	43.0 ± 2.6	50.0 ± 1.1
	14	4	8.41 ± 0.42	52.8 ± 1.0	41.4 ± 1.6
	28	3	7.39 ± 1.06	62.6 ± 2.7	34.8 ± 2.5
25	0	5	4.54 ± 0.35	45.2 ± 1.4	48.9 ± 1.2
	14	5	8.61 ± 0.39	43.0 ± 1.7	52.3 ± 2.6
	28	5	8.05 ± 2.03	40.0 ± 2.2	57.8 ± 2.5
250	0	5	4.60 ± 0.44	44.2 ± 1.2	47.6 ± 0.7
	14	5	8.76 ± 0.33	42.6 ± 2.1	55.8 ± 2.1
	28	5	6.84 ± 0.63	36.6 ± 2.9	62.0 ± 3.3

[a] Guinea pigs were inoculated with bovine serum albumin (BSA) (0.2 mg in complete Freund's adjuvant) on day 0 and boosted (0.2 mg in saline) on day 14.

From Fraser, R. C., Parlovic, S., Kurahara, C. G., Murata, A., Peterson, N. S., Taylor, K. B., and Feigen, G. A. (1978), *Am. J. Clin. Nutr.*, 33, 839. ©American Society for Clinical Nutrition. With permission.

Thurman et al. (1982) observed a marked elevation in the percentage and absolute numbers of lymphocytes in the peripheral blood of their antigenically challenged vitamin C-deficient guinea pigs, which was not seen in similarly challenged animals on a full diet. They also observed a relative decrease in the percentage of PMN leukocytes in the ascorbic acid-deficient animals, as shown in Figure 4. They believe that this lymphocytosis is an attempt by the guinea pigs to compensate for their reduced immunological reactivity by increasing their production and circulation of lymphocytes.

Clearly, more work is needed to clarify the differences between the findings of different workers, but it seems that ascorbic acid deficiency can cause a disturbance of leukocyte production under certain circumstances.

XXII. DECREASED COMPLEMENT ACTIVITY

Marsh (1936) reported that the serum "complement" complex, as it exists in the guinea pig, disappears or suffers a reduction of titer when ascorbic acid is withdrawn completely or partially from the diet. Moreover, the concentration of complement was restored to normal or to a slightly supernormal level by the provision of a diet rich in vitamin C. Horgan (1936) made similar observations and noted that the complement titer returned to normal, within the course of a week, when ascorbic acid-rich foods were restored to the diet of the animals. However, Zilva (1936) reported that the complement activity of guinea pig blood suffers no reduction when the animals are depleted of vitamin C or even when they are in a scorbutic condition. In contrast, Ecker and Pillemer (1940) found that a generous allowance of vitamin C was required to provide maximal complement activity. They noted that guinea pigs can be maintained free from scurvy on a diet containing 1.5 mg of ascorbic acid a day, but 10 to 20 mg a day was needed for full complement activity.

Spink et al. (1941, 1942a, b) reported that intravenous administration of large doses of ascorbic acid to ascorbic acid-deficient human subjects had no effect on the complement titer of their serum. However, they studied blood samples drawn before and immediately

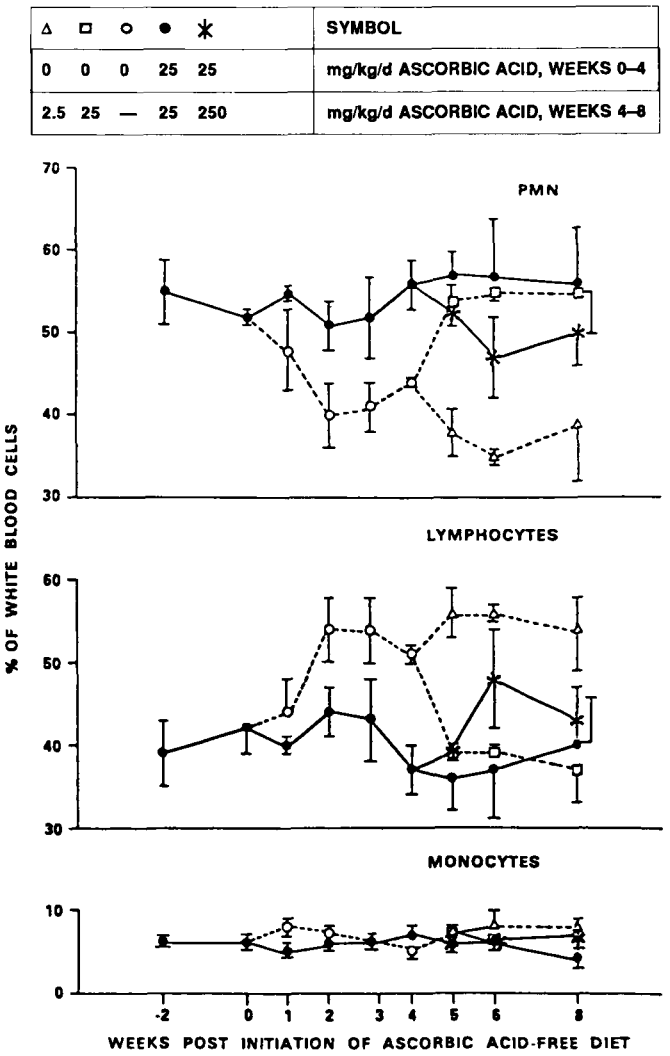

FIGURE 4. Unpublished data showing the differential white blood cell counts of tuberculin-immunized guinea pigs on different ascorbic acid intakes. The guinea pigs were immunized to tuberculin purified protein derivative (PPD) 3 weeks before being initiated on a Reid-Briggs ascorbic acid-free diet. The ascorbic acid-deficient animals showed a marked elevation in the percentage and the absolute number of lymphocytes in the circulating blood. (From Thurman, G. B. [1982], Frederick Cancer Research Center, Frederick, MD, personal communication.)

after injection of the vitamin, allowing no time for the synthesis of complement. Agnew et al. (1942) found no consistent difference between the complement levels of normal and scorbutic guinea pigs, nor did Kodicek and Traub (1943).

Bates et al. (1978) observed a significant reduction of protein-bound hydroxyproline in the serum of guinea pigs in acute vitamin C deficiency ($p < 0.001$), but considered this to be part of a general hypoproteinemia, as they found no change in complement C1 activity, which is Clq dependent. Clearly, decreased complement levels would be more apparent in chronic vitamin C deficiency than in acute scurvy, as decreased synthesis of the protein will only become apparent when existing supplies have been metabolized or degraded.

Sakjamoto et al. (1981) studied the complement systems and complement components in the blood of scorbutic and normal guinea pigs. They reported first a rise, then a late fall in the CH50 complement hemolysis titer. The C1 and C2 levels were also low in scurvy, but C3 showed a rise and C4 did not change. It was suggested that the low level of C1 in scurvy might be due to its Clq component having a collagen-like region in its A chain, constituting most of the N terminal half of the chain and having similar collagen-like regions in the B and C chains. This suggestion has been confirmed by Johnston et al. (1985), who have demonstrated increased hydroxylation of plasma Clq in ascorbic acid-supplemented guinea pigs (Chapter 4, Volume III).

Li and Lovell (1985) studied channel catfish experimentally infected by *Edwardsiella ictaluri*, the bacterium that causes enteric septicemia in these fish. The mortality rates decreased with increases in dietary ascorbic acid doses, ranging from 100% for fish fed an ascorbic acid-deficient pellet diet to 15% for fish fed 300 mg of ascorbic acid per kilogram of diet. There was no mortality among those fed a megadose (3000 mg/kg) ascorbic acid diet. These workers also studied fish immunized with *E. ictaluri* antigen. They found significantly lower levels of complement hemolytic activity, decreased antibody production, and decreased phagocytic activity in fish fed an ascorbic acid-deficient diet, but observed no change in the rate of killing of bacteria after they had been engulfed by the leukocytes. It was concluded that megadose feeding of ascorbic acid enhances the humoral immune responses, antibody production, and complement activity in channel catfish.

Johnston et al. (1987) assayed the plasma complement Clq component in vitamin C-depleted and repleted guinea pigs by three different methods: they measured it indirectly by quantifying protein-bound hydroxyproline and directly, both by hemolytic assay and by immunodiffusion against anti-Clq. Regardless of the method, plasma Clq levels were 30 to 50% higher in the animals fed tissue-saturating quantities of ascorbic acid than in those fed adequate or suboptimal amounts of the vitamin ($p < 0.05$).

XXIII. SECONDARY EFFECTS OF DISTURBED FOLIC ACID METABOLISM

As discussed in more detail in Chapter 4, Volume III, ascorbic acid deficiency causes a disturbance of folate metabolism, involving decreased conversion of folic to folinic acid. This results in a metabolic deficiency of folinic acid or citrovorum factor and causes megaloblastic anemia, which is a well-known feature of scurvy in guinea pigs and monkeys and has often been reported in human infants and adults in association with ascorbic acid deficiency.

Ludovici and Axelrod (1951) discovered that pterolylglutamic acid deficiency in rats causes a severe impairment of antibody response as demonstrated by a challenge with group O, Rh positive human erythrocytes. Indeed, such was the immunological depression of the folic acid-deficient rats that none of them possessed a measurable agglutinin titer. This is confirmed in a review by Beisel (1982) who concluded that folate deficiency leads to a reduction of host resistance and to impaired lymphocytic functions, both in man and in animals.

The relevance of this to the human condition has been clearly demonstrated by the work of Hunt et al. (1984), who studied 199 elderly patients admitted to a geriatric ward in Huddersfield with acute illness. The patients with low initial leukocyte ascorbic acid levels (<15 $\mu g/10^8$ cells) had somewhat lower initial folate levels, which rose after 4 weeks in the group receiving ascorbic acid (200 mg/d), but remained low in the control group. There was a significantly higher mortality rate ($p < 0.05$) during the 24-week study period among those with initially low leukocyte ascorbic acid levels, despite similar mean initial "severity of illness" scores in the two groups. Grouping the subjects in the trial by type of disease, there was a tendency, though not significant, for patients with respiratory infections to fare

better up to 8 weeks in the supplemented group, but the mortality during the 24 weeks of the study followed the initial and not the achieved ascorbic acid levels and was actually higher (not significant) in the treated group. Clearly, any work on the effects of ascorbic acid deficiency and immunity should distinguish between primary ascorbate deficiency and secondary folinic acid deficiency effects. Even so, folinic acid deficiency resulting from such a disturbance of folic acid metabolism can and does occur in vitamin C-deficient humans, monkeys, and guinea pigs on what would otherwise be an adequate folic acid intake, and in such cases it is treatable with ascorbic acid alone; so impairment of immunity due to this disturbance of folate metabolism may properly be considered as a result of ascorbic acid deficiency.

XXIV. PROLONGED UPPER RESPIRATORY INFECTIONS

An extensive literature has accumulated concerning *Vitamin C and the Common Cold,* which is the title of a book by Pauling (1970). Many attempts have been made to answer two questions — one is whether vitamin C deficiency is associated with an increased incidence, severity, or duration of colds and the other is whether large doses of ascorbic acid can decrease the incidence, severity, or duration of colds. In this section, we are concerned only with the first question, which relates to vitamin C-deficient individuals. Thus, we are limited to a consideration of studies where the subjects were known to be vitamin C deficient.

Glazebrook and Thomson (1942) reported a study of 15- to 20-year-old cadets at a naval academy in Scotland, which provided a diet containing only 10 to 15 mg of ascorbic acid per student per day, because the food was cooked and then kept hot too long before it was served. Moreover, many of the cadets came from poor homes where the diet had been deficient in ascorbic acid. Indeed, many of them had severe gingivostomatitis which was known to respond to treatment with vitamin C, as a result of earlier work by Roff and Glazebrook (1939).

A total of 335 cadets were given ascorbic acid supplements, 200 mg daily, for 6 months, and 1100 cadets served as controls. The incidence of colds and tonsillitis was 30.1% in the ascorbic acid-treated group and 34.5% in the control subjects. This is only a 13% reduction in the incidence of colds and tonsillitis and is not statistically significant because there is a 12% probability of such a difference occurring by chance. However, as pointed out by Pauling, 23.0% of the cadets in the ascorbic acid group and 30.5% of the controls were admitted to sick quarters for severe colds and tonsillitis. This is a 25% reduction of admissions in the ascorbic acid-treated group and the probability of it having been due to chance is only 1%, which is a highly significant finding. The average number of days in sick quarters because of infection (corysa, tonsillitis, rheumatic fever, or pneumonia) was 2.5 for the cadets receiving ascorbic acid and 5 for the controls. The most important finding was that there were 17 cases of pneumonia and 16 cases of rheumatic fever among the 1100 controls and no case of either disease among 335 cadets given ascorbic acid supplements. Such a result does not require statistical analysis, but in fact, the probability of its being due to chance is about 0.3%.

In a study of experimental scurvy in man, Bartley et al. (1953) observed just as many colds in the control as in the deprived subjects, but the mean duration of the colds in the deprived subjects was 6.4 d as compared with 3.3 d in nondeprived subjects. This clearly suggests that colds last longer in ascorbic acid-deficient subjects. On its own, it would not prove this, as the results did not quite reach the 5% level of significance, but since it is confirmatory of the earlier findings of Glazebrook and Thomson (1942), it is valuable evidence.

A study of ascorbic acid supplementation, 1 g daily vs. placebo, was carried out on 90

of the students and staff at the University of Strathclyde in Glasgow and was reported by Charleston and Clegg (1972). They observed dramatic results, for there was a 49% reduction in the incidence of colds in the vitamin group, and this was highly significant ($p < 0.002$). The average duration of the colds in the ascorbate group was 3.5 d, as compared with 4.2 d, but this was less significant ($p < 0.05$). However, a subsequent double-blind controlled study by Clegg and MacDonald (1975) showed 34% fewer colds in students taking 1 g a day of D-isoascorbic acid, an isomer of vitamin C, but no benefit to those taking L-ascorbic acid.

Anderson et al. (1972) reported a much larger Canadian study of 1000 volunteers who took part in a very well-controlled double-blind study, of ascorbic acid, 1 g daily, vs. placebo, at the University of Toronto during the winter months. In this study, the incidence of colds was only 9% less in the ascorbate group, and this was not significant. However, there was a statistically significant difference ($p < 0.05$) between the two groups in the number of subjects who remained free of illness throughout the study period. Furthermore, the subjects receiving the vitamin experienced approximately 30% fewer total days of disability (confined to the house or off work) than those receiving the placebo, and this was statistically highly significant ($p < 0.001$). The reduction in disability appeared to be due to a lower incidence of constitutional symptoms such as chills and severe malaise.

Originally 527 dental practitioners and their wives were studied by Cheraskin et al. (1973) using questionnaires concerning their daily vitamin C consumption and the incidence of respiratory symptoms. In subsequent years, 171, 116, 64, and 12 of these subjects completed new questionnaires and attended group seminars on nutrition. A highly significant relationship was found between increasing ascorbic acid intakes and a decreasing incidence of respiratory symptoms, which was very encouraging. Unfortunately, the attrition rate may have caused a bias, even in the first 3 years, for those who appreciated benefit from their diet would be more likely to return than would those who felt no such benefit. Studies concerning the use of ascorbic acid supplements for the prevention or treatment of the common cold have been the subject of reviews by Chalmers (1975), Thomas and Holt (1978), and Editorials (1976, 1979).

Most authors have concluded that ascorbic acid supplementation does not reduce the incidence of the common cold in well-nourished individuals, but many well-controlled studies have shown beneficial effects in reducing the severity and/or duration of cold symptoms; other studies have not, so the literature is said to be contradictory. Another, more reasonable interpretation might be that some studies included an appreciable number of ascorbic acid-deficient subjects and thus showed benefit, while other studies included mostly subjects who were not in need of ascorbic acid supplements.

Not only dietary intake of ascorbic acid, but all of the factors discussed in Volume I affect the blood and tissue levels of ascorbic acid. So ascorbic acid needs vary from person to person, from time to time, and from place to place. Clearly, males, aged persons, smokers, and those with achlorhydria, hemolysis, infection, hemosiderosis, or hypercupremia, and many other conditions need more ascorbic acid than others. For this reason, some, like Pauling, having advocated very large doses of ascorbic acid, 1 g a day for prevention and 4 g a day for treatment of colds, so as to be sure of providing for those with very high needs. In the opinion of the writer, these very high doses will probably not be necessary if sufficient chelating fiber (flavonoid or catechin) is given with ascorbic acid to prevent wastage of the vitamin by oxidation and hydrolysis (see Chapter 11, Volume I).

Indeed, some studies of dietary supplementation with vitamin C in moderate doses, with or without bioflavonoids, have shown just as much benefit as the megadose ascorbic acid studies have done. Franz et al. (1956), studying four groups receiving the bioflavonoid naringin, or ascorbic acid, 195 mg daily, or a combination of naringin and ascorbic acid, or placebo, observed significantly more rapid recovery from corysa in the two ascorbic acid-

treated groups ($p < 0.05$). Similarly, Baird et al. (1979) observed that subjects receiving 80 mg of ascorbic acid a day as orange juice, or 80 mg of ascorbic acid in an orange-flavored drink, had 14 to 21% fewer respiratory symptoms than a control group receiving the orange-flavored drink without ascorbic acid ($p < 0.05$). Incidentally, these two studies involved the nonchelating flavonoids naringin from grapefruit and hesperidin from oranges. Only the smaller amounts of eriodictyol in orange juice would provide chelation and preservation of ascorbic acid to some extent. However, the acidity of the juice undoubtedly retards losses of ascorbic acid oxidation and subsequent hydrolysis.

It is interesting to note that Tebrock et al. (1956), providing ascorbic acid, 50 mg, with and without lemon bioflavonoid complex (or orange bioflavonoid complex), 250 mg four times a day, or a placebo, to patients with coryza, who also received a cold remedy containing an antihistamine, a decongestant, and two analgesics, observed no benefit from ascorbic acid. Were the antihistamine effects of the vitamin concealed by the antihistamine effects of the cold remedy?

XXV. INCREASED BLOOD HISTAMINE LEVELS

Human ascorbic acid deficiency, even of mild degree, is associated with an increased blood histamine level, which is promptly reduced by the administration of ascorbic acid, as shown by Clemetson (1980). The effects of ascorbic acid on histamine metabolism will be discussed more fully in Chapter 1, Volume III, but it is pertinent here to observe that ascorbate-responsive histaminemia is by no means rare, being present in perhaps one tenth or even one third of an apparently healthy active working population. Even if ascorbic acid did not increase resistance to infection, which it does in ascorbate-deficient people, it should be no surprise that decreasing the blood histamine level makes us feel better.

Histamine levels are always increased locally in an area of inflammation and histamine is responsible for much of the vasodilation and congestion of inflamed tissues. Indeed, acute inflammation is in many ways like local scurvy. Ascorbic acid, in addition to its many other virtues, is an effective antihistamine. The fact that ascorbic acid decreases whole blood histamine levels undoubtedly explains the findings of Zuskin et al. (1973) that ascorbic acid reduces the airway constriction induced by the inhalation of histamine in human adults. It also explains the practical findings of Valik and Zuskin (1973), who carried out ventilatory function tests before and at the end of the work shift on workers in a textile factory, who were exposed to flax dust. Ascorbic acid (500 mg daily), given daily before the shift for a week, caused a significant reduction in measured airway constrictor effects of the dust. It also provided subjective improvement in 12 out of 13 byssinotic workers as compared with 2 out of 13 who felt some improvement after receiving placebo tablets.

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Chapter 13

LIVER, BILE, AND GALLSTONES

I. LIVER ASCORBATE DEPLETION

In the majority of mammals, the liver is rich in ascorbic acid because of ascorbate synthesis in that organ. Moreover, in humans, apes, monkeys, and guinea pigs, which cannot synthesize ascorbic acid, the liver receives this vitamin directly from the portal vein blood after absorption from the gastrointestinal tract. The liver normally constitutes the largest store of ascorbic acid in the body, but when dietary ascorbic acid is discontinued, the guinea pig liver ascorbic acid level falls rapidly.

Ginter and Bobek (1981) have observed that the initial rate of ascorbic acid depletion in the guinea pig liver is faster than in other organs (Figure 1); "while in all other tissues the decline of the ascorbate level follows first-order kinetics, in the liver it shows a two-phase pattern — very rapid initially, with a half life of approximately 24 hours, followed by a more moderate decline with a half life of about seven days." Hence, changes in hepatic function appear soon after the vitamin C supply has ceased.

II. HISTOPATHOLOGY

Spellberg and Keeton (1939, 1940) found marked fatty changes in the liver of guinea pigs after vitamin C depletion. Mukherjee and Banerjee (1954), studying female rhesus monkeys, observed hemorrhage in the livers of the scorbutic animals. In chronic scorbutic guinea pigs, they observed fatty degeneration of the hepatic cells surrounding the central veins. Willis (1957) reinvestigated the effects of vitamin C deficiency on the liver in guinea pigs and reported as follows. "Scurvy mainfests itself in the liver by fatty degeneration, acute non-fatty hepato-cellular degenerations, massive necrosis and changes in hepatic reticulin. None of these lesions are prevented by cytsine or choline, alone or in combination. Some of them are reversible with ascorbic acid replacement." Ginter and Bobek (1981) reported that chronic ascorbic acid deficiency in the guinea pig leads to cholesterol and triglyceride accumulation in the liver. During long-term marginal vitamin C deficiency, the liver of the guinea pig becomes enlarged and displays centrilobular fatty degeneration, moderate hyperplasia of the bile ducts, and discrete signs of fibroplasia.

III. TRYPAN BLUE STUDIES

Russell and Callaway (1943) observed pathological changes in the parenchymal cells of the liver in scorbutic guinea pigs. Trypan blue injected subcutaneously into scorbutic guinea pigs was found to be more heavily deposited in the parenchymal cells of the liver and in the proximal convoluted tubules of the kidney than in the corresponding cells of control animals receiving the same amount of dye. This observation was interpreted as indicating a pathological change in these cells as a result of vitamin C deficiency. The scorbutic animals also showed fatty infiltration of the liver cells, particularly in the region of the central veins.

IV. ELECTRON MICROSCOPY

Sheridan and Bourne (1964) made electron microscopic studies of liver specimens from normal, scorbutic, and pair-fed control guinea pigs. Extensive changes were seen in the livers of the vitamin C-deficient animals, but the same ultrastructural changes were seen in

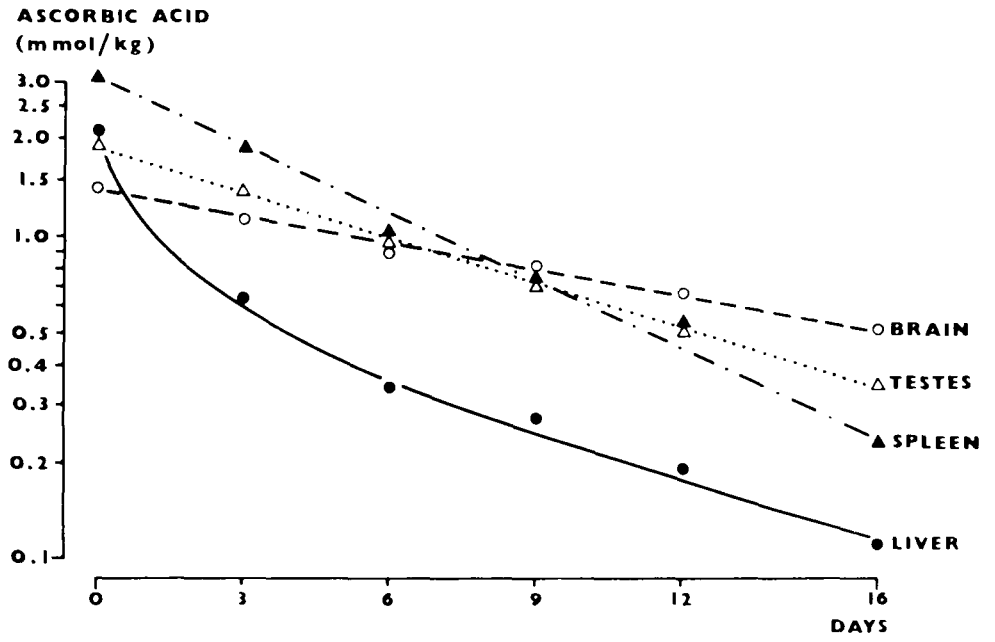

FIGURE 1. While the ascorbic acid concentration in most guinea pig tissues falls according to an exponential curve during ascorbate depletion, and thus gives a straight line on semilogarithmic paper, the concentration of ascorbic acid in the liver falls more rapidly at first. (From Ginter, E. and Bobek, P. [1981], *Vitamin C: Ascorbic Acid*, Counsell, J N. and Hornig, D. H., Eds., Applied Science, London, 299. With permission.)

the half-starved, pair-fed control animals, so none of the changes could be said to be characteristic of vitamin C deficiency. They observed

1. An increase in the number of mitochondria
2. Dilation and vesiculation of the endoplasmic reticulum
3. An increase in the number of small dense cytoplasmic structures in close proximity to the Golgi apparatus
4. The formation of small multilaminar structures

but all these changes were seen to an even greater extent as a result of subacute starvation in pair-fed controls.

Sulkin and Sulkin (1975) examined the fine structure of liver cells from normal, acutely scorbutic, and marginally vitamin C-deficient guinea pigs. "In general sections of liver taken from acutely scorbutic animals (animals on a vitamin-C-free diet for 21—30 days) showed a fairly normal characteristic profile. A few cells exhibited swollen mitochondria and the mitochondrial matrix appeared less dense. Observations in these initial studies revealed that the hepatic cells from the marginally deficient animals (animals on a marginally deficient diet for 90 days or more) differed from hepatic cells of the other two groups. These differences included a marked reduction and displacement of the granular endoplastic reticulum. Whenever present, this organelle usually encircled the mitochondria, the latter having developed a dense matrix. The major alteration observed was a sharp proliferation of smooth endoplasmic reticulum, sometimes occupying large areas of cytoplasm, often to the extent of displacing the other organelles to the periphery of the cell." These changes were found to be due to a significantly increased deposition of cholesterol in the liver.

V. PROTEIN CATABOLISM

Mohanram and Rao (1967) studied guinea pigs which had ceased to grow after 8 weeks

on an ascorbic acid-deficient diet which provided 0.4 mg of ascorbic acid a day; pair-fed and *ad libitum*-fed control animals received 10 mg of ascorbic acid a day. Following intraperitoneal injection of ^{14}C-leucine, there was no impediment to the incorporation of this amino acid into liver proteins, and this initially occurred more rapidly in the ascorbic acid-deficient than in either the pair-fed or normal control animals. However, protein catabolism also occurred faster in the ascorbic acid-deficient animals, so that after 4 h, the ^{14}C-leucine content of the liver was lower in the ascorbic acid-deficient animals. Similar results were obtained in studies of skeletal muscle, skin, intestinal mucosa, and kidney tissue, where catabolism was also greater than anabolism. Free leucine levels in the tissues were higher in the ascorbic acid-deficient than in the pair-fed or the control animals. Free leucine levels in the liver were reported to be 160 μg/g in the ascorbic acid-deficient animals compared with 102 μg/g in the pair-fed and 110 μg/g in the normal controls.

VI. GLYCOGEN DEPLETION

Nadal and Mulay (1954) and many other workers (Chapter 3, Volume III) have observed a decrease in the rate of formation of liver glycogen from precursors in scurvy. Ganguli and Banerjee (1961), investigating the cause of decreased glycogen storage in the liver of the scorbutic guinea pig, observed that the activities of hexokinase, phosphoglucomutase, and phosphohexoisomerase were reduced in scurvy. Maximal depression in activity was observed with phosphoglucomutase. Among the enzymes studied, glucose 6-phosphatase was the only enzyme the activity of which was found to remain unaffected in the liver by scurvy. The dehydrogenases of glucose 6-phosphate and 6-phosphogluconate exhibited increased activity in scorbutic guinea pigs. They postulated that the defect in glycogenesis and the observed glycogenolysis were due to the hypoinsulinism that is associated with scurvy (Chapter 2 of this volume).

VII. FAT ACCUMULATION

Bessey et al. (1934) observed fatty infiltration of the liver in guinea pigs with varying degrees of vitamin C deficiency. This was so marked in the terminal stages of scurvy that the cells of the entire lobule were almost entirely composed of fat. Edema of the periportal connective tissue was observed and irregularly distributed hemorrhages were present in some animals. Richards et al. (1941) found normal guinea pigs to have a liver fat content of 2.6 to 4.2%; this increased to 5.0 to 24.7% after 3 to 4 weeks on a vitamin C-free diet.

VIII. IMPAIRED DRUG AND TOXIN METABOLISM

Richards et al. (1941) found a markedly prolonged sleeping time in scorbutic guinea pigs given nembutal compared with that in normal animals; the administration of ascorbic acid reversed this effect. Overman et al. (1942), Sullivan et al. (1943), and Dayton and Weiner (1961) observed that high doses of ascorbic acid antagonize the action of dicoumarol on the prothrombin times of rabbits, guinea pigs, and human subjects, while Baumann et al. (1942) showed that the administration of ascorbic acid to rats failed to alter either the extent or the duration of the hypoprothrombinemia produced by dicoumarol. It seems that rabbits, like humans and guinea pigs, can be ascorbic acid deficient in this respect, and ascorbic acid deficiency decreases the synthesis of liver microsomal cytochrome P-450 which is needed for the oxidative detoxification of coumarin compounds.

Rosenthal (1971) and Smith et al. (1972) made clinical observations confirming that ascorbic acid can shorten the prothrombin times of patients receiving warfarin sodium (coumadin), but Hume et al. (1972) found no such effect of ascorbic acid, presumably

because his patients were not ascorbic acid deficient. Deckert (1973) confirmed that ascorbic acid-deficient guinea pigs showed a markedly increased sensitivity to warfarin, but found no evidence of altered warfarin metabolism to account for the increased sensitivity. He therefore suggested that some other mechanism must be responsible. Axelrod et al. (1954) reported a threefold increase in the plasma half-life of acetanilide and significant increases in the half-lives of aniline and antipyrine in guinea pigs on the 16th to 20th days of ascorbate depletion. These changes occurred before there were any signs of scurvy and before any significant weight loss had occurred. The increase in the plasma half-life of these substances returned to normal in 4 to 6 d when the animals were given ascorbic acid.

Conney et al. (1961) observed that vitamin C-deficient guinea pigs are more sensitive to the muscle-relaxing drug zoxazolamine than are normal guinea pigs, due to decreased activity of the enzyme system in liver microsomes that metabolizes this drug. While ascorbic acid-supplemented guinea pigs were paralyzed for 156 min by intraperitoneal injection of a standard dose of zoxazolamine (100 mg/kg), vitamin C-deficient guinea pigs (10 to 14 d on a scorbutogenic diet, but not yet showing any obvious signs of scurvy) were paralyzed for 309 min by the same dose. *In vitro* studies revealed that liver microsomes from ascorbic acid-supplemented guinea pigs metabolized an average of 36 μg of zoxazolamine, while microsomes from the vitamin C-deficient animals metabolized an average of only 12 μg of zoxazolamine. "The addition of ascorbic acid *in vitro* to microsomes obtained from vitamin C-deficient guinea pigs did not increase the activity of this enzyme system." Clearly, time is needed for ascorbic acid to stimulate the synthesis of the enzyme *in vivo*.

Degkwitz and Staudinger (1965) observed decreased *p*-hydroxylation of acetanilide by ascorbic acid-deficient guinea pig liver microsomes; this was reversed when the deficient animals were given ascorbic acid. Likewise, the hepatic metabolism of certain barbiturates was so impaired in ascorbic acid-deficient guinea pigs that Degkwitz and Staudinger (1974) had to reduce the dose of phenobarbital administered in their experiments to about 60% of the amount given to normal guinea pigs, since the deficient guinea pigs did not otherwise survive. These workers concluded that the impairment of the monooxygenase function of the liver in scorbutic guinea pigs is due to a reduction of the concentration of cytochrome P-450 and not directly to a deficiency of ascorbic acid. The cytochrome P-450 level fell to about 40% of its initial value in scurvy, possibly due to a defect in the synthesis of heme. They found that the level of cytochrome P-450 and the monooxygenase function of guinea pig liver could be partially restored by D-arabo-ascorbate or by 5-oxo-D-gluconate, without *l*-ascorbic acid. They therefore concluded that ascorbic acid aids, but is not essential for the synthesis of cytochrome P-450. Sato and Zannoni (1974) studied the effects of ascorbic acid on the drug-metabolizing enzymes of the liver mitochondria of 2-week-old weanling guinea pigs maintained for a short time on a vitamin C-deficient diet; being in a rapid growth period, they are more susceptible to a vitamin deficiency. After 8 d on an ascorbic acid-deficient diet, the cytochrome P-450 reductase, *O*-demethylase, and *N*-demethylase activities were significantly lower than in animals on a full diet, but there was no significant decrease in the level of cytochrome P-450. After 15 d of ascorbic acid deficiency, there were further significant reductions in all the enzyme activities compared with controls ($p < 0.001$). Cytochrome P-450 was 31% lower; P-450 reductase was 67% lower; *O*-demethylase was 75% lower; and *N*-demethylase was 36% lower. D-Isoascorbic acid could replace the effect of L-ascorbic acid on some of the drug oxidation reactions, but much larger doses were needed. Not only did Sato and Zannoni observe that ascorbic acid deficiency resulted in decreased drug metabolism, they also found that the activity of the microsomal drug oxidation system increased over basal levels when the animals were given high supplements of the vitamin. NADPH-cytochrome P-450 reductase, *N*-demethylase, and *O*-demethylase activities increased as much as 200%, while the increase in the quantity of cytochrome P-450 was smaller but, nevertheless, significantly greater than control values. By analogy, it was

suggested by these authors that the recommended daily vitamin C allowances for human beings (70 to 75 mg/d) may not be adequate for optimal activity of the microsomal drug enzymes which may be required for detoxification. In a review of this subject, Zannoni and Sato (1975) noted that other laboratories have reported decreased oxidation of zoxazolamine, coumarin, diphenylhydramine, and meperidine in microsomes from the liver of scorbutic guinea pigs.

Feetam et al. (1975) studied the effects of ascorbic acid supplements, in doses of 3, 5, and 10 g/d, on the prothrombin times of patients who had been stabilized on warfarin sodium as long-term therapy. These workers observed that ascorbic acid caused a fall in the plasma total warfarin levels (2 to 40%, mean 17.5%), but no significant changes in the test to control prothrombin ratios of these patients. It was suggested that the intestinal absorption of warfarin may have been impaired by the diarrhea which was experienced by many of the patients receiving megadose ascorbic acid. It would seem that ascorbic acid supplements will reduce the prothrombin times of patients on coumarin therapy only when there are suboptimal liver ascorbic acid stores.

Ascorbic acid is partly converted to ascorbate-2-sulfate in man, so Houston and Levy (1975, 1976) investigated the degree to which high doses of ascorbic acid might compete with acetaminophen and with salicylamide, two widely used analgesics and antipyretics, for sulfation and excretion in man. It was indeed found that substrate competition does occur, so that less of these compounds are excreted in the urine as sulfates when high-dosage ascorbic acid is administered, but the total excretion of these substances was not affected. Houston et al. (1976) cited isoproterenol as another substance, the sulfation and excretion of which might be affected by high-dosage ascorbic acid. No potentiating effect was observed when isoproterenol and the vitamin were given intravenously, but experiments on dogs suggested that high-dosage ascorbic acid might possibly potentiate the effect of any isoproterenol inadvertently swallowed during the use of an inhaler, as sulfation normally occurs during one passage through the wall of the intestine and even before reaching the liver.

Zannoni and Sato (1976) observed that ascorbic acid, alpha tocopherol, and riboflavin are all essential for full activity of the hepatic drug metabolizing systems. Not only did vitamin C deficiency cause a decrease in the quantity of liver microsomal electron transport components such as cytochrome P-450 and NADPH cytochrome P-450 reductase, it also decreased a variety of drug-enzyme reactions such as *N*-demethylation, *O*-demethylation, and steroid hydroxylation.

Wilson et al. (1976) found that 2 weeks of high-dosage ascorbic acid administration (300 to 4800 mg a day) caused no change in the rate of clearance of antipyrine by four healthy human volunteers, presumably because these individuals had not been vitamin C deficient initially. In contrast, Houston (1977) found that a 1 g/d supplement of vitamin C for 7 d caused a statistically significant increase in the total body clearance of antipyrine by five healthy male subjects ($p < 0.005$).

Sikic et al. (1977a,b) also reported the effects of ascorbic acid deficiency and of ascorbic acid supplements on the drug-metabolizing enzymes of guinea pigs. Aminopyrine *N*-demethylation decreased 40% in the liver after 21 d of ascorbic acid depletion and showed considerable recovery after 3 d of ascorbic acid repletion. Glutathione S-aryltransferase activity in the livers of ascorbic acid-depleted guinea pigs decreased to 50% of that in the controls, but had not fully recovered after 14 d of repletion.

Chakraborty et al. (1978a,b) observed that ascorbic acid reduced the extent of both enzymic and histological changes in the livers of rats following prolonged exposure to polychlorinated biphenyl compounds and organophosphates. Both parathion and malathion decreased the acetylcholinesterase activity of the brain. An oral supplement of ascorbic acid (20 mg/100 g of body weight per day) almost halved this effect of these insecticides on the brain and partially reversed the growth-retarding effect of these compounds. Although the adminis-

tration of vitamin C to nondeficient human volunteers does not appear to enhance the metabolism of antipyrine, Smithard and Langman (1977) have shown that the administration of ascorbic acid to elderly ascorbic acid-deficient people does enhance antipyrine metabolism. The metabolic clearance rate (MCR) in 10 deficient people was 25.3 ± 8.8 ml/h/kg, compared with 33.5 ± 11.5 ml/h/kg in 27 nondeficient elderly people. This difference is significant ($p < 0.05$). When eight of the deficient group were given vitamin C for 2 weeks, the MCR improved from 26.1 ± 9.6 to 36.5 ml/h/kg. The difference was again significant ($p < 0.025$). No such improvement could be demonstrated in the nondeficient group. Major changes in the rate of prothrombin synthesis, due to fluctuations in the rate of hydroxylation of coumarin during periods of ascorbate depletion and repletion, may account for the fact that the dosage of coumarin and its analogues can sometimes be so difficult to control and may well be the reason that this group of anticoagulants can be so treacherous.

Studies by Chen and Barnes (1976) and by Chen and Chang (1978) in normal guinea pigs showed that dietary vitamin E and vitamin C decreased liver lipid peroxidation more effectively than did either vitamin given alone. Moreover, vitamin E had a sparing effect on vitamin C, increasing the plasma total and reduced ascorbic acid levels ($p < 0.01$) when there was no vitamin C in the diet. However, vitamin E showed a protective effect against hemolysis which vitamin C did not. Leung et al. (1981) demonstrated that both vitamin C and vitamin E suppressed lipid peroxidation in rat liver *in vitro* and showed that the two vitamins were more effective in this respect than the sum of their individual activities.

Moreover, studies by Kato et al. (1977, 1981) showed that dietary ascorbic acid has an ameliorating effect on the toxic effects of polychlorinated biphenyls (PCB) in guinea pigs. Growth retardation due to dietary PCB (50 ppm) was lessened when the dietary ascorbic acid was increased from 50 to 2000 ppm. Also, increases in serum cholesterol and phospholipid and in hepatic lipid peroxidation due to PCB were markedly suppressed by the larger intake of ascorbic acid. The optimum intake of ascorbic acid in the guinea pigs fed PCB for the changes in growth, serum cholesterol, and lipid peroxidation was provided by 800 to 2000 ppm in the diet.

Ginter et al. (1982) studied guinea pigs on a 0.2% cholesterol diet providing low levels of vitamins C and E. They found that dietary supplements of either vitamin C or vitamin E caused a significant stimulation of the activities of both aniline hydroxylase and *p*-nitroanisole-*O*-demethylase in hepatic microsomes. Moreover, the two vitamins were found to act synergistically on aniline hydroxylase. "Multiple regression analysis showed the effect of vitamin C on the activity of both microsomal enzymes to be more striking than that of vitamin E." It was concluded that an optimum combination of vitamins C and E may substantially enhance the detoxifying ability of the liver. Studies by Kawai-Kobayashi and Yoshida (1986), in guinea pigs, have confirmed that supplementary vitamin C (1000 mg/kg of diet) and vitamin E (2000 mg/kg of diet) afforded greater protective effect against liver damage due to PCB than did modest allowances of these vitamins (200 and 70 mg, respectively) or high-dosage supplements of either. Moreover, high levels of ascorbic acid and vitamin E significantly decreased the serum total cholesterol levels elevated by PCB in guinea pigs.

IX. LIVER ENZYME STUDIES

Staudinger et al. (1961) reported their studies of an ascorbic acid-dependent DPNH oxidase in animal tissues. Discussing the role of ascorbic acid in microsomal electron transport and its relationship to hydroxylation reactions in rat liver, kidney, and adrenals, it was postulated that a flavoprotein such as cytochrome b_5 transports hydrogen or an electron from DPNH to monodehydroascorbic acid. Ascorbic acid was found to be lost by oxidation in the presence of cytochrome b_5 *in vitro*, but when DPNH was also present, this loss by oxidation did not

occur, presumably because the first oxidation product, monodehydroascorbic acid, was promptly reduced by DPNH. Thus, DPNH-monodehydroascorbic acid-transhydrogenase allows ascorbic acid to play an essential role as an oxidant in several hydroxylation reactions.

Degkwitz et al. (1968) observed that the hydroxylation of coumarin by liver microsomes was markedly decreased in scorbutic guinea pigs. They noted that this was not due to starvation and believed that it was not due to decreased oxidation of NADPH nor to a lower concentration of cytochrome P-450 or cytochrome b_5 because the hydroxylation of coumarin could be raised again by increasing the ascorbic acid concentration for a short period *in vivo*. Kato et al. (1969) reported that the cytochrome P-450 and b_5 contents of the liver microsomes of guinea pigs were not significantly altered after 12 d on an ascorbic acid-deficient diet, but the hydroxylation of aniline, hexobarbital, and zoxazolamine was markedly reduced.

Leber et al. (1969) studied the enzyme activities of liver microsomes from normal, scorbutic, and calorie-deficient (starved) guinea pigs. Scorbutic guinea pig liver microsomes showed decreased activities for the demethylation of aminopyrine and for the hydroxylation of acetanilide and also a decreased concentration of cytochrome P-450 relative to the starved control animals. The liver microsomal b_5 concentration was also low in the scorbutic animals, but no lower than in the starved control animals. Studies of scrobutic guinea pigs by Leber et al. (1970) revealed that the cytochrome P-450 content and the aminophenazone-demethylating activity of the liver microsomes could be increased by injection of ascorbic acid, but more than 48 h were required for restoration of full enzyme activities. Phenobarbital induction of these enzyme activities occurred even in scorbutic guinea pigs, but was markedly augmented by ascorbic acid administration.

Wade et al. (1972) found the levels of cytochrome P-450 and b_5 to be lower than normal after 12 d of ascorbic acid deficiency. Likewise, aniline hydroxylation per unit of protein was depressed by ascorbic acid deficiency. Degkwitz et al. (1972) reported that the concentration of cytochrome P-450 in the liver microsomes of guinea pigs decreases within 24 h after omission of vitamin C from the diet and continues to fall to about 50% of the starting value while the vitamin C content of the liver declines. A basal content of about 40% remained even during complete ascorbic acid deficiency. The amount of cytochrome b_5 was also found to decrease at the beginning of L-ascorbic acid deficiency. Luft et al. (1972) confirmed that the specific content of cytochrome P-450 decreases to about 40% of normal in the livers of guinea pigs fed without L-ascorbic acid for a long period of time. The content was increased to normal within 48 h by treatment with either L-ascorbic acid or δ-aminolevulinic acid. It was concluded that vitamin C stimulates the biosynthesis of heme.

Zannoni et al. (1972) studied the *in vitro* enzyme activities of liver microsomes from guinea pigs after 10 and 21 d on a vitamin C-deficient diet. There was no change after 10 d when the liver microsomal ascorbic acid was 50% of normal, but there was a marked decrease in several drug oxiation activities and in the activities of inividual microsomal electron transport components after 21 d when the liver microsomal ascorbic acid had fallen to 30% of normal. The quanitity of cytochrome P-450, cytochrome b_5, and NADPH cytochrome c-reductase were reduced by 33 to 40% and the NADPH cytochrome P-450 reductase was reduced by 85% in the ascorbic acid-deficient animals. Aniline hydroxylation, aminopyrine *N*-demethylation, and *p*-nitroanisole *O*-demethylation were also found to be significantly decreased, and none of these changes was reproduced by starvation.

All of these changes could be reversed by supplying ascorbic acid to the guinea pigs, but several days of treatment were required for full recovery of these enzyme systems. Aniline hydroxylase deficiency was reversed in 6 d and cytochrome P-450 deficiency was reversed in 10 d, but cytochrome c reductase activity remained low even after 16 d of treatment with vitamin C. Clearly, several essential enzymes become depleted long before the stage of frank scurvy, and they seem to require several days of vitamin C treatment for full recovery. Oddly enough, these workers found that the administration of phenobarbital induced the

synthesis of these liver enzymes even in ascorbic acid-deficient guinea pigs and the enzyme levels were restored to normal by phenobarbital even after the ascorbic acid (TAA)* level of the microsomal fraction of the liver had fallen from 71 to 5 μg/g of wet weight.

Degkwitz and Kim (1973) observed that the decreased concentrations of cytochromes P-450 and b_5 in the livers of scorbutic guinea pigs return to normal within 24 to 36 h after the liver ascorbate levels have been restored to normal, and that this could be achieved more rapidly by intraperitoneal injection of ascorbic acid than by oral administration of the vitamin. The amounts of both cytochromes decreased in guinea pigs when D-arabino-ascorbate was substituted for L-ascorbate over the course of 8 d, but were higher than in animals without any ascorbic acid for this time.

All guinea pigs provided with 5-oxo-D-gluconate had the same amounts of cytochromes P-450 and b_5 as animals receiving the same amounts of vitamin C. Moreover, this maintenance of the liver cytochrome levels was achieved even though there was no evidence of any conversion of 5-oxo-D-gluconate to ascorbic acid and the ascorbic acid (TAA) content of the liver fell to scorbutic levels. It is interesting that this substance can substitute for L-ascorbic acid in this function, even though it does not act as an oxidation-reduction system as ascorbic acid does. Clearly, it is no substitute for ascorbic acid in many other functions, as it does not cure scurvy.

Degkwitz et al. (1974) made similar observations of reduced concentrations of microsomal cytochromes P-450, P-454, and b_5 in the adrenals and kidneys and of cytochrome b_5 in the spleens of ascorbic acid-deficient guinea pigs, so it seems to be a general phenomenon which is not restricted to liver microsomes.

Degkwitz and Staudinger (1974) reported "a decrease of the specific activity of the monooxygenase to about 50 per cent of the starting value in liver microsomes of guinea pigs deprived of vitamin C for 14 days. The activity was measured by the hydroxylation of acetanilid and by the demethylation of aminophenazone and of hexobarbital. The monooxygenase activity declines to the same extent as the specific concentration of cytochrome P-450, thus showing that L-ascorbic acid does not function as an electron donor for the enzyme reaction but influences the concentration of cytochrome P-450, the terminal oxidase of the system. The concentration of cytochrome P-450 decreases to about 40 per cent of the starting value during prolonged vitamin deficiency." These workers observed that the decrease starts within 24 h after omission of vitamin C, probably due to both the rapid decline of L-ascorbic acid levels in the liver of guinea pigs and to the short half-life time of the cytochrome P-450. Moreover, in contrast to the findings of Zannoni et al. (1972), they reported that, "when liver levels of L-ascorbate in guinea pigs deprived of vitamin C are restored to normal values the concentration of cytochrome P-450 returns to normal within 24 to 36 hours."

Zannoni and Sato (1975) have reported that ascorbic acid deficiency in the guinea pig is associated with a decrease in both the quantity and the quality of cytochrome P-450 in the microsomes of the liver cells. They have concluded that *O*-demethylation, *N*-demethylation, and hydroxylation reactions as well as individual liver microsomal electron transport components such as cytochrome P-450 and NADPH cytochrome P-450 reductase are decreased in guinea pigs depleted of ascorbic acid.

Sikic (1977b) reported that 21 d of ascorbate depletion caused a 50 to 60% decrease in hepatic cytochrome P-450 levels in guinea pigs, but only 20 to 30% decreases in the renal levels, and no change in the lung cytochrome P-450 level. Omaye and Turnbull (1980) also found decreased hepatic microsomal cytochrome P-450 levels and impaired drug metabolism in scorbutic guinea pigs. The hepatic cytochrome P-450 levels decreased to 86, 65, and 47% of normal after 6, 12, and 19 d, respectively, on a scorbutogenic diet.

Sutton et al. (1983) observed that a dietary supplement of ascorbic acid, 50 mg a day for

* TAA — total ascorbic acid, reduced and oxidized forms.

4 d, caused an increase in the specific activities of both cytochrome P-450 and cytochrome b_5 in the liver microsomes of guinea pigs. However, ascorbic acid supplements exceeding 100 mg/d in guinea pigs decreased the activities of both these hepatic microsomal hemoproteins. These findings are of particular interest; they confirm the findings of Holloway and Rivers (1981) concerning cholesterol-7α-hydroxylation *(vide infra)* and are analogous to the paradoxical findings of Chatterjee et al. (1975), studying carbohydrate metabolism (Chapter 3, Volume III).

Brodfuehrer and Zannoni (1987) studied the effects of ascorbic acid deficiency and ascorbic acid supplementation on the FAD-monooxygenase (FMO) activity and the cytochrome P-450 activity of the livers of guinea pigs, with and without food restriction. It was found that the heme protein cytochrome P-450 was reduced 50% by ascorbic acid deficiency, with or without food restriction, but the FMO activity which was reduced 39% as a result of ascorbic acid deficiency was further reduced 69% when ascorbic acid deficiency was combined with food restriction.

Since the flavin-containing monooxygenase is an enzyme which oxidizes a large number of drugs, including chlorpromazine, benzphetamine, nortriptyline, propylhiouracil, ethylmorphine, and the thioether-containing pesticides amino fluorene and alpha napthylthiourea, these workers concluded that, "a combination of ascorbic acid deficiency and reduced food intake could potentially alter the rate of metabolism of a great variety of pharmaceutical drugs and environmental chemicals."

X. CHOLESTEROL ACCUMULATION

Ginter (1975) observed that ascorbic acid is essential for the 7α-hydroxylation of cholesterol by cytochrome P-450, and that the conversion of cholesterol to bile acids is therefore retarded in ascorbic acid deficiency, as discussed in Chapter 5, Volume III. However, Holloway and Rivers (1981) have reported that not only ascorbic acid deficiency, but also an excessive intake of ascorbic acid can cause a reduction in cytochrome P-450 and cholesterol 7α-hydroxylase activities.

XI. CIRRHOSIS

Ginter (1975) observed that the addition of cholesterol to an ascorbic acid-deficient diet provokes an enormous accumulation of cholesterol in the livers of guinea pigs and that chronic hypovitaminosis C causes fatty cirrhosis of the liver (Figure 2). It seems paradoxical that ascorbic acid deficiency should lead to cirrhosis, as one expects to find defective collagen formation in scurvy. However, Bates (1979) has shown that hepatic collagen synthesis is spared in vitamin C deficiency. Likewise, Sulkin and Sulkin (1975) have observed large concentrations of collagen in between the cells of autonomic ganglia of guinea pigs in prolonged marginal vitamin C deficiency. Beattie and Sherlock (1976) reported a survey of the vitamin C status of 138 patients with liver disease. Significantly low leukocyte ascorbic acid levels were found in 37 patients with alcoholic liver disease ($p < 0.01$) and in 25 patients with primary biliary cirrhosis ($P < 0.05$). Multiple vitamin deficiencies are to be expected in alcoholics, who are notorious for neglecting their food, but the finding of ascorbic acid deficiency in primary biliary cirrhosis was unexpected. There was no evidence of inadequate dietary ascorbic acid intake and no significant relationship to the markedly elevated serum ceruloplasmin levels in primary biliary cirrhosis, which might have accounted for the ascorbate defiency. It is, however, possible that high liver copper levels might have been responsible for a disturbance of ascorbic acid metabolism, as the patients taking cholestyramine for the bile-salt itching of cholestatic jaundice had low vitamin C concentrations and Sherlock has shown that all forms of cholestasis are associated with high liver copper levels.

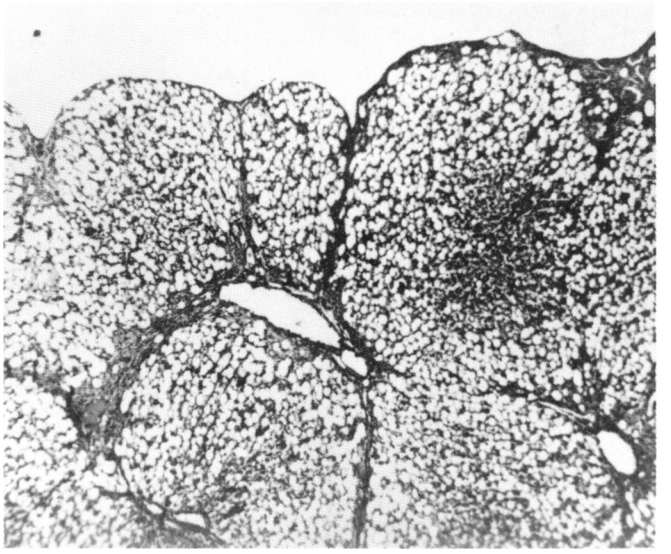

FIGURE 2. Fatty cirrhosis of the liver of a guinea pig which had been fed a cholesterol-enriched, marginally vitamin C-deficient diet for 6 months. (From Ginter, E. and Bobek, P. [1981], *Vitamin C: Ascorbic Acid*, Counsell, J. N. and Hornig, D. H., Eds., Applied Science, London, 299. With permission.)

Morgan et al. (1976) studied 80 patients with chronic nonalcoholic liver disease, 60 with cryptogenic cirrhosis and 20 with chronic aggressive hepatitis. There was no significant difference between these two groups, as regards any of the vitamin levels, so the combined data were presented. Fat-soluble vitamins A, E, and carotene were deficient in 40%, leukocyte total ascorbic acid levels were less than 2 SD below normal in 35%, folic acid deficiency was found in 17%, and 10% of the patients had deficiencies of vitamin B_1 or members of the B_2 complex. Clearly, multiple vitamin deficiencies are common in chronic liver disease and need special attention.

XII. HEPATITIS

Baur and Staub (1954) reported that they had tried 8 methods of treatment for 206 patients with infective hepatitis between the years 1947 and 1953. All eight groups received bed rest, vitamin B complex subcutaneously or intravenously, and similar intakes of carbohydrate, protein, and fat. Other treatments differed.

Of the most recently treated patients, 11 had received 10 g of ascorbic acid in 1 l of saline intravenously for 5 d in addition to the measures already described. The group receiving the ascorbic acid made the quickest recovery, as evidenced by an accelerated decrease in serum bilirubin, reduction in the duration of dysproteinemia, reduction of the period of urinary excretion of bilirubin, urobilin, and urobilinogen, and more rapid improvement in appetite and weight gain. The mechanism of action of ascorbic acid was not known, but the authors discussed a possible virucidal action or a beneficial effect on the metabolism of the liver, improving liver function and favorably affecting inflamed liver tissue. The possibility of an action to improve adrenal function was also suggested. The other methods of treatment with which ascorbic acid was compared were

1. Oral glucose plus subcutaneous insulin
2. Oral levulose

3. Multivitamin preparations or vitamin K intramuscularly, plus niacinamide, vitamin E, or percorten, intramuscularly
4. Aureomycin daily, plus cholic acid, hexamethylene tetramine, methionine, and choline
5. Daily saline infusions
6. Intravenous saline with levulose, plus B complex vitamins, and sometimes additional ascorbic acid or vitamin K
7. Infusions of saline with vitamin B complex while the patient is fasting for 2 or 3 d

An important study of high-dosage ascorbic acid in prophylaxis against hepatitis following blood transfusion was reported by Morishige and Murata (1978), working at the Fukuoka Torikai Hospital in Japan. During the period from 1967 to 1976, there were 12 cases of hepatitis among 170 transfused patients who received little or no ascorbic acid (incidence 7%) and 3 cases, all non-B, among 1367 who received 2g/d or more (incidence 0.2%). These figures could possibly have been weighted to some extent by the fact that the control patients were derived mostly from the earlier years, when testing of blood for type B hepatitis was less sophisticated and less likely to have occurred; "there were very few control patients during the years 1974 to 1976 because by 1974 the value of ascorbic acid had become so clear that the decision was made, for ethical reasons, to give vitamin C in large amounts to essentially every patient." Nevertheless, the low incidence of posttransfusion hepatitis (2 per 1000) achieved in the treated group is so low that the evidence is persuasive. One wonders what effect this treatment might have in preventing the transmission of acquired immune deficiency syndrome (or AIDS).

XIII. EFFECTS OF ENDOTOXIN

A very important study by Fukada and Koyama (1963) demonstrated that pretreatment with ascorbic acid, 500 mg daily, mixed in their soybean curd diet, protected rabbits against the toxic effects of *Salmonella typhosa* endotoxin. Ascorbic acid saturation markedly ameliorated the depletion of liver glycogen after endotoxin, both in intact and in adrenalectomized rabbits, and completely prevented the attendant hypoglycemia. Ascorbic acid saturation also prevented the reduction of blood glutathione (GSH) levels, which otherwise followed endotoxin administration. After ascorbic acid saturation, even adrenalectomized rabbits were able to tolerate a dose of endotoxin which was otherwise 100% fatal for adrenalectomized rabbits.

XIV. BILE

The cholesterol in bile is normally held in micellar solution by bile acids; so it is very pertinent that Vlahcevic et al. (1970) found a markedly reduced total bile acid pool of only 1.29 g in eight patients with gallstones, compared with 2.38 g in nine patients without gallstones ($p < 0.001$). Indeed, there was no overlap between the values in the two groups. Studies by Ginter et al. (1971) and by Jenkins (1977, 1978) have shown that ascorbic acid deficiency diminishes the bile acids to cholesterol ratio (Table 1) and the phospholipids to cholesterol ratio (Table 2) in the gallbladder and the hepatic bile of guinea pigs. This leads to an increased incidence of gallstones (Table 3). The effect is most pronounced in guinea pigs on a cholesterol diet, but also occurs in guinea pigs on an ascorbic acid-deficient diet without cholesterol.

Jenkins (1978) cited evidence that bile acids inhibit cholesterol synthesis. He therefore postulated that ascorbic acid deficiency causes not only decreased conversion of cholesterol to bile acids, but also increased cholesterol synthesis. He expressed the belief that small cholesterol crystals forming in the gallbladder undergo repeated growth and dissolution, and that growth occurs when conditions favor precipitation rather than dissolution. He cited

Table 1
THE INFLUENCE OF CHRONIC MARGINAL VITAMIN C DEFICIENCY (DURATION 25 WEEKS) ON THE COMPOSITION OF THE GALLBLADDER BILE OF MALE GUINEA PIGS FED A DIET WITH 20% BUTTER[a]

Parameter	Control (0.5% AA in the diet)	Vitamin C deficiency (1.0 mg AA/animal/d)
Cholesterol (μmol/l)	84 ± 14	104 ± 5
Chenodeoxycholic acid (mmol/ℓ)	14.1 ± 3.6	8.1 ± 0.9
7α-Ketolithocholic acid (mmol/l)	4.7 ± 1 3	3.1 ± 0.5
Lithocholic acid (mmol/l)	1.9 ± 0.1	1.1 ± 0.4
Total bile acids (mmol/l)	20.7 ± 5.1	12.3 ± 1.6
Chenodeoxycholic acid / Cholesterol	173 ± 37	77 ± 5*
Total bile acids / Cholesterol	256 ± 56	117 ± 13*

Note: Values are mean ± SEM for five guinea pigs in each group; *, significantly lower in comparison with the control group ($p < 0.05$).

[a] The bile acid/cholesterol ratio is markedly reduced by vitamin C deficiency and it seems to be this that predisposes to gallstones.

From Ginter, E. and Bobek, P. (1981), *Vitamin C: Ascorbic Acid*, Counsell, J. N. and Hornig, D. H., Eds., Applied Science, London, 299. With permission.

Table 2
BILE ACID, CHOLESTEROL, AND PHOSPHOLIPID CONTENT OF THE GALLBLADDER BILE OF GUINEA PIGS RECEIVING EITHER 0.5 OR 5 MG OF VITAMIN C DAILY

Group	Daily dosage of vitamin C	Bile acids (BA)	Cholesterol (C)	Phospholipids (P)	Ratio of BA:C	Ratio of P:C
A	0.5	8.99 ± 0.81*	2.11 ± 0.12*	7.19 ± 0.62	4.4*	3.7*
B	5	14.54 ± 0.97	1.22 ± 0.17	8.41 ± 0.82	16.7	9.3

Note: Values are millimoles per liter ± SEM; *, significantly different from group B values at $p < 0.01$ (t-test).

From Jenkins, S. A. (1977), *Experientia*, 33, 1616. With permission.

Osuga et al. (1974) who reached similar conclusions concerning the growth of gallstones in the squirrel monkey (*Saimiri sciureus*). Jenkins (1980) has shown that ascorbic acid also improves the bile acid to cholesterol ratio in the bile of pregnant guinea pigs.

XV. GALLSTONES

Pavel et al. (1969) reported a relationship between gallstones and hypo- or avitaminosis C in guinea pigs on a diet containing liver as a source of protein. Even a poor winter diet, which did not cause the usual signs of scurvy, nevertheless caused cholelithiasis. Most of

Table 3
WEIGHT CHANGES, MORTALITY, AND INCIDENCE OF GALLSTONES IN GUINEA PIGS RECEIVING EITHER 0.5 OR 5 MG OF VITAMIN C DAILY

Group	Daily dosage of vitamin C (mg)	No. of animals		Average weight change (g)	No. of survivors with gallstones
		Started	Survived		
A	0.5	18	14	+2.89	14
B	5	18	17	+25.67	0

From Jenkins, S. A. (1977), *Experientia*, 33, 1616. With permission.

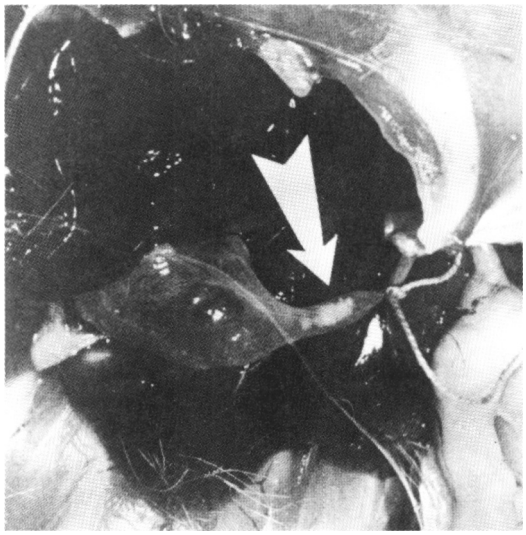

FIGURE 3. Cholesterol gallstone in gallbladder of ascorbate-deficient guinea pig fed a lithogenic diet for 1 month. (From Ginter, E. and Bobek, P. [1981], *Vitamin C: Ascorbic Acid*, Counsell, J. N. and Hornig, D. H., Eds., Applied Science, London, 299. With permission.)

the animals on the vitamin C-deficient diet also developed gastric and/or duodenal ulcers.

Di Filippo and Blumenthal (1972) found gallstones in 9 out of 12 guinea pigs after 4 weeks on an ascorbic acid-deficient diet and in only 1 out of 8 control guinea pigs receiving an ascorbic acid supplement. These were pigmented stones, but they were found on analysis to consist mainly of protein. Bellmann et al. (1974) confirmed the development of cholelithiasis in guinea pigs after 30 d on a vitamin C-free diet and also reported the development of pancreatolithiasis in these animals when deprived of ascorbic acid.

Jenkins (1977, 1978) also reported that the gallbladders of ascorbic acid-deficient guinea pigs often contain gallstones. These stones had a variable compostition, but if the diet of the guinea pigs contained cholesterol, the content of cholesterol in the gallstones may be 50 to 80%. Ginter and Bobek (1981) provided an illustration of such a guinea pig gallstone, which is reproduced in Figure 3.

Ginter and Mikus (1977) have shown that supplementary ascorbic acid (5 g/kg of diet) decreased the incidence of gallstones in golden hamsters on a semipurified, fat-free, high-

glucose diet known to produce cholesterol gallstones. Ascorbic acid also lowered the cholesterol concentration and half-life in the blood plasma and in the liver and accelerated cholesterol transformation to bile acids. Jenkins has made similar observations in repeated studies of guinea pigs. It is not yet known whether this knowledge will be useful in the case of human beings.

Large doses of vitamin C (1 g daily) for 1 or 2 weeks did not alter the chemical composition of the bile in the ten healthy subjects studied by Pedersen (1975), but might be expected to have a beneficial effect in people with latent hypovitaminosis C. It is certainly of interest that Coyne et al. (1976) observed a decreased cytochrome P-450-dependent 7α-hydroxylase activity in liver biopsies from gallstone-forming human subjects. In all probability, long-term adherence to a high-ascorbate diet will be found to be beneficial in reducing the incidence of gallstones, especially if the cholesterol and the carbohydrate intake are controlled, but it is very doubtful if such a diet will do anything to remove gallstones that have already formed.

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