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Vitamin C

Volume I

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

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Classical Scurvy: A Historical Review
Chronic Subclinical Ascorbic Acid Deficiency
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Chapter 1

CLASSICAL SCURVY: A HISTORICAL REVIEW

The suffering and the toll of human lives taken by vitamin C deficiency over the ages cannot even be estimated, but we can be sure, by reading old accounts of scurvy, that it must have been a common catastrophe, especially among sailors on long sea voyages, but also as a winter epidemic among urban populations; it ravaged whole armies and occurred among the inhabitants of besieged cities. It appeared wherever man relied solely on food held in storage. The early literature on scurvy was reviewed by Bourne in 1944; he showed hieroglyphs (Figure 1) from Egyptian papyri as convincing evidence that this disease, characterized by bleeding gums and hemorrhages in the skin, existed about 3000 years ago. He also refers to the writings of Hippocrates (500 B.C.) where there is the description of a disease which can unequivocally be regarded as scurvy. Scurvy occurred at the siege of Damietta in the First Crusade and was described by Jacques de Vitry as follows (Guizot, 1825). "A large number of men in our army were attacked also by a certain pestilence, against which the doctors could not find any remedy in their art. A sudden pain seized their feet and legs; immediately afterwards the gums and teeth were attacked by a sort of gangrene, and the patient could not eat any more. Then the bones of the legs become horribly black, and so, after having continued pain, during which they showed the greatest patience, a large number of Christians went to rest on the bosom of our Lord." There is also a first-hand account by Jean Sire de Joinville in 1250 of scurvy disabling and killing the soldiers of Louis IX near Cairo in the Seventh Crusade; this was published in Menard's 1617 edition of de Joinville's writings and was translated into English by James Hutton in 1910; the account reads as follows: "We had no fish in the camp to eat during Lent except the karmout (a kind of eel), which preyed upon the dead bodies, for they are a gluttonous fish. And in consequence of this misfortune, and of the unhealthiness of the country, where never a drop of rain falls, we were attacked with the army sickness, which was such that our legs shrivelled up and became covered with black spots, and spots of the colour of earth, like an old boot; and in such of us as fell sick the gums became putrid with sores, and no man recovered of that sickness, but all had to die. It was a sure sign of death when the nose began to bleed: there was nothing left then but to die."

Not only did the disease cause great suffering, it influenced the outcome of many land and naval battles and has thus had a profound effect on human history.

It is strange that humans lack the instinct to know what they need when they are beset by this disease. We thirst when we need water, we seek food when we are hungry and we sleep when we are tired, but we seem to have no instinct to seek fresh fruit and vegetables when we are vitamin C deficient.

One could argue that we lack this instinct because, as primates, we have evolved from mammals not needing vitamin C, having the capacity to manufacture it from simple sugars in their livers; but it is more than that. We lack so many other instincts possessed by animals. Foals can get up and run at birth, but human infants take a year to learn to walk. Birds fly south for the winter, but the Arctic Eskimo had no such instinct to migrate southwards. Dogs eat grass when they are sick, but sick human beings do not increase their intake of fresh fruits and vegetables. We have to work out by trial and error, or by logic, so many things that animals know by instinct; however, our naked ignorance at birth has forced us to learn by experiment and to work things out for ourselves; it may be this very ignorance at birth that has led us to become the most experimenting, calculating, and thinking creatures on Earth. There is no need to experiment if you already know. Indeed, "certainty of knowledge is the antithesis of progress." We must always question old tenets and question

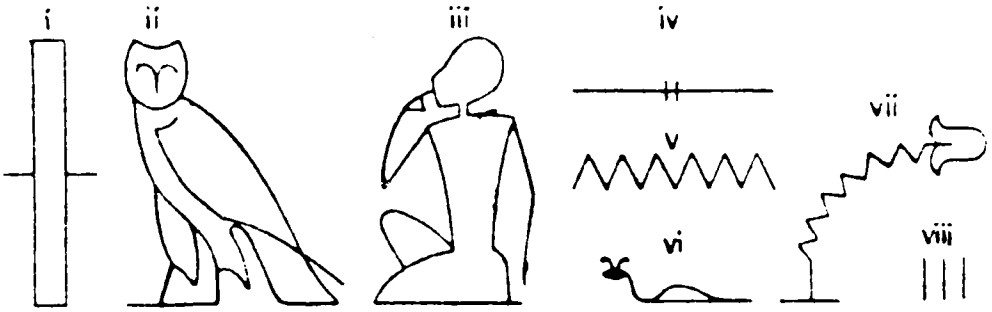

FIGURE 1. Ancient Egyptian hieroglyphs believed by Ebbell (1938) and by Bourne (1944) to represent scurvy; hieroglyphs iv, v, vi, vii, and viii indicate a disease characterized by petechial hemorrhages in the skin. (From Bourne, G. H., [1944], *Proc. R. Soc. Med.*, 37, 512. With permission.)

our own preconceived ideas. We must design experiments to test hypotheses. This we do and thus, we learn, but it has been a slow and painful progress.

Unfortunately sometimes we do not have or we do not recognize the evidence on which to base our conclusions. The Arctic Eskimos saw the sun rise in the east, so they migrated eastwards from the Bering Straits to the Hudson Bay and eventually all the way to Greenland. They did not know that it was warmer to the south. How could they? But the caribou knew by instinct to go south at the end of the summer.

Similarly, people attempting to care for patients with scurvy, did not know what was needed.

In the winter of 1535, the men of the second expedition of Jacques Cartier were on shore in Newfoundland when they were beset by a very serious illness in December; they did not know the nature of the disease, but Cartier's description which follows, was recorded by Lind (1753) and leaves no doubt that it was, indeed, scurvy.

> In the month of December, we understood that the pestilence was come upon the people of Stadacona; and in such sort, that before we knew of it, above fifty of them died. Whereupon we charged them neither to come near our forts, nor about our ships. Notwithstanding which, the said unknown sickness began to spread itself amongst us, after the strangest sort that ever was either heard of or seen; insomuch that some did lose all their strength, and could not stand upon their feet; then did their legs swell, their sinews shrunk, and became as black as a coal. Others had also their skin spotted with spots of blood, of a purple colour. It ascended up their ancles, knees, thighs, shoulders, arms and neck. Their mouth became stinking; their gums so rotten, that all the flesh came away, even to the roots of their teeth; which last did also almost all fall out. This infection spread so about the middle of February, that of a hundred and ten people, there were not ten whole; so that one could not help the other; a most horrible and pitiful case! Eight were already dead; and more than fifty sick, seemingly past all hopes of recovery. This malady being unknown to us, the body of one of our men was opened, to see if by any means possible the occasion of it might be discovered, and the rest of us preserved. But in such sort did the calamity increase, that there were not now above three sound men left. Twenty-five of our best men died; and all the rest were so ill, that we thought they would never recover again: when it pleased God to cast his pitiful eye upon us, and send us the knowledge of a remedy for our health and recovery.
>
> Our Captain considering the deplorable condition of his people, one day went out of the fort, and walking upon the ice, he saw a troop of people coming from Stadacona. Among those was Domagaia, who not above ten or twelve days before laboured under this disease; having his knees swelled as big as a child's head of two years old, his sinews shrunk, his teeth spoiled, and his gums rotten and stinking. The Captain, upon seeing him now whole and sound, was thereat marvellous glad, hoping to know of him how he had cured himself. He acquainted him, that he had taken the juice of the leaves of a certain tree, a singular remedy in this disease. The tree in their language is called *ameda* or *hanneda;* by a decoction of the bark and leaves of which, they were all perfectly recovered in a short time.

Sir Richard Hawkins was clearly well informed, for he is recorded as having effected a cure for scurvy by giving lemon juice to his men in 1593.

The following extract from Chapter 12 of a book published in 1596 by Mr. William Clowes, surgeon to Queen Elizabeth I, enables us to picture the miserable condition of people afflicted with scurvy and the difficulties experienced by others in finding a cure.

The Cure of Two Seafaring Men which Fell Sick at the Sea of the Scorby

I cannot here well pass over this briefe note or observation of the curing two seafaring men, which travelled a long time upon the seas, and there fell sicke of the Scorby, which infection as I gathered by inquiry, was reputed principally unto their rotten and unholsome victuals, for they said their bread was musty and mouldie Bisket, their beer shapre and sower like viniger, their water corrupt and stinking, their best drink they had, they called Beucridge, halfe wine and halfe putrified water mingled together, and yet a very small and short allowance, their beefe and porke was likewise, by reason of the corruption thereof, of a most lothsome and filthy taste and savor, insomuch that they were constrained to stop their noses, when they did eat and drinke thereof: moreover their bacon was restie, their fish, butter and cheese wonderfull bad, and so consequently all the rest of their victuals: by means hereof, and likewise lacke of convenient exercise, cleane keeping and thrift of apparell, and again being in an ill disposed climate, and want of good aire: these causes and such like were the only means they fell in to Scorby, for their gums were rotten even to the very roots of their teeth, and their cheeks hard and swolen, their teeth were loose neere readie to fall out, their jawes very painfull, their breath of a filthy savor, that at what time I dress their gums, and washed their mouthes, the savor was so odious that I was scarse able to staie and abide it: in like maner their legs were feeble, and so weake, that they were scarse able to carrie their bodies: moreover, they were full of aches and paines, with some blewish and reddish staines or spots, some broad and some small like flea bitings, or the graines of a Pomegranate, likewise their legs were colde, hard, and swolen, which caused me to fear a Gangraena, for colones in such extremities being in corupt bodies full of evill juice, doth challenge putrifaction, which disease or sickness, although it be in some safely cured, yet experience daily proveth that a number also die. Now the first thing that required helpe by Chirurgery was their gums, and their legs, being the conjoined cause, but for that I will proceede as orderly as I can in my writing. I will begin with the antecedent cause inwardly, which was done and performed by the advise and counsel of learned Physitians* who very confidently set me down their opinions for their maner and order of purging with other remedies, as hereafter followeth: First as I said, evacuation going before, to diminish the humors sore abounding, it was therefore thought most meete to begin with blood letting in the middle vain on the left arme, . . .

* N.B. Mr. William Clowes was a Chirurgion (not a Physitian).

Records of the first voyage from England to the East Indies by ships of the newly formed East India Company in 1600 provided an excellent example of the value of lemon juice. A squadron of four ships was sent under the command of Captain James Lancaster who sailed in the Dragon. When they reached the Cape of Good Hope, all the men except those aboard the Dragon were so ill with scurvy that they were hardly able to bring their ships to anchor. But there was no scurvy on the flagship because of the lemon juice which Captain Lancaster had brought to sea in bottles and of which he had issued three teaspoonsful every morning to those who showed the slightest signs of scurvy; 105 of the 424 men on this expedition died of scurvy, but none died aboard the Dragon.

Dr. John Woodall, a surgeon of the East India Company, certainly knew that lemons provided an excellent treatment for scurvy, and he also knew that lemon juice could be used as a preventative, for he wrote as follows in his chapter on scurvy in his book, *The Surgeons Mate,* published in 1639: "Some Chirurgeons also give of this juice daily to the men in health as a preservative, which course is good if they have store, otherwise it were best to keep if for need." But he had no concept of scurvy as a deficiency disease, for he also wrote:

Truly, the causes of this disease are so infinite and unsearchable as they farre pass my capacity to search them all out . . . Some charge Bisket as a cause of the scurvie but I am not of their opinion; some say inordinate watchings are the cause thereof; some say extreme labour wanting due nourishment; some also affirme cares and griefe to be some cause thereof; others affirme the very heat of the aire, resolving the spirits; but what shall I amplify further? for it is also true that they which have all the helpes that can be had for mony, and take as much care as men can devise are even by the evil disposition of the aire, and the course of nature, strook with a scurvie, yea and die thereof at sea and land both.

. . . The juyce of Lemmons is a precious medicine and well tried, being sound and good, let it have the chiefe place, for it will deserve it, the use whereof is: It is to be taken each morning two or three spoonfulls, and fast after it two hours, and if you add one spoonfull of Aquavitae thereto to a cold stomach, it is better. Also if you take a little thereof at night it is good to mix therewith some sugar, or to take the syrup thereof is not amisse . . . In want whereof, use the juyce of Limes, Oranges or Citrons, or the pulpe of Tamarinds.

Bächström (1734) however, recognized it as a deficiency disease, for he wrote:

From want of proper attention to the history of scurvy, its causes have been generally, though wrongfully, supposed to be, cold in northern climates, sea air, the use of salt meats, etc.: whereas this evil is solely owing to a total abstinence from fresh vegetable food and greens; which is alone the primary cause of the disease.

He is quoted by Lind (1753) with the following tale:

A sailor in the Greenland ships was so over-run and disabled with the scurvy, that his companions put him into a boat, and sent him on shore; leaving him there to perish, without the least expectation of recovery. The poor wretch had quite lost the use of his limbs; he could only crawl about on the ground. This he found covered with a plant, which he, continually grasing like a beast of the field, plucked up with his teeth. In a short time he was by this means perfectly recovered; and, upon his return home, it was found to be the herb scurvy grass.

But several more years were to pass before Bächström's ideas were tested, and many more still before they were put into general use.

During Lord Anson's voyage around the world from 1740 to 1744, more than half of the men on the Centurion, and on the Gloucester, died of scurvy during the 3-month journey from Cape Horn to the Islands of Juan Fernandez (Anson 1748).

Lest we should think of scurvy as only a nautical disease, we should be reminded of Dr. John Cook's letter to Dr. James Lind in which he described conditions in eastern Europe from his own experience there in 1738 and 1739. At Taverhoff, where the Verona (r. Voronezh) joins the Don, south of Moscow, at Astrakhan on the estuary of the Volga by the Caspian Sea, and at Riga in Livonia (Latvia) on the Baltic, he reported that scurvy was an "endemic and dreadful disease" in all those areas during the long winters (Lind 1753).

It was James Lind who conducted a controlled trial of various remedies for scurvy and thus proved that oranges and lemons could cure this condition within 6 to 26 d. In fact, although Lind rediscovered and described a practical cure and a preventative for scurvy, his main fame should be as one of the first persons recorded as having conducted a controlled clinical trial; his experiment was carried out under very difficult circumstances, on board a rolling 50-gun, 3-masted sailing ship, in the 3rd month at sea in the English Channel, at which time Lind himself may not have been in the best of health; but his work in its brilliant simplicity stands, along with Harvey's discovery of the circulation of the blood and Jenner's discovery of vaccination, as one of the foundation stones at the beginning of the new area of "learning by experiment", the beginning of the modern scientific era.

In his *Treatise of the Scurvy* (1753), Lind states:

On the 20th of May 1747, I took twelve patients in the scurvy, on board the *Salisbury* at sea. Their cases were as similar as I could have them. They all in general had putrid gums, the spots and lassitude, with weakness of their knees. They lay together in one place, being a proper apartment for the sick in the fore-hold; and had one diet common to all, *viz*, watergruel sweetened with sugar in the morning; fresh mutton-broth often times for dinner; at other times puddings, boiled biscuit with sugar, &c.; and for supper, barley and raisin, rice and currants, sago and wine, or the like. Two of these were ordered each a quart of cyder a-day. Two others took twenty-five gutts of *elixir vitriol* three times a-day, upon an empty stomach; using a gargle strongly acidulated with it for their mouths. Two others took two spoonfuls of vinegar three times a-day, upon an empty stomach; having their gruels and their other food well acidulated with it, as also the gargle for their mouth. Two of the worst patients, with the tendons in the ham rigid, (a symptom none of the rest had), were put under a course of sea-water. Of this they drank half a pint every day, and sometimes more or less as it operated, by way of gentle physic. Two others had each two oranges and one lemon given them every day. These they eat with greediness, at different times, upon an empty stomach. They continued but six days under this course, having consumed the quantity that could be

spared. The two remaining patients, took the bigness of a nutmeg three times a-day, of an electuary recommended by an hospital-surgeon, made of garlic, mustard-seed, *rad. raphan.* balsam of Peru, and gum myrrh; using for common drink, barley-water well acidulated with tamarinds; by a decoction of which, with the addition of *cremor tartar,* they were gently purged three or four times during the course.

The consequence was, that the most sudden and visible good effects were perceived from the use of the oranges and lemons; one of those who had taken them, being at the end of six days fit for duty. The spots were not indeed at that time quite off his body, nor his gums sound; but without any other medicine, than a gargarism of *elixir vitriol,* he became quite healthy before we came into Plymouth, which was on the 16th of June. The other was the best recovered of any in his condition; and being now deemed pretty well, was appointed nurse to the rest of the sick.

Lind believed that other factors accelerated the onset of the disease. He recorded two spring voyages of 10 and 11 weeks aboard H.M.S. Salisbury in the English Channel, from April to June in 1746 and 1747, in which scurvy began to rage after only 1 month at sea, and another 12-week voyage from August 10 to October 28 during which only one sailor was afflicted with scurvy. He attributed this to cold, damp, close, and foggy weather during the spring voyages and mainly fair weather during the autumn voyage. Certainly the stress of bad weather may have played a part, but one must also consider the fact that human vitamin C stores are lowest in the spring, after a long winter of consuming stored foods; they are highest in the fall, when all the fresh fruits and vegetables have been available. So the sailors probably had very low vitamin C stores when they set sail in April.

Lind was by no means the first to use oranges and lemons in the treatment of scurvy; he recorded their successful use by Admiral Sir Richard Hawkins (1662) and by others. Lind was the first to compare different treatments and to record his observations. The problem was that so many treatments had been recommended, and most, like scurvy grass, were effective only when freshly harvested.

Scurvy grass (*Cochlearia officinalis*) is a member of the order Cruciferae, so its curative powers were supposed by superstition and religious belief to be due to its four petals forming the sign of a cross. Of course this plant, when dried and stored in the apothecary jar, or boiled to prepare a potion, is quite ineffective. It was this rapid loss of activity on storage that caused so much confusion about which particular plant was, and which was not, effective in the treatment of scurvy.

Captain James Cook demonstrated the effectiveness of James Lind's ideas. During 3 years at sea on his second voyage "towards the South Pole and around the World," from 1772 to 1775, he did not have one sailor sick with scurvy. Not only did he take every opportunity to bring fresh fruits and vegetables aboard for his men, he also took care to improve their living conditions, as recommended by Lind. In 1776 Captain Cook was elected a Fellow of the Royal Society and he was subsequently awarded the Copley Gold Meal for "preventing scurvy."

However, it was not until many years later that the Royal Navy made a regular issue of 1 oz of lemon juice mandatory for every sailor after 2 weeks at sea. Sir Gilbert Blane in 1830 clamined that the use of lemon juice in the Royal Navy was begun in 1795, and scurvy was "totally rooted out" within 2 years.

However, there was no regular and general issue of lemon juice in the Navy in 1795. In fact, the seamen knew of the value of lemon juice by that date and resented it not being supplied to them. Indeed, Admiral Waldegrave wrote a letter to the Admiralty on November 24, 1797, requesting payment for some vegetables and lemon juice he had bought for the men of H.M.S. Pluto and was refused (Smith 1919a). He wrote again on December 2, explaining that these provisions had helped the men from joining the "mutinous proceedings of the Latona..."

There is a marginal note on this letter, which may be in Sir Gilbert Blane's own handwriting as he was then one of the Commissioners of the Sick and Wounded Board of the Admiralty.

"December 4: Let me see whether he received the second order about confining the use of lemon juice and sugar to those on the surgeon's list."

As reported by Smith (1919b), even in 1801, it was only after some correspondence with the Sick and Wounded Board, of which Sir Gilbert Blane was still a member, and after overcoming obstruction, that Dr. Baird, surgeon on board Lord St. Vincent's flagship, got that full issue of lemon juice to the fleet during the siege of Brest that secured for it the exceptional record of health triumphantly cited by Sir Gilbert Blane.

It was only in August 1804 that representations of Dr. Baird to the Lords Commissioners achieved the order that lemon juice and sugar should be issued regularly to the Channel Fleet. Previously it was given only to ships going on foreign service and for the use of the sick.

Professor George Budd recognized scurvy, rickets, and keratomalacia as diseases due to deficiencies of specific elementary principles in the diet and recommended the provision of better diets in ships, prisons, and garrisons. In 1842 he published a series of five articles, based on his lectures at King's College, entitled "Disorders Resulting from Defective Nutriment". Speaking of scurvy, he said that, "men had not yet perceived that the disease had its real origin, not in the cold of our rigorous climate, but in abstinence from fresh fruit and vegetables."

When practically extinct in the Royal Navy, scurvy was still a scourge in the Mercantile Marine, especially in ships sailing to and from the East: it was reduced when the use of lemon juice was made compulsory by Act of Parliament in 1844, largely at the instigation of Dr. Budd. Again, when the Merchant Shipping Amendment Act of 1867 doubled the compulsory issue from 1/2 to 1 oz daily, and ensured that the juice supplied should reach a certain standard of quality, there was a significant drop in the number of cases received into the Seaman's Hospital, according to Curnow and Smith (1891) and Smith (1896).

However, sporadic reports of scurvy still occurred when injured or decayed fruit was used to prepare the juice, and also when the lemon juice was adulterated, embezzled, or substituted. Pereira (1853) reported, "Lemon juice has long been regarded as an invaluable antiscorbutic; but, on account of the difficulty of preserving it, crystallized citric acid is usually substituted."

There was much confusion arising from the assumption that the acidity of citrus fruit juices was a measure of antiscorbutic activity. When Sir James Ross' polar expedition of 1848 returned in 1849 with a report of a serious outbreak of scurvy, the lemon juice aboard his ships was studied and was found to "lack nine tenths of the proper acid content." A Board of Inquiry was formed, and as a result, fresher, more acid supplies of lemon juice were sought. The tests of quality were always chemical, of course, not therapeutic, and consisted principally of ascertaining the amount of alkali that was neutralized by a given amount of juice. Of course, it was a mistake to suppose that acidity was the important element. Pure citric acid had been known since 1820 to compare unfavorably with the fruit juice in the treatment of scurvy. Yet by some confusion surgeons then often spoke of their fresh lemon juice as "citric acid"; somehow Mediterranean lemon juice (of *Citrus medica* var. *limonum*) from Malta became known as lime juice, and English sailors became known as "Limeys".

Later, West Indian lime juice (of *C. medica* var. *acidicum*) from Montserrat was substituted, either in error or because of its greater acidity; this change is believed to have been the cause of scurvy among the members of Sir George Nares' Arctic expedition in 1875, and much later among British troops in the Middle East during World War I. It was Alice Henderson Smith (1918, 1919a, b) who searched old records and discovered the change from lemons (and some sweet Mediterranean limes) to sour West Indian limes, which had occurred many years earlier in the 1860s. Harriette Chick, Margaret Hume, and Ruth Skelton (1918) reported fresh West Indian lime juice to have only about one quarter the potency of

fresh lemon juice. Moreover, they reported that lemon juice retains its antiscorbutic value longer than lime juice. Some specimens of stored lime juice were found to be completely devoid of antiscorbutic activity. These comparative assays of different samples of lime juice and lemon juice were made possible by the important discovery of Holst and Frölich, at the University of Christiania in Norway (1907, 1912), that the guinea pig is a vitamin C-dependent animal and can be used for measuring the essential nutrient that decays so rapidly.

Scurvy was present in the British army in the Crimean War; Dr. Linton (1858) described the suffering of a grenadier, aged 23, with a fractured humerus. He had fallen and broken his arm while carrying a log across some frozen snow. In the hospital, old ulcers on his leg opened up and his gums became spongy; the callus which formed at the fracture site was unusually small; only when his diet was improved did his ulcers heal.

Scurvy affected the Confederate Armies during the American Civil War; it complicated wounds and interfered with surgical operations, as recounted by Eve (1866).

Turkish soldiers suffered severely from scurvy in the World War I, and in those who developed the disease, both flesh wounds and fractures healed poorly as recounted by Lobmayer (1918).

Outbreaks of scurvy among British troops in the Middle East during World War I prompted the British government to fund studies of scurvy at the Lister Institute in London in order to determine the relative antiscorbutic values of different foods and to study the storability and transportability of antiscorbutic foods. They used guinea pigs and monkeys as experimental animals and confirmed the work of Fürst (1912) that dry cereals and legumes (oats, barley, peas, beans, and lentils) which have no antiscorbutic value, develop it during germination (Chick and Hume 1917). Moreover, these seeds are readily transportable in the dry state and can be germinated easily when needed. Thousands of pounds of dried peas were available on board ship when sailors were dying of scurvy, but they did not know that their lives could be saved by allowing them to germinate.

Studies of the chemical nature of vitamin C were pursued at the Lister Institute for many years, using frequent animal assays (Zilva 1924[a,b], 1929, 1930). By 1930, Zilva had achieved a 300-fold concentration of the active substance from lemon juice and knew that the vitamin was water-soluble, contained no nitrogen, and was probably a derivative of a hexose; he also knew that it was highly susceptible to oxidation, especially in alkaline solutions.

In 1928, Albert Szent-Györgyi, a Hungarian chemist working at Cambridge University on oxidation-reduction systems, isolated a six-carbon substance with strong reducing properties from the cortex of the adrenal gland of the ox and also from oranges and cabbages. At first, not knowing what it was, but believing it to be a sugar, he jokingly called it "Godnose"; later, "hexuronic acid".

Progress was greatly aided by the discovery of Tillmans and co-workers in Frankfurt that the antiscorbutic principle was a reducing agent and could be estimated by titration with the dye 2,6-dichloroindophenol. Using this knowledge, Tillmans and Hirsch (1932) and others were able to distinguish active from inactive substances.

Then, Svirbely and Szent-Györgyi (1932a, b), at the University Szeged in Hungary, and King and Waugh (1932), at the University of Pittsburgh, almost simultaneously demonstrated that "hexuronic acid", the oxidation-reduction factor found in the adrenal cortex, oranges, and cabbage is the antiscorbutic principle, vitamin C (Waugh and King 1932a, b).

Haworth and Szent-Györgyi decided to call it ascorbic acid. Vedder (1932), of the U.S. Army, also isolated pure crystals of the vitamin.

Svirbely and Szent-Györgyi (1933) showed that the ascorbic acid content of the adrenal glands of guinea pigs fell from a mean of 0.9 mg/g on a basal diet that was liberally supplemented with fresh spinach to 0.03 mg/g after 20 d on the basal, ascorbic acid deficient diet, alone. Moreover, they found that guinea pigs receiving just enough lemon juice (1.5 cc daily), or ascorbic acid from paprika juice (0.5 mg daily), to prevent scurvy, nevertheless, had low adrenal ascorbic acid levels of 0.13 and 0.09 mg/g, respectively.

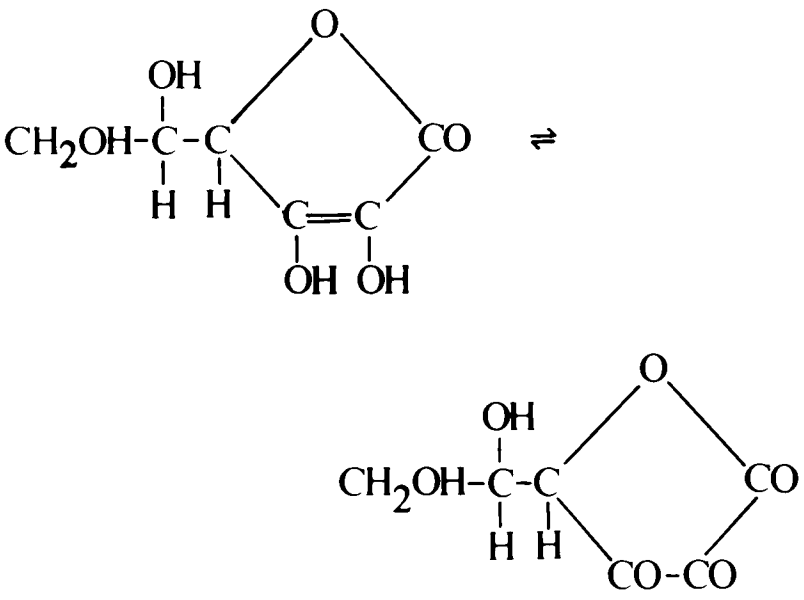

FIGURE 2. The structures of ascorbic acid (above) and its reversible oxidation product dehydroascorbic acid (below). (From Haworth, W. N. [1933], *J. Soc. Chem. Ind.*, 52, 482. With permission.)

They concluded that, "there is a wide limit between health and scurvy and that animals fed on restricted amounts of the vitamin, though not showing signs of scurvy, are greatly depleted of their vitamin store."

The chemical structure of vitamin C, shown in Figure 2, was established in 1933 by Haworth, Hirst, Percival, Reynolds, Smith, and Cox, working at Birmingham University, and in the same year by Karrer et al., by Micheel and Kraft, and by von Euler and Klussman.

The vitamin was synthesized in 1933 by the Nobel Prize winner, Professor Tadeus von Reichstein, and his co-workers in Switzerland, who confirmed its structure on the basis of its preparation from xylose or from sorbose. It was synthesized independently in the same year by Ault et al. of Haworth's group in England. Further work by Reichstein and Grüssner (1934) led to the industrial production of ascorbic acid on a commercial scale by the Roche Company in Basel.

Those interested in delving more deeply into the history of scurvy are referred to excellent accounts by Bourne (1944), Major (1945), Lorenz (1953), and Stewart and Guthrie (1953).

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Chapter 2

CHRONIC SUBCLINICAL ASCORBIC ACID DEFICIENCY

Frank scurvy, due to a complete lack of fresh fruits and vegetables, is seldom seen nowadays, but lesser degrees of ascorbic acid deficiency are still very common; moreover, evidence is accumulating which leads one to believe that prolonged borderline deficiency of this vitamin may have less obvious, but more permanent effects, doing harm to many organs and tissues and accelerating age changes in the heart and the brain. There is evidence that chronic subclinical ascorbic acid deficiency predisposes to atherosclerosis, to amyloidosis, and to diabetes mellitus, which slowly and progressively damage the blood vessels throughout the body.

It seems that the enzyme L-gulonolactone oxidase, which permits the synthesis of ascorbic acid from simple sugars, developed in amphibians as these aquatic vertebrates began to leave the water (Figure 1). Chatterjee et al. (1975) have suggested that the ability to synthesize ascorbic acid may have afforded some protection from the higher oxygen content of the air; indeed, ascorbic acid does protect against oxygen toxicity in man (Chapter 24 of this volume), and it is interesting to note that some people live to a great age in certain mountainous regions of the world where the oxygen tension is low (Chapter 5 of this volume).

All mammals except the guinea pig, the higher apes, and certain bats are able to synthesize ascorbic acid in the liver (Figure 2), while amphibians and reptiles synthesize this substance in the kidney (Table 1).

Clearly, the lack of this enzyme was no tragedy for the guinea pig as this animal obtains a plentiful supply of vitamin C by eating grass. Similarly, the higher apes and the flying mammals manage by feeding on fresh greens, berries, and fruit. However, for us the loss or suppression of this enzyme becomes a very serious problem when we try to survive the winter on stored foods. Indeed, we are defective mammals, lacking fur and lacking the enzyme needed for the synthesis of ascorbic acid; we should take as much care to make up for this enzyme defect as we do in providing ourselves with clothing and housing to make up for our lack of fur.

It is becoming apparent that disorders of ascorbic acid metabolism are much more common than a simple dietary deficiency of this vitamin; moreover, a substandard dietary intake and a disturbance of ascorbic acid metabolism often occur together and each compounds the other. The whole of Section II of this volume is devoted to the many intrinsic and extrinsic factors which seem to affect ascorbic acid metabolism; it may be noted that most of the factors having adverse effects on ascorbic acid metabolism are known to be conductive to vascular disease.

One would never think of treating a patient with sickle cell disease or cystic fibrosis of the pancreas without taking those inborn errors of metabolism into account. Likewise, no human being should ever be treated without regard to our common metabolic defect.

Throughout this book special emphasis has been placed on certain heavy metals which have two valency states and the ions of which catalyze the oxidation of ascorbic acid. Such oxidation is important because the oxidized form of this vitamin — dehydroascorbic acid — is an unstable compound with a short half-life, readily undergoing hydrolysis with loss of vitamin activity. It is presumably the electron acceptance involved in the valency change from cupric to cuprous, ferric to ferrous, mercuric to mercurous, and manganic to manganous, that catalyzes the oxidation of ascorbic acid *in vitro;* somewhat similar changes seem to be brought about by certain iron and copper metalo-enzymes in the body (Chapter 10 of this volume).

In hard-water regions, deposits of insoluble salts form inside household water pipes and

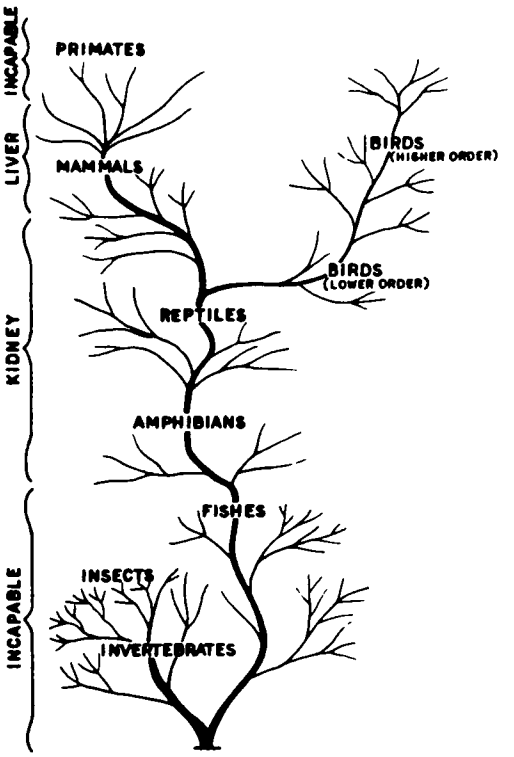

FIGURE 1. Schematic representation of ascorbic acid synthesizing abilities of various species of animals in relation to their phylogeny. (From Chatterjee, I. B. [1973], *Science*, 182, 1271. With permission.)

prevent them from eroding, but in soft-water areas there is a tendency for copper and iron pipes to become eroded, and a very high iron or copper content may be found in the household water supply. In soft-water regions the copper level often reaches 2 ppm in the first water drawn from copper pipes in the morning. Thus, the ascorbic acid in food can be partially oxidized, hydrolyzed, and lost, even before it is consumed, if it is mixed, or especially if it is cooked with such water. Moreover, ascorbic acid is mutagenic in the presence of copper, as observed by Stich et al. (1976), presumably due to release of "ascorbate-free-radical" or monodehydroascorbic acid.

It is, therefore, very important that ascorbic acid should not be taken as such, with tap water, but should always be taken with the chelating food fibre of plants, which binds and inactivates heavy metal catalysts. The indirect antioxidant activities of chelating bioflavonoids, tannins, and catechins are, therefore, reported in detail in this book and reasons are given for suggesting that D-catechin (or +catechin), known as vitamin C_2 by earlier French writers, is the substance of choice.

Controlled experiments by many workers will be cited, showing that chronic borderline ascorbic acid deficiency predisposes to atherosclerosis, to amyloid degeneration, and to diabetes mellitus in guinea pigs. All of these lead to degenerative vascular disease, so it is very pertinent that the death rates from all forms of vascular disease have been found to be higher in soft-water areas than in hard-water areas in the U.S., the U.K., and in Japan where they have been studied (Chapter 10 of this volume).

Chronic subclinical ascorbic acid deficiency is no respecter of persons: it affects middle class individuals just as it does the poor. In some people it very slowly and surreptitiously damages the β-cells of the islets of Langerhans, leading to temporary or permanent diabetes

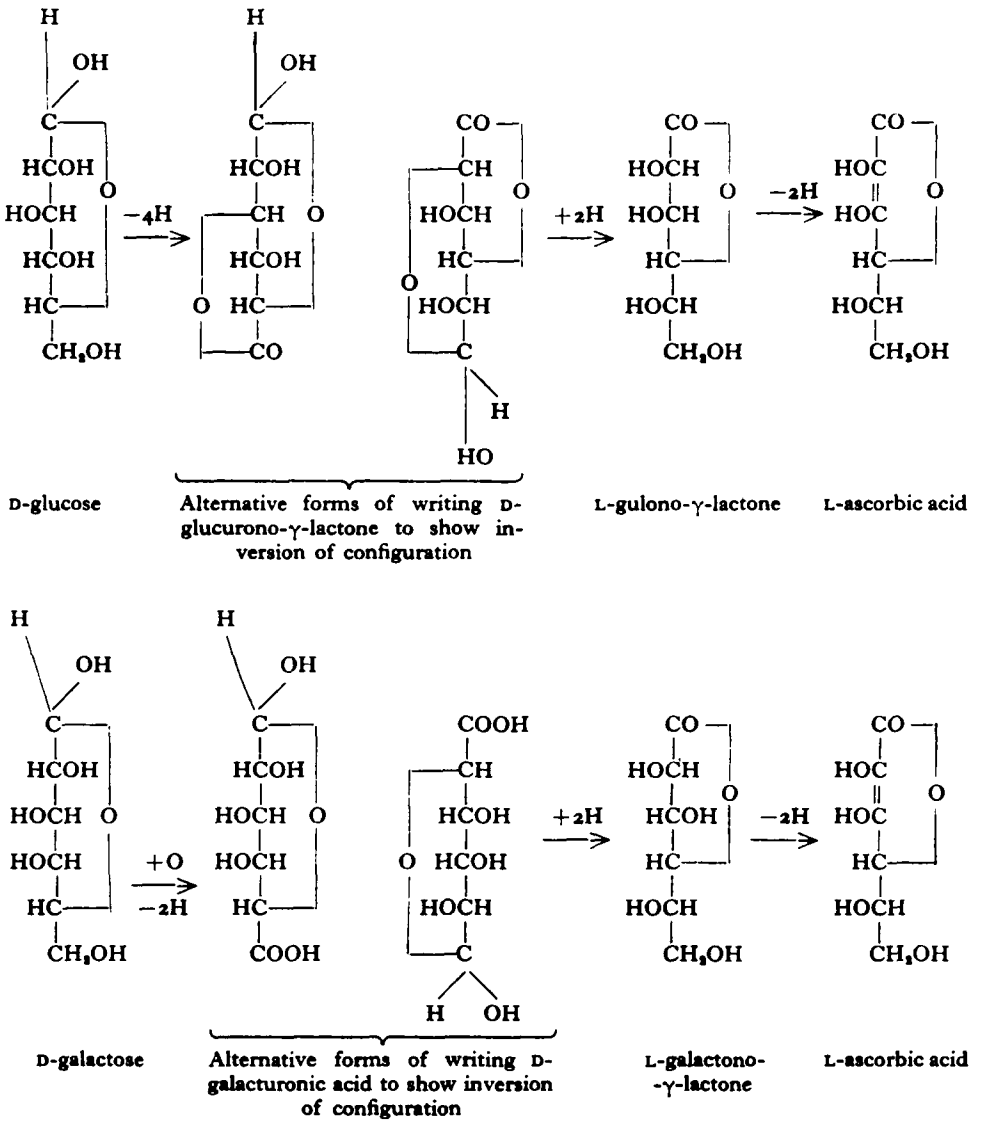

FIGURE 2. Synthesis of L-ascorbic acid in rats. (From Isherwood, F. A. [1953], *Proc. Nutr. Soc.*, 12, 335. With permission.)

mellitus. In some it damages the intima of the arteries and facilitates the subendothelial deposition of cholesterol. In others it may facilitate amyloid degeneration. Needless to say, some people develop atherosclerosis, amyloid degeneration, and diabetes mellitus — all three.

The possible roles of subclinical ascorbic acid deficiency in peptic ulcer disease, in gallstone formation, in deep vein thrombosis, in mental depression, in arthritis, in osteoporosis, and in many other serious disease states will be discussed in this book, but it seems clear that these are all multifactorial disorders; ascorbic acid deficiency simply tips the scales in someone already predisposed to the disease. Even so, a proper supply of ascorbic acid containing foods, or correction of ascorbic acid-metabolism, can often tip the scale the other way and can prevent ill health or restore good health.

Undoubtedly, there are many factors, including smoking, stress, heredity, and cholesterol

Table 1
ASCORBIC ACID SYNTHESIS FROM L-GULONO-1,4-LACTONE IN MICROSOMAL FRACTIONS FROM TISSUES OF DIFFERENT SPECIES OF ANIMALS

Animals	Ascorbic Acid Synthesized (μg/mg protein/h)	
	Kidney	Liver
Insects	—	—
Invertebrates	—	—
Fishes	—	—
Amphibians		
Toad (*Buto melanostictus*)	144 ± 10	—
Frog (*Rana tigrina*)	115 ± 10	—
Reptiles		
Turtle (*Lissemys punctata*)	98 ± 8	—
Bloodsucker (*Caloter versicolor*)	50 ± 5	—
House lizard (*Hemidactylus flaviviridis*)	46 ± 6	—
Common Indian Monitor (*Varanus monitor*)	32 ± 4	—
Angani (*Mabuya carinata*)	25 ± 4	—
Snake (*Natrix piscator*)	18 ± 2	—
Tortoise (*Testudo elegans*)	14 ± 2	—
Mammals		
Goat	—	68 ± 6
Cow	—	50 ± 6
Sheep	—	43 ± 4
Rat	—	39 ± 4
Mouse	—	35 ± 4
Squirrel	—	30 ± 4
Gerbil	—	26 ± 4
Rabbit	—	23 ± 2
Cat	—	5 ± 1
Dog	—	5 ± 1
Guinea pig	—	—
Flying mammals		
Indian fruit bat (*Pteropus medius*)	—	—
Indian pipistrel (*Vesperugo abramus*)	—	—
Primates		
Monkey (*Macaca mulatta*)	—	—
Man	—	—

From Chatterjee, I. B., Majumder, A. K., Nandi, B. K., and Subramanian, N. (1975), *Ann. N.Y. Acad. Sci.*, 258, 24. With permission.

intake which can predispose to atherosclerosis, but chronic subclinical ascorbic acid deficiency and disorders of ascorbate metabolism are especially important because they are among the factors that one can hope to do something to change.

In this book, the roles of ascorbic acid deficiency in various tissues and in various diseases will be discussed under separate subject headings, so no further attempt will be made to outline them here.

Historical Note

While testimonial evidence is of little value in establishing the value of any treatment, and we can only rely on controlled experiments to find the truth, it is nevertheless interesting in retrospect to recall that the concepts of (1) latent ascorbic acid deficiency; (2) affecting

middle class individuals; (3) causing diabetes mellitus; and (4) possibly resulting in permanent harm, were all contained in an article by Van der Loo (1938) which Rev. Msgr. William Kwaaitaal of Pineville, Louisiana, has kindly translated as follows:

Experience from Practice. Diabetes Mellitus and Vitamin C by C. J. Van der Loo (Family Doctor)

This information concerns two diabetes mellitus patients who through the use of an overdose of ascorbic acid, improved so much that threatening invalidity became avoided.

In a district meeting Dr. Labberte told that through the use of ascorbic acid he had overcome a weariness and fatigue he had been plagued with already for several years.

Testing himself with the reagent "dichlorophenolindophenol" he had found out that he had a large deficiency of Vitamin C in his urine.

After this information by Dr. Labberte, I (Dr. Van der Loo, the writer of the article) ordered immediately the reagents. I used my own urine to study the reaction and came to the surprising result of a total deficiency of Vitamin C. I started the use of overdoses of ascorbic acid — till the vitamin content became normal. (I myself was the first patient).

My second patient had *no* Vitamin C in his urine either.

He uses at the moment 450 mg ascorbic acid a day and has much improved. In both patients the existing diabetes mellitus improved-and the tolerance of starch increased.

Must I conclude that Vitamin C is a cure for diabetes?

In both patients there were symptoms of scurvy due to lack of Vitamin C. These improved by our treatment.

Therefore, I wondered whether the sugar regulating system (pancreas-brain-thyroid gland, etc.) might be sick due to Vitamin C deficiency.

If this supposition is correct, then Vitamin C can never be a complete cure for diabetes. If half of the sugar regulating system is destroyed then this half can never be restored; in that case the remaining half only can be prevented from destruction. If the patient is in a stage that he can only be kept alive with insulin injections, then the use of Vitamin C won't make the insulin therapy superfluous.

If my theory is correct then only patients who have not yet reached the point for needed insulin therapy can be prevented from those injections, by Vitamin C treatment.

Follow the history of two patients — the first one being myself.

Patient 1 (60 years old) healthy, never tired till he was 33 — then spells of fatigue attributed to very increased activities. In 1913, after much night work he collapsed. Sugar in the urine.

Felt pretty good with a diet of two slices of bread a day and 2 potatoes — with butter, meat, eggs, fruit, vegetables, cheese.

However, sometimes spells of fatigue and some sugar in the urine.

A few fast days corrected that again, though fatigue and sugar again at times. Reduced his work activities, drove a car the last two years. Pain in upper right leg. Called it an auto-leg. Aspirin did not help. Also always a disagreeable feeling of cold in both upper legs.

At the beginning of last year again sugar in the urine. Very difficult to correct. Fatigues and pains increased. In April to Wiestbaden for warm baths and massages. The typical auto-leg improved — but back home the pains became worse. *Right* femur — right fibula — also pains in left upper leg. Pains all during the nights. For diet he took only one slice of bread in the morning, one apple at night. Still some sugar in urine. He dreaded the threatening insulin-injections and invalidity.

Then he heard about the experience of Dr. Labberte (about himself) and began to use overdoses of Vitamin C (300 mg a day) and noticed already an improvement after a few days. After a month all pain and cold feelings disappeared. No sugar. And at the moment he is on his former diet. Feels strong again and able to do all work.

Patient 2 (62 years old).

Eight years ago the patient consulted a doctor in Amsterdam. 5% sugar was discovered; a diet was prescribed; advised to use alcohol.

September, 1935, the patient collapsed. Coma diabeticum.

Released two weeks later. Advised to inject 10 units of insulin every other day.

December, 1935. The patient came to me (Dr. C. Van der Loo — family doctor) for treatment.

Most of the time I found sugar in the urine. Daily insulin injections became necessary.

Sometimes in the afternoon he got spells of yawning and fatigue.

His diet: two thin slices of bread, 50 Gr. potatoes, butter, eggs, vegetables, fruit, cheese.

In April 1937 the patient stayed away.

On December 7, 1937, the family brought me his urine. His bad temper had become intolerable. In his night urine: more than 3/4% sugar; no Vitamin C. Diet the same, but he no more gave himself insulin injections. I prescribed 3 ascorbic acid tablets three times a day.

Dec. 24. Night urine: almost 3/4% sugar. Vitamin C still too low. Temper had improved. Felt better and more vital.

January 6, 1938. No sugar in night urine. Day urine 1/4%. Vitamin C almost normal.
Patient had improved again; temper normal.
January 20, 1938. No sugar in urine. Vitamin content more than sufficient. Patient felt fine. These were to middle class patients who where subjectively and objectively improved through overdoses of Vitamin C.
I thought these cases important enough to be published.

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Factors Affecting the Economy of Ascorbic Acid

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Chapter 3

INADEQUATE ASCORBIC ACID INTAKE

Many workers studying the vitamin C requirements of human subjects, monkeys, and guinea pigs have been struck by the wide disparity between the findings in different laboratories. Some find guinea pigs do well on 1 or 2 mg of ascorbic acid a day, while others find them to have a requirement of 20 mg/d. Some of the reasons for these wide disparities are now evident, as ascorbic acid can be lost by oxidation and subsequent hydrolysis when traces of heavy metals like copper are present in the drinking water; conversely ascorbic acid can be preserved when there is sufficient protein or chelating fiber in the food to bind and inactivate heavy metals.

Prolonged storage or cooking may destroy vitamin C in food before it is consumed. There are also many factors which can alter the requirements of an individual animal or an individual patient, so it is difficult to define an adequate or an inadequate intake of vitamin C.

Parsons (1938) observed that, "occasionally scurvy occurs even when the diet contains considerable quantities of vitamin C; an infant admitted to the Children's Hospital with frank scurvy was having daily orange juice containing 60 mg of ascorbic acid. We have suspected that this might be due to lack of absorption, though we have not been able to demonstrate this chemically. Indeed, in the case to which I have just referred, cure was actually obtained by giving large doses of ascorbic acid by mouth. It is probable that some children require more vitamin than others."

Kline and Eheart (1944) studied nine normal young women living in the same dormitory and eating an experimental diet; they reported that the ascorbic acid intake requirements to achieve saturation in these women mostly ranged between 1.4 and 1.8 mg/kg/d, but one subject had a requirement of only 0.6 mg or less per kilogram, while two had ascorbic acid requirements of 2.2 mg/kg or above.

One of the world's most experienced investigators of vitamin C deficiency must surely be Dr. John Crandon of Boston, Massachusetts, who abstained from all foods containing ascorbic acid for nearly 7 months; he took daily supplements of all other known vitamins to make sure that he developed a pure vitamin C deficiency and that the clinical picture was not confused by other deficiencies, as is so often the case with naturally occurring maladies. Crandon et al. (1940) made a detailed report of all the laboratory and clinical findings in this experiment. Ascorbic acid (reduced form), estimated by the method of Mindlin and Butler (1938), was no longer detectable in the blood plasma after 41 d on the diet, at which time the white blood cell and blood platelet (buffy coat) ascorbic acid level had fallen from 28 to 10 mg/100 g. Thereafter, the plasma readings remained at 0, whereas the buffy coat readings did not reach 0 until 121 d had elapsed; 2 weeks later, small perifollicular hyperkeratotic papules (raised reddened hair follicles) began to appear over the buttocks. Not until after 161 d of the diet, or after the plasma ascorbic acid had been 0 for 120 d and the buffy coat 0 for 40 d, did the perifollicular hemorrhages, so characteristic of scurvy, appear over the lower legs. Then, 3 weeks later an experimental wound made in the back of the subject showed no signs of healing after 10 d. Previously, a similar wound made at the end of 3 months of diet, after the plasma had been 0 for 44 days and the buffy coat reading was 4 mg/100 g, showed reasonably good healing both clinically and microscopically.

Subsequently, Crandon et al. (1953), reviewing the results of this experiment wrote as follows: "Although ultimate failure of wound healing was the more striking result of this experiment, equally significant were the results of fatigue tests performed just before the cessation of the diet. In the completely scorbutic state the subject was able to run on a motor-driven tread-mill at a rate of seven m.p.h. for only 16 sec, at the end of which time

he was completely exhausted, with a sensation of impending collapse. Following ascorbic-acid therapy, the subject was able to run for 66 sec, without the sensation of impending disaster experienced in the first test. The subjective phenomena associated with the pre-scorbutic state were vague and difficult to describe. There was certainly an increased lassitude and desire for sleep. There was a marked disinclination to exertion."

These authors also reported at the same time a study of blood samples from 561 selected surgical patients at the Boston City Hospital. Here they measured total ascorbate (by the method of Roe and Kuether, 1943) in blood plasma, in the buffy coat, and in specimens of abdominal muscle sheath; they found blood plasma ascorbate levels to be closely related to tissue levels and to be more valuable than buffy coat levels in prediction of wound healing. Ideal blood plasma ascorbate levels range from 1.0 to 1.3 mg/100 ml and may be found in healthy young persons receiving 75 to 100 mg of ascorbic acid a day in their diet (viz., one orange and one tomato a day). When the diet contains only 15 to 25 mg/day, plasma values range from 0.1 to 0.3 mg/100 ml: in this study they found 24 patients with plasma ascorbate levels below 0.2 mg/100 ml and were able to follow 18 of them for 2 years; 12 of the 18 had serious wound complications including evisceration in 1, massive bleeding into the wound in 1, wound dehisced down to the peritoneum in 2, incisional hernia in 3, poor or no healing in 2, and persistent draining sinus in 3. There is no proof that all of these wound complications were due to a lack of ascorbic acid, but the very high rate of wound complications would seem to be more than coincidental.

Among patients with almost totally deficient plasma, who were receiving no vitamin C, a precipitous drop in buffy coat ascorbic acid was frequently noted with the onset of infection, evisceration, or other serious complications. It is thus evident that there may develop a vicious cycle wherein the problem may compound itself. Ascorbic acid deficiency predisposes to serious complications, and these in turn may hasten depletion of ascorbic acid stores.

Crandon et al. studied seven patients with frank scurvy. All seven showed traces of ascorbate in the buffy coat, and five showed traces in the plasma as well. They concluded that scurvy, in many cases, appears to be a manifestation not of complete absence of ascorbic acid alone, but of marked ascorbic acid deficiency plus local tissue stress.

Actually, the difference between the original findings of Crandon et al. (1940), where ascorbic acid disappeared from both plasma and platelets before the onset of scurvy, and the subsequent studies of Crandon et al. (1953), where small amounts of ascorbic acid were still found in the blood of patients with scurvy, may be partly due to the different methods of chemical analysis used. The method of Mindlin and Butler measures only the reduced (most active) form of the vitamin, while the method of Roe and Kuether measures the reduced form plus the small amount of the oxidized form, dehydroascorbic acid (DHAA), which is normally present in the blood, as well as possibly traces of inactive diketogulonic acid derived from the latter. Only DHAA is detectable in the blood plasma and tissues in scurvy.

Lind (1753) also believed that stress and various other factors in the form of hardships would hasten the onset of scurvy and noted that half of Lord Anson's crew died of scurvy rounding Cape Horn, even though they had only been at sea for 3 months. Of course, we do not know how their diet had been before they set sail, but it is undoubtedly true that many facotrs, even smoking, as noted by Maynwaringe (1668), can hasten the onset of scurvy.

However, Crandon speaks of local tissue stress, which raises the very interesting question as to whether a damaged or infected tissue or organ can develop scurvy while the rest of the body remains relatively healthy. There is insufficient evidence on this point and it does deserve further study. Indeed, the recently discovered inverse relationship between histamine and ascorbic acid makes it conceivable that inflammation is in one respect similar to local scurvy.

Dodds and MacLeod (1947) studied 41 healthy young women students at the University of Tennessee in Knoxville; all the women were maintained on a low-ascorbic acid diet containing approximately 7 mg of ascorbic acid a day, and all received an ascorbic acid supplement increasing stepwise from 25 to 50 to 75 and to 100 mg/d at 7- to 10-d intervals, with a view to establishing the plasma ascorbic acid level associated with each intake level. However, it was observed that there was a wide range in the plasma ascorbic acid values at each of the controlled intake levels; the authors concluded that any estimation of the vitamin intake of a population based on a plasma ascorbic acid survey cannot be narrowly defined.

Salomon (1957) studied the biological half-life of ascorbic acid in nine guinea pigs and found a range from 70 to 144 h. Likewise, Williams and Deason (1967) reported great variability in the amount of ascorbic acid required to maintain the body weight of guinea pigs under experimental conditions and gave reasons for believing that there may be an even greater variation between the needs of individual human beings. These authors postulated that guinea pigs, monkeys, and human beings may have varying abilities (hitherto undetected) to produce ascorbic acid endogenously, but cannot produce it fast enough to maintain health. Yew (1973) made use of seven different methods to assess the dietary ascorbic acid needs of young guinea pigs. Growth rates both before and after surgical stress, recovery times after pentothal anesthesia, scab formation, wound healing, and the production of hydroxyproline and hydroxylysine during wound healing all supported the conclusion that young guinea pigs need an ascorbic acid intake of about 5 mg/100 g of body weight daily, which is about ten times the amount required to prevent the development of scurvy. He observed greater variation between the growth rates of guinea pigs at lower levels of ascorbic acid intake. Yew concluded that the individual needs of guinea pigs vary greatly, but when they are given relatively high levels of ascorbic acid, the whole population appears to be adequately supplied, with the result that their performance is more nearly uniform.

People who take plenty of exercise seem to have higher serum ascorbic acid levels than sedentary individuals (Chapter 24 of this volume), while bedridden paraplegics and tetraplegics tend to have lower than normal ascorbic acid stores, as shown by Hunter and Rajan (1971). Moreover, these observations are of clinical importance, for low ascorbic acid levels have been shown to impair the healing of pressure sores and high ascorbic acid levels have been shown to accelerate their healing (Chapter 4, Volume II). There also seem to be wide variations between individuals in the rate at which they lose ascorbic acid, depending on age, sex, hormonal state, injury, infection, and other disease states, so it follows that different people need different amounts of ascorbic acid. It would be a simple matter if all individuals had the same daily intake requirements for vitamin C, or even if each category of person — man, woman, child, pregnant woman, or old person had a known requirement for this vitamin, for then the stated government nutritional standards wound have more precise meaning.

Unfortunately it is not so simple; in addition to age, size, sex, and pregnancy, there are many other variables which affect ascorbic acid requirements. Notable among these are stress, smoking, infection, injury, and variations between individuals, but other more subtle factors including the intake and storage of certain heavy metals, such as copper and iron, may also have a profound influence, as we shall see.

Why should there be such variability in the intake requirement for this vitamin? There is no simple answer to such a question, but it is probably because it is unstable in neutral or alkaline solutions and oxidizes to form DHAA or "ascorbone" which tends to hydrolyze to diketogulonic acid with loss of vitamin activity.

DHAA does possess vitamin activity when taken by mouth, because it can be reduced to ascorbic acid in the body, but DHAA has a higher intake requirement than ascorbic acid because of its short half-life and tendency to hydrolyze. It is worthy of note that DHAA is

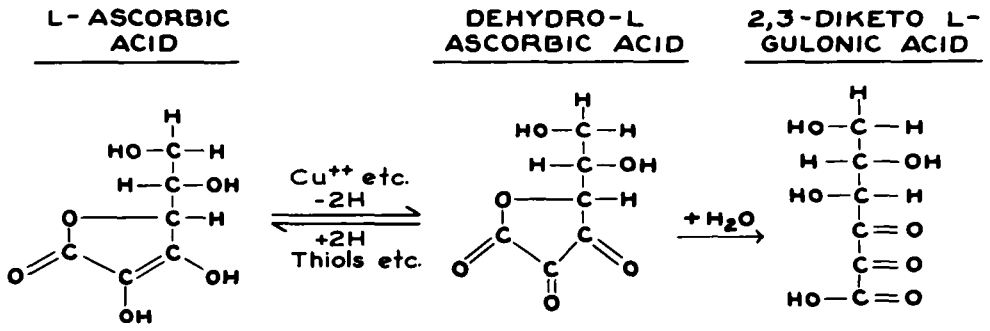

FIGURE 1. Showing the reversible equilibrium between the reduced and oxidized forms of L-ascorbic acid and the loss of activity by hydrolysis of the unstable dehydro form to diketogulonic acid. The latter becomes disassociated to form oxalic acid and a four-carbon compound; monodehydroascorbic acid or "ascorbate-free-radical" is a compound intermediate between ascorbic acid and dehydroascorbic acid; it is a highly reactive substance and is almost certainly responsible for the mutagenicity of ascorbic acid in the presence of copper.

very toxic when given by injection, even in very small doses, causing diabetes mellitus and hypertension; it may be that the integrity of the capillaries and their continuation as the endothelium, or inner lining of the larger blood vessels, may be more dependent on the oxidation-reduction state of this vitamin than on its total quantity. In normal health, plasma ascorbic acid is mostly in the reduced form, while in scurvy it is mostly, if not all, in the oxidized form.

This relationship is indicated by the chemical equation shown in Figure 1; it may be noted that oxidation of ascorbic acid is accelerated by certain heavy metal ions such as copper, manganese, and iron (King 1961) as well as by the copper protein ceruloplasmin; reduction is promoted by sulfhydryl compounds, such as reduced glutathione, which is present on or in many cells in the body.

Ascorbic acid is fairly stable in the dry state at room temperature and also in acid solutions, such as are provided by citric acid in citrus fruit, but oxidizes rapidly in neutral or weakly basic solutions which contain oxygen and traces of heavy metal catalysts such as iron or copper, which may be found in tap water. Moreover, at body temperature, the resultant DHAA is lost by hydrolysis as fast as it is formed, as shown in Figure 2.

Fortunately, this rapid loss of ascorbic acid does not usually occur in the human body because of the acid present in the stomach, because of protein binding of the heavy metals in the blood, and because of reducing agents in various tissues. However, there are many factors which cause individual variations in the economy of ascorbic acid. Indeed, Yew (1975), reviewing the literature on this subject, concluded that biological variation may contribute significantly to the difficulty of determining the ascorbic acid needs of individuals.

An elegant study by Subotičanec-Buzina et al. (1984) in Zagreb, Yugoslavia, demonstrated a highly significant increase in the physical working capacity of elementary school boys as a result of ascorbic acid supplementation (70 mg/d for 2 months ($p < 0.01$). Both test and control groups received dietary supplements of riboflavin and pyridoxin. The mean plasma vitamin C in the experimental group (n = 49) rose from 0.33 to 1.49 mg/dl and the prevalence of deficient vitamin C values (<0.20 mg/dl) decreased from 52.3 to 0%. Clearly, the ascorbic acid content of the food of these boys must have been inadequate for their needs at the time of the study; even though they had appeared to be in normal health before the study, they showed a clear-cut and measurable benefit from the vitamin supplement. At the beginning of the study the maximal oxygen uptake (VO_2 max) for the two groups averaged 2.9 l/min. There were 47.6% of the subjects in the experimental group and 55% of the control group with values below that cut-off point. In the experimental group, subjects with values below

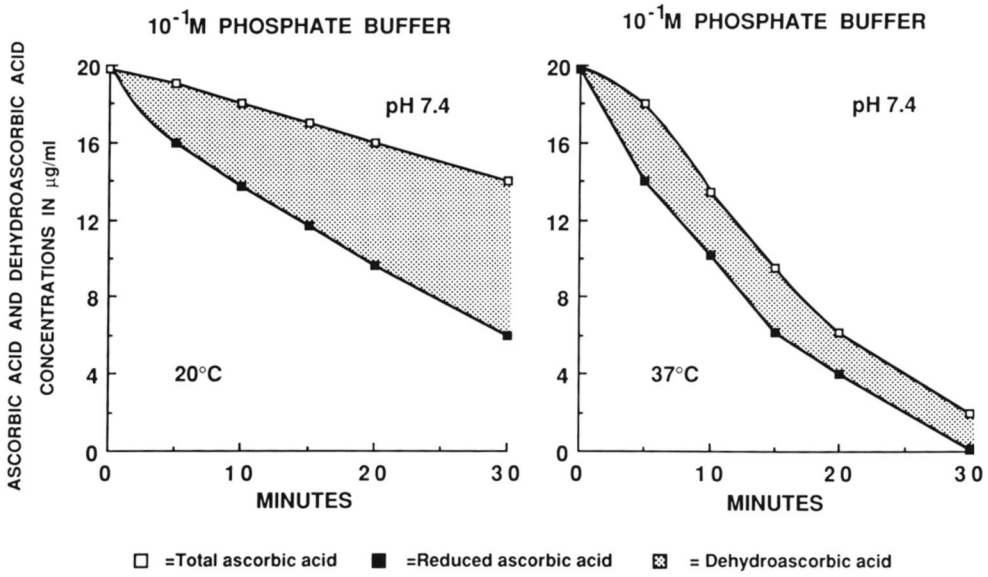

FIGURE 2. Studies of the oxidation of ascorbic acid in one tenth molar sodium phosphate buffer at room temperature and at body temperature in the presence of dissolved oxygen: 2 ml of a freshly prepared solution of ascorbic acid 1 mg/ml (at about 5°C) was added to 100 ml of the buffer at 20 or 37°C, to give an initial ascorbic acid concentration of 19.6 g/ml. Samples were withdrawn at 5-min intervals for 30 min and were analyzed by the Hughes (1956) homocysteine method. Oxidation of samples to be analyzed for ascorbic acid plus dehydroascorbic acid was arrested by addition of homocysteine at pH 7.4 for 10 min before acidifying, while oxidation of samples to be analyzed for ascorbic acid alone was arrested by acidifying directly; 6 ml of 3% HPO_3 was added to each 4-ml sample. Analytical reagent grade dibasic and monobasic sodium phosphate salts were dissolved in glass-distilled water to prepare the buffer solutions used in these experiments. Nevertheless, the oxidation of ascorbic acid was markedly accelerated by such traces of heavy metals impurities as were present in these salts, as proven by subsequent experiments using heavy metal chelating agents. (From Clemetson, C. A. B. and Andersen, L. [1966], *Ann. N. Y. Acad. Sci.*, 136, 339. With permission.)

2.9 l/min increased their VO_2 max by 0.53 l/min compared with an increase of 0.07 l/min in the subjects with initial values above 2.9 l/min ($p < 0.01$). Clearly, about half the boys in the supplemented group showed a very marked increase in working capacity, such as would likely make the difference between losing and winning their next soccer game. Moreover, their improved ascorbate status could lift their spirits (Chapter 9, Volume II) and would most probably improve their resistance to infection (Chapter 14 of this volume).

The reason for this improvement in the maximal oxygen uptake resulting from ascorbic acid supplementation is not known for certain; but it may well be related to the histaminemia of ascorbic acid deficiency and the antihistamine activity of ascorbic acid.

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Chapter 4

SMOKING

As early as 1941 Harmsen reported finding low blood levels of vitamin C in heavy smokers. In 1952, McCormick reported that cigarette smoking rapidly destroys vitamin C, and in the same year Scott speculated on the roles of vitamins B_1 and B complex deficiency in the malnutrition associated with tobacco amblyopia.

Durand et al. (1962) confirmed that the ascorbic acid content of the blood of smokers is markedly reduced and found it in general to be about half that of nonsmokers.

Venulet and Danysz (1955) reported that the vitamin C level in the milk of women who smoke is markedly lower than in the milk of women who do not smoke; they found that the vitamin C level of the milk rose faster if the women stopped smoking than if they took a vitamin C supplement, but continued smoking; these authors were of the opinion that lack of vitamin C in a woman smoker during pregnancy and breast feeding injures the fetus and then the infant. Due to seasonal variations in the availability of vitamin C, they stated that smoking by mothers is particularly injurious in winter and spring; they expressed their belief that administration of vitamin C does not prevent the injurious effect of tobacco intoxication.

Calder et al. (1963) studied 91 nonsmokers, 83 moderate smokers, and 31 heavy smokers and reported highly significant differences between their mean plasma ascorbic acid levels (0.91, 0.73, and 0.52 mg/100 ml, respectively). Their white blood cell and platelet ascorbic acid levels (29.1, 24.8, and 21.7 $\mu g/10^8$ cells) were also significantly different. These authors were able to show in the laboratory that tobacco smoke destroys vitamin C in solution.

Forty healthy young Polish soldiers aged 20 to 23 were studied by Rupniewska (1964). The mean fasting plasma ascorbic acid level of 22 who smoked cigarettes (0.159 mg/100 ml) was significantly lower than that of 18 who were nonsmokers (0.224 mg/100 ml).

Pelletier (1968) reviewed this subject and found many studies showing lower blood ascorbate levels in smokers, but only one poorly documented study showing no difference. Pelletier confirmed the influence of smoking in a detailed experiment involving five cigarette smokers and five nonsmokers. The smokers all had low vitamin C levels in plasma and in whole blood (0.29 and 0.30 mg/100 ml) compared with nonsmokers (0.66 and 0.73 mg/100 ml). Plasma dehydroascorbic acid (DHAA) was also determined and yielded values of approximately 0.1 mg/100 ml in both the smokers and in the nonsmokers. Because of the lower total ascorbate content in the smokers, the proportion of DHAA was 20% in the smokers and 12% in the nonsmokers. It may be noted that, as is usual, the ratio of reduced to oxidized ascorbic acid is low when the total ascorbate is low; this suggests that the ascorbate level may be low because it is being oxidized. Urinary excretion of ascorbic acid was much lower in the smokers than in nonsmokers during a 5-day period of saturation, in which the subjects received 2 g of ascorbic acid per day; the smokers retained 18.5 mg of ascorbic acid per kg body weight, while the nonsmokers retained only 5.2 mg/kg. This indicates the degree of desaturation or low body stores of ascorbic acid in the smokers. Both smokers and nonsmokers had similar whole blood and plasma ascorbic acid levels 3 d after saturation.

Evans et al. (1967) exposed two groups of 15 male guinea pigs each to cigarette smoke for two 10-min periods each day for 28 d and found that the "smoker group" gained weight less rapidly than the control group and had lower vitamin C levels in the adrenals (22.0 ± 7.9 mg/100 g vs. 31.1 ± 5.4 mg/100 g). Similar results were reported by Hughes et al. (1970) who observed a reduction of the adrenal ascorbic acid, but no change in the ascorbic acid content of the testes or spleen of guinea pigs exposed to smoke for periods up to 20 min/d for 4 to 18 d. Pelletier (1968) administered nicotine by mouth to guinea pigs for 28

days and studied the vitamin C content of their organs at the end of this time. His results showed a marked reduction of the mean ascorbic acid content of the adrenals from 30.2 to 15.3 mg/100 ml. However, adrenal ascorbic acid depletion cannot be considered as a specific effect; one can obtain similar results as a result of almost any kind of stress, or by administering the adrenocorticotrophic hormone. In the opinion of the writer, the effect of smoking on ascorbic acid levels may be due to the carbon particles of the smoke, for carbon particles absorb oxygen and then rapidly oxidize ascorbic acid under laboratory conditions, so they may well do the same thing in the lung. In fact, finely powdered "heat activated" charcoal is used to convert all ascorbic acid (AA)* to DHAA in the Roe and Kuether (1943) method of analyzing blood for total ascorbic acid (TAA)**; the oxidation takes place in a matter of seconds *in vitro*. If this also happens in the lungs, then the present trend towards low tar and nicotine cigarettes will not help to decrease the effect of smoking on ascorbic acid metabolism.

Further papers on the effects of cigarette smoking on vitamin C metabolism have been published by Brook and Grimshaw (1968), Faltot et al. (1968), Elwood et al. (1970), and by Pelletier (1970, 1975); the effect has been amply confirmed in heavy smokers.

Bailey et al. (1970) found no significant difference between the plasma AA levels of 20 young men, at the University of Saskatchewan, who smoked cigarettes (mean 0.61 mg/100 ml) and 20 who did not smoke (mean 0.66 mg/100 ml). The subjects were mostly students of physical education, and smoking was defined as 10 to 20 cigarettes a day, so clearly they were not heavy smokers.

Burr et al. (1974), in a study of elderly men and women in a small town in South Wales, observed highly significant effects of male sex, increasing age, and smoking habit on the plasma and leukocyte ascorbic acid levels, but found that these levels showed a closer correlation with frequency of fruit intake than with any other variable; finding that smokers ate fruit less often than nonsmokers, they concluded that the lower ascorbate levels of the smokers were partly, but not entirely, due to their lower dietary intake of fruit.

Albanese et al. (1975) studying hospital workers in Rye, New York, found leukocyte ascorbic acid (TAA) levels to be 34% lower in 18 smokers than in 34 nonsmokers (18.9 and 29.1 $\mu g/10^8$ cells, respectively). The plasma TAA levels of their nonsmoking subjects were disproportionately low (mean 0.18 mg/100 ml), but those of the smokers were even lower (mean 0.13 mg/100 ml).

Kevany et al. (1975) reported white blood cell ascorbic acid (TAA) levels of 50 μg in 18 men who were nonsmokers and 39 $\mu g/10^8$ cells in 23 men who smoked ($p < 0.05$). They also observed a significant negative correlation between white cell ascorbic acid concentrations and serum cholesterol levels in smokers, which was not present in nonsmokers. They drew a parallel between these findings and those of Ginter et al. (1969, 1971) who showed that the catabolism of cholesterol to bile acids was significantly reduced in male guinea pigs with chronic ascorbic acid deficiency; they suggested that the lower ascorbic acid levels of smokers may well account for the significantly higher serum cholesterol levels of male smokers than nonsmokers, in the age range 20 to 50 years, as reported by Van Houte and Kesteloot (1972), using regression analysis in a study of 42,000 men. Furthermore, they quoted the work of Willis (1953) and of Banjerjee and Singh (1958) showing that ascorbic acid deficiency in guinea pigs causes hypercholesterolemia and accelerates the development of atherosclerosis; they suggested that ascorbic acid deficiency might account for the greater incidence of atherosclerosis in men who smoke, an opinion previously expressed by Rupniewska (1964).

McClean et al. (1976) studied the vitamin C levels of 178 healthy male hospital employees and blood donors in New Zealand. They found both plasma and leukocyte ascorbic acid

* AA—ascorbic acid, reduced form.

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

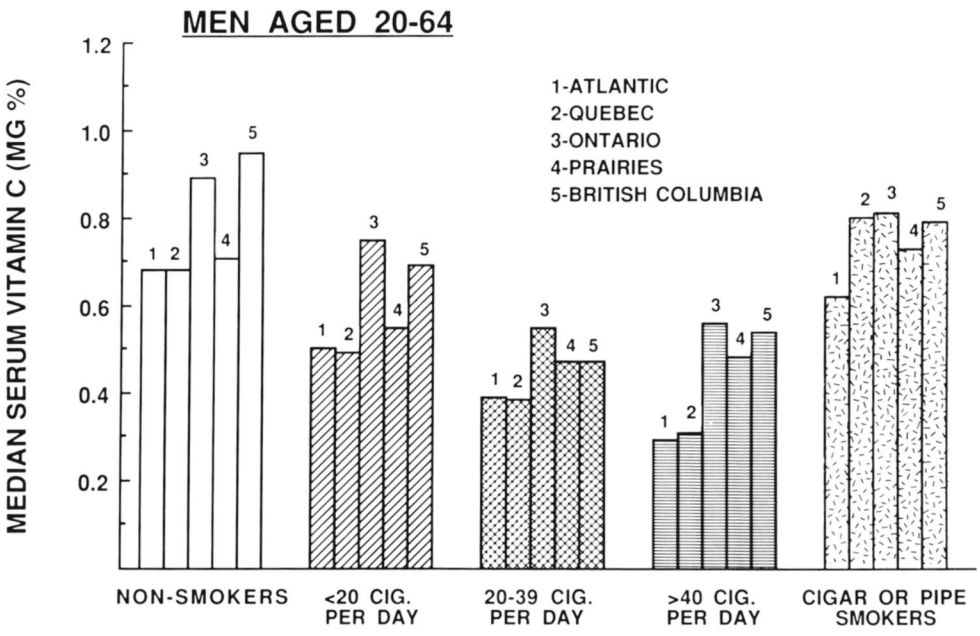

FIGURE 1. Median serum vitamin C levels in men aged 20 to 64 in relation to smoking habits according to Nutrition Canada study. (From Pelletier, O. [1977], *Int. J. Vitam. Nutr. Res. Suppl.*, 16, 147. With permission.)

(TAA) levels to be significantly lower in smokers than nonsmokers in the younger age groups, 17 to 29 and 30 to 39, but not in the older men. They did not think that the smokers and nonsmokers differed greatly in their intakes of vitamin C-rich foods and concluded that smoking per se affects the vitamin C levels.

Pelletier (1977) found that cigarette smokers as a whole have median serum ascorbic acid levels 30% lower than nonsmokers, and for those who smoke more than 20 cigarettes a day, the median is 40% lower than normal. However, he found that the median serum ascorbic acid level of men who smoke cigars or pipes was similar to that of nonsmokers. This is particularly interesting because the health hazards of cigar and pipe smoking are known to be much less than those due to cigarette smoking. Undoubtedly the nicotine content of cigar and pipe smoke is very high, so nicotine is absorbed from the tongue and the mouth by pipe and cigar smokers, but carbon particles do not reach their lungs to the same extent as in cigarette smokers because they do not inhale the smoke. There is no doubt that cigarette smoking decreases the serum ascorbic acid levels, as shown in Figure 1, for the data reported by Pelletier (1977) from the Nutrition Canada National Survey included 4600 men and women from 5 different regions, and the difference was observed in both men and women matched for age, region, and dietary ascorbic acid intake.

Solomon et al. (1968) conducted a dental survey including men and women who had never smoked and those who had smoked only cigarettes. They found periodontal disease, with gingival recession and alveolar resorption to be much more common in smokers than in nonsmokers and also more common in men than in women. They found that women aged 20 to 39 have about twice as great a chance of having periodontoclasia or becoming edentulous if they smoke cigarettes; they cited the opinion that this could not be attributed entirely to the deposition of calculus. One can only speculate as to the role that lower ascorbic acid levels of smokers may play in this increased incidence of periodontal disease.

Daniell (1976) studied 72 women between the ages of 40 and 70 who presented with symptomatic and radiographically proven compression fractures of weight bearing vertebrae. Over 2/3 of those with spinal osteoporosis (no recognizable trauma) were heavy smokers,

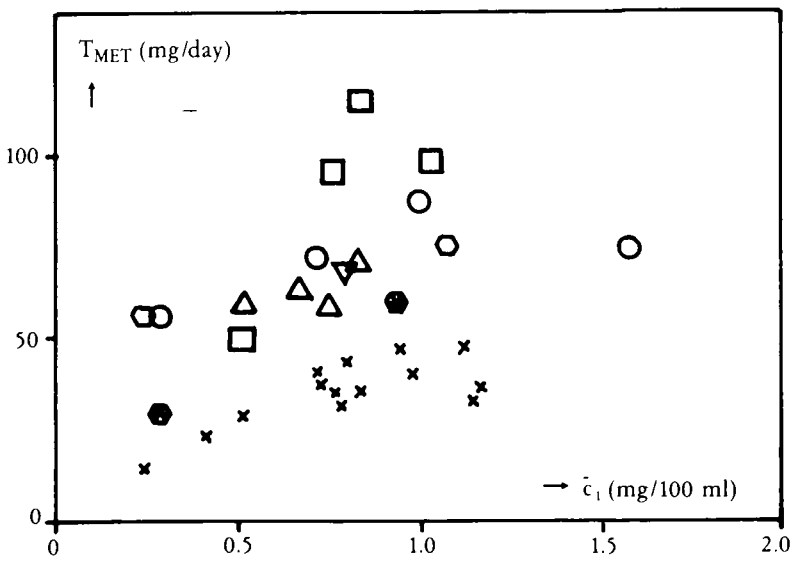

FIGURE 2. Metabolic turnover of ascorbic acid at different plasma ascorbic acid levels, in smokers (geometric patterns) and nonsmokers (×) at ascorbic acid intakes ranging from 30 to 180 mg/day. (From Kallner, A. B., Hartman, D., and Hornig, D. H. [1981], *Am. J. Clin. Nutr.*, 34, 1347. ©American Society for Clinical Nutrition. With permission.)

compared with 2/5 of women in a control population and 35% of a group of women whose spinal fractures had followed trauma. Daniell found that the smokers had an earlier menopause than the nonsmokers, but the osteoporosis of the smokers was greater than could be accounted for by the earlier menopause alone. The smokers had apparently lost cortical bone from their metacarpals at a rate of 1.02% per postmenopausal year ($p < 0.001$). The decreased tissue and plasma ascorbic acid levels may well be the cause of the osteoporosis and the premature menopause which are so prevalent in these slender white women who smoke too much.

Smokers have more coronary heart attacks than nonsmokers, as shown by Mann et al. (1975). They also have more cerebrovascular accidents (strokes), more retinal hemorrhages, and more peripheral vascular disease than nonsmokers. In fact, it used to be said that a man who has had one leg amputated for gangrene, due to peripheral vascular disease, will surely lose the other leg before long, unless he gives up smoking.

One cannot help but wonder to what extent the ascorbic acid deficiency of cigarette smokers contributes to development of these cardiovascular diseases, as eight other factors predisposing to cardiovascular disease are also associated with ascorbic acid deficiency (Clemetson, 1979).

The absorption, plasma level, turnover rate, apparent volume distribution, total body pool, renal excretion, and half-life of ascorbic acid have been studied in nonsmokers and in smokers by Kallner et al. (1979 and 1981); they gave traces of ascorbic acid 1-^{14}C to men, with oral intakes ranging from 30 to 180 mg/d. The absorption, distribution, and renal handling of ascorbic acid were found to be similar in the smokers and the nonsmokers, but the smokers had a higher metabolic turnover than the nonsmokers (Figure 2); the half-life of ascorbic acid was markedly reduced in the smokers (Figure 3). It was concluded that a daily intake of at least 140 mg of ascorbic acid is required for smokers to reach steady-state concentrations and total body ascorbate stores comparable to nonsmokers for whom a daily intake of 100 mg was considered to be appropriate.

A study of teenage girls by Keith and Mossholder (1986) showed much lower plasma

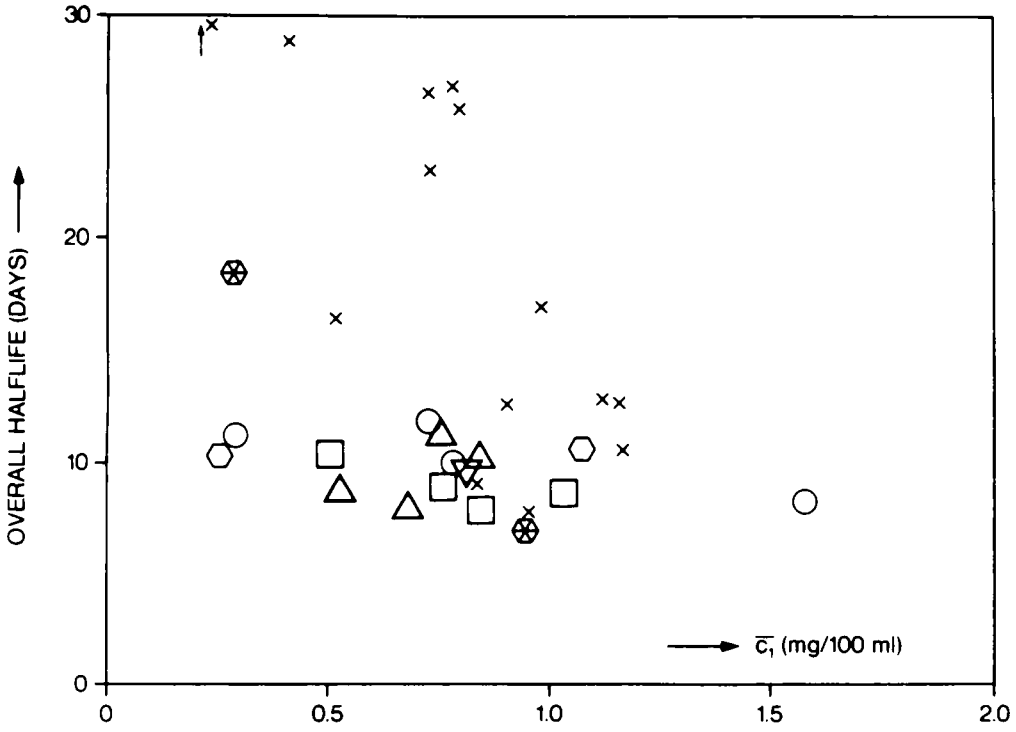

FIGURE 3. Half-life of ascorbic acid at different plasma ascorbic acid levels, in nonsmokers (×) and smokers (other symbols). (From Kallner, A. B., Hartman, D., and Hornig, D. H. [1981], *Am. J. Clin. Nutr.*, 34, 1347. ©American Society for Clinical Nutrition. With permission.)

ascorbic acid (TAA) levels (0.32 ± 0.19 mg/dl) in smokers than in nonsmokers (1.46 ± 0.69 mg/dl). This difference was partially due to a greater dietary vitamin C intake by the nonsmokers, but even when plasma ascorbic acid levels were adjusted for vitamin C intake, smokers were still found to have significantly lower plasma ascorbic acid levels than nonsmokers (mean 0.48 mg/dl vs. 1.38 mg/dl).

If it is the "heat-activated" carbon of inhaled smoke that destroys ascorbic acid in the blood, as it passes through the lungs, this might explain why fire fighters are prone to develop coronary heart disease; indeed, New York City fire fighters who develop heart disease have special pension provisions, and the disease is assumed to be attributable to their work.

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Chapter 5

AGING

I. HUMAN STUDIES

Using a silver nitrate staining method to demonstrate ascorbic acid Gough (1934) reported that the anterior lobe of the pituitary of the ox has an even higher concentration of this vitamin than the adrenal or the corpus luteum; this was confirmed by biological assay in several species. Studying human pituitaries obtained at autopsy, he observed that the silver nitrate reaction was strong in the young and the middle aged, in whom the general body nourishment was normal, but the reaction was less in the aged and in those who died after a long illness.

Yavorsky et al. (1934) analyzed extracts of finely ground human tissues obtained within 24 h of death; dichloroindophenol titration revealed that tissue ascorbic acid (AA)* levels decreased with age, as shown in Table 1. They concluded that latent scurvy was fairly common, especially in older people, and that it was seldom suspected before death.

Sendroy and Schultz (1936) reached the conclusion that ascorbic acid utilization decreases with age between 4 and 36 years, as shown in Figure 1. Measuring urinary ascorbic acid excretion on an intake of 250 mg/d for 1 week in convalescent hospital patients they calculated the difference between intake and excretion and called it "ascorbate utilization". Expressing this on a body weight basis, they proposed an ascorbic acid utilization coefficient.

$$\text{Utilization coefficient} = \frac{\begin{array}{c}\text{7 d oral AA intake (mg)} \\ \text{minus 7 d urinary AA output (mg)}\end{array}}{\text{Body weight (kg)}}$$

Their data showed an inverse relationship between the square root of the age and the utilization coefficient (C) as below:

$$C \times \sqrt{\text{age}_{\text{yrs}}} = 67.5$$

At first glance one might think that these findings run counter to those of Yavorsky et al., for decreased utilization might be thought to imply plentiful stores of ascorbic acid, but that is only because of our acceptance of the word "utilization". If we substitute the words "ascorbate retaining ability", we might conclude that it is the ability to retain ascorbic acid that decreases with age. Indeed, either or both may be true, for children may retain more and utilize more ascorbic acid than adults, but only blood and tissue levels will tell us about blood and tissue ascorbate saturation. In practice we use blood, serum or plasma, and leukocyte ascorbic acid levels to represent the blood and tissue levels of living subjects.

Aykroyd et al. (1949) reported that 28% of the people surveyed in Newfoundland, both in 1944 and again in 1948, had serum ascorbic acid levels of less than 0.2 mg/100 ml; the mean ascorbic acid levels were found to decrease with age, so the children were less affected than the adults.

Elderly people are often found to have low ascorbic acid levels, but it is difficult to know to what extent this tendency is due to an inadequate diet and to what extent it is due to age

* AA — ascorbic acid, reduced form.

Table 1
VITAMIN C CONTENT OF TISSUES IN DIFFERENT AGE GROUPS (mg/g)

Age group:	1—30 d	1—12 months	1—10 years	11—45 years	48—77 years
No. of cases:	11	9	11	17	19
Adrenal	0.581	0.525	0.550	0.393	0.230
Brain	0.460[a]	0.189[a]	0.433[a]		0.110[a]
Pancreas	0.365	0.304	0.225	0.152	0.095
Liver	0.149	0.148	0.163	0.135	0.064
Spleen	0.153	0.112	0.157	0.127	0.081
Kidney	0.153	0.122	0.098	0.098	0.047
Lung	0.126	0.057[b]	0.058	0.065[b]	0.045[b]
Heart	0.076	0.049	0.042	0.042	0.021
Thymus	0.304	0.319	0.190		0.046[a]

Note: The ascorbic acid concentrations of human tissues were found to decline with advancing age.

[a] Average of two specimens available for analysis in this group.
[b] Average of six specimens available for analysis.

From Yavorski, M., Almaden, P., and King, C. G. (1934), *J. Biol. Chem.*, 106, 525. With permission.

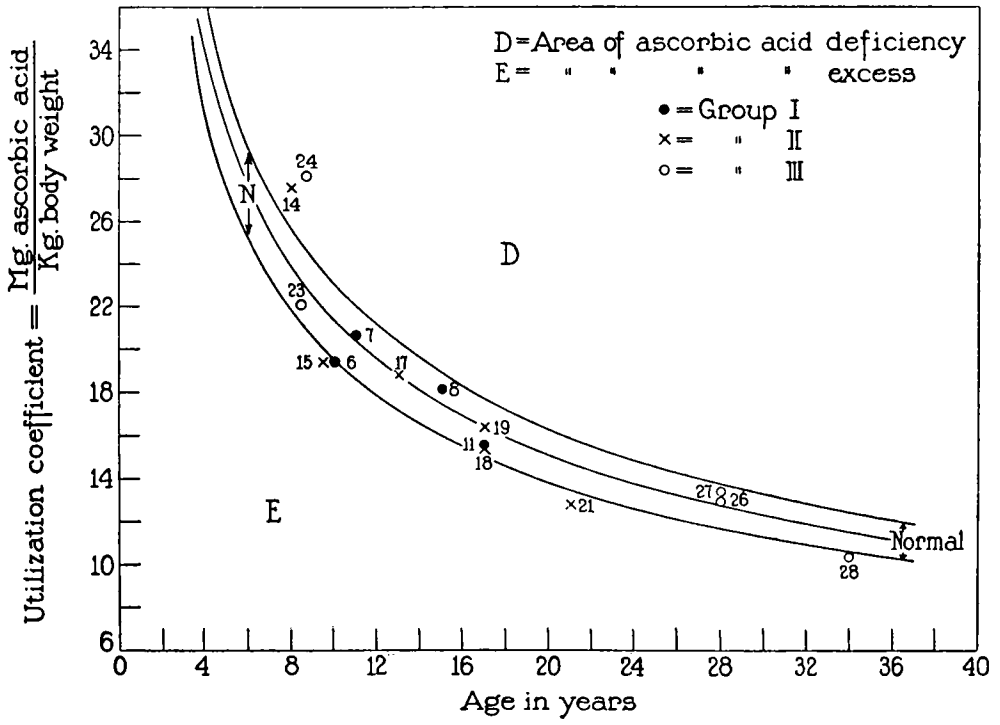

FIGURE 1. Variation in the utilization of ascorbic acid by people of different age groups. For details see original text. (From Sendroy, J. and Schultz, M. P. [1936], *J. Clin. Invest.*, 15, 369. ©American Society for Clinical Investigation. With permission.)

itself. Kirk and Chieffi (1953) reported whole blood ascorbic acid levels in 61 men and 81 women living in an institution on the same diet which supplied about 45 mg of ascorbic acid a day; they found a significant decline with age in men, but no significant change in women. Morgan et al. (1955) found no such correlation between age and serum ascorbic acid levels in men and women living at home on diets assessed as containing much higher levels of ascorbic acid (means 76 to 136 mg/d), but they found women at all ages to have higher serum ascorbic acid levels than men.

A more extensive study of human tissue ascorbic acid levels at different ages was conducted by Schaus (1957) who studied 69 samples of pituitary, 71 samples of cerebral cortex, 67 samples of myocardium, and 63 samples of pectoral muscle obtained at autopsy. A statistically significant decrease of ascorbic acid (TAA)* concentration with age was observed in the pituitary, where it fell from 0.89 mg/g in the 1st decade to 0.46 mg/g in the 8th decade, and in the cerebral cortex where it fell from 0.45 mg/g to 0.1 mg/g in the same 8 decades, both in males and females. The ascorbic acid levels in the myocardium and in the pectoral muscle fell in the first 2 decades, but remained unchanged thereafter. Comparing the sexes, the mean ascorbic acid level of the female pituitary was 55% higher and the myocardium 24% higher than that of the male.

Denson and Bowers (1961) found uniformly low white blood cell ascorbic acid (TAA) levels in 50 elderly people living in the London area. The mean level was 13.4 $\mu g/10^8$ cells as compared to 35 $\mu g/10^8$ cells in 25 healthy adults of working age. Franklin (1963) also drew attention to scurvy in the aged.

Surveys by Bowers and Kubik (1965), by Kataria et al. (1965) and by Andrews et al. (1966) have shown very low plasma and leukocyte ascorbic acid levels in old people. Bowers and Kubik studied 50 elderly people (mean age of 76.2 years) living at home in the industrial midlands of England and found an average plasma ascorbic acid (TAA) level of only 0.23 mg/100 ml and a mean white blood cell level of 12.3 $\mu g/10^8$ cells; 200 younger people with an average age of 35 years had a mean white blood cell level of 26.2 $\mu g/10^8$ cells. They reported that white blood cell ascorbic acid levels in their elderly subjects could be raised in 2 weeks to the same levels as in younger people by giving them 120 mg of vitamin C daily.

Kataria et al. (1965) studied 25 elderly people (mean age 75.8) living at home in the London area, and found low plasma ascorbic acid (TAA) levels of 0.49 mg/100 ml (range 0.1 to 1.1 mg/100 ml), but their mean leukocyte ascorbic acid level was 26 $\mu g/10^8$ cells, which was the same as younger (35-year-old) subjects in their study. In 15 hospitalized elderly subjects and in 16 old people under institutional care, they found low plasma and white blood cell levels. The plasma level in the hospital patients averaged 0.18 mg/100 ml (range 0.1 to 0.2), and the average white blood cell content was 14 (range 5 to 22) $\mu g/10^8$ cells. The figures for the institutionalized patients were even lower, 0.09 mg/100 ml (range 0.04 to 0.15) in plasma, and 8 (range 4 to 16) $\mu g/10^8$ cells in white blood cells.

Griffiths et al. (1966) reported that among elderly patients admitted to hospital, 41% were deficient in ascorbic acid and 59% deficient in thiamine; in elderly people living at home and not ill, 27% were deficient in vitamin C.

O'Sullivan et al. (1968) found that a very high proportion of elderly people in Cork had an inadequate ascorbic acid intake; age and poor diet often compound one another in the elderly.

Brook and Grimshaw (1968) studied 32 men and 50 women nonsmokers who were all healthy and doing full-time office or manufacturing work in food and pharmaceutical factories; their data showed a significant decline in the plasma vitamin C concentration with

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

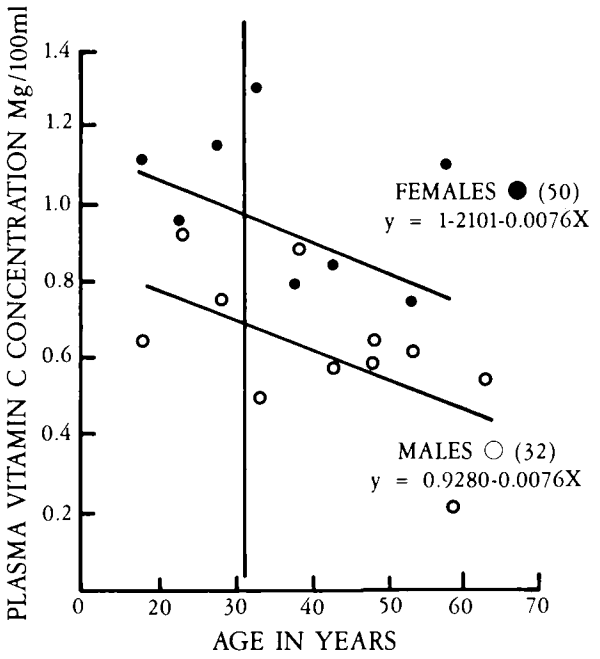

FIGURE 2. Regression of plasma vitamin C (TAA) concentration with age for 32 men and 50 women nonsmokers. (From Brook, M. and Grimshaw, J. J. [1968], *Am. J. Clin. Nutr.*, 21, 1254. ©American Society for Clinical Nutrition. With permission.)

increasing age in the men and in the women, as shown in Figure 2, but the leukocyte ascorbate concentration did not decline significantly with age.

Loh and Wilson (1971b) observed a significant inverse relationship between age and leukocyte ascorbic acid concentration; this was more pronounced in women than in men.

Studying 204 men and 247 women aged 62 to 94 years living at home in Edinburgh, Milne et al. (1971) found a significant decrease in the mean leukocyte ascorbic acid (TAA) levels of women with increasing age, viz., 26.4, 23.2, and 16.5 $\mu g/10^8$ cells in the 60- to 70-, 70- to 80-, and over 80-year age groups, respectively. No such fall with age was evident in the men, whose mean leukocyte ascorbate levels of 17.7, 19.8, and 14.6 $\mu g/10^8$ cells were already deplorably low in the 60- to 70-year age group.

Burr et al. (1974a, b), studying 830 elderly people living at home in a small town in South Wales, found leukocyte ascorbic acid (TAA) levels to fall significantly with age to the extent of 2.2 $\mu g/10^8$ cells in men and 2.5 $\mu g/10^8$ cells in women in a 10-year period, in people over 65 years of age ($p < 0.001$ for each). They also found increasing age to be accompanied by decreasing plasma ascorbic acid (AA) levels in both men ($p < 0.01$) and women ($p < 0.001$). In a study of young and elderly nuns living in a convent they found plasma ascorbic acid levels to be significantly lower for a given ascorbic acid intake in the elderly.

Wilson (1974) also found lower leukocyte ascorbic acid levels in the elderly than in the young, as shown in Table 2.

McClean et al. (1976), studying 178 people aged 17 to 68 who were either blood donors or hospital employees in New Zealand, also observed a tendency for the leukocyte and plasma ascorbic acid (TAA) levels of nonsmokers to decline with age.

Bates et al. (1977) recorded low plasma ascorbic acid levels below 11 $\mu mol/l$ (0.2 mg/100 ml) in 10 out of 23 elderly people living at home in England. They observed a strong

Table 2
MEAN LEUKOCYTE ASCORBIC ACID (TAA) CONCENTRATION ($\mu g/10^8$ WHITE BLOOD CELLS) FOR MALES AND FEMALES OF VARIOUS AGE GROUPS

Healthy controls (age in years)	Leukocyte concentrations (mean and standard deviations)	
	Male	Female
4—12	56.4 ± 21.9	—
11—18	36.6 ± 9.1	42.8 ± 12.6
22—49	30.4 ± 8.7	34.0 ± 10.9
56—87	24.4 ± 13.6	23.4 ± 11.3

From Wilson, C. W. M. (1974), *Practitioner*, 212, 481. With permission.

positive correlation between plasma and "buffy coat" (leukocyte and platelet) vitamin C levels in elderly subjects without dietary supplements. It was therefore suggested by these workers that measurement of the plasma ascorbate level, which requires less blood and is easier to perform, is likely to be quite adequate to identify individuals "at risk", although the extent of tissue desaturation, at the lower end of the scale, is better defined by the buffy coat level.

Schorah (1981) reported plasma and leukocyte ascorbic acid levels to be lower in elderly (65 to 75 years old) institutionalized patients (0.13 mg/100 ml and 8.2 $\mu g/10^8$ cells) than in elderly people of the same age group living at home (0.48 mg/100 ml and 24.1 $\mu g/10^8$ cells), but even these levels were low compared with the levels found in blood donors aged 19 to 55 years (1.03 mg/100 ml and 31.5 $\mu g/10^8$ cells).

Havivi et al. (1985) found that 5% of elderly people on a collective farm in Israel had low leukocyte ascorbic acid levels (below 15 $\mu g/10^8$ cells), even though an adequate diet was available to them on the kibbutzim where they lived. These authors reaffirmed their earlier finding that dietary supplementation with vitamin C elevated both the serum iron and the hemoglobin levels of the people they studied.

Low plasma and leukocyte ascorbic acid levels in the elderly may be partly due to inadequate vitamin C intake, but there seems little doubt that age itself is also a factor. Nevertheless, Suter and Russell (1987) express the very conservative opinion that, "At present, there is little evidence that the 1980 recommended daily allowance for vitamin C should be altered for the elderly."

II. ANIMAL STUDIES

A. Guinea Pigs

The enzyme glutathione:dehydroascorbate oxidoreductase (GDOR), which catalyses the reduction of dehydroascorbic acid (DHAA) to ascorbic acid by reduced glutathione, has been studied by Grimble and Hughes (1968).

$$2\ GSH + DHAA \rightarrow GSSG + AA$$

They found that the adrenals, stomach, brain, and erythrocytes from young guinea pigs possessed a greater DHAA reducing capacity than those from older animals. They postulate

that decreased levels of the enzyme GDOR may account for the decreased ascorbic acid stores of elderly people.

Hughes and Jones (1971) studied guinea pigs of different ages, all receiving ascorbic acid intakes proportional to their body weight (0.5 mg/100 g body weight) and observed that the older guinea pigs, aged 485 days, had significantly lower tissue ascorbic acid (AA) levels in the adrenal glands, spleen, and eye lens than younger animals, aged 60 days ($p < 0.01$). In contrast, the brain ascorbic acid level was found to be somewhat higher in the older animals ($p < 0.01$).

B. Cockerels

Dieter and Breitenbach (1968) studied the ascorbic acid and DHAA concentrations of lymphoid tissues obtained during maturation of cockerels; they found that a progressive fall in the concentration of ascorbic acid (TAA) in the thymus and spleen was associated with a decrease in the ratio of reduced to oxidized ascorbic acid. There was also a marked decrease in the concentration of ascorbic acid (TAA) in the adrenal glands of cockerels during maturation, but this was not associated with any consistent change in the AA/DHAA ratio of that organ.

C. Rats

Morehouse and Guerrant (1952) observed a gradual decrease in both the liver and plasma ascorbic acid concentrations in rats with advancing age. This may be due to a gradual decrease in the ability of the rat liver to synthesize ascorbic acid, or it may represent a decreasing ability of the animal to store ascorbic acid as it ages.

Patnaik and Kanungo (1964) have measured the *in vitro* uptake of ascorbic acid by bone homogenates from rats and have found that the ascorbate uptake shows a marked decline with age (Figure 3). They relate ascorbate uptake to collagen synthesis and give reasons for believing that the decreased AA uptake in older rats is due to a decreased synthesis of collagen.

Further evidence of the effect of aging on ascorbic acid metabolism was provided by Patnaik and Kanungo (1966) when they showed an age-related decrease of the AA concentration in various organs in the rat.

Patnaik (1971) stated that the concentration of ascorbic acid has been reported as decreasing with age in the rat eye lens, adrenal, brain, testis, and blood and states that changes in the rates of synthesis, permeability, utilization, or differential transport might be responsible for these findings. The fact that the rate of decline of ascorbic acid content varies from organ to organ does suggest that it is the ability of various tissues to retain ascorbic acid, rather than the rate of synthesis, which determines the differential aging effect. Patnaik cites the theory of Bjorksten and Andrews (1964) in which aging involves cross-linkage of proteins; Patnaik suggests that decreased storage of ascorbic acid may be related to such changes.

III. CLINICAL ASSOCIATIONS, CLINICAL TRIALS

Purpura (extensive bruising) and hemorrhages under the tongue are common in elderly people. Tattersal and Seville (1950) reported purpura in 60 out of 809 elderly patients admitted to hospital; the bruises were most commonly seen on the anterior aspects of their arms and forearms and on the backs of their hands. Both these patients and the controls were ascorbate deficient, as judged by ascorbic acid loading tests; skin biopsies from those with purpura showed degeneration of the collagen fibres of the dermis, but they were not responsive to 3 weeks of treatment with ascorbic acid (300 mg) or with rutin (150 mg daily). The usual combination of ascorbic acid and rutin was not tested. The only etiological factor, besides age, suggested by these authors was exposure of the forearms to radiation, the men to sunlight at work, and the women to radiant heat in the course of their domestic duties.

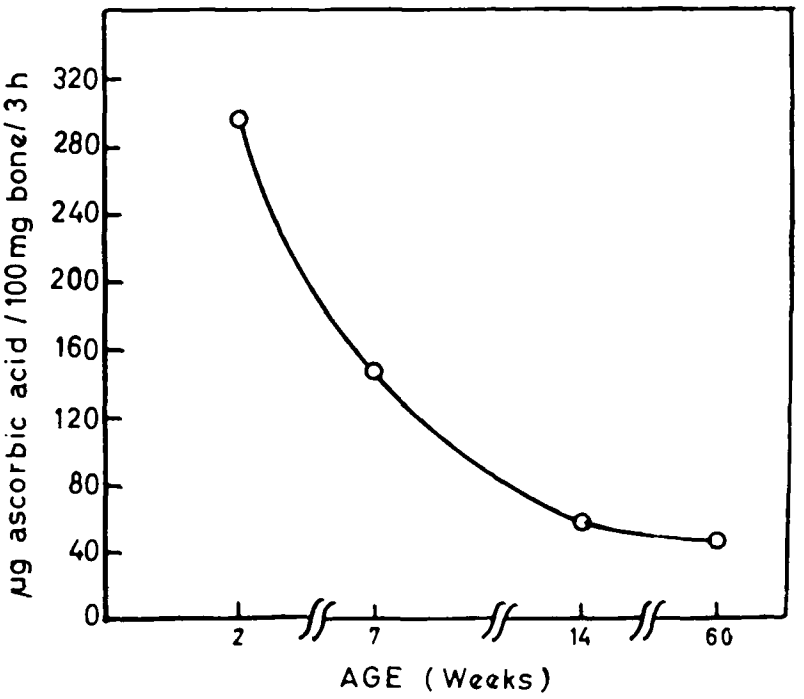

FIGURE 3. Uptake of ascorbic acid by bone (μg/100 mg) of rats in different age groups. An average of seven experiments was taken for each of the age groups. (From Patnaik, B. K. and Kanungo, M. S. [1964/1965], *Gerontologia*, 10, 155. S. Karger AG, Basel. With permission.)

Several visible vascular lesions were described by Bean (1956) who observed that the incidence of dome-shaped varicose enlargments of sublingual veins, sometimes known as "caviar lesions", increases with age.

Andrews and Brook (1966) found hemorrhages under the tongue in 29 of 92 patients over the age of 60; 5 of the 29 had received supplements of ascorbic acid for up to 2 months, and 4 of these had normal leukocyte ascorbic acid levels. Yet despite their prolonged supplementation, the hemorrhages under the tongue persisted. In the remaining 24 with sublingual hemorrhages, the mean white blood cell ascorbic acid content was lower than in those 65 who had no such hemorrhages. The levels were 10.23 (range 5.1 to 21.3) $\mu g/10^8$ cells in those with and 17.67 (range 6.4 to 47.9) $\mu g/10^8$ cells in those without hemorrhages under the tongue. Senile purpura occurred in 30 of 64 old people studied, but in contrast to the findings in sublingual hemorrhages, no difference in the white blood cell ascorbic acid content was found between those with and without the purpura.

Ninety-four long-stay elderly patients with senile purpura and sublingual hemorrhages were studied by Arthur et al. (1967). They were given 1000 mg of ascorbic acid every day for 1 week, continuing with 50 mg daily for a period of a further 5 weeks; neither the hemorrhages nor the purpura showed any real improvement. Moreover, Disselduff and Murphy (1968), in a subsequent study of 16 elderly patients, found no relationship between leukocyte ascorbic acid levels and senile purpura or sublingual hemorrhages; nor did these workers find any relationship between dietary intake and leukocyte ascorbic acid levels in this small sample.

Taylor (1968) observed that the "caviar-like" sublingual varicosities, being thin walled and lacking collagen support, are often associated with small submucous hemorrhages and may be mistaken for hemorrhages themselves. Andrews et al. (1969) confirmed that they are venular dilations.

Table 3

	Active tablets	Inactive tablets	Total
Deteriorated	0	5	5
Not deteriorated	20	22	42
Total	20	27	47

Note: This table shows the results of Eddy's examination of randomized photographs of sublingual varicosities and ecchymoses in elderly patients before and after 12 to 18 months of treatment with a multivitamin preparation containing ascorbic acid (50 mg) or placebo in the trial conducted by Berry and Darke (1972). While the sublingual lesions were not cured, the data do suggest that the vitamin supplements may have prevented deterioration.

From Eddy, T. P. and Taylor, G. F. (1977), *Age Ageing*, 6, 6. With permission.

Taylor et al. (1971) found that these lesions show a significant ($p < 0.025$) negative correlation with the frequency of consumption of fruit and vegetables and a positive correlation ($p < 0.01$) with subcutaneous petechiae and follicular and other skin hemorrhages. These sublingual varicosities were first photographed and described histologically by Mendes da Costa and Cremer (1930) who called them "caviar like grains under the tongue."

Eddy and Taylor (1977) pointed out that the first medical records of varicose veins under the tongue appear to be James Lind's reference in his *Treatise of the Scurvy* (Lind, 1753) to Herman Boerhaave's (Aphorisumus 1148 de scorbuto, 1708) and Joan (Ecthii de Scorbuto, 1541), where they were stated to be signs of scurvy. These authors, reviewing their own careful photographic studies and those of others, conclude that vitamin supplements including ascorbic acid (50 mg daily) will not cure these sublingual varicosities, but do prevent deterioration, which is more commonly observed in untreated controls, as shown in Table 3. They propose that previous, perhaps recurrent, vitamin deficiencies may lead to irreversible changes in the elderly that cannot subsequently be changed by vitamin therapy. They suggest that an epidemiological comparison between populations who have long maintained a high vitamin status, and those whose vitamin status has been variable, may be more informative than therapeutic trials of the effect of vitamins on these lesions. In this context Eddy and Taylor report that 22 elderly pepole who had been vegetarians for many years had high ascorbic acid concentrations in plasma (1.02 mg/100 ml) and leukocytes (35.9 $\mu g/10^8$ WBC). Visual and photographic examination of their tongues showed a much lower incidence of sublingual petechiae and varicosities than is general in elderly people.

There are reasons for believing that other vitamins, especially pyridoxine and riboflavin, may be important in aiding tissue storage of ascorbic acid for cystathionine synthetase and cystathionase are pyridoxine-dependent enzymes which respectively catalyze the synthesis and cleavage of cystathionine, an intermediate in the metabolism of methionine to cysteine. Moreover, riboflavin is a glutathione reductase, so pyridoxine and riboflavin supplements may accelerate the formation of sulfhydryl compounds which aid tissue storage of ascorbic acid by reducing DHAA to ascorbic acid within the cell. This may explain why the Farnborough trial, reported by Brocklehurst et al. (1968), Griffiths (1968), Taylor (1968), and Taylor et al. (1971) was so successful, for both pyridoxine and riboflavin were included. In that study, 40 geriatric hospital patients received a daily tablet containing thiamine (15 mg), riboflavin (15 mg), nicotinamide (50 mg), pyridoxine (10 mg), and ascorbic acid (200 mg), and 40 others received placebos.

Table 4
COMPARISONS OF OBSERVATIONS AFTER 1 YEAR OF TREATMENT IN VITAMIN-SUPPLEMENTED AND UNSUPPLEMENTED GERIATRIC HOSPITAL PATIENTS

	0 months	3 months	6 months	9 months	12 months
Skin hemorrhages					
Active	0.77 ± 0.33	1.90 ± 0.54	2.05 ± 0.67	0.78 ± 0.27	0.78 ± 0.41
Control	0.76 ± 0.27	1.75 ± 0.70	4.06 ± 1.56	3.13 ± 1.19	3.37 ± 1.38
Hess test: petechial count					
Active	4.00 ± 0.89	8.75 ± 1.31	10.08 ± 1.12	9.97 ± 2.11	8.99 ± 1.89
Control	6.48 ± 1.21	13.96 ± 1.02	24.68 ± 2.35	25.80 ± 3.46	32.33 ± 1.92
Myotatic hyperirritability					
Active	2.83 ± 0.46	3.02 ± 0.45	1.58 ± 0.42	1.22 ± 0.43	1.09 ± 0.27
Control	2.72 ± 0.49	2.78 ± 0.45	3.96 ± 0.41	5.59 ± 1.66	4.23 ± 0.41

Note: Means ± standard errors. Supplement: ascorbic acid 200 mg, thiamine 15 mg, riboflavin 15 mg, nicotinamide 50 mg, pyridoxine 10 mg daily (vs. placebo).

From Brocklehurst, J. C., Griffiths, L. L., Taylor, G. F., Marks, J., Scott, D. L., and Blackley, J. (1968), *Gerontol. Clin.*, 10, 309. S. Karger AG, Basel. With permission.

Biochemical studies showed leukocyte ascorbic acid (TAA) levels in the deficiency range disappeared from all but two of the treated group, but persisted in most of the untreated patients. The untreated group showed an increased number of skin hemorrhages and petechiae during the year, while the number of petechiae and skin hemorrhages remained fairly constant in the treated group, as shown in Table 4. At the end of the year, Taylor was able to allot the survivors in this blind controlled trial into groups of "treated" and "untreated", simply by observation, with only one mistake.

Brocklehurst et al. (1968) found that 9 out of 29 untreated patients developed bedsores, while only 2 out of 33 patients receiving the multivitamin supplement including 200 mg of ascorbic acid every day were similarly affected ($p < 0.01$).

Andrews et al. (1969) reported very variable leukocyte ascorbic acid responses to dietary supplementation in different elderly individuals, as shown in Figure 4. They observed that some elderly people required 9 months of supplementation, with 40 to 80 mg of ascorbic acid daily, before their leukocyte ascorbic acid levels reached those found in younger people (Figure 5); they also reported that the leukocyte ascorbic acid levels of the elderly fell again in 3 months when the dietary supplement was discontinued.

Such a very slow increase in the accumulation of ascorbic acid by the leukocytes requires an explanation; it could perhaps be the result of a gradual reduction in the level of copper in the liver and other tissues following ascorbic acid supplementation, as was shown by Hamilton-Smith and Bidlack (1980) in the liver copper of guinea pigs supplemented with ascorbic acid. If so, one could hasten the removal of copper and, thus, the storage of ascorbic acid by the administration of chelating flavonoids or catechins along with the ascorbic acid.

Windsor and Williams (1970) conducted a very interesting study of urinary hydroxyproline excretion in elderly patients; they found that ascorbic acid supplements (1 g daily for 6 d) caused a significant increase in the excretion of hydroxyproline by those whose leukocyte total ascorbic acid levels were less than 15 $\mu g/10^8$ cells on admission to hospital, as shown in Figure 6. Patients whose initial ascorbic acid levels were normal, on the other hand, showed no change in hydroxyproline excretion after loading with ascorbic acid. This certainly suggests that the elderly patients with low ascorbic acid levels had defective collagen metabolism which was rectified by treatment.

Berry and Darke (1972) observed no improvement in the sublingual lesions of elderly

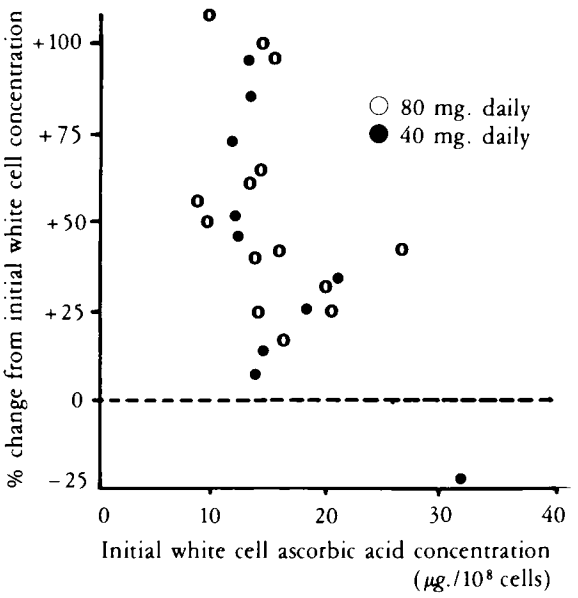

FIGURE 4. Showing the great variability of individual leukocyte ascorbic acid responses by elderly people to vitamin C supplementation for 17 months. One might have expected that the people most deficient in vitamin C would have shown the greatest response, but it is evident that this was not so. (From Andrews, J., Letcher, M., and Brook, M. [1969], *Br. Med. J.*, 2, 416. With permission.)

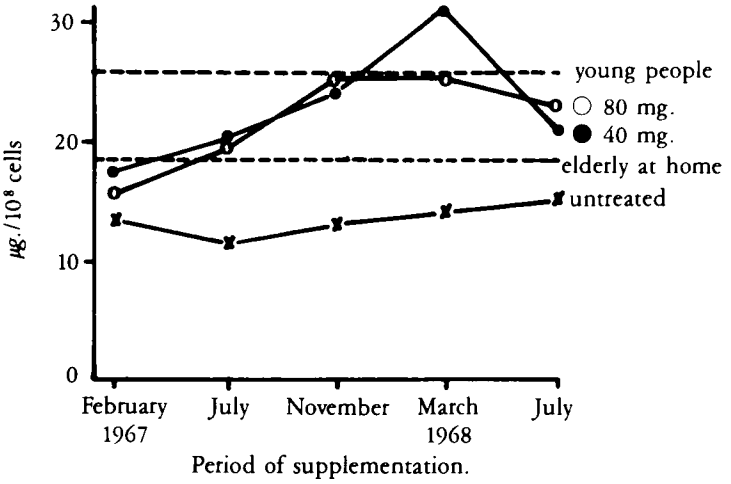

FIGURE 5. Mean concentrations of ascorbic acid in the leukocytes of elderly people, over 65 years of age, during a 17-month period of supplementation with ascorbic acid, 40 mg daily and 80 mg daily, compared with untreated controls. (From Andrews, J., Letcher, M., and Brook, M. [1969], *Br. Med. J.*, 2, 416. With permission.)

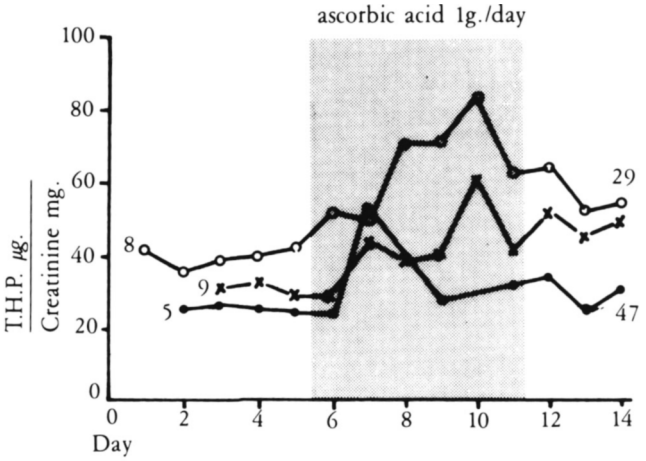

FIGURE 6. Effect of an ascorbic acid supplement on the urinary hydroxyproline/creatinine ratios in three elderly patients with low leukocyte ascorbic acid levels. The figures on each graph indicate the initial and final leukocyte ascorbic acid levels in $\mu g/10^8$ cells. (From Windsor, A. C. W. and Williams, C. B. [1970], *Br. Med. J.*, 1, 732. With permission.)

Table 5
LEUKOCYTE TOTAL ASCORBIC ACID LEVELS ($\mu g/10^8$ WBC)

	Mean vitamin C levels			
	Below 12 μg	**12—18.5 μg**	**18.6—25 μg**	**Over 25 μg**
Number of samples	32	38	40	49
Number of deaths	14	14	10	5
Mortality as % of samples	47	37	25	10

Note: Mortality at 4 weeks following admission.

From Wilson, T. S., Weeks, M. M., Mukherjee, S. K., Murrell, J. S., and Andrews, C. T. (1972), *Gerontol. Clin.*, 14, 17. S. Karger AG, Basel. With permission.

hospital patients who received ascorbic acid (50 mg daily), with or without B vitamins for a year.

Wilson et al. (1972) reported their findings in 159 patients who had been admitted to an acute geriatric unit in 1968. They found that those with low leukocyte ascorbic acid (TAA) levels (<12 $\mu g/10^8$ cells) had a significantly higher mortality, within 4 weeks of admission, than those with high levels (>25 $\mu g/10^8$ cells) as shown in Table 5.

A controlled trial of dietary supplementation with ascorbic acid (200 mg daily), vs. placebo, for patients admitted to a geriatric hospital was therefore conducted and was reported by Wilson et al. (1973). As before, low vitamin C levels in the women on admission were associated with an increased mortality at 4 weeks following admission. This mortality was not significantly influenced by the administration of 200 mg daily of ascorbic acid. A variable response in leukocyte ascorbic acid was noted in the group receiving the "active" tablets. Those who responded with a rise in vitamin C levels had a significantly lower 4-week mortality than those who showed no response. Low levels were found especially in patients with respiratory infections and with bedsores.

Taylor (1973) has given reasons for believing that many elderly people would benefit

from supplements of the B group vitamins and C and has expressed the opinion that all hospital patients should receive such supplements.

A study of vitamin C supplements for elderly people living in the community was reported by Burr et al. (1975). They gave 150 mg of ascorbic acid daily for 12 weeks and then 50 mg daily for 2 years. The blood plasma and leukocyte ascorbic acid levels were elevated by the dietary supplement, but unfortunately this study did not show any change in mortality or morbidity as a result of this treatment; those elderly people whose initial leukocyte ascorbic acid (TAA) levels were below 15 $\mu g/10^8$ cells still had a significantly higher mortality rate than those with higher levels.

It would therefore seem that the low leukocyte ascorbic acid levels are due to a disturbance of ascorbic acid metabolism associated with disease states, rather than to a simple dietary deficiency, and that this cannot be remedied by ascorbic acid alone, at least in such doses as have been tried.

Dubin et al. (1978) have shown that old people with low leukocyte ascorbic acid levels do not have impaired adrenal function and that treatment with ascorbic acid does not alter their adrenal function.

A double-blind controlled prospective trial of vitamin C (1 g/d) vs. placebo was conducted by Schorah et al. (1981) at the General Infirmary in Leeds (U.K.) on 94 elderly "long-term" geriatric in-patients who were known to have low initial plasma and leukocyte ascorbic acid levels (mean 0.17 mg/100 ml plasma and 10.1 $\mu g/10^8$ leukocytes). After 2 months of treatment both plasma and leukocyte levels had increased substantially in those receiving vitamin C supplements, and in this group there were slight but significant increases in the mean body weight (0.41 kg), plasma albumen (0.46 g/l), and prealbumen (25.4 mg/l) compared with those receiving placebo therapy, who showed decreases of 0.60 kg, 0.5 g/l, and 7.0 mg/l, respectively. There were also reductions in the clinical signs of purpura and petechial hemorrhages in those receiving vitamin C.

IV. OXIDANT THEORY

There are many theories as to the cause of aging, but one of the most logical is that proposed by Tappel of the University of California at Davis (1968). He believes that aging is due to oxygen. Clearly since oxygen is essential for life, it follows that aging is inevitable, but there may be ways of slowing the process.

Could low oxygen tension be the reason, or one of the reasons, for the longevity of people living in the mountainous regions of Abkhazia in southern Russia, in the land of the Hunza in Kashmir, and in the Andean village of Vilcabamba in Equador where people are reported to live longer than usual, many exceeding 100 years and some even claiming to be 130 years old, as reviewed by Leaf (1973). Tappel suggests that aging is due to peroxidation of polyunsaturated fats. Age pigments accumulate in the brain and Tappel believes them to be peroxidized lipids complexed with protein. Tappel points out that peroxidation can be slowed by biological antioxidants such as alpha tocopherol (vitamin E), vitamin C, sulfhydryl amino acids, and by the trace element selenium.

Jamieson and Van den Brenk (1964) found that ascorbic acid and other antioxidants afford protection against high-pressure oxygen toxicity in the lungs of rats, so it is certainly reasonable to suppose that ascorbic acid might have a similar protective effect against any much slower damage which might be due to breathing 20% oxygen of air at atmospheric pressure. Comfort et al. (1971) demonstrated increased longevity in mice when their diet was supplemented with the antioxidant ethoxyquin.

Tappel's daily diet for slowing the clock of aging includes 0.5 to 1 g of vitamin E, 200 mg of vitamin C, 1 g of methionine, and also hydroxytoluene. Of course, this is only a theory, and there is as yet no way of knowing whether this diet would have any effect in

slowing down the aging process. Even if Tappel's theory is correct, it could be that the prooxidant effect of a high concentration of copper in the drinking water, or some other factor, might vitiate or reduce the benefits of such a diet.

Kratzing and Willis (1980) found that exposure of mice to ozone (200 ppm) caused the loss of 50% of the ascorbic acid from their lungs in 30 min and gave reasons for believing that ascorbic acid acts as an extracellular antioxidant in the lungs. Nevertheless, the ascorbic acid is easily overwhelmed by ozone which causes premalignant changes in the lungs of mice according to Werthamer et al. (1972).

Radiation damage seems to be like an accelerated version of the aging process; it also is believed to be due to peroxidation. Radiation damage is increased by oxygen and is directly related to tissue copper concentration. It is decreased by antioxidants like vitamin E, by sulfhydryl reducing agents or ascorbic acid, by copper chelating bioflavonoids, and especially by chelating and reducing agents like penicillamine.

V. FUTURE RESEARCH

The increased mortality of elderly people with low leukocyte ascorbic acid levels does not seem to be correctable with modest ascorbic acid supplements, presumably because the elderly have a disturbance of ascorbic acid metabolism, rather than a simple ascorbic acid deficiency. Moreover, it is entirely possible that correcting the abnormality of ascorbate metabolism might not affect their mortality; nevertheless, attempts at this are certainly worthwhile.

The success of the Farnborough study suggests that inclusion of other vitamins, particularly pyridoxine and riboflavin, as well as 200 mg of ascorbic acid daily, may be beneficial; these vitamins could in theory aid storage of ascorbic acid.

If high tissue copper levels are responsible for preventing storage of ascorbic acid, then high-dosage ascorbic acid or, better still, ascorbic acid supplements given with chelating flavonoids or catechins may be needed and are certainly worthy of trial.

Indeed, catechin coating of all ascorbic acid tablets to prevent the development of mutagenicity by ascorbic acid in the presence of copper (Chapter 11 of this volume) may have the added benefit of reducing tissue copper levels by withdrawing copper into the lumen of the bowel for excretion.

Other substances which may be given with ascorbic acid in different trials include antioxidants such as alpha tocopherol, reducing agents like cysteine, or reducing and chelating agents like penicillamine.

Certainly copper should not be included in vitamin supplements.

Estimation of the whole blood histamine levels of elderly patients on admission to hospital and observation of the rapidity with which these levels fall, with and without ascorbic acid supplements, may prove to be a valuable guide to recovery of health and may also serve to convince all doubters of the need for ascorbic acid supplements in the elderly. We all like to have something we can measure as an indicator of response to the treatment we are giving. Since elevated whole blood histamine levels usually respond dramatically to ascorbic acid within 24 h, such tests would serve well to benefit elderly patients by encouraging their medical attendants.

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Chapter 6

SEX

I. GUINEA PIGS

It has long been suspected that female guinea pigs may be less susceptible to scurvy than males.

Bourne (1935) observed some protection from scurvy in pregnant guinea pigs and also in female guinea pigs which had been treated with gonadotrophins to luteinize their ovaries. He studied pregnant, nonpregnant treated, and nonpregnant control guinea pigs; all three groups lost weight, but the pregnant and the treated groups remained healthy and active after the nonpregnant controls had died of scurvy. Using his silver staining histological technique, he demonstrated plentiful ascorbic acid in the corpus luteum of the ovary and suggested that this organ might be capable of synthesising ascorbic acid.

Odumosu and Wilson (1971, 1973a) reported that some female guinea pigs of the Hartley Williams strain live much longer (60 to 154 d) than males (24 to 30 d) on the same scorbutogenic diet and also reported the finding of gulonolactone oxidase in the livers of the longest surviving animals by a histochemical technique; these same authors (1973b) also reported that intraperitoneal injection of gulonolactone prolonged the lives of male and, even more so, female guinea pigs on an ascorbic acid-free diet. They interpreted these findings as indicating that the genes controlling the synthesis of ascorbic acid are repressed and not completely missing in the guinea pig, and that some female guinea pigs retain a slight capacity to synthesize ascorbic acid.

Hughes et al. (1971) conducted a study of tissue ascorbic acid depletion rates in albino guinea pigs by loading them with ascorbic acid for 25 d and then analyzing their tissues at set intervals after the commencement of a vitamin C-free diet. It was found that the ascorbic acid levels in the adrenal glands, spleen, and the aqueous humour of the eye were depleted faster than those of the eye lens and the brain, but there was no difference between the rates of depletion of the tissues of the male and female animals. Moreover, Jones et al. (1973) studying Dunkin-Hartley "Pirbright" strain guinea pigs found no difference between the ascorbic acid levels in the adrenals, spleens, or brains of male and female animals on the same diet, and no difference between the survival times of male and female guinea pigs on a scorbutogenic diet, with or without the addition of 0.3% cholesterol to the diet. Furthermore, Barnes et al. (1973) studying "Frant" strain albino guinea pigs were not able to confirm the findings of Odumosu and Wilson; they studied 34 male and 35 female guinea pigs on a scorbutogenic diet and found no difference between the sexes; the mean survival time was 23 d for males and females alike.

Possibly certain strains of female guinea pigs do retain the capacity to synthesize ascorbic acid to a limited degree, as suggested by Odumosu and Wilson. Another possible explanation could be that the diet used by Odumosu and Wilson contained a small amount of ascorbic acid. If so, some of the females fared much better than the males on this small amount of the vitamin.

II. RATS

Clearly the rat represents an entirely different situation, having a liver that is known to be able to synthesize ascorbic acid from simple sugars; so the findings of Todhunter and McMillan (1946), of Morehouse and Guerrant (1952), and of Ehmke et al. (1956) that ascorbic acid levels are higher in the whole blood, plasma, leukocytes, liver, and kidneys

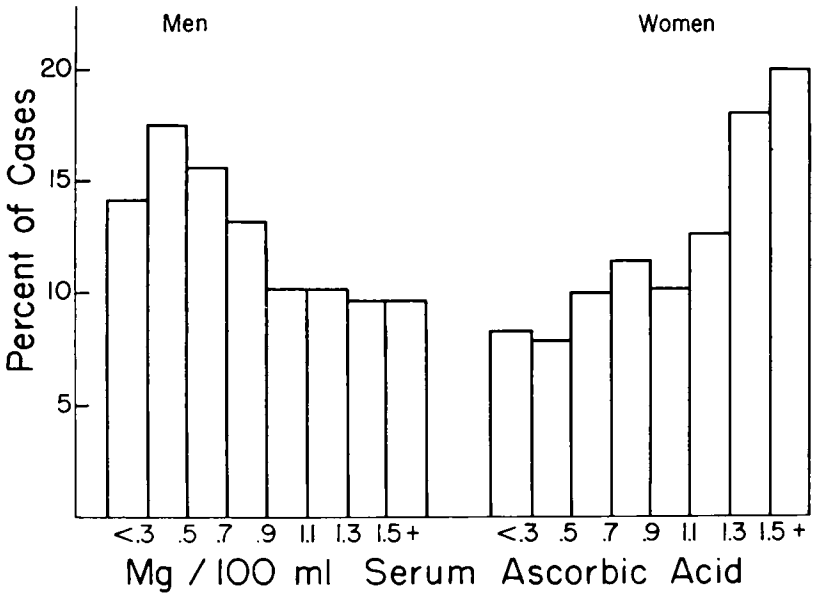

FIGURE 1. Distribution of serum ascorbic acid (TAA) levels of 232 men and 293 women over 50 years of age living in their homes. (From Morgan, A. F., Gillum, H. L., and Williams, R. I. [1955], *J. Nutr.*, 55, 431. ©American Institute of Nutrition. With permission.)

of sexually mature male than female rats, and the existence of higher levels of the enzymes necessary for the synthesis of ascorbic acid in the livers of male rats (Stubbs and McKernan, 1967), are not relevant to human beings.

III. HUMANS

Brown (1951) reported 43 patients admitted to hospital with scurvy in Glasgow between 1944 and 1949. He remarked that most of them were men who had been living alone for at least a year, in poor conditions in a lodging house, and most were over 50 years of age. Many writers have observed this preponderance of men over women with scurvy and wondered whether it is entirely due to the men being bad at providing for themselves or whether there may not be an innate tendency for men to have lower ascorbic acid levels than women.

Kirk and Chieffi (1953) reported somewhat lower mean whole blood total ascorbic acid (TAA,* levels in men (0.82 mg/100 ml) than in women (1.02 mg/100 ml) between the ages of 16 and 39 years, but this difference was not found between older men and women; they observed that season, age, and state of health were important variables.

In the San Mateo study of men and women over 50 years of age, living in their own homes, Morgan et al. (1955) reported that the serum ascorbic acid (TAA) levels were lower in men (0.83 ± 0.08 SE) than in women (1.07 ± 0.08 mg/100 ml), in spite of higher intakes (Figure 1).

Franz et al. (1956), studying nursing students and medical students at Dartmouth Medical School, found the blood ascorbic acid levels of the young men to be significantly lower than those of the young women, both initially (mean 0.95 vs. 1.32 mg/100 ml) and also after dietary supplementation with ascorbic acid, 333 mg three times a day for 3 months (1.41 vs. 1.72 mg/100 ml).

Dodds (1959) reported that men studied in her laboratory on a controlled dietary intake

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

Table 1
WHOLE BLOOD AND PLASMA ASCORBIC ACID (TAA) LEVELS (mg/100 ml) AND WHOLE BLOOD/PLASMA ASCORBATE RATIOS OF 11 MALE AND 7 FEMALE MEDICAL STUDENTS

Analysis of Results on the Control Subjects

	Males			Females				
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	t	*p*
Blood	11	0.507	0.211	7	0.884	0.265	3.349	.01—.001
Plasma	11	0.476	0.227	7	0.897	0.318	3.291	.01—.001
Ratios	11	1.104	0.143	7	1.001	0.072	1.753	.1—.05

Note: The ages of the two groups were similar and they were leading comparable lives. At least one meal a day was taken in the hospital and there were no obvious differences in the dietary intake of ascorbic acid. Nevertheless, the whole blood and plasma ascorbic acid concentrations were significantly higher in the women than in the men ($p < 0.01$).

From Spathis, G. J. and Hallpike, J. F. (1961), *Guy's Hosp. Rep.*, 110, 148. With permission.

of 75 mg of ascorbic acid daily maintained plasma levels of approximately 0.5 mg/100 ml and had a small constant urinary excretion of about 5 mg of ascorbic acid a day; women on the same ascorbic acid intake were found to have plasma levels of 0.8 to 0.9 mg/100 ml with urinary excretion of about 12 mg/d. She felt that these differences were more than could be attributed to the greater blood volume of the men.

A study of 11 male and 7 female medical students at Guy's Hospital by Spathis and Hallpike (1961) showed significantly higher whole blood plasma ascorbic acid (TAA) levels in the women than in the men, even though there was no obvious difference in their diets. The results of that study, shown in Table 1, also demonstrate a slightly higher mean whole blood/plasma TAA ratio in the men, which is almost certainly due to the lower blood levels of the men. Roe et al. (1947) observed that in healthy human subjects with whole blood TAA levels below 0.6 mg/100 ml, the plasma TAA level is lower, while above 0.9 mg/100 ml it is higher than the whole blood level.

In the nutritional study of aged people in Onondaga County, New York, reported by Brin et al. (1965), the men as a group were younger (mean age 69 years) than the women (mean age 74 years). Nevertheless, the mean plasma ascorbic acid (TAA) concentration was significantly higher in the 103 women (0.84 ± 0.43 SD) than in the 122 men (0.55 ± 0.35 mg/100 ml; $p < 0.001$).

Griffiths et al. (1967) studied 100 hospital workers between the ages of 16 and 66 and found the mean whole blood ascorbic acid (TAA) level of the women to be significantly higher than that of the men.

56 women	0.983 ± 0.30 mg/100 ml
44 men	0.822 ± 0.28 mg/100 ml

These workers also studied 118 geriatric patients and found ascorbic acid deficiency to be significantly more common in men than in women; thiamine deficiency, on the other hand, was evenly distributed between the sexes.

Brook and Grimshaw (1968) studied 32 healthy men and 50 healthy women nonsmokers (aged 17 to 63) doing clerical or manufacturing work and found that both the plasma and the leukocyte vitamin C (TAA) concentrations of the men were significantly lower ($p < 0.01$) than those of the women (see Table 2). Dodds (1969) showed that a sex difference became

Table 2
PLASMA AND LEUKOCYTE ASCORBIC ACID (TAA) LEVELS OF MEN AND WOMEN NONSMOKERS

	Men (32)			Women (50)		
Age (years):	20	39.9[a]	60	20	32.1[a]	60
Plasma ascorbic acid: mg/100 ml	0.78	0.62	0.47	1.06	0.97	0.75
Standard error	±0.12	±0.07	±0.12	±0.07	±0.05	±0.12
Leukocyte ascorbic acid $\mu g/10^8$ cells		24.6			30.7	
Standard error		±1.3			±1.4	

[a] Mean age of sample.

From Brook, M. and Grimshaw, J. J. (1968), *Am. J. Clin. Nutr.*, 21, 1254. ©American Society for Clinical Nutrition. With permission.

evident at puberty; for her youngest group (4 to 12 years of age), there was little difference in either intake or serum ascorbic acid between boys and girls; among adolescents and college students (13 to 20 years of age), the males had higher vitamin C intake, yet their serum ascorbic acid levels were found to be below those of the females (Figure 2).

Woodhill (1970), in a nutritional survey of 15 elderly men and 14 elderly women in New South Wales, found the men to have significantly lower plasma ascorbic acid levels than the women with similar dietary ascorbic acid intake levels, but this was not a fair comparison because the two groups were not strictly comparable. Of the men, 9 were in the rehabilitation unit of an acute care hospital and the other 6 men were living at home receiving food from "Meals on Wheels", which had been kept hot for a long time, while the 14 women were all living in two church homes. The mean plasma ascorbic acid level of the elderly men (aged 78 to 86) receiving "Meals on Wheels" was dangerously low (0.076 mg/100 ml), even though they were supposedly receiving 19 mg of ascorbic acid a day in their food, but the eight elderly women (aged 72 to 89) living in one of the church homes on a similar ascorbic acid intake of 20 mg/d, had a mean plasma ascorbic acid level (0.315 mg/100 ml) which was considerably better.

Loh and Wilson (1971a), measuring total ascorbic acid (TAA) in plasma and leukocytes, reported a sex difference in the metabolism of ascorbic acid in the reproductive age, but they found that this difference disappeared in old age. However, studying university students in Dublin, Loh (1972a, b) found leukocyte ascorbic acid concentrations which did not conform to the males lower precept, even though the vitamin C intake of the male students was lower than that of the females; when studying the plasma/leukocyte ascorbic acid regression coefficients, he found them to differ between the sexes in the university students and in the geriatric group, but not in the adult group.

Loh et al. (1974) have found no difference between men and women in the ability of leukocytes to take up ascorbic acid from plasma *in vitro*; they have also reported that men and women do not differ in their maximum leukocyte ascorbic acid-storing capacity after ascorbic acid loading *in vivo*. Moreover, they found no significant difference in leukocyte or plasma ascorbic acid (TAA) levels between men and women in any age group. However, on comparison of the regression lines, relating plasma and leukocyte ascorbic acid (TAA) concentrations to ascorbic acid dietary intake, they demonstrated significant differences between the regression lines of adult men and women in both the leukocyte and plasma parameters.

Burr et al. (1974) reported mean plasma ascorbic acid levels of 0.24 and 0.37 mg/100

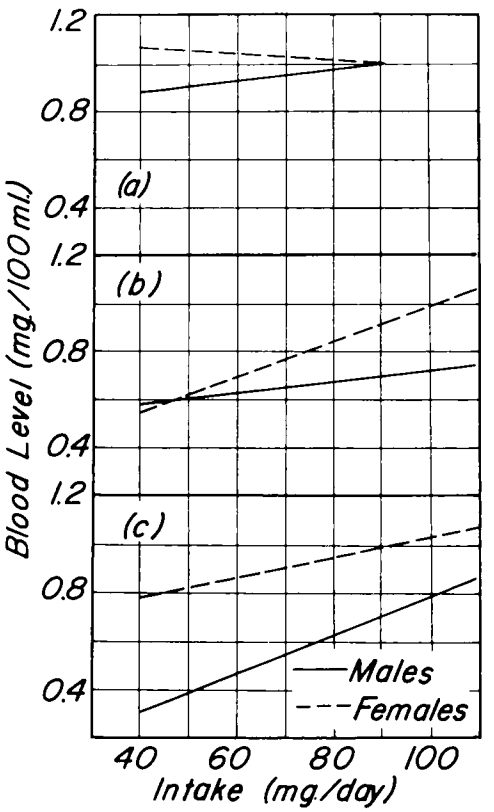

FIGURE 2. Regressions of ascorbic acid blood levels with ascorbic acid intake, males and females, (a) 4 to 12 years of age; (b) 13 to 20 years; and (c) 20 years and over. (From Dodds, M. L. [1969], *J. Am. Diet. Assoc.*, 54, 32. ©American Dietetic Association. With permission.)

ml in 202 men and 429 women aged 75 years and over. Their mean leukocyte total ascorbic acid levels were 16.6 and 21.4 $\mu g/10^8$ cells, respectively. However, dietary histories revealed that the men ate fruit less frequently than the women; it was concluded that the lower plasma and leukocyte ascorbic acid levels of these elderly men were partly, but not entirely, due to their having a lower ascorbic acid intake than the women.

The most useful data allowing comparison between the serum ascorbic acid levels of men and women are those of The Nutrition Canada National Survey, which includes dietary histories and blood analyses of a little over 2000 men and 2600 women in 5 regions of Canada, as reported by Pelletier (1977). The large numbers allow for analysis of each variable: age, sex, dietary ascorbic acid intake, smoking habit, and province, independent of the others. Although the 40- to 64-year-old men had a median vitamin C intake comparable to that of women of the same age, their median serum ascorbic acid levels were 25% lower. The 20- to 39-year-old men had an average ascorbic acid intake 35% higher than women of the same age, but had similar ascorbic acid levels. Presumably the higher ascorbic acid intake was enough to counteract the sex difference in this age group. When the data are broken down into ascorbic acid intake groups, as in Figure 3, it may be seen that women nonsmokers had higher serum ascorbic acid levels than men nonsmokers of each intake group in nearly all of the regions studied.

It has often been stated that scurvy is more common in men than in women; some have suggested that this is because elderly bachelors and widowers provide poorly for their own

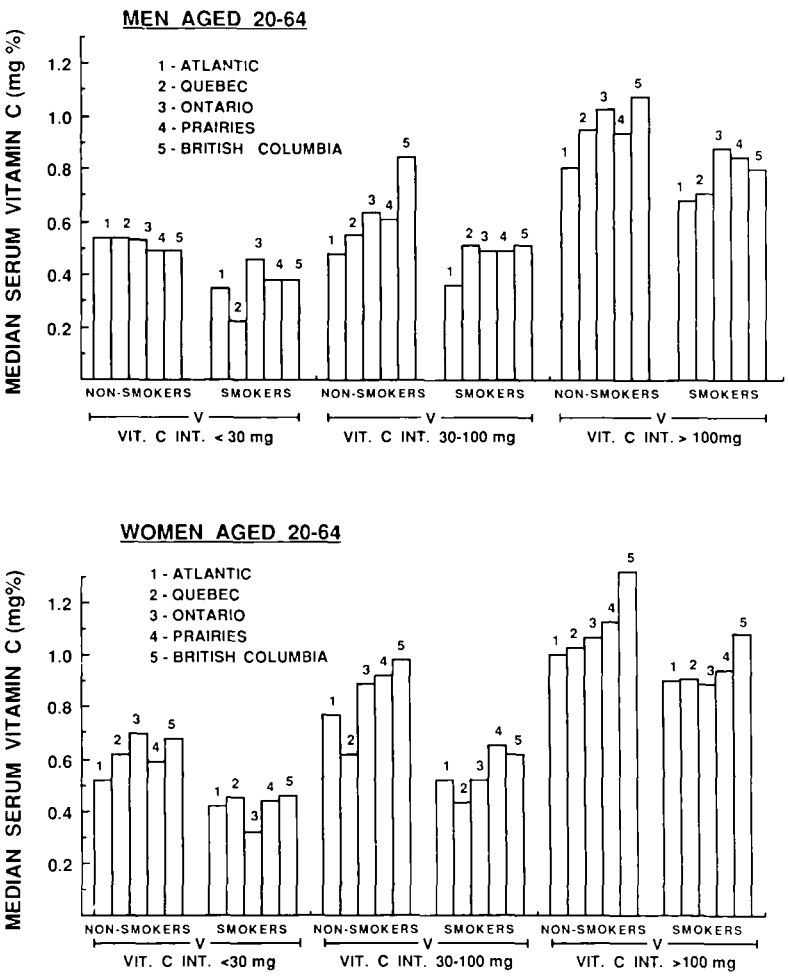

FIGURE 3. Women nonsmokers had higher serum ascorbic acid levels than men for each ascorbic acid intake group in nearly all of the regions studied. (From Pelletier, O. [1977], *Int. J. Vitam. Nutr. Res.*, 16, 147. With permission.)

dietary needs, some of them trying to subsist on bread and margarine with perhaps a little fish or meat paste and tea.

Cutforth (1958) reported on 11 patients with scurvy at King's College Hospital, London, and 10 of the 11 were men. However, Walker (1968) reported seven patients, and Mitra (1970) four patients, with scurvy, and all were women.

The general consensus of opinion seems to be that there is a difference between the sexes in their metabolism of ascorbic acid, favoring women, which may be hormonally induced by androgens or may be due to the decreased iron stores of women, resulting from menstrual blood loss during the reproductive years. These factors are discussed more fully in the sections of this book dealing with hormones (Chapter 13 of this volume) and heavy metals (Chapter 10 of this volume). Iron storage can decrease leukocyte ascorbic acid levels (Loh and Wilson, 1971b). Moreover, the inverse relationship known to exist between hemoglobin levels and ascorbic acid concentrations in hemochromatosis and hemosiderosis also occurs under apparently normal conditions in the female adolescent menstruating population and to a lesser extent in the adult female population (Loh and Wilson, 1971a). So the lower hemoglobin levels and lower liver iron stores of women could perhaps account for their higher ascorbic acid levels.

Some writers, like Wilson (1974), have suggested that women develop less severe and fewer scorbutic symptoms than men in times of stress and during vitamin C deficiency. This may be so, but the reports of Walker and of Mitra make it clear that women are not immune from scurvy. Moreover, they often develop excessive menstrual bleeding associated with bruising and bleeding gums when they have a borderline deficiency or a disturbance of ascorbic acid metabolism, as discussed in the chapter of this book devoted to menorrhagia (Chapter 11, Volume III).

Garry et al. (1982) conducted a 5-year study of the vitamin C status of 270 free-living, healthy men and women over 60 years of age, residing in and around Albuquerque, New Mexico. Mostly well-educated, health-conscious people volunteered for this study, so most of them had a high dietary vitamin C intake. Comparing dietary information and plasma ascorbic acid levels, these authors determined that men had significantly lower plasma ascorbic acid levels than women on the same dietary intake ($p < 0.0004$). It was estimated that the intakes needed to maintain a plasma ascorbic acid level of 1.0 mg/d would be 75 mg/d for women and 150 mg/d for men, but no account was taken of smokers vs. nonsmokers in this study.

If men really do have lower ascorbic acid levels than women, as it seems they do, this could possibly play a contributory role in the higher incidence of coronary heart disease in men than in women (Chapter 20, Volume III).

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Chapter 7

MENSTRUAL CYCLE, ESTRUS CYCLE, OVULATION

I. OVARY

Ascorbic acid was demonstrated in the ovaries, as well as the adrenals and the anterior pituitary glands, of human beings, dogs, rats, and guinea pigs by Gough and Zilva (1933) who observed blackened areas due to silver deposits in sections of these organs after immersion for 15 min in 0.4% silver nitrate solution.

The ascorbic acid (AA)* contents of the ovaries of guinea pigs, rats, rabbits, cats, hedgehogs, and cows were studied by Giroud and Leblond (1934, 1935, 1939), both by a histochemical technique which involved the infusion of a silver nitrate solution into blood vessels and by chemical analysis using 2,6-dichloroindophenol titration; they reported that there was not enough ascorbic acid to demonstrate in the Graafian follicles, but the corpora lutea were as rich in ascorbic acid as the adrenal cortex and accumulated silver granules which seemed to be associated with the mitochondria. The interstitial cells of the ovary were also found to contain the vitamin; the ascorbic acid concentration of the corpus luteum was highest when that organ was mature and decreased as it involuted. Bourne (1935) suggested that the corpus luteum might even be able to synthesize ascorbic acid for he observed prolonged survival of guinea pigs on a scorbutogenic diet when their ovaries had been luteinized by subcutaneous injection of antuitrin S. However, this does not seem to be so, for Giroud and Leblond (1935) observed no blackening of the corpora lutea or the interstitial cells of the ovaries of pregnant guinea pigs after they had been fed for 9 d on a vitamin C-deficient diet. Moreover, Kramer et al. (1933) observed a marked degeneration in the histological appearance of the corpora lutea and the Graafian follicles in the ovaries of ascorbic acid-deficient guinea pigs, such that they could not become pregnant. Also, Deb and Banerjee (1957) observed a pronounced reduction in the DNA content of the ovaries of scorbutic guinea pigs; so it would seem that the ovary is not able even to protect itself against vitamin C deficiency.

Biskind and Glick (1936) confirmed that the corpora lutea of cows contain abundant amounts of vitamin C and suggested a correlation with the production of progesterone. Israel and Meranze (1941) reported that injection of large doses of vitamin C had a progesterone-like effect on the endometrium of infantile rabbits which had been primed by daily injections of estrogen. Di Cio and Schteingart (1942) noted that a combination of vitamin C and "gonadotrophic hormone", when injected into young rats, caused a much greater increase in the weight of the female reproductive tract than did injection of the hormone alone. So it seemed that ascorbic acid supplements could affect the estrus cycle, even in animals like the rabbit and the rat which make their own ascorbic acid.

Studies by Hoch-Ligeti and Bourne (1948) revealed that rat ovaries show cyclic changes in the concentration and histological distribution of ascorbic acid during the estrus cycle; the ascorbic acid content and concentration of the ovaries were lowest at estrus.

Miller and Everett (1948) observed a significant increase in ascorbic acid and cholesterol storage by the corpora lutea of pregnancy in rats 18 h after the subcutaneous injection of luteinizing hormone (LH) on the 5th d of pregnancy.

Claesson et al. (1949) showed a rapid marked decrease, followed by a slow increase in the ascorbic acid (TAA)** content of the interstitial cells of the ovaries of day-12 pregnant

* AA — ascorbic acid, reduced form.

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

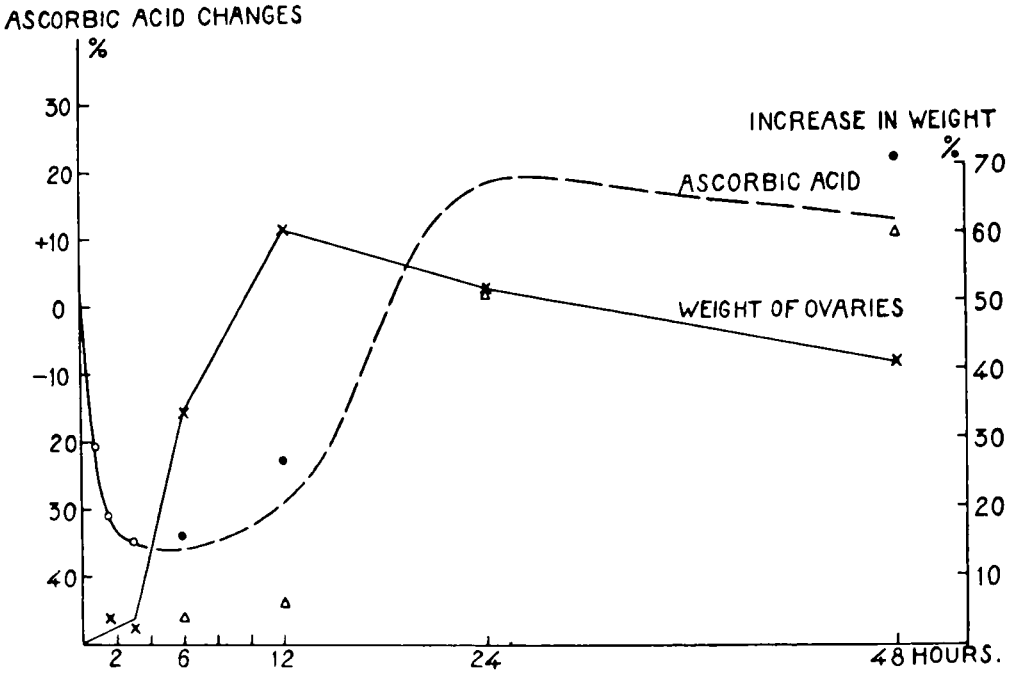

FIGURE 1. Changes in the weight and ascorbic acid (TAA) content of the interstitial tissue of rabbit ovaries following intravenous injection of gonadotrophin (PMS) on the 12th d of pregnancy. The corpora lutea were removed, and all large follicles were punctured before the ovaries were weighed and analyzed. The ascorbic acid content of the interstitial cells was rapidly reduced (21% after 45 min, 31% after $1^1/_2$ h, and 35% after 3 to 6 h) following gonadotrophic stimulation. Later, there was a slow increase in the ascorbic acid content to normal or higher values after 24 to 48 h. (From Claesson, L., Hillarp, N. Å., Högberg, B., and Hökfelt, B. [1949], *Acta Endocrinol.*, 2, 249. With permission.)

rabbits, following intravenous injection of pregnant mare's serum (PMS) gonadotrophin (Figure 1). Similarly, Hökfelt (1950), studying the effect of PMS gonadotrophin on the corpora lutea at the same stage of pregnancy in rabbits, observed a 25% fall in the ascorbic acid (TAA) content of the corpora lutea, maximal 3 h after injection of PMS, followed by a major increase in ascorbic acid content, maximal at 24 h, by which time the weight of the corpora lutea had virtually returned to normal (Figure 2).

Deane (1952) described changes in the silver nitrate staining reactions of various tissues in the ovary of the rat during the estrus cycle; the cells of the theca interna of normal follicles, the lutein cells, the interstitial cells, and the granulosa cells of atretic follicles showed silver strippling indicative of ascorbic acid. She concluded that ascorbic acid was especially rich at the sites of ovarian steroid formation.

Coste et al (1953) showed a pronounced reduction in the ascorbic acid content of the ovaries of rats in late proestrus, just before ovulation. They conjectured that it was the result of stimulation by gonadotrophins, either FSH or FSH and LH.

Noach and van Rees (1958) studied the weight and ascorbic acid content of one ovary from each of eight adult rats before, and the other, 4 h after the injection of chorionic gonadotrophin. They also studied eight adult female rats as controls before and after saline injections. In their first experiment there was a significant reduction in both the content and concentration of ascorbic acid in the ovaries following the administration of gonadotrophin, but they could not confirm these results in a similar experiment a year later. Subsequently they confirmed the first experiment, using immature rats and then most consistently with hypophysectomized immature rats that had been primed with low doses of chorionic gonadotrophin.

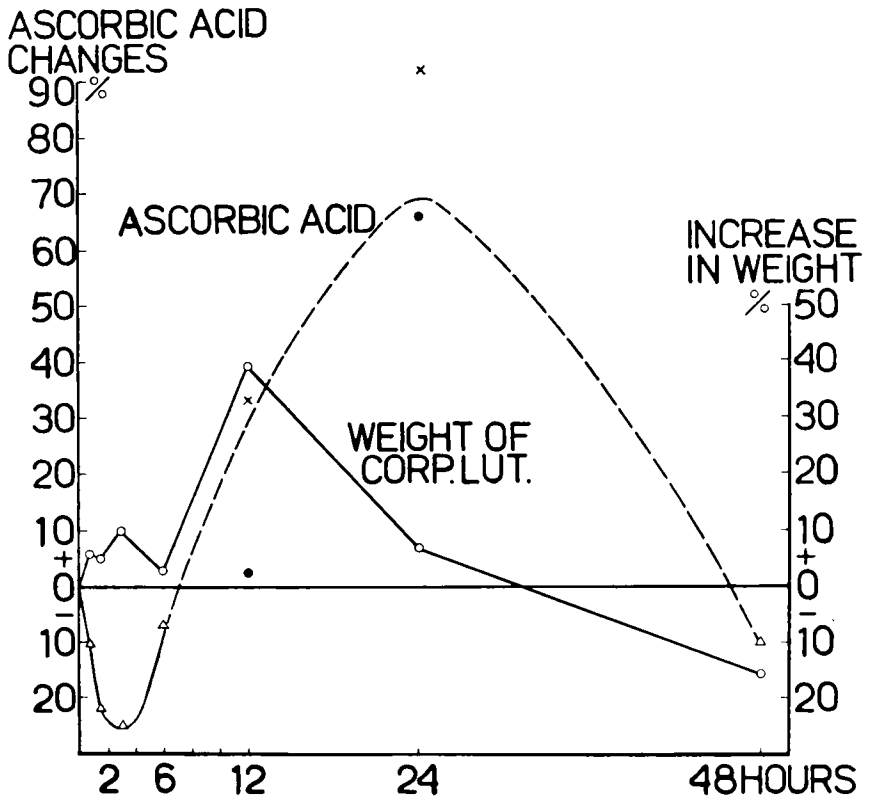

FIGURE 2. Showing the changes in the weight of the corpora lutea of pregnancy and the changes in their ascorbic acid (TAA) content following the intravenous injection of gonadotrophin (PMS) on the 12th d of pregnancy in rabbits. There is first a fall in the ascorbic acid content of the corpora lutea, reaching 25% at 3 h, and then a pronounced increase in the ascorbic acid content which is maximal at 24 h. (From Hökfelt, B. [1950], *Acta Physiol. Scand.*, 20, 172. With permission.)

It would seem that ovarian ascorbate depletion by gonadotrophins cannot be demonstrated when the ovaries are already fully stimulated by endogenous gonadotrophins; it is best demonstrated in the ovaries of immature or hypophysectomized rats that have been primed with small doses of gonadotrophin daily for 1 week before the larger test dose is administered.

Parlow (1958, 1961), McCann and Taleisnik (1960), Albert et al. (1961), and Foreman (1963) observed that the injection of LH or chorionic gonadotrophin into primed immature female rats caused a rapid dose-related depletion of ovarian ascorbic acid. This effect became so well established that it was used as the standard method for the assay of LH until radioimmunoassay became available. The ovary, which is rich in ascorbic acid, can lose 40% of its ascorbic acid within 1 h after the injection of LH.

McCann and Taleisnik demonstrated that neither FSH, nor LTH, nor ACTH caused any significant depletion of ovarian ascorbic acid. Moreover, FSH had no synergistic effect; that is to say, it did not increase the ovarian ascorbic acid-depleting effect of LH. They found that injection of vasopressin did cause an appreciable depletion of ovarian AA, but endogenous vasopressin release did not. They concluded that vasopressin is not likely to interfere with the assay of LH in body fluids by this method.

Mills and Schwartz (1961) observed a cyclic variation in the concentration of ascorbic acid in the ovaries of rats, highest in metestrus and lowest on the afternoon of proestrus (following LH release which occurs at about 2 p.m.).

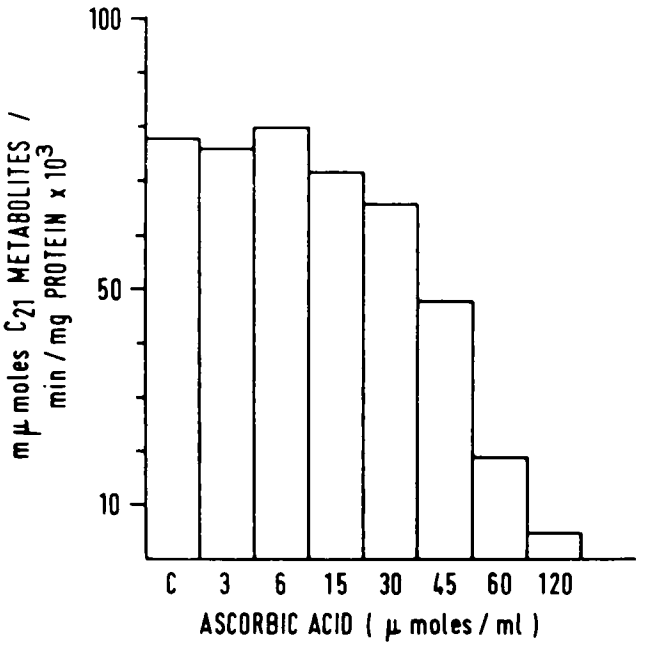

FIGURE 3. The effect of different concentrations of ascorbic acid on the conversion of (4-^{14}C) cholesterol to (4-^{14}C) pregnenolone, (4-^{14}C) progesterone, and (4-^{14}C) 20α hydroxypregn-4-en-3-one in a mitochondrial preparation from luteinized rat ovaries. The cholesterol side-chain cleavage enzymes were inhibited by concentrations of ascorbic acid similar to those found in the resting ovary. A decrease in the ascorbic acid content released the side-chain cleavage reaction from inhibition. It was therefore suggested that ascorbic acid acts as a brake on progesterone synthesis and that this brake is released when ascorbic acid is released from the ovary following LH stimulation. (From Sulimovici, S. and Boyd, G. S. [1968], *Steroids*, 12, 127. With permission.)

Marsh and Savard (1964) demonstrated that LH increased progesterone production by slices of bovine corpora lutea of pregnancy, presumably from cholesterol. Sulimovici and Boyd (1968) studied the cholesterol side chain cleaving enzyme system in the mitochondria of rat ovaries. These workers presented evidence that this enzyme system is inhibited by concentrations of ascorbic acid comparable to those observed in the "resting" ovary (Figure 3). They concluded that reduction of the ascorbic acid concentration to a level similar to that observed in the LH-stimulated gland results in release of this inhibition and thus increases steroid production. These authors reported that immature rat ovarian mitochondria stimulated by LH contained little ascorbic acid, but that ascorbic acid seems to be metabolized by these organelles.

Paeschke (1969a, b) studied the ascorbic acid concentration in the ovaries of guinea pigs throughout the estrus cycle and found a small dip, followed by a high ovulatory peak, at estrus as shown in Figure 4. Smith (1972) published an improved method for the measurement of ovarian ascorbic acid.

Labhsetwar (1970, 1971) observed elevated pituitary LH stores and ovulation following the injection of prostaglandin $F_{2\alpha}$ in pregnant rats and hamsters. Sato et al. (1972) reported that prostaglandins increased the LH concentration in the serum and induced ovarian ascorbic acid (AA) depletion in intact and, to a less extent, in hypophysectomized rats. However, they found that prostaglandins failed to produce ovulation or luteinization in hypophysectomized immature rats pretreated with PMS; thus, prostaglandins seemed to have an LH-

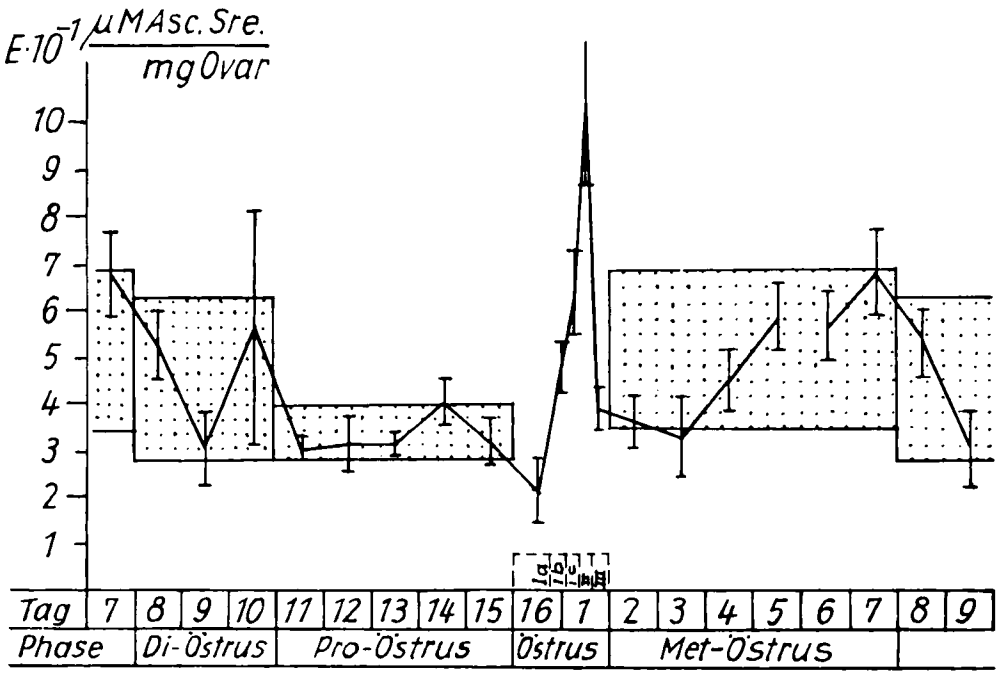

FIGURE 4. Ovarian ascorbic acid content during the estrus cycles of guinea-pigs. (From Paeschke, K. D. [1969], *Zentralbl. Gynaekol.*, 91, 1129. With permission.)

releasing effect as well as a direct and an indirect ovarian ascorbic acid-depleting effect, but did not possess an LH-like effect. However, Armstrong et al. (1973) observed that indomethacin, a prostaglandin synthesis inhibitor, was effective in blocking LH-induced ovulation in rabbits, but did not prevent luteinization of those follicles where ovulation was blocked, nor steroidogenesis by the ovarian interstitial cells. They concluded that prostaglandins may be involved in the process of ovulation, possibly as mediators of the response to LH.

Le Maire et al. (1973) have shown that both prostaglandins E and F are present in rabbit Graafian follicles and that their concentrations increase towards the time of ovulation. Moreover, Sharma et al. (1976) have demonstrated that the guinea pig ovary is capable of synthesizing both prostaglandins E and F from endogenous precursors and have shown that ovarian prostaglandin levels are markedly increased at estrus. Thus, a whole new approach to the study of ovulation is being developed, but the exact role of ascorbic acid is still unknown.

The facts that intravenous injection of copper acetate induces ovulation in rabbits and that large doses of ascorbic acid can be used to inhibit this effect, as shown by Sharma et al. (1976) lend support to the concept that ovarian ascorbic acid may act as a brake on ovarian function.

A study of the protein binding of L-ascorbic acid (1-^{14}C) by a protein extract from the ovaries of cows was conducted by Sharma and Wilson (1978). With a protein dilution of 1 in 3 they found approximately 65% of the ascorbic acid ^{14}C to be bound to protein. As might be expected, this protein binding was inversely related to the total (unlabeled) ascorbic acid present, and fell to 20% with higher levels of ascorbic acid, such as may be found in the ovary. The ovarian protein binding of ascorbic acid was not affected by estradiol, progesterone, oxytocin, vasopressin, prostaglandin E_2 or $F_{2\alpha}$. The effects of gonadotrophins on ovarian protein ascorbate binding were not reported in that paper, but the authors conjectured that it might play an important role in the regulation of ovulation.

II. URINE

Pillay (1940a, b, 1942, 1946) reported a decreased urinary ascorbic acid (AA) concentration on the day of ovulation in women who had been given ascorbic acid, 300 mg daily by mouth, 3 to 6 h before collection of each urine specimen. This finding was confirmed by Guggisberg (1948), by Paeschke and Vasterling (1968), and by Nakajima (1959).

Hartzler (1945), on the other hand, observed very low urinary ascorbic acid (AA) excretion during the menses in one woman who was studied for 12 weeks on a diet containing 75 mg of AA daily on week days and 300 mg on Sundays; one wonders whether possible contamination of the urine by hemolysed blood may not have accelerated the losses of AA by oxidation in the 24-h samples of menstrual urine in this experiment, even though the specimens were collected in brown bottles containing oxalic acid, as there was no mention of refrigeration.

Loh and Wilson (1971) observed a pattern of urinary total ascorbic acid (TAA) excretion during the menstrual cycle, somewhat similar to that of Pillay and others, in first morning urine samples from women receiving 500 mg of ascorbic acid every morning at breakfast. TAA excretion began to fall 3 d before ovulation; it reached a minimum concentration on the day before the rise of basal body temperature and increased as the temperature rose following ovulation. They likened it to the pattern of excretion of LH, as shown in Figure 5, which is taken from their work.

Likewise, Kameter et al. (1975), studying both LH levels and urinary ascorbic acid excretion in 18 women taking ascorbic acid, 500 mg every evening, observed a trough of vitamin C excretion 2 d before ovulation, and a peak of excretion on the day before ovulation; they suggested that this preovulatory peak of excretion may be due to the rising estrogen level.

III. BLOOD

A. Women

Neuweiler (1937) found no typical pattern to the vitamin C concentration in the blood of women during the menstrual cycle.

Mickelsen et al. (1943) observed midcycle peaks in the plasma ascorbic acid (AA) levels of four out of eight women they studied.

Hauck (1947) reported no consistent menstrual pattern in the urine or plasma ascorbic acid levels of ten women studied for periods of 4 to 6 weeks, but this was hardly a fair test, as they had all been saturated with ascorbic acid at the beginning of the study at different stages of the menstrual cycle and all showed blood and urine levels which fell progressively throughout the period of study. Moreover, no attempt was made to record the basal body temperatures of the subjects in order to find out if and when they were ovulating. Clearly this was an unsuccessful attempt to obtain additional data from an experiment designed for another purpose.

Kofoed et al. (1965) found little change in the total ascorbic acid, but recorded profound changes in the ratio of ascorbic acid to dehydroascorbic acid (DHAA) in the serum of women, lowest at midcycle, as shown in Table 1A.

On the other hand, Fujino et al. (1966) reported that the whole blood ascorbic acid (TAA) levels of healthy young women were 13% lower during menstruation; they found a very significant inverse relationship between whole blood ascorbic acid and total iron-binding capacity (transferrin) levels, the latter being 12% higher during menstruation. They suggested a close metabolic relationship between transferrin and ascorbic acid. Plasma iron itself was decreased 32% during mensturation.

Kenny (1970) reported the results of analysis of blood samples drawn twice weekly from

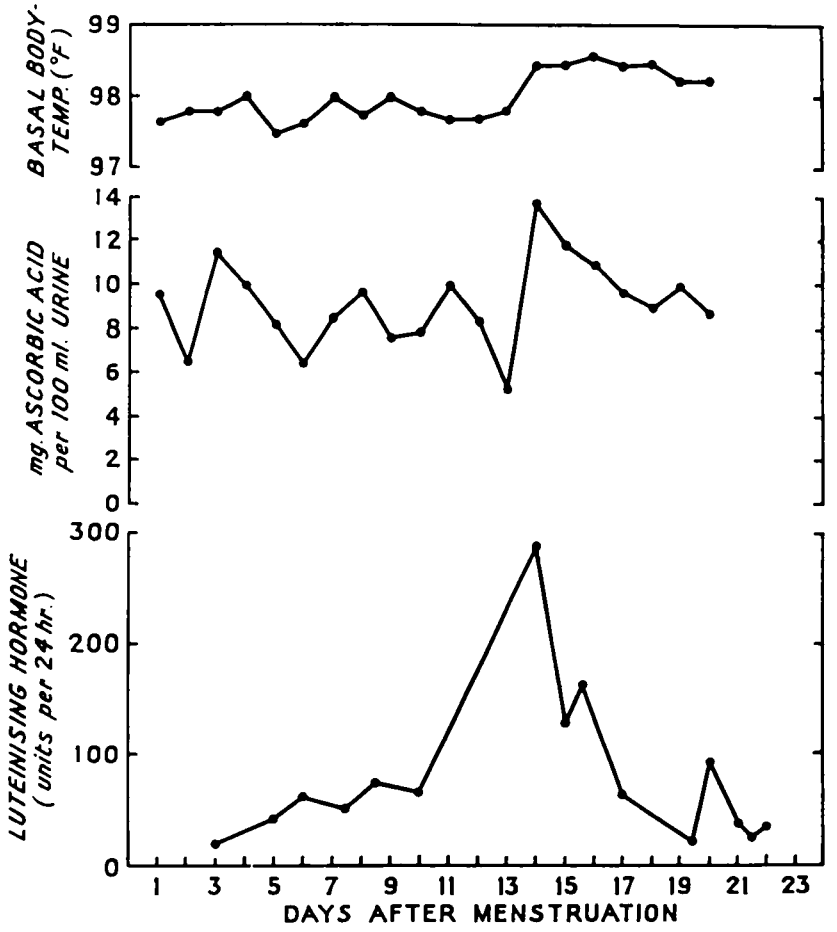

FIGURE 5. Basal body temperatures and mean morning urine ascorbic acid concentrations of five women with ovulatory menstrual cycles, who were receiving a daily dietary supplement of 500 mg of ascorbic acid. Values for the excretion of luteinizing hormone (LH) in morning urine specimens are shown for comparison of the timing of the ascorbate dip and the LH peak. (From Loh, H. S. and Wilson, C. W. M. [1971], *Lancet*, 1, 110. With permission.)

eight women maintained on a low ascorbic acid intake (37 mg/d) for 70 d; she found the ratio of ascorbic acid (AA) to total ascorbic acid (TAA) to be higher in the leukocytes during the ovulatory phase of the menstrual cycle in five out of six women with ovulatory cycles; this is just the opposite of the changes reported by Kofoed et al. in the serum. Kenny found no relationship between the menstrual cycle and ascorbate levels in the blood plasma or the urine.

Rivers and Devine (1972) observed markedly increased plasma total (TAA) and reduced (AA) levels at midcycle on the day or the day following ovulation in two healthy young women.

Studies of the serum total copper levels of one woman during the menstrual cycle by von Studnitz and Berezin (1958) suggested cyclic changes, but these were not confirmed by the studies of Hagenfeldt et al. (1973) who found significant cyclic changes in the endometrial copper concentration, but not in the plasma copper levels of seven women at various stages of the menstrual cycle.

There is no doubt that high-dosage or high-potency estrogens do elevate the plasma copper levels of women, as discussed in Chapter 13 of this volume, and that more than 95% of

Table 1

	TAA[a]	DHAA + DKG[b]	AA[c]	AA/DHAA + DKG ratio
A. Serum Ascorbic Acid mg/100 ml				
Menstrual	1.4	0.1	1.3	13.0
Midcycle	1.4	0.5	0.9	1.8
Late cycle	1.5	0.1	1.4	14.0
B. Cervical Mucus Ascorbic Acid mg/100 ml				
Postmenstrual	4.2	2.5	1.7	0.68
Midcycle	5.2	4.3	0.9	0.21
Late cycle	4.1	2.3	1.8	0.78

[a] TAA = Total ascorbic acid by Roe and Kuether (1943) method.
[b] DHAA + DKG = Dehydroascorbic acid + diketogulonic acid and other preexistent Roe-Kuether reacting uronic acids.
[c] AA = Reduced ascorbic acid obtained by difference between TAA and DHAA + DKG.

Data derived from Kofoed, J. A., Blumenkrantz, N., Houssay, A. B., and Yamauchi, E. Y. (1965), *Am. J. Obstet. Gynecol.*, 91, 95.

this copper is in the form of ceruloplasmin, which is an ascorbate oxidase. This accounts for the fact that women were found to have a higher mean blood copper level (124 μg/100 ml) than men (107 μg/100 ml). Women taking the old high-dosage estrogen-progestagen birth control pills had high blood copper levels (216 μg/100 ml), and pregnant women at term have such high blood copper levels (226 μg/100 ml), as recorded in the data of Clemetson (1968). However, the changes due to endogenous estrogens during the menstrual cycle are clearly modest and would, therefore, be demonstrable only in a study involving a large number of women with regular ovulatory cycles. The same probably applies to plasma ascorbate levels where so many factors, other than the estrogen levels, affect the blood ascorbic acid at any time of the cycle.

B. Baboons

Boots et al. (1976), studying five baboons under controlled conditions in captivity, observed no significant menstrual rhythm in their serum ascorbic acid levels, but did find a slight tendency for the ascorbic acid levels to be lower during the periovulatory period and to rise during the luteal phase. They found no difference in the disappearance rates of ascorbic acid from the serum after intramuscular injections of AA given in the follicular, ovulatory, or luteal phases of the menstrual cycle.

In a subsequent study, Boots et al. (1983) maintained ten baboons on a complete ascorbic acid-rich diet (AA, 2000 ppm) and analyzed blood samples from each animal every week throughout one complete menstrual cycle; these workers again found the serum ascorbic acid levels to be significantly elevated during the luteal phase of the cycle.

C. Cows

Phillips et al. (1941) demonstrated an increase in the plasma ascorbic acid levels of cows at estrus; it was only a temporary increase, for the ascorbic acid level had returned to normal within 14 h. Some of the infertile cows were found to have low plasma ascorbate levels and lacked these plasma ascorbic acid peaks until they had been treated with parenteral ascorbic acid. These workers and others reported considerable success in the treatment of infertile cows by injection of vitamin C just before breeding. The writer has obtained a

similar impression of increased fertility in women following oral administration of ascorbic acid and bioflavonoids for the treatment of menorrhagia; indeed, capillary fragility studies conducted before and during pregnancy in two of them are shown in Figure 8 of Chapter 14 of this volume. Another 40-year-old woman who had not taken any precautions for 10 years was quite upset when she became pregnant while on treatment; she said we should have warned her.

D. Cervical Mucus

Studies of the ascorbic acid content of the mucus from the endocervix of the human uterus during the menstrual cycle were reported by Kofoed et al. (1965); they used the method of Roe and Kuether (1943), which estimates DHAA plus diketogulonic acid (DKG), but can also be used to estimate total ascorbic acid (TAA) after conversion of all AA to DHAA by the use of activated charcoal. Ascorbic acid (AA) can then be obtained by difference. They found that the total ascorbic acid (TAA) concentration of cervical mucus gradually rose from 4.2 to 5.2 mg/100 ml at midcycle and fell again to 4.1 mg/100 ml before menstruation. However, the reduced ascorbic acid (AA) concentration fell from 1.7 to 0.9 mg/100 ml at midcycle, and rose again to 1.8 mg/100 ml before menstruation, as shown in Table 1B.

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Chapter 8

INFECTION

I. ASCORBATE DEPLETION

An outbreak of scurvy was reported among the inmates of an asylum in Victoria, Australia, by W. A. T. Lind in 1919, which serves to illustrate the part played by infection in the onset of scurvy. There had been no alteration in the diet of the patients for years, but an infectious disease (the nature of which was not determined) caused a febrile illness and led to widespread scurvy among the patients.

Not only does infection deplete ascorbic acid stores, but ascorbic acid deficiency lowers resistance to infection, so each compounds the other. It is for this reason that naturally occurring scurvy is so often accompanied by serious infections and foul-smelling, bleeding gums, while experimental scurvy, produced by vitamin C deficiency in volunteers, is very slow in onset, and the affliction of the gums is not so virulent.

This relationship between vitamin C deficiency and infection was recognized by Hess (1922) in his Cutter lecture at Harvard Medical School, when he stated, "The main factors determining the nutrition of the body are diet and environment and infection. This is the triad, which, working hand in hand, or at cross purposes, brings about a physiologic or a pathologic state." Ascorbic acid had not yet been isolated and there was no way of knowing that blood ascorbic acid levels were decreased by infection. Even so, Hess already suspected that the antiscorbutic nutritional requirements were influenced by infection, for he stated, "Clinically it is by no means easy to determine the respective role of infection and nutrition when confronted with disease. Either can result in secondary involvement of the other." In the same address in 1922 Hess stated,

> This interrelationship of infection and nutrition was exemplified in 1913, when, as the result of a diet of pasteurized milk, latent scurvy developed among a group of infants under my care. This scorbutic taint was followed by a widespread grip infection, with hemorrhagic skin manifestations, which disappeared only in part on the administration of orange juice. For some years it was difficult to know how to interpret this peculiar clinical picture, whether to regard the epidemic as due to scurvy, or to infection. As the result of subsequent experience I realized some years later, that it had been due to both causes — the result of a primary nutritional disturbance and a secondary bacterial invasion. It is probable that the overt and classical signs of scurvy — those which are described in the textbook — for example, the hemorrhage of the gums, are not always purely scorbutic or nutritional, but the result of the secondary infection which comes about, sooner or later, in conjunction with that disorder. . . . The three main factors in nutrition — diet, infection and environment — probably are so inextricably bound together that it will be impossible to appraise the role of each in the disorders of metabolism in which they are concerned.

These words ring true today, but now, with the aid of chemical analyses, we do attempt a better understanding of the way in which each of these factors affects the others.

Woringer and Sala (1928) observed four bottle-fed infants in whom whooping cough led on to the development of scurvy which was cured by supplements of lemon and tomato juice. Three of the four infants developed scurvy while in hospital after being on a standard diet for several weeks. The vitamin C content of the fresh cow's milk may not have been ideal, due to pasteurization, but it was sufficient to prevent scurvy in all the other infants on an identical diet.

Martin (1931) reported scurvy in a patient who had been on an ulcer diet and alkalis for six months for duodenal ulcer; the condition was precipitated by an attack of influenza. Here we see a low-ascorbic acid diet, made worse by alkalis and then frank scurvy brought on by infection.

Yavorsky et al. (1934) studied the ascorbic acid content of the tissues of people who had

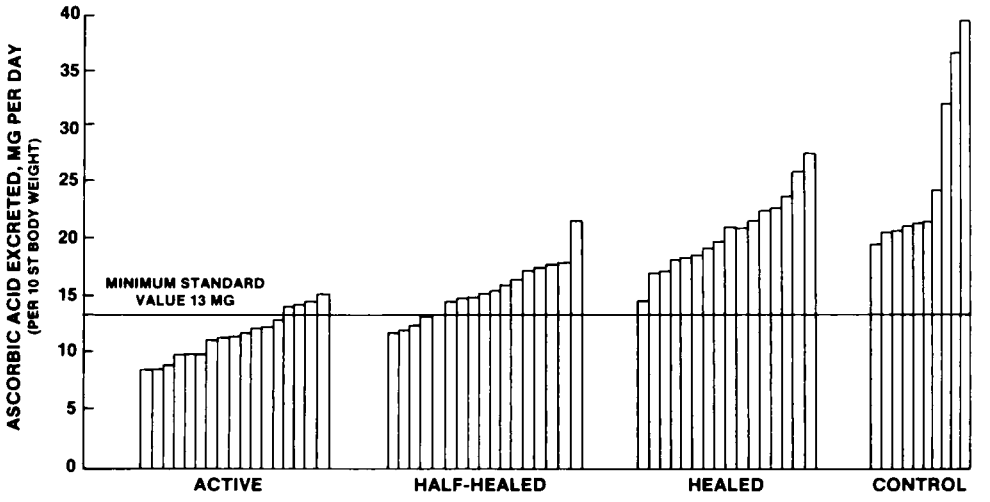

FIGURE 1. Decreased excretion of vitamin C in osteomyelitis. The heights of the columns represent the daily excretions of vitamin C on a controlled diet by 17 patients with active osteomyelitis, 17 with "half healed" disease, 16 whose disease was healed, and 10 controls. The broken horizontal line represents the minimum standard excretion, 13 mg/d (From Abbasy, M. A., Harris, L. J., and Gray Hill, N. [1937], *Lancet*, 233 (2), 177. With permission.)

died of various causes; they noted a diminution of vitamin C in the majority of those who had died of generalized infections.

Harde and Benjamin (1935) observed that the adrenals of guinea pigs dying of diphtheria toxin did not reduce silver nitrate, while those of control animals were rapidly blackened. In subsequent experiments they estimated the ascorbic acid content of guinea pig tissues by titration with 2,6-dichloroindophenol; they found that those guinea pigs showing a severe reaction following sublethal doses of diphtheria toxin had decreased ascorbic acid levels in the liver and adrenals. Later the same year, Harde et al. (1935), measuring urinary ascorbic acid levels following oral administration of ascorbic acid, found evidence of hypovitaminosis C in several patients with pneumonia, but they were unable to say whether the vitamin C deficiency preceded or followed the pneumonia.

Lyman and King (1936) noted a marked loss of ascorbic acid (AA)* from the adrenal glands, pancreas, and kidneys of guinea pigs which had received injections of diphtheria toxin. In 39 animals given toxin, the average losses of vitamin C from these 3 organs were 38, 25, and 21%, respectively, as compared with 39 control injected animals. In some experiments the liver also showed a loss of vitamin C, but in other experiments there was an increase in liver AA, suggesting mobilization.

Abbasy et al. (1936) studied 193 patients with acute rheumatism (rheumatic fever) and 64 control subjects and found the urinary ascorbic acid excretion to be markedly reduced in the patients.

Abbasy et al. (1937) reported decreased excretion of ascorbic acid in patients with osteomyelitis, compared with control subjects, all restricted to one orange a day as their sole source of vitamin C (Figure 1); they also observed decreased excretion of ascorbic acid in corysa and in influenza.

Abbasy et al. (1937) reported that the excretion of vitamin C is reduced in tuberculosis; the responses to test doses of ascorbic acid by patients with active, healing, and quiescent pulmonary tuberculosis are shown in Figure 2.

Experiments with guinea pigs on a constant ascorbic acid intake by Harris et al. (1937) showed that several different acute infections decreased their adrenal ascorbic acid content

* AA — ascorbic acid, reduced form.

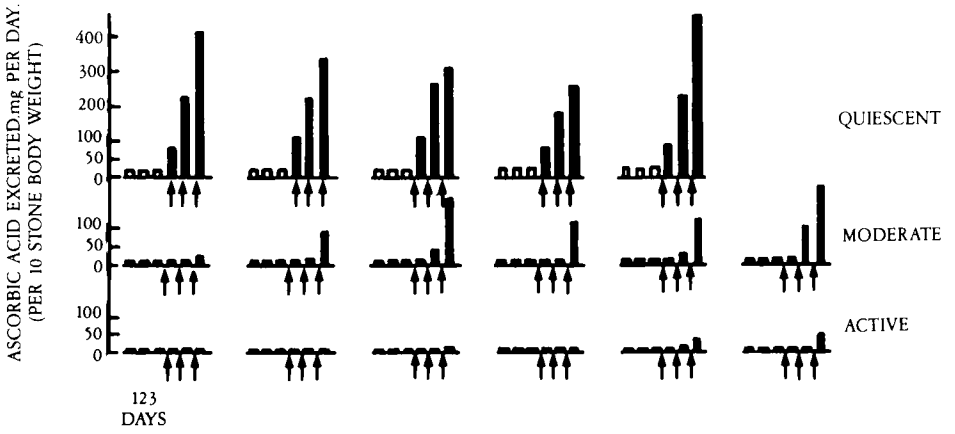

FIGURE 2. Pulmonary tuberculosis. Responses to test doses of ascorbic acid by six patients with "active", six with "moderate", and five with "quiescent" disease. Patients with "active" tuberculosis showed evidence of a very low state of ascorbic acid saturation by giving little or no response, while those with "quiescent" disease gave good responses, and those assessed as moderate gave intermediate readings. Test doses of ascorbic acid were given on three successive days, as marked by the arrows. (From Abassy, M. A., Harris, L. J., and Ellman, P. [1937], *Lancet*, 233 (2), 181. With permission.)

and concentration. Chronic infection with mycobacterium tuberculosis in partially immunized animals decreased the ascorbic acid content and concentration of the liver as well as the adrenals.

Kendall and Chinn (1938) isolated from the gastric contents of certain patients with achlorhydria, and from feces, certain strains of bacteria which were capable of fermenting and destroying ascorbic acid, even in an anaerobic environment. They were mostly members of the *Mucosus capsulatus* and *Enteroccus* groups, but all would ferment glucose in preference to ascorbic acid. Nevertheless, the authors suggested that these ascorbic acid-fermenting strains may gain ascendency in the alimentary canals of certain persons, leading in them to a detectable ascorbic acid deficit.

Banerjee et al. (1940), studying 9 normal individuals and 16 patients with acute pulmonary tuberculosis, confirmed that the mean urinary total ascorbic acid excretion was much lower (17 mg/24 h) in the patients than in normal subjects (68 mg/24 h). They also reported that the percentage of the urinary ascorbate in the "combined" form was increased in the tuberculosis subjects.

A study was made of the relationship of vitamin C to the clinical course of scarlet fever by Abt et al. (1942); the initial plasma ascorbic acid levels were significantly lower in 76 children with scarlet fever than in 110 controls. However, the levels in rheumatic fever, chorea, and diphtheria were not found to be significantly different from normal in that study.

Further evidence for the existence of a disturbance of ascorbate metabolism in patients with various kinds of serious illnesses was provided by Roe et al. (1947). These workers compared blood and plasma ascorbic acid levels in blood samples obtained 15 h after food from 50 healthy individuals and noted that with whole blood levels above 0.9 mg/100 ml, all but 1 of 19 had plasma levels which were higher than the whole blood levels; with whole blood levels below 0.6 mg/100 ml, the plasma concentration was always lower than the whole blood level; with whole blood levels between 0.9 and 0.6 mg/100 ml, the plasma level was either equal to or slightly greater or less than the whole blood concentration, as shown in Figure 3. Studies of guinea pig blood samples showed a similar pattern. Blood samples were also obtained from 50 patients in the hospital for treatment of varius serious illnesses including pulmonary tuberculosis, hyperthyroidism, syphilis, gonorrhea, glomerulonephritis, portal cirrhosis, hepatitis, diabetes mellitus, carcinoma, pernicious anemia with

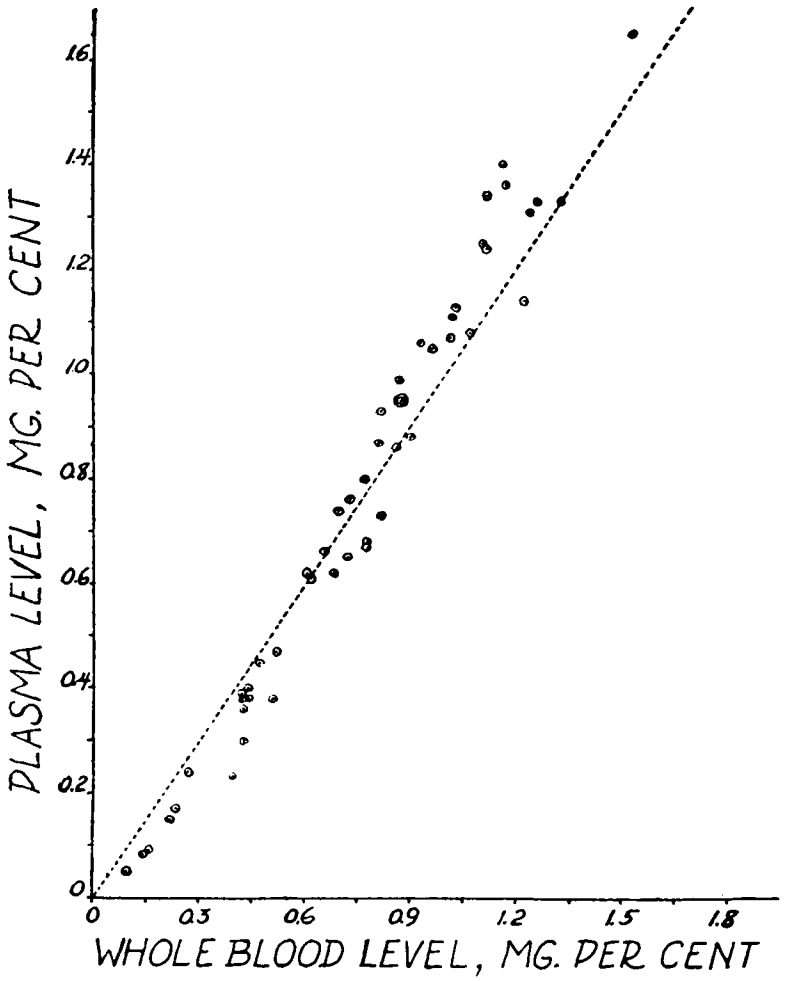

FIGURE 3. The relation of the level of ascorbic acid in the whole blood to that in the plasma in 50 healthy human subjects. (From Roe, J. H., Kuether, C. A., and Zimler, R. G. [1947], *J. Clin. Invest.*, 26, 355. ©American Society for Clinical Investigation. With permission.)

cellulitis, cystitis, pyelonephritis, duodenal ulcer, Addison's disease, rheumatoid arthritis, and cardiovascular disease. All of these had plasma ascorbic acid levels below the whole blood level, which would be expected in those with blood levels below 0.6 mg/100 ml, but even those 15, whose ascorbic acid levels were above 0.6 mg/100 ml, all had plasma levels lower than the whole blood levels, as shown in Figure 4. They interpreted these findings as indicating that patients with serious illnesses have a greater need for ascorbic acid, because of the underlying pathological processes, and this need is supplied by a more rapid withdrawal of the vitamin from the plasma than occurs in healthy subjects.

Karlson et al. (1959) studied both plasma ascorbic acid levels and intradermal dichloroindophenol dye clearance tests on human subjects. They noted that the dye clearance time was prolonged during infections and returned to normal when the infection subsided.

Studies by Kechik and Sykes (1979) at Wye College demonstrated decreased ascorbic acid (TAA)* levels in the blood plasma, duodenum, jejunum, ileum, liver, and adrenal

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

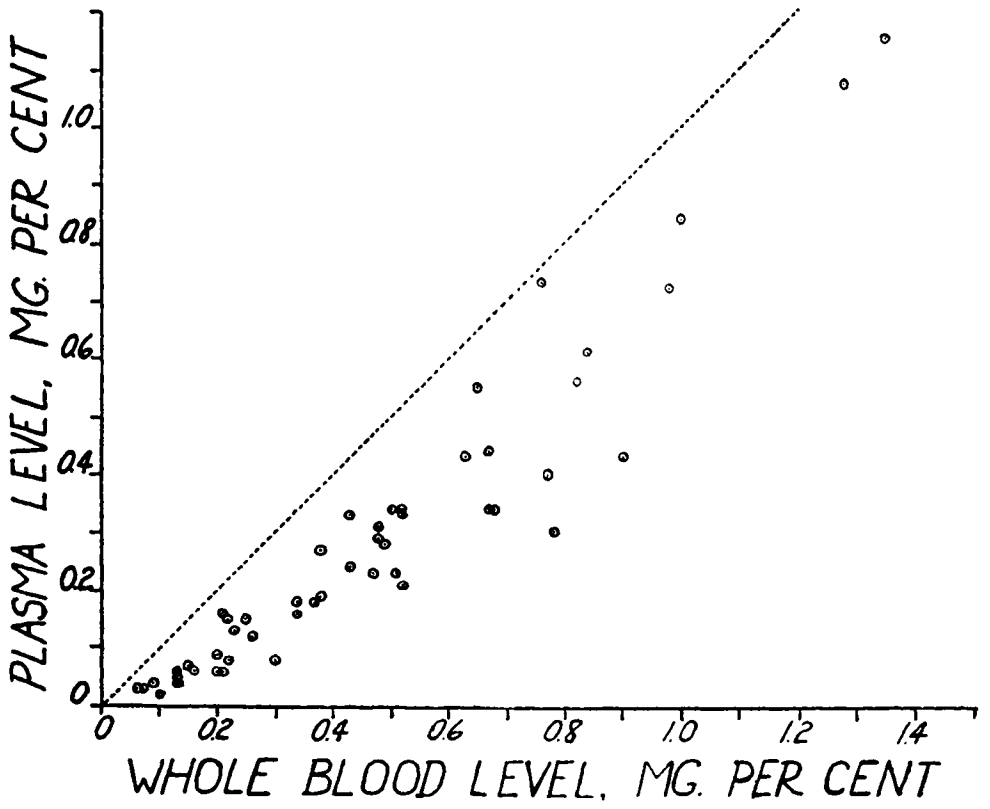

FIGURE 4. The relation of the level of ascorbic acid in the whole blood to that in the plasma in 50 patients. (From Roe, J. H., Keuther, C. A., and Zimler, R. G. [1947], *J. Clin. Invest.*, 26, 355. ©American Society for Clinical Investigation. With permission.)

glands of chicks infected with coccidiosis (*Eimeria acervulina*), compared with pair-fed controls. This depletion commenced 4 to 5 d after oral inoculation with the oocysts and is believed to indicate a rate of utilization or destruction of the vitamin exceeding the rate of synthesis.

Bacelar et al. (1982), studying patients with schistosomiasis in northeastern Brazil, found no significant difference from normal in the plasma or the leukocyte ascorbic acid levels of their patients with hepatosplenic disease, but they did note that those with gastro-intestinal hemorrhage had lower than normal plasma ascorbic acid levels (0.25 mg/100 ml).

Recent work by Aleo and Padh (1985) has shown that the endotoxin produced by *Escherichia coli* inhibits the uptake of (1-^{14}C)-L-ascorbic acid by mouse fibroblasts in tissue culture. This inhibition by endotoxin was found to take place only in the presence of unheated serum. Furthermore, polymyxin B, which is known to bind the lipid A portion of endotoxin, did not prevent the inhibition of ascorbic acid uptake caused by endotoxin, so the inhibition of ascorbate uptake would seem to be a function of the carbohydrate moiety of the toxin. This work may have very important implications as regards the clinical management of septic shock or endotoxin shock, which can still be lethal, even in patients treated with modern antibiotics.

II. ASCORBATE OXIDATION

Nyden (1948) measured both ascorbic acid (AA) and dehydroascorbic acid (DHAA) in rats. She observed a significant reduction in the AA content, associated with a decrease in

Table 1
DEHYDROASCORBIC ACID AND ASCORBIC ACID LEVELS OF BLOOD OF MEN IN HEALTH AND WITH TYPHOID FEVER

Group	No. of persons	Blood-ascorbic acid (mg per 100 ml)	Blood-dehydro-ascorbic acid (mg per 100 ml)
Normal persons	21	0.88 ± 0.03[a]	0.09 ± 0.01
Persons with typhoid fever	41	0.54 ± 0.06	0.27 ± 0.03
Persons convalescent from typhoid fever	11	0.72 ± 0.001	0.16 ± 0.003

[a] Mean and standard error.

From Banerjee, S. and Belavady, B. (1953), *Lancet*, 2, 912. With permission.

the AA/DHAA ratio in the liver, spleen, and adrenals of rats infected with *Trypanosoma hippicum*. However, there was at the same time a doubling of both AA and DHAA concentrations in the blood plasma. This rise in the plasma ascorbic acid level of infected rats is just the opposite of what one finds in humans or guinea pigs with infection. Since rats can synthesize ascorbic acid in the liver, it would seem that there was increased AA utilization by the tissues and increased release of synthesized ascorbic acid from the liver in these rats with trypanosomiasis; it is our misfortune that we cannot respond to infection in the same way.

Banerjee and Belavady (1953) estimated the ascorbic acid (AA) and DHAA levels in the blood of 41 patients with typhoid fever and of 21 normal healthy controls, using 2,6-dichloroindophenol titration before and after treatment with hydrogen sulfide and removal of excess gas. They found normal blood to contain 0.88 mg of AA and 0.09 mg of DHAA per 100 ml. The AA level of the blood of patients with typhoid fever was 0.54 and the DHAA level was 0.27 mg/100 ml, as shown in Table 1. Thus, not only was the ascorbic acid level of the blood decreased, but also the DHAA level was increased, so that about one third of the total ascorbic acid in the blood was in the dehydro form. In normal people, on the other hand, the DHAA constituted about $^1/_{11}$ of the total ascorbic acid of the blood. When patients were recovering from typhoid fever, the ascorbic acid level of the blood increased, and the DHAA level decreased, but they did not reach the normal values.

Ascorbic acid is normally present in the blood and tissues mostly in the reduced form, and there are only traces of DHAA, but it seems that in infections like typhoid fever the tissues have a lessened capacity to reduce DHAA formed in the body; thus, DHAA accumulates in the blood.

Chakrabarti and Banerjee (1955) also found that the blood levels of DHAA are increased and ascorbic acid is decreased in meningococcal meningitis, tetanus, acute lobar pneumonia, typhoid fever, and tuberculous meningitis, indicating an accelerated oxidation of ascorbic acid. They noted that the patients whose blood DHAA level was highest were those who most frequently died, as shown in Table 2.

III. CERULOPLASMIN AS AN ASCORBATE OXIDASE

One of the factors which may affect ascorbic acid metabolism in chronic infections is the increased blood copper level which is associated with many human disease states, including infection, as reported by Lahey et al. (1953) and Gubler (1956). More than 95% of this copper, both in health and in the presence of infection, is bound as part of the blue copper-protein ceruloplasmin, synthesized in the liver, which has eight atoms of copper per molecule

Table 2
DEHYDROASCORBIC ACID AND ASCORBIC ACID CONCENTRATIONS IN BLOOD (mg/100 ml)

Subjects	Dehydroascorbic acid	Ascorbic acid
Normal (28)[a]	.06 ± .01[b]	.87 ± .02
Meningococcal meningitis		
Acute cases who did not survive (8)	.95 ± 0.6	.27 ± .03
Acute cases who survived (17)	.61 ± .04	.43 ± .02
Convalescent cases (11)	.19 ± .02	.53 ± .01
Tetanus		
Acute cases who did not survive (13)	.73 ± .04	.36 ± .01
Acute cases who survived (12)	.41 ± .03	.52 ± .02
Convalescent cases	.15 ± .02	.74 ± .02
Lobar pneumonia		
Acute cases who did not survive (7)	.68 ± .04	.30 ± .02
Acute cases who survived (15)	.40 ± .02	.43 ± .01
Convalescent cases (13)	.16 ± .01	.59 ± .02
Typhoid fever		
Acute cases who did not survive (4)	.56 ± .07	.24 ± .01
Acute cases who survived (19)	.35 ± .02	.45 ± .02
Convalescent cases (15)	.15 ± .01	.68 ± .03
Tubercular meningitis — chronic (17)	.33 ± .07	.50 ± .03

[a] Figures in parentheses indicate number of subjects.
[b] Mean ± standard error of mean.

From Chakrabarti, B. and Banerjee, S. (1955), *Proc. Soc. Exp. Biol. Med.*, 88, 581. With permission.

and is an amine oxidase and a polyphenol oxidase (Gubler et al., 1953). Humoller et al. (1960), using physiological concentrations of ascorbic acid have shown that ceruloplasmin is an enzyme which is not only capable of catalyzing the oxidation of amines such as epinephrine or serotonin, but is also a true oxidase for ascorbic acid. They found that the ascorbic oxidase activity of plasma is dependent on four less firmly held copper atoms and is abolished when these are chelated with versene, but is not due to nonceruloplasmin copper since added inorganic copper is inactive in the presence of albumen. On the other hand, they found that the amine oxidase activity of ceruloplasmin is reduced 50% by versene and is therefore considered to be a property of both the four loosely bound and the four firmly bound atoms of the molecule.

The ascorbate oxidase activity of ceruloplasmin has been confirmed by Osaki et al. (1963, 1964) so it is possible that the ascorbic acid deficiency of infection may be related to the increase in plasma copper which averaged 40% per cent in 18 patients observed by Gubler (1956).

IV. LEUKOCYTE ASCORBATE UPTAKE AND RELEASE

Ascorbic acid is transported from place to place in the leukocytes, platelets, red cells, and plasma of the blood, mainly in the reduced (AA) form, as confirmed by Majumdar et al. (1964) in cats, but it is transported across cell membranes into and out of cells in the nonionic DHAA form, as shown by Lloyd (1950), Panteleeva (1950), and Lloyd and Sinclair (1953). Inside the erythrocytes DHAA is reduced to ascorbic acid, as shown by Lloyd and Parry (1954).

Mohanram and Srikantia (1967) have shown that leukocytes from different subjects differ widely in their ability to take up ascorbic acid *in vitro* when exposed to the vitamin: it may

Table 3
CHANGES IN LEUKOCYTE ASCORBIC ACID DURING THE COMMON COLD

	Leukocyte ascorbic acid/10^8 WBC								
Day	Subject 1	Subject 2	Subject 3	Subject 4	Subject 5	Subject 6	Subject 7	Mean	Standard deviation
Precold	24.5	18.6	—	16.8	—	20.0	—	20.0	3.3
1	10.5	9.7	10.3	—	10.1	10.4	10.8	10.3	0.3
2	8.4	8.1	11.9	15.3	6.8	—	—	10.1	3.5
3	14.6	11.7	14.4	16.3	13.4	14.8	17.1	14.6	1.8
4	21.5	15.9	—	17.1	—	19.7	20.9	19.0	2.4
5	28.1	—	—	—	—	14.7	28.0	23.6	7.7
10	26.6	22.1	20.2	17.1	23.8	21.3	37.2	24.0	6.5

From Hume, R. and Weyers, E. (1973), *Scott. Med. J.*, 18, 3. With permission.

be postulated that in any cell or tissue (group of cells) more permeable to DHAA than to AA, its ability to store AA will depend upon its ability to reduce DHAA to AA, thereby allowing more DHAA to diffuse into it. Conversely, any tissue where oxidation of AA predominates will fail to store AA.

Many workers have studied the ascorbate status of patients with infection, but few have had the opportunity to study human subjects before, during and after infection, for the simple reason that patients do not present themselves until they are already ill. However, Hume and Weyers (1973), when studying leukocyte ascorbic acid (TAA) levels during the common cold, were fortunate enough to have obtained and analyzed blood samples from four out of seven volunteers during the week before the onset of symptoms. The results are shown in Table 3 and Figure 5, where it is seen that the leukocyte ascorbic acid level fell from a normal value of 20.0 to a low level of 10.3 $\mu g/10^8$ WBC on the first day of symptoms; it rose again to normal as the symptoms subsided. In subsequent studies of high-dose vitamin C, four colds were experienced by three individuals who took 1 g of vitamin C a day throughout the winter months and 6 g/d for 3 d at the onset of the symptoms of a cold. Even so, the leukocyte ascorbic acid level fell, as shown in Figure 6, being lowest on the day 3 instead of day 1 but the lowest recorded levels were still within the normal range.

The mechanism by which infection causes such profound effects on ascorbic acid metabolism is not known, but some possibilities spring to mind. One is the transfer of ascorbic acid to the infected area by leukocytes, where they migrate through the wall of the capillary by diapedesis, give up their ascorbate load by osmosis, engulf bacteria and other debris by phagocytosis, and return via the lymph vessels to rejoin the circulating blood as ascorbate-deficient leukocytes.

Lewin (1976) believes that chemotaxis plays a major role in directing ascorbate transport by leukocytes; he believes that a compound of histamine and ascorbic acid, histaminium ascorbate, is formed in the diseased or damaged area.

Chatterjee et al. (1975a) have shown that autoxidation of ascorbic acid in the presence of histamine results in histamine breakdown. Since oxidized ascorbic acid (DHAA) is a very unstable compound with a short half-life, it is rapidly hydrolyzed and destroyed unless it is rapidly reduced back to ascorbic acid (AA) by sulfhydryls.

Healthy cells have a negative surface charge or zeta potential, due to acidic surface groups, a negative transmembrane potential, due to their high intracellular K^+, and a reducing capacity toward ascorbic acid due to reduced glutathione and other sulfhydryl groups, both inside and on their outer surface. However, damaged cells have a positive injury potential, no transmembrane potential, and they release oxidants (like oxyhemoglobin) into the region. We may conjecture that the positive injury potential attracts the negatively charged leuko-

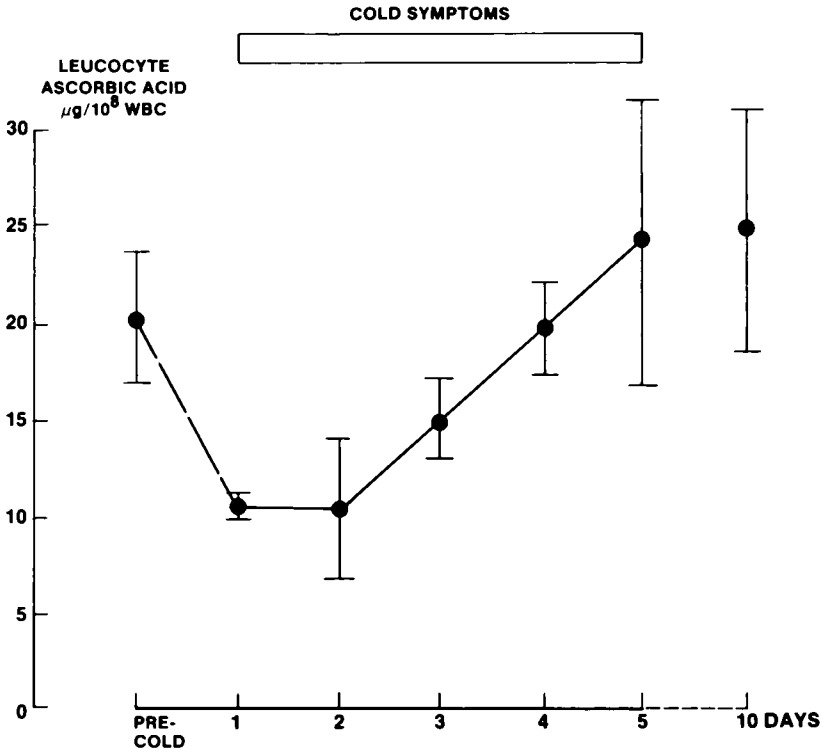

FIGURE 5. Mean leukocyte total ascorbic acid (TAA) levels in μg/10^8 white blood cells ± SD of four subjects, before and after a cold, and in three more during and after a cold. For full data, see Table 3. (From Hume, R. and Weyers, E. [1973], *Scott. Med. J.*, 18, 3. With permission.)

cytes, laden with ascorbic acid, into the area, where they release ascorbic acid, which tends to restore the normal redox potential, but is itself destroyed in the process. Indeed, in many ways it seems that inflammation may resemble a form of local scurvy.

V. LEUKOCYTE ASCORBIC ACID DILUTION

Loh et al. (1974) have observed that a rise in the total white cell count is normally associated with a fall in the luekocyte ascorbic acid concentration and vice versa, both in men and in women. So clearly the leukocytosis of infection may lead to a reduction of the leukocyte ascorbic acid level simply as a dilutional effect. But this dilutional effect is only one of the causes of the reduction in the leukocyte ascorbate level, for it occurs even in the presence of abundant ascorbic acid supplies.

Stankova et al. (1975) observed that the total ascorbate content of human polymorphonuclear leukocytes was reduced by 12% during phagocytosis. The AA content of the leukocytes fell from 54.9 to 40.0 and the DHAA content rose from 23.2 to 28.6 nmol/10^8 cells during phagocytosis of latex particles.

MacLennan and Hamilton (1977), studying elderly men and women admitted to a geriatric unit, categorized them as being in stable or unstable condition, according to whether they had only a chronic illness or an acute illness such as bronchopneumonia in addition to their chronic disease. They found that the unstable group had significantly lower leukocyte ascorbic

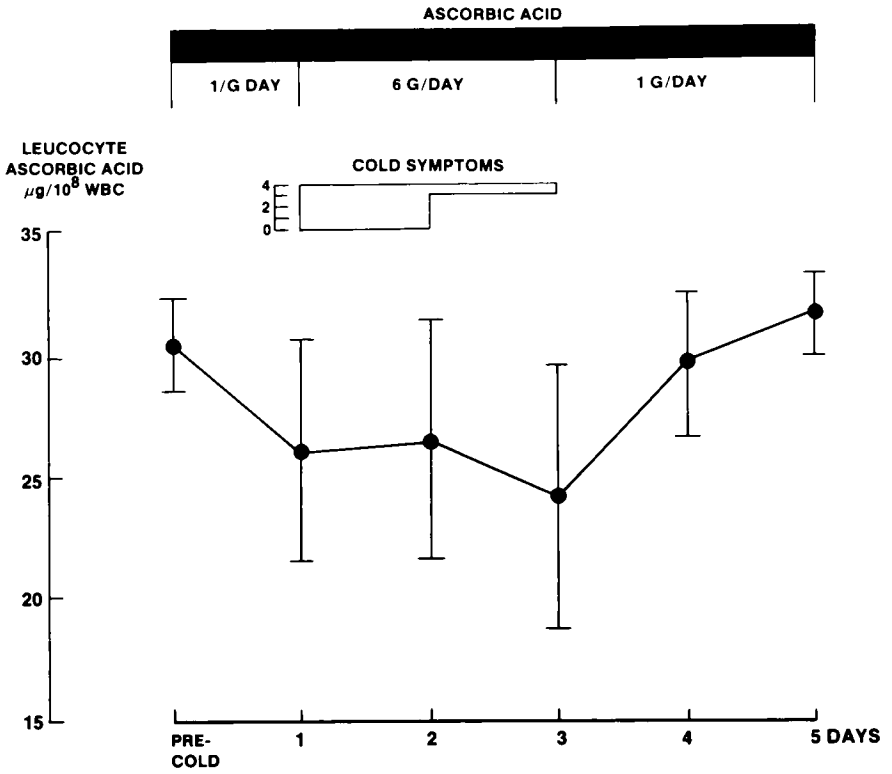

FIGURE 6. Changes in leukocyte ascorbic acid during the common cold while ingesting supplements of ascorbic acid. Means and one SD. (From Hume, R. and Weyers, E. [1973], *Scott. Med. J.*, 18, 3. With permission.)

acid (TAA) levels than the stable group. Acute illness was often accompanied by first a rise, then a fall in leukocyte ascorbate levels, but plasma ascorbate levels did not change significantly. They noted that those with low leukocyte ascorbate levels had a bad prognosis, but took this to be a marker rather than a cause of their deterioration.

VI. INFECTION COMPOUNDING EFFECTS OF ASCORBATE DEPLETION

In this section we are concerned with the effects of infection on ascorbic acid metabolism; however, the fact that ascorbic acid deficiency markedly decreases resistance to infection, as described in Chapter 12 of Volume II, is clearly pertinent to this discussion. Worsening of the infection can further deplete ascorbate stores and, thus, compound the infection, as in a downward spiral vortex.

Parsons (1938) observed:

> Despite the long latent period, the symptoms of acute infantile scurvy appear suddenly and it is much more probable that some infection, perhaps only slight, is the real exciting factor — the onset of an infection fanning the smouldering embers of the latent disease into the blazing fire of frank scurvy . . . An interesting parallel to the sudden onset of the symptoms of acute scurvy is presented by their rapid cure when large doses of ascorbic acid are administered; the paralysed child crying with apprehension and pain will in 48 hours move its legs freely and allow them to be handled almost with impunity.

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Chapter 9

TRAUMA, SURGERY, AND BURNS

Studies by Uzbekov (1937) demonstrated decreased ascorbic acid levels in the adrenal cortex of guinea pigs following burns. Adrenal ascorbic acid depletion is now a well-recognized effect of any kind of stress, so we must look at the blood and other tissues if we wish to study the more general effects of trauma on ascorbic acid metabolism.

Lund (1939) studied the plasma ascorbic acid (AA)* levels of 43 patients before and after major surgery. He found that in those with normal preoperative levels, the vitamin level was reduced postoperatively by about 30 to 50%, but those with initial levels below 0.2 mg/100 ml were already so low that no further drop could be detected.

	Preoperative plasma ascorbic acid levels (mg/100 ml)		**Postoperative plasma ascorbic acid levels (mg/100 ml) third-day mean**
	Range	**Mean**	
n(9)	0.8 +	1.0	0.6
n(10)	0.5—0.8	0.6	0.4
n(11)	0.2—0.5	0.3	0.2
n(13)	0.0—0.2	0.1	0.1

Low blood ascorbic acid (AA) levels in surgical patients were also observed by Wolfer and Hoebel (1939); they stated that, "After operation normal patients may show a drop (of the blood ascorbic acid) to scurvy levels because of long periods of intravenous therapy without food by mouth, because of abnormal bowel physiology, and because of the increased utilization of vitamin C that accompanies infections and operative procedures." These workers recommended large doses of ascorbic acid (1 g daily for 10 d) in order to achieve tissue saturation for proper wound healing.

Bartlett et al. (1940) observed a definite fall in the vitamin C (AA) level of the blood plasma of most patients following major surgery, as shown in Figure 1. This fall was more marked in those who had a high initial level, and its degree was somewhat dependent on the extent of the surgical procedure. They did not observe any significant change in the pre- and postoperative urinary ascorbic acid excretion levels. These same workers did, however, observe that the plasma ascorbic acid clearance curves following an intravenous dose of 1000 mg of ascorbic acid often showed a marked change in contour following surgery. There was a lowering of the peak to which the blood level rose after the injection of vitamin C and a more rapid fall towards the starting level, such that the whole curve became flattened and lost the characteristic shape seen in preoperative and control curves. They concluded that the changes in vitamin C metabolism, which occur following surgery, are dependent on the extent of the procedure and represent increased destruction, utilization, or storage of vitamin C.

The following case history is taken from the work of Bartlett et al.

Case 2. — A male, age 23 was admitted to the hospital, for an obstructing duodenal ulcer. His physical condition was good except for a recent loss of five pounds in weight. Blood examination showed a red blood cell count of 4,350,000 with a hemoglobin of 80 per cent. His blood cevitamic acid level was low (0.07 mg per cent), and the pre-operative clearance curve was normal. A posterior gastro-enterostomy was performed under nitrous oxide-ether anesthesia. During the first 12 days after operation, ten doses of 1,000 mg of vitamin C were given intravenously.

* AA — ascorbic acid, reduced form.

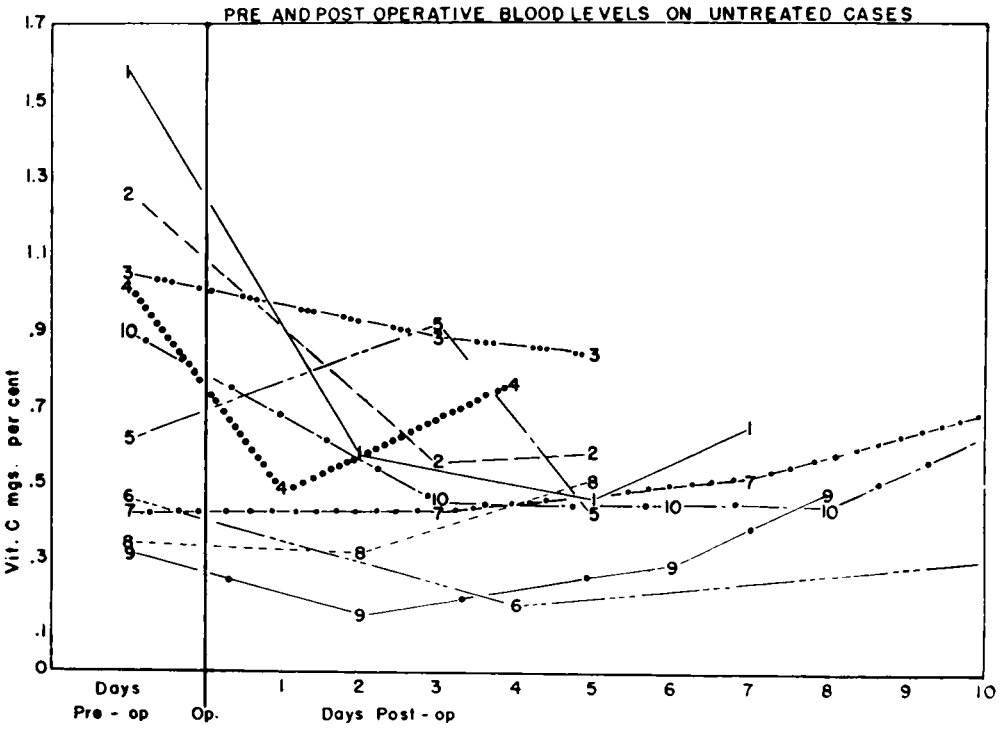

FIGURE 1. Blood vitamin C determinations on ten patients before and at various intervals after operation are shown. Most of them show a definite drop in the blood vitamin C level after operation. This seems more marked when the initial level is higher, and its degree seems to be somewhat dependent upon the extent of the surgical procedure. The operative procedures on these patients, numbered to correspond with the numbers on the graph are: (1) lobectomy, (2) incision and drainage of chronic empyema, (3) herniorrhaphy, (4) cholecystectomy, (5) herniorrhaphy, (6) abdominoperineal resection of rectum, (7) gastric resection, (8) posterior gastroenterostomy, (9) gastric resection, and (10) gastric resection. (From Bartlett, M. K., Jones, C. M., and Ryan, A. E. [1940], *Ann. Surg.*, 111, 1. With permission.)

The curves on the second and third days after operation show marked flattening, while that on the fifth day has returned essentially to normal (Figure 2).

Clark and Rossiter (1944) studied the effects of thermal injury by scalding anesthetized rabbits; they clipped the fur and then immersed the backs of the animals in water at 70°C for 30 s. Following this burning, there was a significant hyperglycemia lasting for a few hours and a fall in the liver glycogen content. They also noted a significant fall in the adrenal ascorbic acid concentration from 290 mg/100 g to 109 mg at 4 h and to 160 mg at 24 h, but no significant change in the liver ascorbic acid levels which were 120, 130, and 160 mg/100 g, respectively. The liver, by virtue of its mass is one of the main body stores of ascorbic acid, but is better studied in an animal which cannot synthesize ascorbic acid in response to need.

Bourne (1944) studied wound healing in guinea pigs on different ascorbic acid intakes and wrote as follows.

"It is apparent from the concentration of vitamin C in healing tissue that the more extensive the injury the more vitamin C will be required. It seems also since the tissue in greatest need of vitamin C appears to deplete other organs to obtain its requirements, that even if a wound heals fairly well, other parts of the body may become deficient in the vitamin, and this suggests that the routine administration of vitamin C to all injured persons would be desirable."

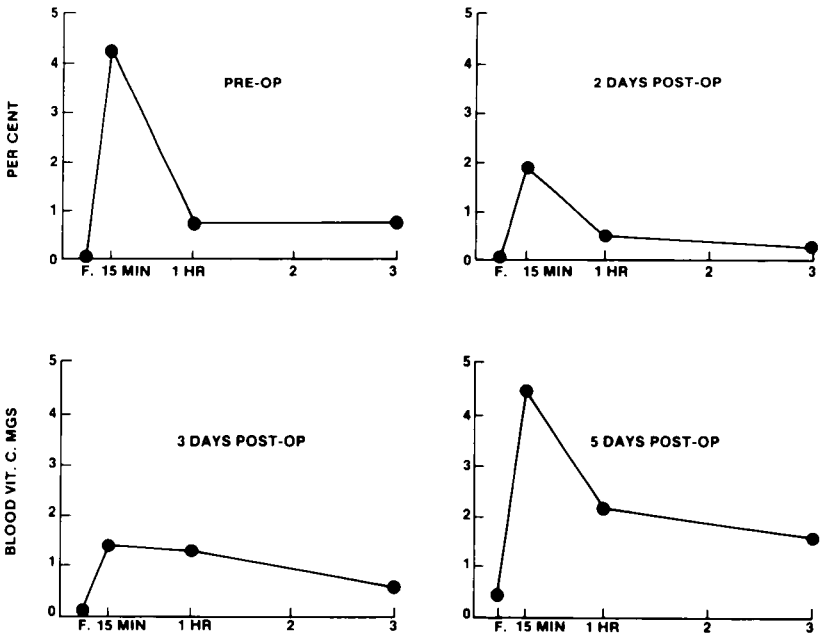

FIGURE 2. Case 2: the vitamin C clearance curves before operation and on the second, third and fifth postoperative days are shown. A marked change in contour occurs on the second and third postoperative day, but on the fifth day after operation the curve has returned essentially to normal. (From Bartlett, M. K., Jones, C. M., and Ryan, A. E. [1940], *Ann. Surg.*, 111, 1. With permission.)

The work of Levenson et al. (1946) revealed that five out of six patients with severe acute surgical conditions (including two gunshot wounds, two crush injuries, one hematemesis, and one perforated gastric ulcer) had plasma ascorbic acid (AA) levels which fell to zero at the time when they were most severely ill. They recommended giving large doses of ascorbic acid, thiamin, riboflavin and nicotinic acid to people who are acutely ill.

Andreae and Browne (1946) carried out careful ascorbic acid balance studies on 7 patients with burns, 14 patients with fractures, and 3 normal control subjects. They, too, found a profound disturbance of ascorbic acid metabolism, with increased ascorbic acid utilization soon after injury. Repeated blood analyses on three burn patients, on admission to hospital and again 20 h later, showed that their whole blood ascorbic acid levels had fallen from 0.30, 0.56, and 0.34 mg/100 ml to 0.08, 0.10, and 0.03 mg/100 ml, respectively. All were previously well-nourished individuals. These authors commented that in Crandon's (1940) dietary deficiency experiment, it took 82 d before the white cell ascorbic acid fell to 4.0 mg/100 g; they frequently encountered such low values as soon as 24 h after injury; most of their patients needed to receive 500 to 700 mg of ascorbic acid daily for 6 d before saturation was achieved, almost as though they had scurvy.

Lund et al. (1947) observed that patients with extensive deep burns have low plasma ascorbic acid (AA) levels which require large doses of ascorbic acid to rectify and maintain a normal level. They suggested as a result of their studies that 1 to 2 g of ascorbic acid, 10 to 20 mg each of thiamine and riboflavin, and 150 to 250 mg of nicotinic acid be given daily to severely burned patients and that these doses may be needed for long periods.

Levenson et al. (1950) reaffirmed the existence of abnormalities of vitamin C metabolism following acute trauma; they reported a fall in the plasma ascorbic acid concentration, a decrease in urinary ascorbic acid excretion, and a decreased response to "load tests".

Pronounced metabolic changes are known to follow trauma, surgery, burns, and scalds;

nitrogen balance studies have shown a catabolic phase following injury, which usually ceases after a week and is followed after a few weeks by anabolism during the recuperative phase, which may continue for 2 or 3 months; changes in calcium metabolism are very similar. It is true that increased urinary losses of both nitrogen and calcium may occur following bed rest alone, but the metabolic changes following injury are much greater and they are related to the severity of the trauma; they are also affected by the preexisting nutritional state of the individual.

Changes in ascorbic acid metabolism are no less marked. Beattie (1947) observed that burns seemed to have an especially profound effect on the time taken to achieve tissue saturation with ascorbic acid. All of his surgical patients received daily supplements of 500 mg of ascorbic acid from the day of operation or from the day after accidental injury for periods up to 25 d.

> During the first five days of administration no trace of ascorbic acid could be detected in the urine. In the second five-day period the operation cases were all excreting between 50 and 100 mg of the vitamin. The vitamin was not excreted in the cases of accidental injury. During the third five-day period the ascorbic acid output of the operation cases rose to between 176 and 234 mg a day. No vitamin was detected in the urine of the burned patients by the 15th day. They were given supplements of 500 mg daily for a further 10 days. On the 20th day the fracture patients were excreting more than 150 mg, but no ascorbic acid appeared in the cases of the burns. These showed traces of the vitamin on the 23rd day, and on the 25th day excreted less than 100 mg.

Levenson et al. (1957) studied the healing of laparotomy wounds in burned and unburned guinea pigs on restricted ascorbic acid intake regimens. They concluded that the seriously injured individual behaves biochemically and physiologically like a scorbutic.

Crandon et al. (1958), working in the Surgical Service of Tufts University at the Boston City Hospital, studied the ascorbic acid levels of acute surgical patients excluding those suffering from burns or severe trauma. Analyzing blood samples taken immediately before and immediately after surgery from 105 patients, they found an average fall of 17% in the plasma and 20% in the buffy coat ascorbic acid (TAA)* levels during surgery (Tables 1 and 2). The fall was greater in those who had high initial ascorbic acid levels and less in those with low initial ascorbic acid levels.They suggested that this observed reduction in the ascorbate levels might be partly due to dilution of the circulating blood with new plasma, white cells, and platelets less rich in ascorbic acid.

Crandon et al. (1961) confirmed their earlier finding of a fall in the buffy coat (leukocyte and platelet) ascorbic acid level during major surgery, but found no correlation between the degree of fall, the type and duration of operation, or the type of anesthesia. In ten patients they measured the ascorbic acid content of the rectus abdominis muscle and fascia at the beginning and at the end of surgery, but found no appreciable change. Moreover, they observed that intravenous administration of 100 mg of ascorbic acid during surgery caused a marked increase in the plasma ascorbate and a small increase in the buffy coat ascorbate, but no change in the ascorbic acid content of the wound. They suggested that to raise the tissue ascorbic acid level for surgery, the vitamin should be given for some time beforehand.

Radioisotope studies by von Schuching et al. (1960) and by Abt and von Schuching (1961) included measurement of total body, urine, and tissue radioactivity, as well as expired air $^{14}CO_2$, following the injection of L-ascorbic-1-^{14}C acid into guinea pigs. The tissues of scorbutic guinea pigs took up a greater percentage of radioactive ascorbic acid than did the tissues of guinea pigs on higher vitamin C diets. Immediately following the administration of labeled ascorbic acid a high rate of $^{14}CO_2$ expiration occurred, falling to a constant rate after approximately 48 h. However, the lowest conversion of ascorbic acid occurred in animals on a scorbutic diet, and higher conversions of ascorbic acid to $^{14}CO_2$ were observed in animals on diets containing increasing amounts of vitamin C.

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

Table 1
EFFECT OF OPERATION ON PLASMA ASCORBIC ACID

Data		Plasma ascorbic acid: Preoperative value (mg/100 ml)	Plasma ascorbic acid: Postoperative value (mg/100 ml)	Difference (mg/100 ml)		
All cases	n = 105					
Mean		0.428	0.356	−0.072	t = 4.93	$p < 0.01$
Standard error of mean		0.0356	0.0280	0.0146		(significant)
95% confidence limits		0.36—0.50 ± 0.07	0.30—0.41 ± 0.055	−0.10—−0.04		
"Low" group (plasma <0.2, buffy coat <8)	n = 22					
Mean		0.110	0.091	−0.019	t = 1.46	$p > 0.05$
Standard error of mean		0.0107	0.0116	0.0130		(not significant)
95% confidence limits		0.09—0.13 ± 0.02	0.07—0.12 ± 0.025	−0.05—+0.01		
"High" group (plasma >0.2, buffy coat >8)	n = 83					
Mean		0.512	0.426	−0.086	t = 4.80	$p < 0.01$
Standard error of mean		0.0402	0.0310	0.0179		(significant)
95% confidence limits		0.43—0.59 ± 0.08	0.37—0.49 ± 0.06	−0.12—−0.05		

Note: Blood samples taken immediately before and immediately after surgery showed a 17% fall in the mean plasma total ascorbic acid level during surgery.

From Crandon, J. H., Landau, B., Mikal, S., Belmanno, J., Jefferson, M., and Mahoney, N. (1958), *N. Engl. J. Med.*, 258, 105. With permission.

Table 2
EFFECT OF OPERATION ON BUFFY COAT ASCORBIC ACID

Data		Buffy coat ascorbic acid: Preoperative value (mg/100 g)	Buffy coat ascorbic acid: Postoperative value (mg/100 g)	Buffy coat ascorbic acid: Difference (mg/100 g)		
All cases	n = 105					
Mean		16.34	13.04	−3.30	t = 5.48	$p < 0.01$
Standard error of mean		1.034	0.764	0.602		(significant)
95% confidence limits		14.29—18.39	11.53—14.55	−4.49—−2.11		
		± 2.05	± 1.50			
"Low" group (plasma <0, 2, buffy coat <8)	n = 22					
Mean		5.99	4.41	−1.58	t = 2.12	$p < 0.01$
Standard error of mean		0.851	0.637	0.744		(significant)
95% confidence limits		4.22—7.76	3.09—5.73	−3.13—+0.03		
"High" group (plasma >0.2, buffy coat >8)	n = 83					
Mean		19.08	15.32	−3.76	t = 5.16	$p < 0.01$
Standard error of mean		1.108	0.778	0.729		(significant)
95% confidence limits		16.89—21.27	13.78—16.86	−5.20—−2.32		

Note: Blood samples taken immediately before and immediately after surgery showed a 20% fall in the mean "buffy coat" (leukocyte and platelet) total ascorbic acid level during surgery.

From Crandon, J. H., Landau, B., Mikal, S., Belmanno, J., Jefferson, M., and Mahoney, N. (1958), *N. Engl. J. Med.*, 258, 105. With permission.

Injection of labeled ascorbic acid into guinea pigs on scorbutic, maintenance, and high-ascorbic acid diets, before and after surgery, revealed that the wounded animals had a lower $^{14}CO_2$ excretion in the early postoperative period than in the corresponding preoperative period (Figure 3). This diminution in the amount of ascorbate-derived CO_2 in the expired air during the early excretory period in the operated animals coincided with, and was almost certainly due to, the increased deposition of ascorbic acid in the connective tissue of the healing wound, as observed in earlier studies by Abt et al. (1959b, 1960). They state that the guinea pig, regardless of diet, reacts to wounding with the same basic pattern; namely, an increased deposition of ascorbic acid in the scar tissue, plus a slight increase in urinary excretion of the vitamin.

Coon (1962) studied the whole blood and buffy coat ascorbic acid levels of 130 patients undergoing major abdominal surgery and observed the characteristic drop in the blood levels during the first several postoperative days. From studies of six groups of patients receiving 0, 75, 100, 150, 200, or 300 mg of ascorbic acid by subcutaneous injection, he concluded that 200 mg of ascorbic acid must be given daily by parenteral injection to achieve blood levels of 0.4 mg/100 ml or above in 95% of individuals.

Shukla (1969) studied plasma and urinary ascorbic acid levels following abdominal surgery and found that even though 500 mg of vitamin C was administered daily to these patients, there was a progressive fall in the plasma concentration in the early period of wound healing.

Studies of human skin obtained at autopsy from burned patients by Barton et al. (1972) showed that ascorbic acid becomes concentrated in a burned area of skin. They found mean ascorbic acid (TAA) levels of 8.1 mg/100 g in the skin of control cadavers, while the mean TAA levels of skin from burn victims were 10.8, 19.4, and 30.5 mg/100 g in the intact skin, the edge of the burn, and the burned skin areas, respectively. These authors found no fall in the leukocyte ascorbic acid levels of burn victims after admission to hospital, but of course they had no knowledge of the leukocyte ascorbic acid levels of their patients before they were burned. They did find a reduction in the leukocyte ascorbic acid (TAA) levels of some patients after skin grafting, suggesting that ascorbic acid may be used up during grafting.

McGin and Hamilton (1976) studied the ascorbic acid content of blood from 11 healthy volunteers, while in storage under "blood bank" conditions at 4°C for 21 to 28 d; the mean leukocyte ascorbic acid (TAA) level fell from 23.7 to 13.5 $\mu g/10^8$ cells after 7 days, but there was little if any change in the plasma ascorbic acid (TAA) level, the mean value of which fell from 1.20 to 0.98 mg/100 ml after 28 d. They also studied the plasma and leukocyte total ascorbic acid levels before, during, and after surgery in five patients undergoing elective operations for benign peptic ulceration and in four patients undergoing emergency surgery for bleeding peptic ulcers after receiving an average of 10 blood transfusions each. There was a fall in the leukocyte and plasma ascorbic acid level in both groups of patients. This fall had returned to normal by 7 days in the controls, but the leukocyte ascorbic acid level remained deficient in those who had received the massive blood transfusions (Figure 4); they concluded that, "deficient ascorbic acid in stored blood may contribute to low leucocyte ascorbic acid levels in patients after blood transfusion and may contribute to the complication rate when surgery is undertaken in these patients."

Irvin et al. (1978) studied the leukocyte ascorbic acid levels before and after surgery in patients grouped according to the severity of the surgical trauma. The leukocyte ascorbic acid concentrations were significantly reduced after surgery, but the reductions did not seem to be related to the severity of the trauma of the operation. They did, however, find a correlation between the reduction of the leukocyte ascorbic acid level and the white cell increase. They concluded that the postoperative leukocytosis due to surgical trauma involves a release by the bone marrow of leukocytes with a low ascorbic acid content, and that this could account for part of the 42% reduction in leukocyte ascorbic acid concentration which they observed on the 3rd d after surgery.

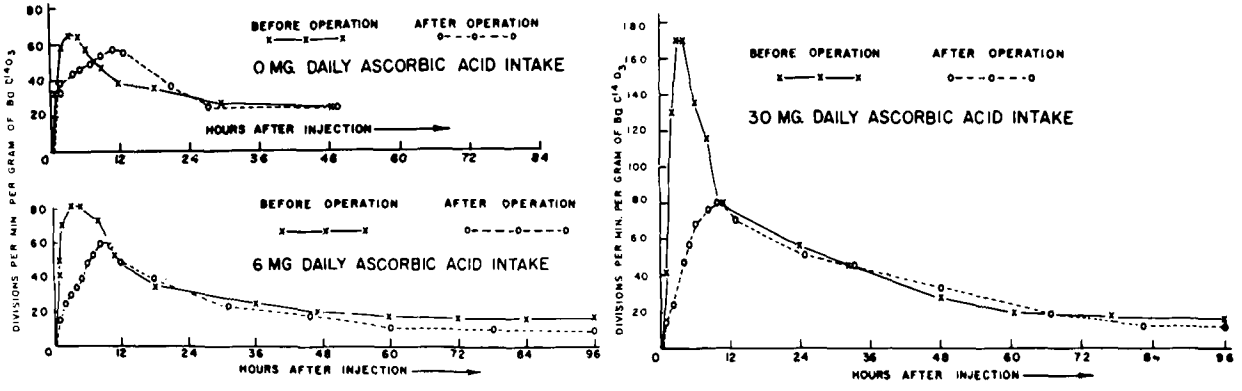

FIGURE 3. Comparison of hourly specific activities of expired $^{14}CO_2$, following the injection of a standard dose of L-ascorbic-1-^{14}C acid, before and after surgery, of guinea-pigs receiving 0, 6, and 30 mg daily ascorbic acid intakes. The wounded animals showed a lower $^{14}CO_2$ excretion in the early postoperative than in the corresponding preoperative excretion period. (From von Schuching, S., Enns, T., and Abt, A. F. [1960], *Am. J. Physiol.*, 199, 423. With permission.)

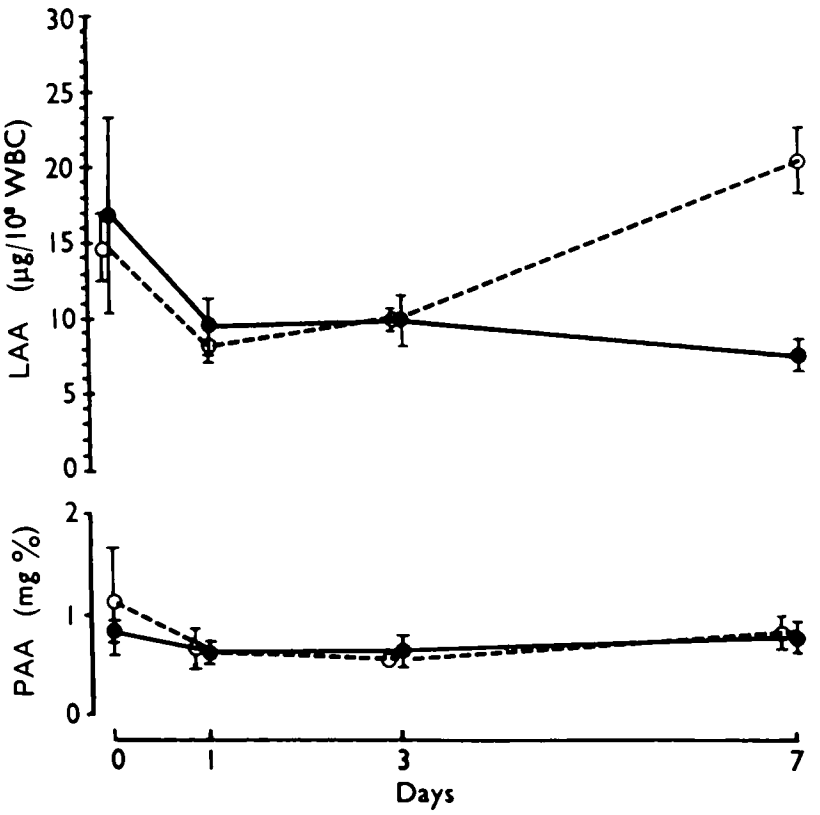

FIGURE 4. Ascorbic acid levels in leukocytes and plasma of patients before and after peptic ulcer surgery; —, after blood transfusion; ----, no blood transfusion. (From McGin, F. P. and Hamilton, J. C. [1976], *Br. J. Surg.*, 63, 505. ©Butterworth & Co., Ltd. With permission.)

Vallance (1979) reported that granulocytes contain about half as much ascorbic acid as lymphocytes, and that the platelet ascorbic acid concentration falls in between the two. He suggests that the inverse correlation between the leukocyte ascorbic acid concentration and the leukocyte count, observed by Irvin et al. following surgery, may be due to the increasing proportion of granulocytes, and that a true fall of leukocyte ascorbic acid does not occur. However, in studies of ascorbic acid levels following myocardial infarction, Vallance conceded that when the acute "stress" has subsided and the leukocyte count has returned to normal, the leukocyte ascorbic acid concentrations and the serum ascorbic acid concentration remain low, presumably because of tissue depletion of ascorbic acid; he found that both may remain low for up to 56 d (Vallance et al., 1978).

Irvin (1981, 1982) confirmed the existence of a marked reduction in the leukocyte ascorbic acid concentration of patients on the first day after surgery and expressed the belief that there is a real reduction of blood ascorbic acid levels after surgery. In ten patients undergoing major surgical procedures, the mean white blood cell ascorbic acid content per liter of blood on the third postoperative day was reduced to 42% of what it had been before surgery.

Mukherjee et al. (1982) observed a precipitous fall in plasma ascorbic acid levels, accompanied by a significant rise in blood dehydroascorbate, following major trauma like severe head injury, burns, or lacerations. However, this change was temporary and normal vitamin C status was regained after recovery. A similar change in plasma ascorbate was also found following major surgery. The change was not due to lack of reduction of de-

hydroascorbic acid to ascorbic acid, but to a high turnover of ascorbic acid in trauma. Supplements of ascorbic acid in trauma resulted in a temporary increase in plasma ascorbic acid.

Moore and Jones (1983), working at the Denver General Hospital, devised a method for the assessment of the severity of abdominal trauma, including blunt injuries, knife wounds, and gunshot wounds, in order to identify the high-risk patients. In a prospective randomized study, they divided the high-risk trauma patients into an enteral-fed group and a control group. The enteral group had a needle catheter jejunostomy placed at the time of laparotomy and received Vivonex® HN (Norwich-Eaton Pharmaceutical) first at 50 ml/h and gradually increasing, each 8 h, to 125 ml/h, through this catheter during the first five postoperative days, while the controls received an intravenous drip of 5% dextrose in water, for the first 5 d, followed by parenteral nutrition by central vein, if not tolerating an oral diet by then. Their preliminary results suggest that the severely injured patient receiving early nutritional support has a lower incidence of septic complications. Septic complications occurred in 29% (4/14) of the control group and in none (0/12) of the enteral-fed group who received complete nutrition, including 100 mg of ascorbic acid a day, from the time of their initial surgery.

Presumably the presence of all the essential amino acids and other vitamins in this commercially prepared diet may have helped to increase the availability of the ascorbic acid, as both proteins and amino acids act as indirect antioxidants or preservatives for ascorbic acid by chelating heavy metal catalysts. Also the sulphydryl amino acids act as reducing agents (Chapter 12 of this volume). Nevertheless, earlier studies have suggested that the ascorbic acid need of the severely injured patient is more likely to be about 1 g/d.

Stanya et al. (1984) reported enhanced body weight recovery in the first week after wounding in guinea pigs receiving higher dose ascorbic acid supplements ($p < 0.005$), even though no such growth effect had been evident in these mature guinea pigs before the surgical trauma. Guinea pigs receiving 0.5 and 5.0 mg of ascorbic acid a day lost weight following surgery, while those receiving 50 and 100 mg/d gained weight. The weight losses suggest a disinclination to eat following surgery, as the wound was a trephine bur hole in the anterior maxilla. We may conjecture that improved wound healing or decreased inflammatory pain and swelling in the animals receiving the higher dose ascorbic acid supplements allowed them to eat sooner and more freely following surgery.

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Chapter 10

HEAVY METALS, WATER SUPPLIES: COPPER, IRON, MANGANESE, MERCURY, AND COBALT

I. *IN VITRO* — CATALYTIC

As long ago as 1922, Hess demonstrated that milk pasteurized in glass containers was a more effective antiscorbutic agent than milk submitted to the same treatment in copper vessels; he suggested the possibility that the vitamin might be destroyed by the catalytic action of metals. In 1933 Euler discovered that pure ascorbic acid took up oxygen more readily in the presence of traces of iron or copper, and especially the latter. Mawson (1935) studied the prooxidant effects of copper and iron and the antioxidant effects of liver extracts and of glutathione, using indophenol titration to estimate residual ascorbic acid (AA)*. He found that copper would inhibit the protective effects of liver or glutathione at lower concentrations than iron, but that a combination of copper and iron had a greater catalytic effect than either alone. It seems that copper potentiates the oxidative effect of iron. Tin had no effect. London tap water was found to be very effective in destroying ascorbic acid *in vitro*. Kellie and Zilva (1936) made a thorough study of this matter; they confirmed the catalytic action of copper and iron and showed that the heavy metals present in distilled water from a tin-lined copper still were enough to cause the complete disappearance of 15 mg of ascorbic acid from 40 ml of such water in a 100-ml flask in 24 h at 37°C, at pH 7.4. The rate of disappearance of ascorbic acid (AA by indophenol titration) in tap water, or in laboratory distilled water, was fairly rapid, but the loss by oxidation in glass-distilled or quartz-distilled water was very much slower. Only by distilling the water three times in glass and then using it immediately could the low rate of oxidation be observed. When oxygen was bubbled for 1 h through this water, the rate of oxidation of the vitamin was not significantly elevated. These results suggested the presence of traces of metal catalysts, and this was subsequently proven to be correct. They found that copper catalyzes the oxidation of ascorbic acid and that chloride ions slow this copper-catalyzed oxidation. Blood plasma, intact red blood cells, and also animal tissues were found to slow the oxidation of ascorbic acid, but hemolysed red cells were found to cause a rapid loss of ascorbic acid, which will be discussed further in Chapter 15 of this volume.

Fresh milk	20.1 mg
After 3 days at a temperature of 20°C	11.3 mg
After pasteurization at 62—63°C for 30 min	11.0 mg
After pasteurization if copper is present	1.7 mg

Sharp (1936) gave the following values for ascorbic acid per liter of cow's milk:

Heinemann (1941) observed that contact with mercury causes significant destruction of ascorbic acid.

Mystkowski (1942) studied the copper-catalyzed oxidation of ascorbic acid and found that it was inhibited by the presence of proteins and amino acids or by chloride ions. NaCl decreased the activation caused by $CuSO_4$; the decrease was proportional to the concentration of NaCl and inversely proportional to the concentration of $CuSO_4$. Activation by ferric salts alone was quite insignificant and never exceeded 10%; it was completely inhibited by NaCl. Weissberger et al. (1943) reported that the copper-catalyzed oxidation of ascorbic acid was proportional to the oxygen concentration. Weissberger and Lu Valle (1944) concluded that

* AA— ascorbic acid, reduced form.

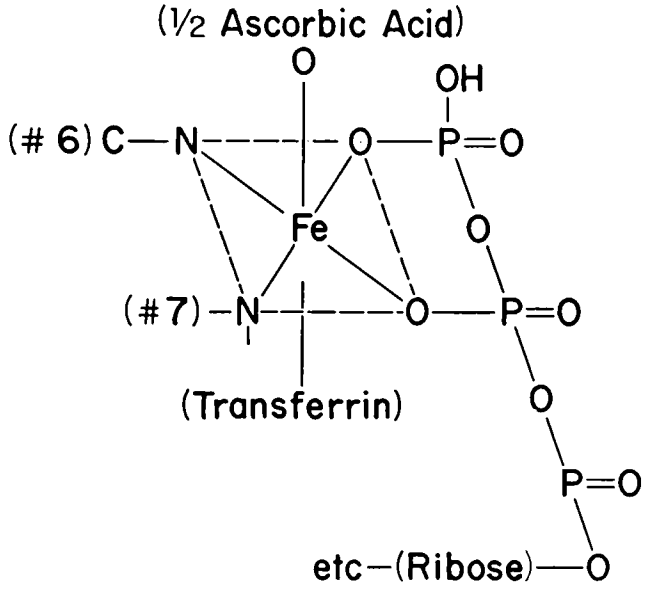

FIGURE 1. Complex of ascorbic acid, ferric iron, and ATP postulated by Mazur et al. (1960) as an essential intermediary in the transfer of plasma iron of transferrin to liver ferritin. Fe^{+++} is shown in the form of a hexadentate structure involving two oxygen atoms of the pyrophosphate linkages of ATP, the N atom attached to carbon atom 6 and N7 of the adenine rings, one of the oxygen atoms of ascorbic acid which is capable of electron transfer, and finally, the unknown linkage of the iron atom with transferrin. (From Mazur, A., Green, S., and Carleton, A. [1960], *J. Biol. Chem.*, 235, 595. With permission.)

in the presence of copper 1 mol of dehydroascorbic acid (DHAA) and 1 mol of hydrogen peroxide is formed from 1 mol of ascorbic acid and oxygen.

Scott (1966) reported catalytic oxidation of ascorbic acid by cupric and mercuric ions, but only very slowly by plumbous ions. He stated that magnesium, calcium, and ferrous ions had no effect, but plumbic, zinc, and cadmium ions release cupric ions from chelation by EDTA (and presumably from proteins and amino acids, etc.). Also, many divalent ions such as cadmium, zinc, nickel, copper, manganese, and cobalt will replace mercury, and the oxidation of ascorbic acid is related stoichiometrically to the amount of mercuric ion released.

Hatano and Kato (1970) made a detailed study of the action of the copper ion on ascorbic acid in the presence of chloride ions.

II. *IN VITRO* — ENZYMATIC

Mazur et al. (1960) found that Cu^{++} and Co^{++} catalyze the oxidation of ascorbic acid, but do not take the place of Fe^{+++} in the oxidation of ascorbic acid by rat liver slices; also that adenine triphosphate (ATP) and ascorbic acid are essential for the incorporation of iron into ferritin in the liver. They assumed that the function of the ascorbic acid is to act as a reducing agent for the ferric iron of transferrin and that ascorbic acid is oxidized in the process. They postulate the formation of a complex involving 2 mol of ATP, 1 mol of ascorbic acid, and the iron-transferrin protein of plasma, as shown in Figure 1.

Table 1
THE SIGNIFICANT REDUCTION IN LIVER ASCORBIC ACID OF GUINEA PIGS OVERLOADED WITH IRON BY IRON-DEXTRAN INJECTIONS 26 WEEKS PREVIOUSLY[a]

Means and standard deviations	Control guinea pigs	Iron-overloaded guinea pigs	Statistical significance *(p)*
Liver iron concentration μg/g wet weight	308 ± 156	5111 ± 1535	<0.001
Liver ascorbic acid concentration mg/100 g	29.4 ± 3.1	0.7 ± 0.98	<0.001

[a] Data from Wapnick et al. (1971).

III. *IN VIVO* — ANIMAL

King (1953) reported the oxidation of ascorbic acid in plant and animal cells by Mn^{+++} and Fe^{+++} as well as by copper proteins.

Szoke et al. (1963) found that oral administration of copper (as cupric sulfate) reduced the ascorbic acid content of the liver and adrenals of guinea pigs.

Wapnick et al. (1971) studied guinea pigs which they had overloaded with iron by injection of iron-dextran (Imferon®) and control animals which they had injected with dextran alone. When the animals were killed 6 months later, the mean liver ascorbic acid level of the iron-overloaded animals was less than 3% of that in the control injected group, and this difference was highly significant (Table 1). These results are analogous to the clinical condition of Bantu with hemosiderosis and scurvy due to excessive consumption of iron-rich beer.

It was therefore surprising when Smith and Bidlack (1980) found increased blood and liver ascorbic acid levels in guinea pigs receiving high dietary iron supplements in their drinking water. However, the explanation of this paradox may be that both test and control groups of guinea pigs were receiving 500 μg of copper a day in their food. The iron caused a highly significant displacement of copper from the livers of the iron-supplemented animals. The liver iron was increased threefold and the liver copper level was reduced to one third as a result of the iron supplement (Table 2). It would seem to the writer that liver copper is a more potent oxidant for ascorbic acid than liver iron, so the exchange was beneficial for ascorbic acid storage. Another difference between the experiments of Wapnick et al. and of Smith and Bidlack is that the total liver iron, of the iron-overloaded group, was ten times as high in the former experiment (c.f. Tables 1 and 2).

Chatterjee et al. (1975) reported that the synthesis of L-ascorbic acid from L-gulonolactone in the liver of the rat was stimulated by Cr^{3+}, W^{6+}, Mn^{2+}, and Co^{2+}, but was significantly reduced by dietary excesses of Zn^{2+}, Cu^{2+}, and Cd^{2+}; however, this can be of no concern in human metabolism unless some human beings are proven to retain some slight ability to synthesize ascorbic acid.

IV. *IN VIVO* — HUMAN

O'Shea (1918) knew that the administration of mercury was highly injurious to a patient with scurvy.

Farmer (1940) noted that patients receiving ferrous sulfate tablets in doses of 6 gr or more daily developed a sharp drop in the plasma ascorbic acid level and a rise in the hemoglobin level.

Fox (1936) and Bernstein and Weiner (1937) observed vitamin C deficiency among mine workers in South Africa; this was confirmed by Fox (1940) and by Fox and Dangerfield

Table 2
BLOOD ASCORBIC ACID CONCENTRATION IN GUINEA PIGS RECEIVING IRON SUPPLEMENT IN THEIR DRINKING WATER[a]

Means ± S.E.M.	(n)	Control guinea pigs	(n)	Iron-supplemented guinea pigs	Statistical significance of difference (*p*)
Whole blood iron mg/100 ml	6	45.6 ± 2.5	6	40.8 ± 1.9	
Whole blood copper μg/100 ml	6	302 ± 24	6	294 ± 30	
Liver total iron μg/g	6	158 ± 16	6	511 ± 50	↑ <0.005
Liver total copper μg/g	6	20.5 ± 3.3	6	7.0 ± 0.9	↓ <0.005
Blood total ascorbic acid mg/100 ml	6	0.18 ± 0.022	6	0.46 ± 0.050	↑ <0.005
Liver total ascorbic acid mg/100 g	6	19.8 ± 2.1	6	24.3 ± 2.2	

Note: This paradoxical result may have been due to the fact that both groups of guinea pigs were receiving 500 μg of copper per guinea pig per day in their diet. The iron supplement caused a threefold increase in the total liver iron and a highly significant decrease, to one third, in total liver copper. Liver copper seems to destroy ascorbic acid more rapidly than liver iron.

[a] Data from Smith and Bidlack (1980).

(1940) who observed a scurvy season from November to April. Grusin and Kincaid-Smith (1954) reported on 30 cases of scurvy among urban Bantu; there were 2 patients with chronic changes in the skin and subcutaneous tissue of the legs, such that the skin was tightly attached to the underlying tissues; there were the usual bleeding hypertrophic gums of scurvy and large subcutaneous hemorrhages. There were some cases of isolated hemorrhage from the bowel, kidneys, and serous cavities, and they also described severe hemosiderosis (excess iron storage) in the liver, spleen, and duodenum at autopsy in the one patient who died.

Further descriptions of osteoporosis and scurvy among the Bantu by Grusin (1956) and by Grusin and Samuel (1957) led on to a paper by Schulz and Swanepoel (1962) in which the connection between hemosiderosis and scurvy in the Bantu was fully recognized. The latter authors found one patient who could not be saturated with vitamin C either by oral or by parenteral administration of ascorbic acid. They postulated that excessive iron stores in the duodenum and the jejunum were destroying much of the ascorbic acid before it could be absorbed and that excessive liver iron stores were destroying that which was administered parenterally. They quoted Breslow and Lukens (1960) who, using a nonenzymatic hydroxylating system, had given their reasons for believing that ferric iron oxidizes ascorbic acid to DHAA. No doubt this unstable substance is then hydrolyzed to diketogulonic acid with loss of vitamin activity and further degraded, as neither ascorbic acid nor diketogulonic acid can be recovered from the urine in any quantity after administration of large quantities of ascorbic acid to these patients.

Further papers on this combination of osteoporosis, scurvy, pseudoscleroderma of the legs, and siderosis in the Bantu by Smith and Swanepoel (1962), Schulz and Swanepoel (1962), Seftel et al. (1964), Seftel et al. (1966), and by Lynch et al. (1967a, b) have led to the conclusion that there is accelerated oxidative catabolism of ascorbic acid in siderotic Bantu (Figure 2). Further studies by Wapnick et al. (1968), Lategan (1971), Charlton and Bothwell (1971), Robins (1972), Hankes et al. (1974), and Charlton and Bothwell (1976) have concluded that hemosiderosis, which is so common in Bantu-speaking South African blacks, is due to excessive and prolonged intake of home-brewed alcoholic beverages with a high iron content, and that this causes the ascorbic acid deficiency with all of its attendant problems.

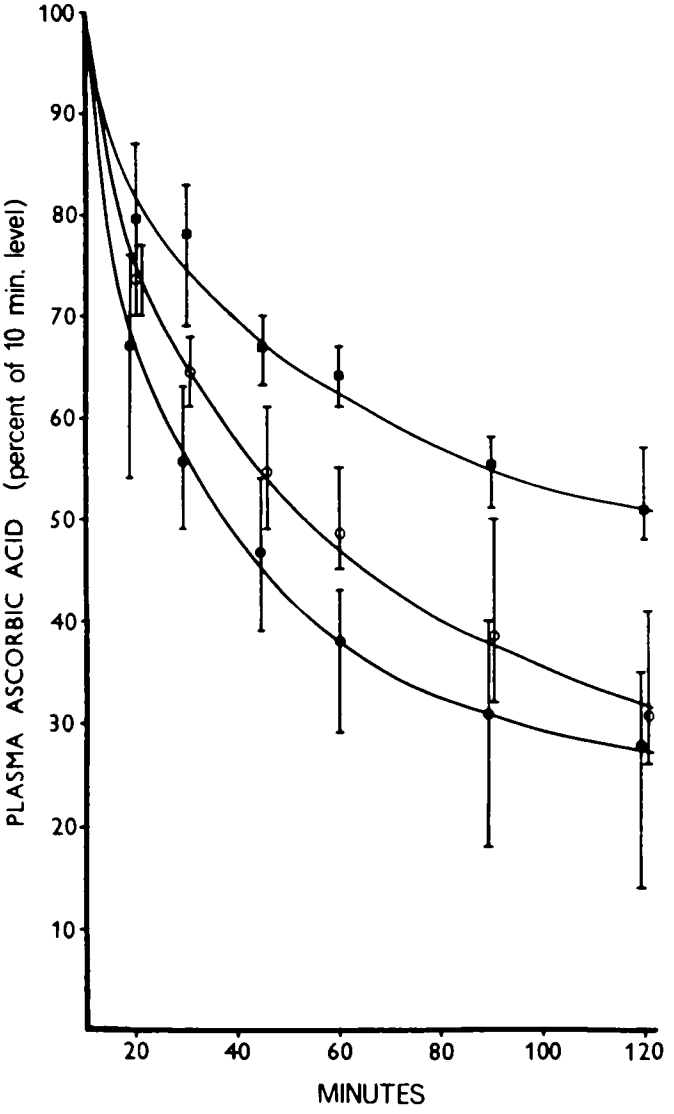

FIGURE 2. The clearance of ascorbic acid (mean ± range) from the plasma following the administration of a single intravenous injection of ascorbic acid in a dose of 15 mg/kg body weight. The concentrations are expressed as percentages of the 10-min figure. Closed squares (upper curve) — normal subjects, closed circles (lower curve) — siderotic subjects, open circles (middle curve) — siderotic subjects after a 3-week course of ascorbic acid (total 10 g). (From Lynch, S. R., Seftel, H. C., Torrance, J. D., Charlton, R. W., and Bothwell, T. H. [1967b], *Am. J. Clin. Nutr.*, 20, 641. American Society for Clinical Nutrition. With permission.)

In an interesting article, Murray et al. (1978) reported on the treatment of iron deficiency anemia among the milk-drinking Somali nomads at refugee camps in the Ogaden region of Ethiopia. Contrary to expectation, they found that treatment of the anemia using ferrous sulfate tablets (900 mg daily for 30 d) led to a highly significant increase in the incidence of infection, including malaria, tuberculosis, and brucellosis in the treated group. There were 36 episodes of infection among the 71 nomads treated for anemia and only 7 episodes of infection among 66 anemic controls who received placebo tablets ($p < 0.001$). The mean

Table 3
LEUKOCYTE ASCORBIC ACID CONCENTRATIONS IN MALE AND FEMALE GERIATRIC PATIENTS DURING DAILY ADMINISTRATION OF 105 mg OF IRON OVER A 14-WEEK PERIOD[a]

	Week of supplement	M	F
Initial leukocyte ascorbic acid concentration in $\mu g/10^8$ cells	0	26 ± 17	24 ± 9
Percentages of original leukocyte ascorbic acid concentration	1	104	97
	2	102	121
	4	91	95
	6	91	62
	8	87	84
	10	92	89
	14	84	133

Note: Daily administration of iron alone to old men resulted in a uniform fall in their leukocyte ascorbic acid concentrations during a 14-week period. In old women a similar dose of iron, after causing a rise in leukocyte ascorbic acid concentrations, was associated with a fall in the ascorbic acid values to 62% of their initial concentrations during the 6th week. By the 14th week, the value had increased again to 133% of the initial value, in the absence of any alteration in dietary intake of vitamin C.

[a] Data from Loh and Wilson (1971a).

serum iron level of the treated group rose from 3.6 to 13.1 μmol/l and the hemoglobin level rose from 8.3 to 12.3 g/100 ml, but their resistance to infection was markedly reduced instead of being elevated. One cannot help but wonder what happened to the ascorbic acid levels of these malnourished refugees as a result of the iron administration; ascorbic acid deficiency would certainly account for their decreased resistance to infection. It is interesting to note that the incidence of infection was highest between the 22nd and the 30th day of iron treatment, after a large quantity of iron had been administered.

Decreased leukocyte ascorbic acid concentrations have been recorded by Loh and Wilson (1971a) in elderly men during 16 weeks of oral iron administration as shown in Table 3. These authors also concluded that their results indicated an increased demand for ascorbic acid during iron therapy in women, but the leukocyte ascorbate levels of the women rose, fell, and later rose again, as shown in the table.

Even though iron can sometimes depress the plasma level of ascorbic acid, Albers (1952), Moore (1955), and others have shown that ascorbic acid aids the absorption of iron. In a study of 20 iron-deficient infants and children, Gorten and Bradley (1954) showed that daily administration of 500 to 750 mg of ascorbic acid increased the absorption of orally administered iron. The reticulocyte peak was 96% higher, the hemoglobin rise was 69% higher, and the calculated iron utilization was 55% better than in the control group. In seven adults they recorded no such benefit, but Layrisse et al. (1974), McLean Baird et al. (1974), Disler et al. (1975a), and Cook and Monsen (1977) have found that ascorbic acid markedly increases iron absorption in adults. Ascorbic acid probably increases absorption of dietary iron by promoting the reduction of ferric iron to the ferrous form. Other reducing agents, such as cysteine, have a similar action. Ascorbic acid seems to increase iron absorption most in iron-deficient subjects. Of course, ascorbic acid is oxidized as iron is reduced and ascorbic acid is very unstable in its oxidized form, so some of it is lost by hydrolysis in the process. It is for this reason that most manufacturers of iron and vitamin preparations arrange for separation of iron and ascorbic acid by the use of enteric-coated granules.

In practice, when one wishes to supply iron and ascorbic acid to a patient, it may be best to give ascorbic acid with orange juice at breakfast in the hope that it will be absorbed; ferrous iron and ascorbic acid can then be given together at noon and again in the evening. Some of the ferrous iron would normally be oxidized to ferric by oxygen in the stomach, but this will be prevented by ascorbic acid, and only ferrous iron will be presented to the intestinal villi for absorption.

Studies of the mineral content of municipal drinking water supplies in different regions of the U.S., Britain, Sweden, and Japan have shown significant correlations between "soft water" and increased death rates from hypertensive and arteriosclerotic heart disease. However, no positive correlation has been found between any constituent of the water and these death rates. Schroeder (1966) suggests that one possible explanation of this phenomenon involves the corrosive quality of soft water for metal pipes in dwellings. Schroeder found 1.4 ppm of copper in soft spring water that had stood overnight in a copper pipe, and only 0.04 ppm in hard well water after similar treatment; he used clean segments of pipe in these comparisons. One must appreciate that the difference must be even greater in practice, as copper pipes often have an inner coating of "fur" in hard water areas and have no such coating in soft water regions.

Needless to say, the iron content of water will also be higher in soft water areas in dwellings with iron pipes.

Clearly, the extra copper or iron content of drinking water could over many years have an adverse effect on the ascorbic acid metabolism of people living in soft water areas, and a resultant relative ascorbate deficiency could be the cause of the increased incidence of atherosclerosis, as discussed elsewhere in this book. This becomes even more probable in view of the facts that (1) even mild ascorbate deficiency is associated with high blood histamine levels, as shown by Clemetson (1980); (2) histamine has been incriminated in the etiology of atherosclerosis by Owens and Hollis (1979) and De Forest and Hollis (1980); and (3) antihistamines and anti-inflammatory drugs have been shown to be protective against atherosclerosis in animals (Harman, 1962 and Hollander et al., 1974).

The derangement of ascorbic acid metabolism in thalassemics with hemochromatosis due to repeated blood transfusions is severe and presents a difficult problem. These patients are ascorbate deficient, as shown by Chatterjee et al. (1980) and Cohen et al. (1981), and they need ascorbic acid during desferoxamine chelation therapy to release iron from the cardiac, hepatic, and reticuloendothelial deposits, so that it can be excreted (see Table 4). However, ascorbic acid can be toxic to these patients during desferal therapy, causing deterioration of cardiac function, as shown by Henry (1979) and reviewed by Nienhuis (1981). Carbohydrate intolerance and insulin-dependent diabetes mellitus are also common in thalassemics, as reported by Lassman et al. (1974) and Costin et al. (1977), especially in those who begin chelation therapy too late (Graziano and Piomelli, 1980/1). This is probably because ascorbic acid is so rapidly oxidized by the iron as it is mobilized; DHAA is known to have toxic effects, to damage the beta cells of the islets of Langerhans, and to cause diabetes mellitus when given intravenously to rats (Chapter 3, Volume III). The benefits of using antioxidants like vitamin E with ascorbic acid in an attempt to resolve this problem are discussed in Chapter 15 of this volume.

Cohen and Schwartz (1980) reported very encouraging results in two 10-year-old boys with thalassemia major whose serum ferritin levels had fallen progressively during 32 months of chelation and ascorbate therapy. As shown in Table 5, their liver functions also improved; eventually their vitamin C levels returned to normal without the need for any ascorbic acid supplement. These authors state, "Finally, the loss of dependence on vitamin C supplementation, for maximal desferoxamine-induced urinary iron excretion, indicates that iron stores have been reduced sufficiently so that increased destruction of dietary ascorbic acid is no longer a prominent feature in these children."

Table 4
CHANGES IN CHEMICAL ANALYSIS OF IRON STORES WITH VITAMIN C THERAPY

Determination	Before therapy (6/5/78)	During therapy[a] (7/17/78)	After therapy (8/14/78)
Serum iron (μg 100 ml)[b]	44	188	192
Iron-binding capacity (μg/100 ml)[b]	220	196	276
Transferrin saturation (%)	20	96	70
Ferritin (μg/l)	1720	6270	3440
Urinary iron excretion with 18-h subcutaneous infusion of 2.0 g of deferoxamine (mg/24 h)	4.1	44.7	29.2

Note: Chemical analyses of serum and urine from a 26-year-old woman with β-thalassemia and scurvy due to iron overload following multiple blood transfusions. The data before, during, and after treatment with ascorbic acid demonstrate that the serum iron level and transferrin saturation were unexpectedly low during clinical scurvy. Before treatment of the scurvy, 2 g of desferoxamine, given subcutaneously over 18 h, resulted in the excretion of only 4.1 mg of iron in the urine in 24 h, but during treatment with vitamin C (50 mg daily for 2 weeks and 100 mg daily for 4 weeks), iron mobilization was markedly increased. Transferrin became saturated, ferritin rose above 6000 μg/l and 44.7 mg of iron was excreted after a similar challenge with the chelating agent. No ascorbic acid could be detected in the leukocytes of this patient at the completion of therapy, in spite of the administration of 3.6 g of ascorbic acid.

[a] Therapy consisted of 100 mg of vitamin C per day.
[b] To convert to micromoles per liter, multiply by 0.179.

From Cohen, A., Cohen, I. J., and Schwartz, E. (1981), *N. Engl. J. Med.*, 304, 158. With permission.

Table 5
FERRITIN LEVELS AND LIVER FUNCTION TESTS DURING CHRONIC IRON CHELATION[a]

	Before chelation	16 months	26 months	32 months
Patient A				
Ferritin (ng/ml)	3460	506	286	159
SGOT (mU/ml)	154	57	41	37
SGPT (mU/ml)	264	76	34	15
Patient B				
Ferritin (ng/ml)	—	1280	523	252
SGOT (mU/ml)	58	56	42	41
SGPT (mU/ml)	68	45	32	24

Note: The progressive reduction of serum ferritin levels in these two patients with β-thalassaemia during prolonged treatment with desferoxamine suggests that the excessive iron stores can be depleted. Moreover, these two patients were no longer dependent on dietary supplements of ascorbic acid after 32 months of chelation therapy.

[a] SGOT, serum glutamic oxalacetic transaminase; SGPT, serum glutamic pyruvic transaminase.

From Cohen, A. and Schwartz, E. (1980), *Ann. N.Y. Acad. Sci.*, 344, 405. With permission.

These beneficial effects of chelation therapy on ascorbic acid metabolism encourage speculation by the writer, as to whether the delayed benefits often observed following prolonged treatment with ascorbic acid and bioflavonoids, tannins, catechins, and other chelating food fibers (Chapter 11 of this volume) may not also be due to the removal of heavy metals from the body by adsorption and chelation by these insoluble substances in the lumen of the intestine.

Reinhold et al. (1981) have investigated the binding of iron by the fiber of wheat and maize. These authors suggest that the promotion of iron absorption by vitamin C may depend, in part, upon its ability to release iron from combination with dietary fiber.

Likewise, Lynch (1981) has observed that the tannins and fiber components of vegetable foods bind iron and impair its absorption, but ascorbic acid facilitates the absorption of iron by forming a chelate with the ferric iron.

However, this beneficial effect of ascorbic acid can go too far, for McLaran et al. (1982) have reported a 29-year-old man in Brisbane, Australia, who died with congestive cardiomyopathy and hemochromatosis, possibly accelerated by excessive ingestion of ascorbic acid[e] had consumed 1 g ascorbic acid tablets daily and artificial orange juice containing added ascorbic acid for 12 months. We may conjecture that this man also had an unusual source of iron and not enough chelating fiber. Presumably it was a profound disturbance of the AA/DHAA ratio in his blood that led to his cardiomyopathy. It seems reasonable to assume that he would have been better served by a smaller dietary supplement of ascorbic acid, 200 mg with 200 mg of catechin, than by the daily 1-g dose of ascorbic acid alone.

Roeser (1983) has reviewed the two-way process whereby "variations in tissue iron stores have an equally profound effect on ascorbic acid metabolism as do variations of tissue ascorbic acid status on iron metabolism."

Hallbert and Rossander (1984) used cauliflower as a source of ascorbic acid to increase the absorption of iron from a simple Latin American-type meal of maize, rice, and black beans. Iron isotope absorption was increased three to four times by the addition of a piece of boiled cauliflower containing 65 mg of ascorbic acid and three times by adding pure ascorbic acid (50 mg).

Lohman et al. (1986) have investigated the effects of iron, copper, calcium, and magnesium ions on the permeation of ascorbate across membranes of dipalmitoyl lecithin and concluded that only ferric and cupric ions affect the permeation rate. However, they concluded that the mechanisms of action of these two ions appear to differ.

The situation is undoubtedly complex, for ascorbic acid aids the absorption of iron and yet excessive iron stores seem to destroy ascorbic acid. It would seem that we are fortunate if we can remain in balance. Moreover, the balance point as regards ascorbic acid and iron is different for men and women (Chapter 6 of this volume) because of the monthly blood loss of women of reproductive age, leading to lower iron stores in women.

As described in more detail in the section of this book devoted to hemolysis (Chapter 15 of this volume), De Alarcon et al. (1979) have conducted radioactive iron tracer absorption studies of patients with thalassemia major and have shown that tea taken as a beverage, with an iron containing meal, causes a pronounced reduction of iron absorption. This confirmed the earlier work of Disler et al. (1975b) and Derman et al. (1977) who demonstrated that tea decreases iron absorption in normal human volunteers.

This effect is almost certainly due to chelation of iron by the catechins and tannins of the tea. Catechins are equally capable of chelating copper; it remains for future research to find out whether D-catechin, formerly known as vitamin C_2 by French workers, is capable of removing excess copper from the liver and other tissues of the body, for this may be the mechanism of its action, both in radiation protection and in improvement of ascorbic acid metabolism and storage. If correct, this hypothesis would explain the 2-month delay in response noted by Clemetson when vitamin C and bioflavonoids were used in the treatment of menorrhagia associated with easy bruising (Chapter 11, Volume III).

Jacob et al. (1987), studying healthy young men in a metabolic unit, have observed that ascorbic acid supplementation (600mg/d) caused a 21% decrease in the serum ceruloplasmin oxidase activity, but no change in the copper absorption or copper retention, nor in the total serum copper or ceruloplasmin protein levels. It would seem that the oxidase activity of the enzyme is reduced when the oxidation-reduction potential of the plasma is reduced by a high ascorbic acid level *in vivo*.

Liver biopsies are too hazardous to use simply for research purposes, but perhaps some method will soon be devised whereby individual heavy metal contents can be estimated by a noninvasive technique such as nuclear magnetic resonance or neutron activation analysis. Experiments can then be designed to find out whether chelating food fiber acts by preventing excessive accumulation of heavy metals like copper, which can deplete ascorbic acid stores and may also accelerate lipid peroxidation and aging.

V. HISTORICAL NOTE

In his recent book entitled *The History of Scurvy and Vitamin C*, Carpenter (1986) has recorded the existence of a document which is very pertinent to the subject of heavy metals and ascorbic acid: "In 1757, John Travis, a surgeon practicing at the English seaport of Scarborough, presented the hypothesis that the scurvy so prevalent in the British Navy was caused by copper poisoning. He argued that men on merchant ships, which generally had iron boilers for cooking their food, were much less affected than those on naval ships, which always had copper boilers, even though the provisions on the latter were usually of higher quality. He believed that the motion of a large quantity of food in a briskly boiling copper pot caused the metal surface to react with salt to form verdigris (a copper salt, actually copper actate), which dissolved in the grease that was usually present."

Travis (1762) wrote that "the acrimonious mineral corpuscles are sheathed by the oil so that when the food is eaten they do not cause vomiting or griping and are then absorbed into the tissues—tearing the extremities of the capillary arteries, irritating, and wounding the nerves. Though the quantity taken at once be so small as not to do any sensible mischief, yet being daily repeated, it gradually, and insidiously, brings on a train of the most dreadful complaints."

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Chapter 11

BIOFLAVONOIDS

The bioflavonoids comprise a large group of naturally occurring plant phenolic compounds with the general structure $C_6{\cdot}C_3{\cdot}C_6$, including the flavones, flavanones, flavonols, flavanonols, flavanes (or catechins), chalcones, dihydrochalcones, aurones, anthocyanidins, and their glycosides. They occur in all parts of higher plants: roots, stems, leaves, flowers, pollen, fruit, seeds, wood, and bark. The anthocyanins are the pigments of fruits, flowers, and leaves; condensed tannins are flavonoid polymers.

Many of these plant polyphenols which are sparingly soluble in water have, nevertheless, been found to possess biological activity and to afford protection against capillary fragility and radiation damage in guinea pigs. They have also been found in some studies to be beneficial when given with ascorbic acid in the treatment of various bruising and bleeding disorders, such as Henoch-Schönlein purpura in children and adults. Griffith et al. (1955) have reported decreased capillary fragility and decreased mortality in patients with hypertension, retinal hemorrhage, and cerebral hemorrhage following treatment with rutin or quercetin.

Bioflavonoids have been found beneficial when given with ascorbic acid in the treatment of menorrhagia by Jersild (1938), Grasset et al. (1953), Preuter (1961), Clemetson and Blair (1962), in the treatment of recurrent abortion by Javert (1955) and Greenblatt (1955), and in rheumatic fever by Rinehart (1955). They have also been reported as reducing the capillary fragility state in preeclampsia by Dieckmann et al. (1949), Burger (1951), and Sauramo (1952).

A large world literature has built up concerning the biological activities of the flavonoids; many physicians have observed manifest clinical benefits from treatment with vitamin C and certain bioflavonoids, while others have reported no benefits beyond those which could be attributed to vitamin C alone.

Interest in the bioflavonoids began when Szent-Györgyi and co-workers (Armentano et al., 1936, Bruckner and Szent-Györgyi, 1936) and others (Elmby and Warburg, 1937, Glazunov and Povolotskaya, 1934) found that pure white crystalline ascorbic acid which had recently been isolated (Waugh and King, 1932a, b, c, Svirbely and Szent-Györgyi, 1932a, b, Tillmans and Hirsch, 1932, Vedder, 1932) was not as effective in the treatment of nonthrombocytopenic purpura, as the original crude yellow powder containing vitamin C, which could be extracted from oranges, lemons, or paprika. The existence of two antiscorbutic vitamins had already been proposed by Randoin and Lecoq in 1927; the properties of a vitamin were now attributed to the yellow substance, which Bruckner and Szent-Györgyi (1936) called "citrin" and which Bentsath and Szent-Györgyi called "vitamin P", indicating an "anti-permeability" vitamin for the capillaries. However, Bentsath and Szent-Györgyi (1937) soon conceded that their vitamin P was active only in the presence of traces of vitamin C. "In the entire absence of ascorbic acid, vitamin P is inactive." Zacho (1939) reported that the capillary strength of guinea pigs, on a very low ascorbic acid dietary intake, fell progressively; that pure ascorbic acid did not correct it, but that, on the other hand, a natural extract resembling citrin was effective. At first the flavanone glycoside hesperidin was thought to be the active ingredient of citrin (but pure hesperidin is white); later flavonoid extracts from oranges were found to contain an eriodictyol glycoside called eriodictin, hesperidin methyl chalcone, and neohesperidin, as well as hesperidin.

Subsequently, workers in the U.S. found rutin, a yellow flavonol glycoside obtained from buckwheat, to be more effective than citrin, and workers in France concentrated on D-catechin ((+)catechin) which they dubbed vitamin C2. Workers in Russia studied D-catechin,

fruit and spruce needle polyphenols, and also tannins. A profusion of literature developed; some authors, like Bacharach et al. (1942) and Bourne (1943), finding that guinea pigs fed a scorbutogenic diet with 5 or 10 mg of vitamin C a day, plus citrin, had significantly higher capillary resistance (capillary strength) than those given the same diet with the same dose of vitamin C alone, other workers finding no such benefit.

Parrot and Richet (1945) observed that vitamin C deficiency causes a markedly increased sensitivity to histamine in guinea pigs. The dose of histamine dihydrochloride killing 50% of the animals (LD_{50}) was reduced from 8 mg/kg to 2.5 mg/kg after 15 d on a scorbutogenic diet. However, they found that animals fed the same deficient diet for the same number of days could be protected from this increased sensitivity by intraperitoneal injection of 2.5 mg of D-catechin within 24 h before the histamine challenge.

In vitamin C bioassay studies Crampton (1947) observed the need for some kind of vegetable roughage as well as all the known vitamins and essential minerals in a guinea pig diet. He observed 80% of successful pregnancies and normal growth of the young when dried, long-stored grass clippings were included; whereas when the roughage was omitted, only 66% of pregnancies were successful and there was some, though not a marked, slowing of growth of the young to maturity. Some refer to the insoluble parts of vegetable food as "fiber"; it includes bioflavonoids, catechins, and tannins as well as cellulose and other substances. The present writer believes it may be the copper-chelating capacity of this roughage which is beneficial in ascorbic acid metabolism.

Radiation damage is known to be proportional to the copper content of the tissues, but studies of bioflavonoids in radiation protection gave confusing and conflicting results because of the different substances used, the different modes of administration, and the different experimental designs. Griffith et al. (1947), using subcutaneous pellets of rutin, Clark et al. (1948), using lemon flavonoids given orally, Rekers and Field (1948), using rutin given by mouth three times a day starting 1 week before irradiation, and Sokoloff et al. (1950), using a water-soluble citrus vitamin P derivative (CVP) in drinking water, all reported definite protection against radiation injury in rats, guinea pigs, dogs, and rats, respectively, while others reported negative results.

Field and Rekers (1949) studied several flavonoids. They gave beagle dogs 50 mg of a flavonoid by mouth three times a day for 1 week before and for 28 d after total body irradiation (350 rad) in small treatment groups of 5 to 12 dogs each. The following flavonoids were found to be effective in lowering the mortality following whole body irradiation: rutin, hesperidin, epimerized D-catechin, homoeriodictyol, and morin. The following were considered to be inactive: hesperidin-methylchalcone, esculin, quercetin, quercitrin, and naringin. Quercetin was effective when given with ascorbic acid (100 mg daily). This is particularly interesting because we now have evidence that dogs may not make enough ascorbic acid for all their needs; moreover, beagles do sometimes develop spinal disease, which is treated with ascorbic acid.

Sevestre et al. (1951) and Fabianek et al. (1952) showed that guinea pigs could be kept alive and healthy for as long as 1 year on a flavonoid-free diet, if each animal received 20 mg of ascorbic acid a day, so the concept of bioflavonoids as vitamins was no longer tenable. However, 20 mg of ascorbic acid per guinea pig per day is an enormous requirement of vitamin C for such a small animal, equivalent to about 2 g/d for a human adult.

Parrot and Cotereau (1946) showed that D-catechin retards the oxidation of ascorbic acid. Gero (1947a, b) confirmed this antioxidant effect of D-catechin and showed similar activity by rutin. He concluded that certain flavonoids, with a catechol configuration in the B-ring, protect ascorbic acid against oxidation, no only by oxygen, but also against photochemical oxidation by methylene blue.

Cotereau et al. (1948) found that dietary supplements of D-catechin increased the storage of ascorbic acid in the liver, spleen, kidneys, and adrenals of guinea pigs. Increased storage

of ascorbic acid in the tissues after dietary supplementation with various plant polyphenols was confirmed by Kursanov et al. (1950), by Durmishidze et al. (1951) and Erofeeva (1959), using D-catechin, by Shamrai et al. (1959), using fruit polyphenols, by Siim (1959), using rutin, and by Verkhratskii (1959), using spruce needle polyphenols. It was partially confirmed by others (Papageorge and Mitchell, 1949, using rutin, and by Nikonova, 1959, using a tannin), but was not found by others (Yarusova et al., 1959, using tea catechins; Rusznyák et al., 1960, using rutin, and Fabianek, 1961, using tannins). Thus, flavonoids seem to have an indirect action, which is dependent on the presence or absence of other factors, rather than a direct action of their own. The significance of this becomes evident when we find that certain flavonoids are indirect antioxidants for ascorbic acid by virtue of chelating and inactivating heavy metal catalysts like copper. Cupric ions rapidly destroy ascorbic acid, so the presence or absence of copper in the drinking water can profoundly affect the results of guinea pig feeding experiments. Even copper water spouts on glass bottles can increase the copper content of soft water in the spout to 2 ppm after standing overnight.

Suzuki and Mori (1951) demonstrated the antioxidant activity of certain flavonoids for ascorbic acid at pH 7.4 *in vitro*. They dissolved the flavonoids in propyleneglycol and concluded that rutin, 3′,4′,5′-trihydroxyflavone, quercitrin, myricetin, quercetin, daphnetin, and a commercial tannin possessed antioxidant activity, but hesperidin, umbelliferon, tea catechol, and tea tannin did not.

However, Masquelier (1951) tested leukocyanidin, rutin, and esculetin for inhibitory action on the oxidation of ascorbic acid by cupric ions at 40°C and found a clearly inhibitory action exerted only by leukocyanidin; antioxidant activity by rutin would have been observed if higher concentrations of this flavonoid in suspension had been studied.

Parrot and Gazave (1951a, b) and Parrot et al. (1953) produced evidence that D-catechin potentiates the reduction of dehydroascorbic acid by glutathione.

Von Euler (1956) reported his findings of antioxidant activity by catechin in alkaline solutions.

Shamrai et al. (1959) reported their belief that a chemical reaction occurs between ascorbic acid and certain plant polyphenols which results in their mutual stabilization.

Heimann and Heinrich (1959) studied the oxygen uptake by ascorbic acid during its copper-catalyzed oxidation and concluded that quercetin acts as a good chelating agent for copper, that it slows the oxidation of ascorbic acid at pH 6.4 to 6.5, and that rutin is less active. Catechol and dihydroquercetin were found inactive in these experiments.

Davidek (1960) reported that flavonoids do not stabilize pure ascorbic acid, but certain of them slow the copper-catalyzed oxidation of ascorbic acid. Rutin and quercitrin gave the best stabilization, while naringin was less active.

Hermann and Grossman (1960) reported that rutin retards the oxidation of ascorbic acid at pH 7 and 8 and hesperidin at pH 6, 7, and 8. However, it should be noted that the hesperidin used by these authors was apparently yellow, while pure hesperidin is white. Naringin had no protective effect at pH 5 to 7 and only minor protective effect at pH 8.

The variety of methods used by different workers in attempts to dissolve the flavonoids, and the contradictory results that they obtained, made it quite clear that further studies were needed. Clemetson and Andersen (1966), therefore, studied the activity of a wide range of flavonoids and related substances in a weakly alkaline medium of pH 7.4 at 37°C. Ascorbic acid oxidation is slow in acid media and losses by oxidation are most likely to present a problem in alkaline media, such as are present in the jejunum or in the stomach of patients with achlorhydria. The rate of oxidation of strong solutions of ascorbic acid (50 mg/100 ml) is affected by the size of the air space in the container. One must therefore either bubble air through the solution to replenish oxygen as it is used or else study the rate of oxidation of dilute solutions (2 mg/100 ml), when an excess of oxygen will be available in the water. The latter method is most convenient and was adopted in the studies to be described.

It is neither necessary nor desirable to ensure that the flavonoids dissolve completely in the test solution, for there is no evidence that they dissolve completely in the stomach or in the jejunum. Moreover, many of them seem to act as excellent antioxidants in suspension. No heavy metal catalysts were added, since traces of impurities in the dibasic and monobasic sodium phosphate, of which the buffer was composed, resulted in rapid oxidation of ascorbic acid. Light affects the rate of oxidation of ascorbic acid, but variations due to this factor were eliminated by ensuring that test and control samples were always run under the same conditions at the same time.

Studies of the antioxidant activities of suspensions of different flavonoids and their glycosides for ascorbic acid at different concentrations in 10^{-1} *M* phosphate buffer at pH 7.4 and 37°C are shown in Figure 1. All the analyses were carried out by a buffered 2.6-dichlorindophenol photometric method, using $^1/_2$- and 1-min optical density readings to extrapolate back to that for zero time. For full details of these experiments the reader is referred to Clemetson and Andersen (1966).

Analysis of the results obtained with a 10^{-3} *M* suspension of each flavonoid, as listed in Table 1 and Table 2, shows that antioxidant activity is possessed by those flavones, flavonones, flavonols, flavanonols, catechins, anthocyanidins, and their glycosides with a 3 hydroxyl, 4 carbonyl couplet in the gamma pyrone ring, and/or a 3′,4′ catechol couplet in the B-ring.

This is well exemplified by the pronounced antioxidant effect of rutin at 20°C and at 37°C at pH 7.4 in Figure 2.

Figure 3 shows that pretreatment of the phosphate buffer with D-catechin in suspension, provides potent antioxidant activity, even when most of the catechin is removed by filtration before the experiment. This is because catechin (rutin or quercetin) carries the heavy metals with it. There was no evidence that D-catechin affects the rate of hydrolysis of dehydroascorbic acid (Figure 3).

In studies of the reduction of dehydroascorbic acid to ascorbic acid by reduced glutathione at pH 7.4 and 20°C in phosphate buffer, it was found that the initial rate of reduction was the same with or without rutin, so the greater yield of ascorbic acid, found later with rutin, is almost certainly due to preservation of ascorbic acid after it has been formed (Figure 4).

Studies of hydroxycinnamic acid fragments of the flavonoid molecule showed that only caffeic acid with a catechol configuration and also an unsaturated side chain possessed antioxidant activity for ascorbic acid (Figure 1H); dihydrocaffeic acid, with a saturated side chain, was found to be inactive.

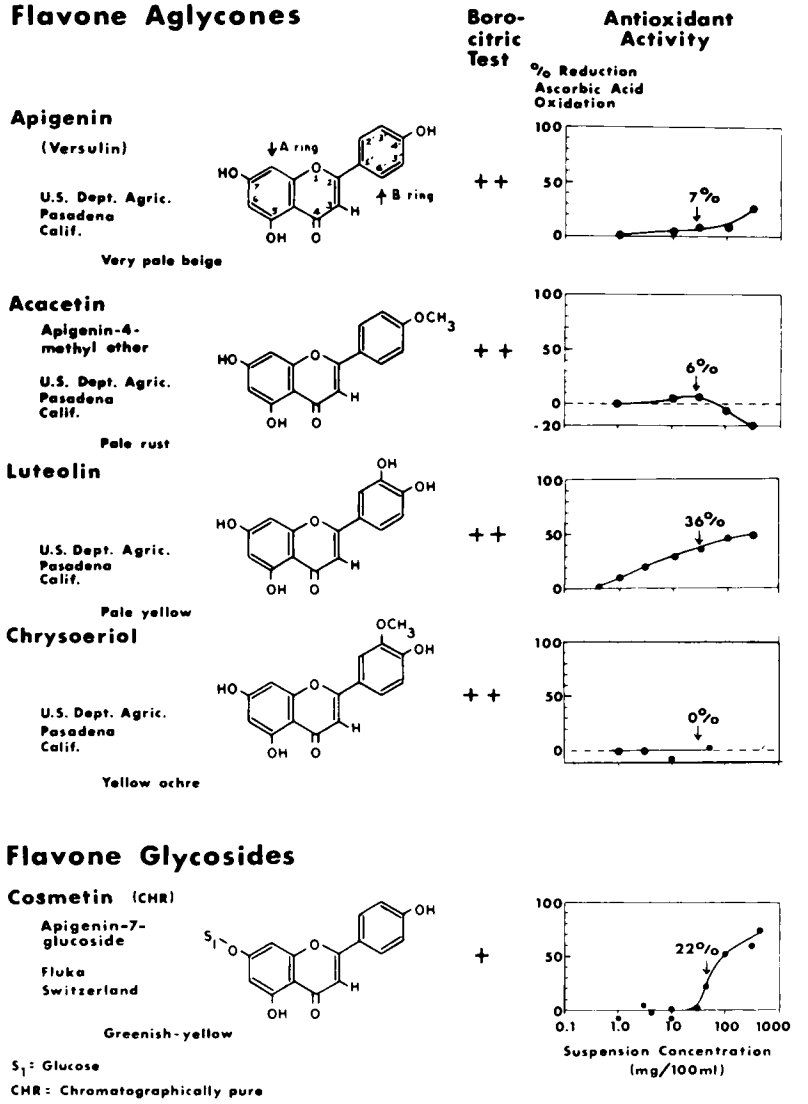
Moreover, the yellow color of the flavones and flavonols, which is absent from the flavanones and flavanonols, is undoubtedly due to their cinnamoyl resonance and is dependent on carbons 2 and 3 of the gamma pyrone ring being unsaturated. Pure hesperidin is a white flavanone glycoside; it has no chelatogenic couplets and has no antioxidant activity for ascorbic acid. Cream-colored commercial hesperidin possesses some antioxidant activity, probably due to contamination with another compound, possibly eriodictyol, although that substance itself has only weak antioxidant activity.

Studies of the antioxidant activity of caffeic acid in phosphate buffer, before and after adding cupric sulfate, demonstrated an increase in the amount of caffeic acid required for a given antioxidant effect (Figure 5). This supports the view that the antioxidant activity of these flavonoids is due to chelation of heavy metal catalysts.

Studies of the effects of suspensions of rutin at pH 5.0 and quercetin at pH 4.0 on the oxidation of ascorbic acid in acetate buffers (Figure 6) demonstrated that these substances possess antioxidant activity even in acid solutions where oxidation is much slower.

Quercetin is envisaged as chelating copper, as shown in Figure 7, and as precipitating iron from ferric chloride as in Figure 8. Certain it is that they can remove these metals from solution.

CHEMICAL STRUCTURES OF SUBSTANCES WHOSE ANTIOXIDANT ACTIVITY FOR ASCORBIC ACID WAS TESTED (IN PHOSPHATE BUFFER AT pH 7.4, 37°C), SHOWING RELATIONSHIP OF STRUCTURE TO ACTIVITY



A

FIGURE 1. Antioxidant activities are given as percentage reduction of ascorbic acid loss, and are plotted against suspension concentrations shown on a logarithimic scale. Arrows indicate 10^{-3}-*M* suspensions, and the numbers next to the arrows indicate the antioxidant activity of a 10^{-3}-*M* suspension of each substance. The results of Wilson's boro-citric test applied to each substance (with control) are given as −, or 1 to 4+, depending on the intensity of the yellow color developed in 1 min. It becomes evident that a positive result to this test is dependent on the presence of a double bond between carbons 2 and 3 of the flavones and flavonols, or related substances such as the chalcones. Flavanones with a single bond between carbon 2 and 3 give a negative result in the boro-citric test. The positive results obtained with certain flavanonols are almost certainly due to their partial oxidation to flavonols. (From Clemetson, C. A. B. and Andersen, L. [1966], *Ann. N.Y. Acad., Sci.*, 136, 339. With permission.)

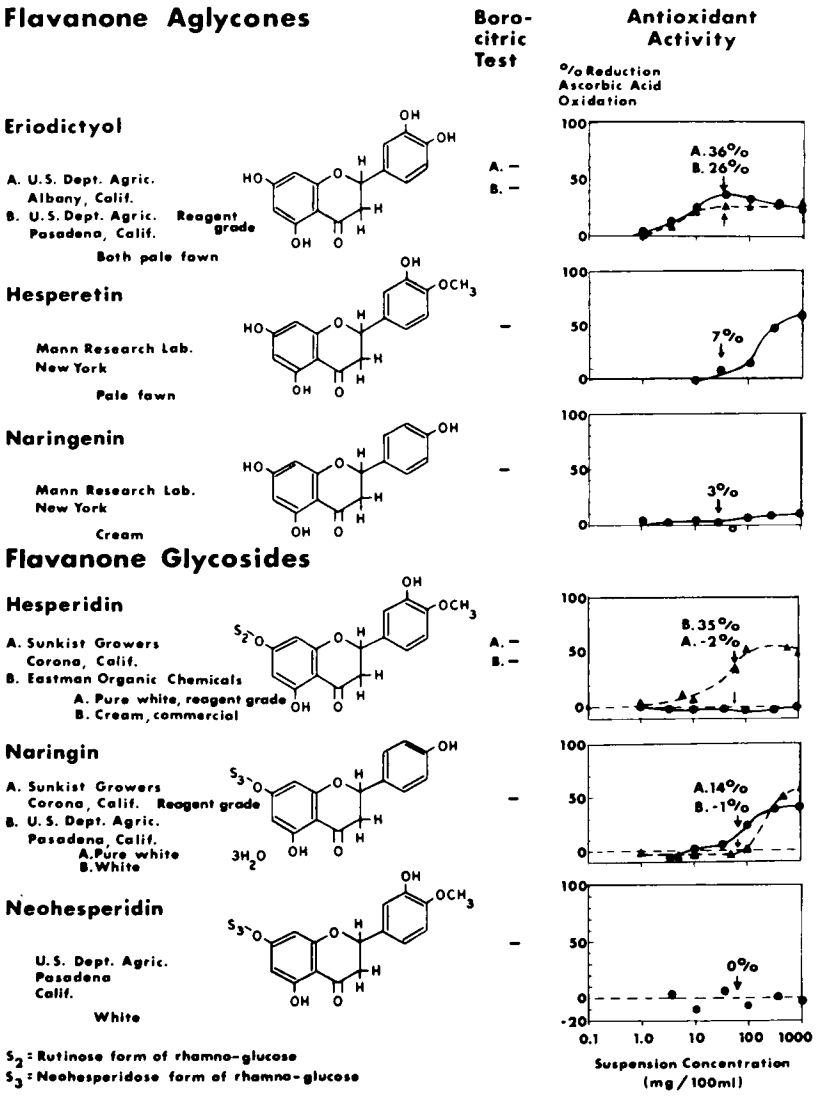


FIGURE 1B

Thus, it is now evident that bioflavonoids, like rutin, quercetin, D-catechin, and probably tannins and other chelating fiber in foods can act as preservatives for ascorbic acid both before and after it is eaten. They are especially valuable for patients who have little or no hydrochloric acid in the stomach (hypochlorhydria or achlorhydria), but may also be useful for all to prevent oxidation of ascorbic acid in the alkaline medium of the jejunum.

As long ago as 1934, Schultzer demonstrated an association between absence of gastric acid and capillary fragility. In fact, 10 out of 42 of his patients with lowered capillary resistance had either achlorhydria or hypochlorhydria. He found these patients were less responsive to vitamin C than his other patients with capillary fragility and suggested that destruction of ascorbic acid in the gastrointestinal canal might be the basis of the problem. He concluded as follows: "It may be that there is a new therapeutic conquest to make in relieving the vitamin C deficiency that may be present in achylics."

Now knowing that radiation damage is accentuated by the copper content of the tissues, it is evident that the copper-chelating effect of these flavonoids accounts for their radiation

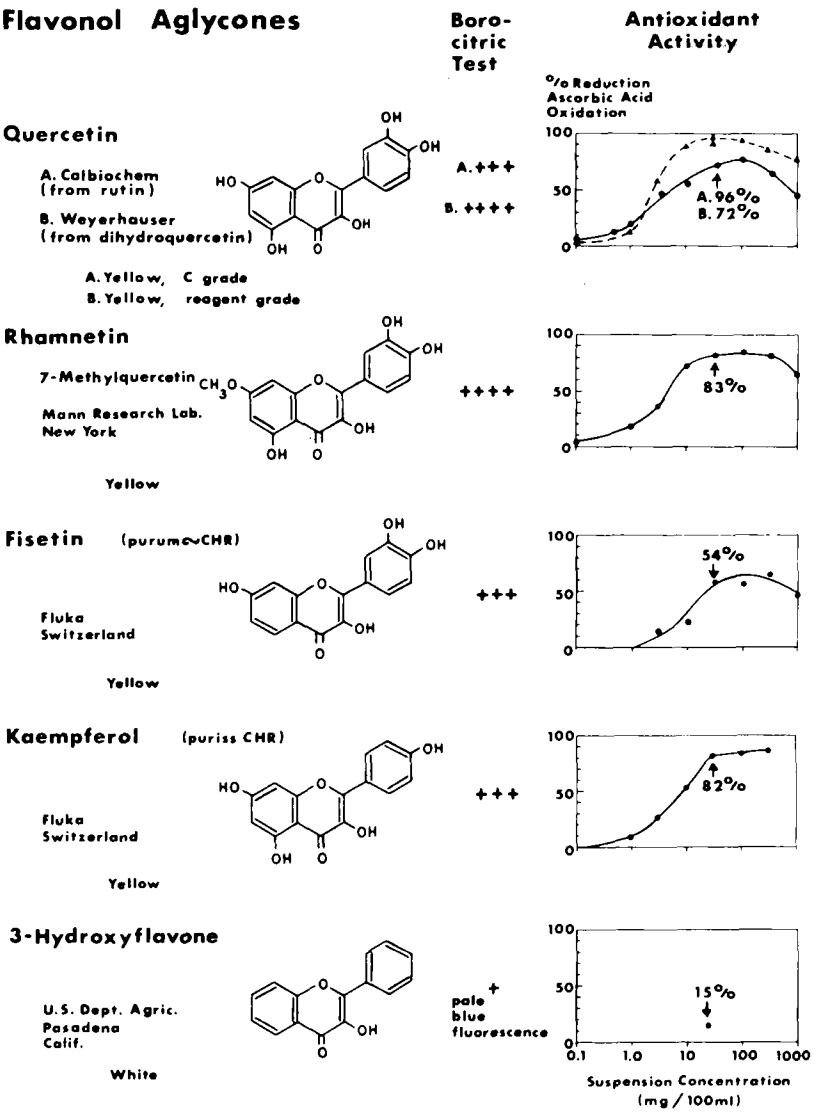


FIGURE 1C

protective effect; it is characteristic of reducing and/or chelating agents to protect against radiation.

We know from the work of Clark and MacKay (1950) that flavonoids are poorly absorbed from the intestine. Booth et al. (1956) and De Eds (1958) did isolate flavonoid metabolites in the urine of rabbits after feeding 2 g of quercetin, but no doubt most insoluble chelating fiber in food remains in the gastrointestinal tract and may carry heavy metals like copper out of the body. Indeed this may be one of the main benefits of flavonoids; they may be able to withdraw excess copper from the body.

Several authors (Spannuth et al., 1946, Richardson et al., 1947, Lea and Swoboda, 1956, and Kelley and Watts, 1957) have found that certain flavonoids, as well as alpha-tocopherol, possess antioxidant activity for unsaturated fats and oils. The originators of this idea may have been the American Indians. According to Spannuth they used the bark of trees for retarding the rancidity of bear grease. Or perhaps credit is due to the first woman who used

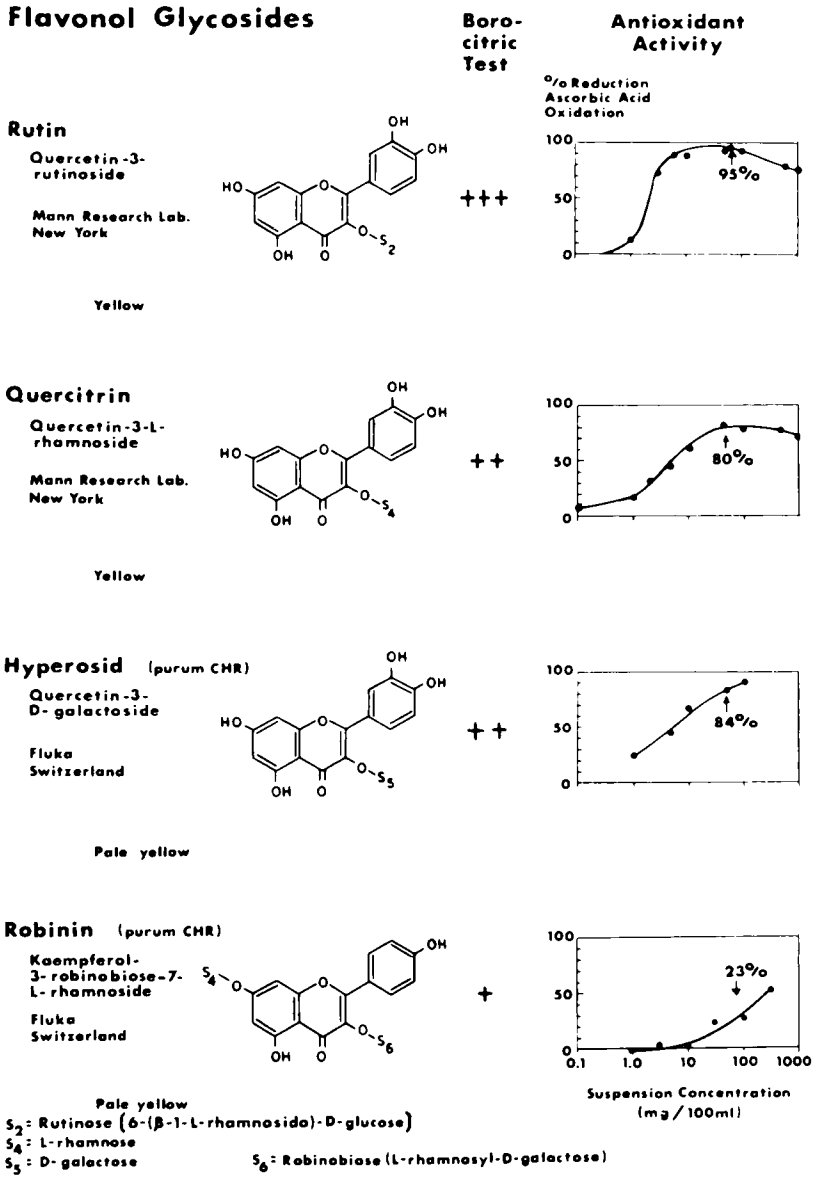


FIGURE 1D

sage and onions for dressing meat. Chipault and associates (1952) have studied the antioxidant activity of herbs, and Lewis and Watts (1958) studied the antioxidant and copper-chelating properties of onions, which they attribute to flavonoids.

The effect of flavonoids on total body copper in animals and in human beings does seem to be an area which has not yet been sufficiently studied. Heavy metal balance studies, with and without orally administered flavonoids, catechins, tannins, or other kinds of chelating fiber are very much needed.

Indeed, 95% of the copper in blood plasma exists as part of the copper-protein cerulo-plasmin molecule; about 5% is loosely bound to albumen and is more labile, but the principal reservoir of copper is the liver.

It is interesting to observe that it is the very instability of vitamin C which causes it to disappear from stored food, which made it so difficult to isolate and which also caused the whole bioflavonoid conundrum.

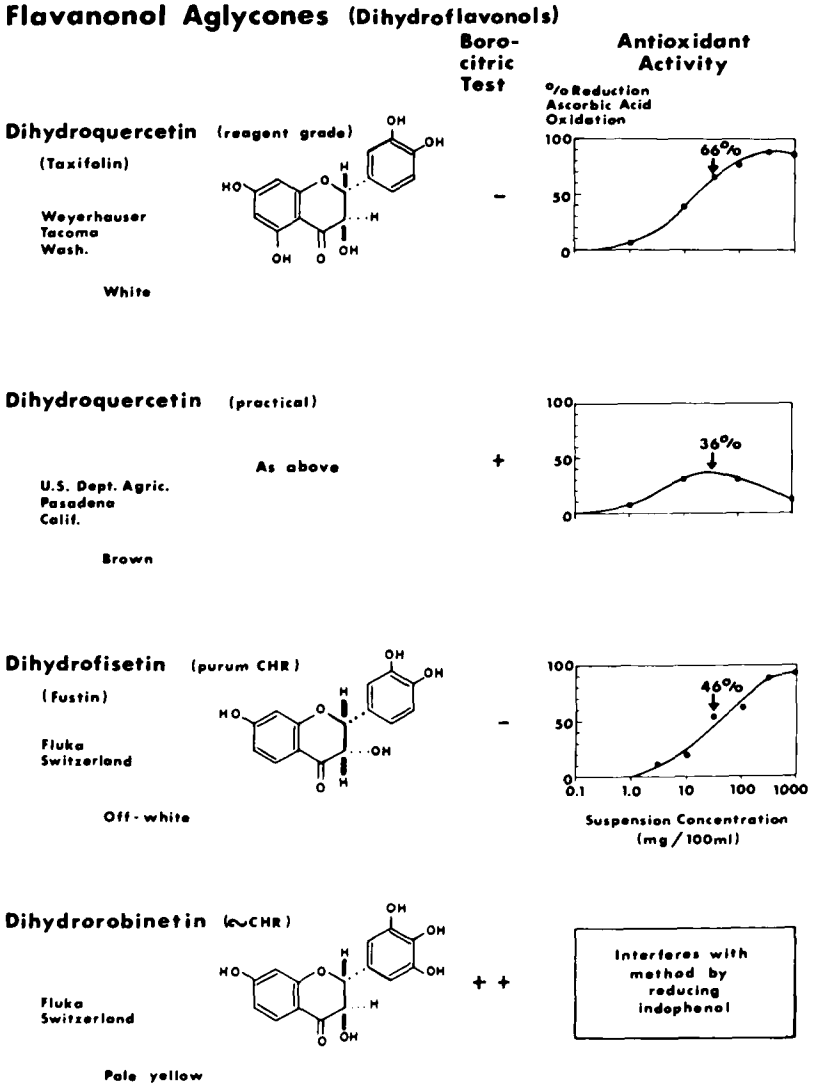


FIGURE 1E

Vitamin C is relatively stable in the dry state and in acid solutions, but is rapidly oxidized to dehydroascorbic acid and hydrolyzed to diketogulonic acid in neutral or basic solutions in the presence of oxygen, and this oxidation is catalyzed by the smallest traces of copper. This copper-catalyzed oxidation is markedly slowed by several chelating bioflavonoids.

Harper et al. (1969) have observed that quercetin acts as an antioxidant for ascorbic acid, both with and without added copper ions, even in a citrate buffer at pH 2.9.

Hughes and Jones (1971) compared the effects of feeding three groups of ten young growing guinea pigs, all on the same vitamin C-free diet, with supplements of natural fruit juices or the same amount of vitamin C as a solution of pure ascorbic acid. After 23 d, not only was the ascorbic acid concentration in the spleen and the adrenal glands significantly higher in the animals receiving black currant concentrate ($p < 0.01$) and somewhat higher in those receiving acerola cherry juice powder ($p < 0.05$) than in those receiving pure ascorbic acid, the growth rates of the animals were also proportionately greater in those receiving vitamin C from natural sources, even though they all received the same daily allowance of

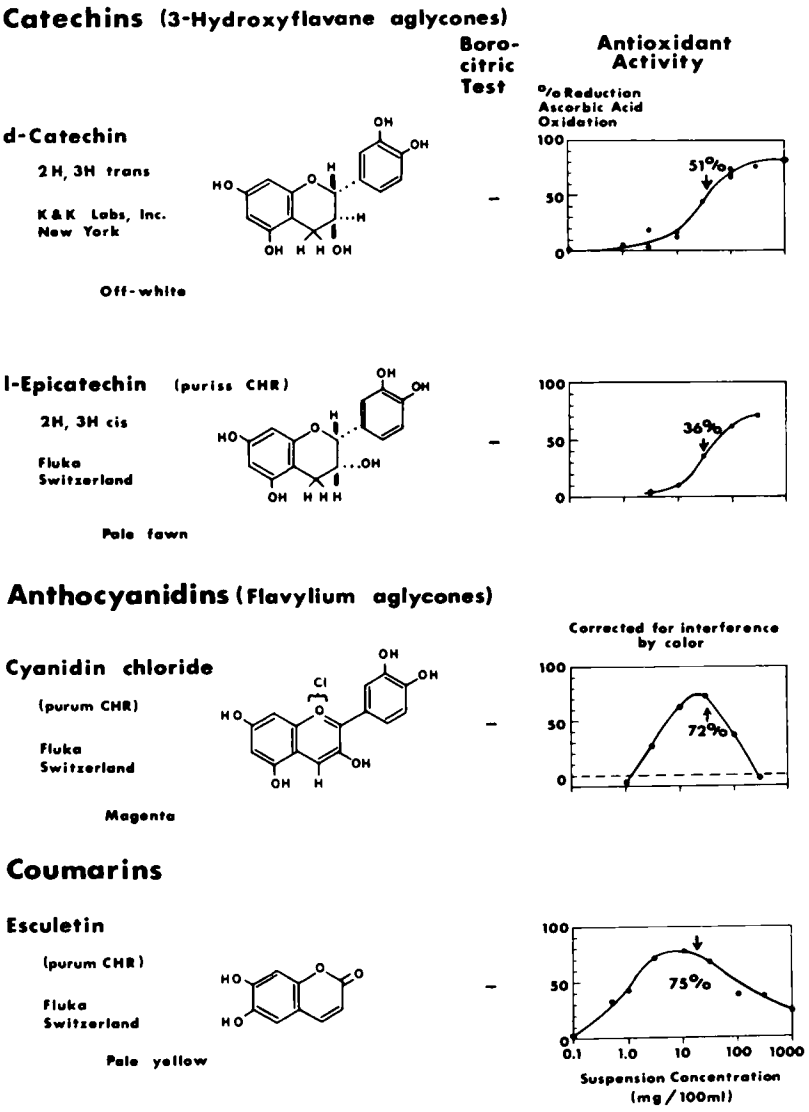


FIGURE 1F

ascorbic acid (0.5 mg/100 g body weight). Similar studies of young guinea pigs fed a diet containing ascorbic acid 0.1 mg/100 g body weight, by Wilson et al. (1976) demonstrated a marked increase in the growth rate ($p < 0.05$) and significantly increased storage of ascorbic acid in the adrenals ($p < 0.01$), leukocytes ($p < 0.01$), and spleen ($p < 0.02$) of guinea pigs receiving a dietary supplement of a flavonoid-rich extract of orange peel.

Stich et al. (1976) have found that ascorbic acid becomes mutagenic in the presence of copper. This is almost certainly due to the release of "ascorbate-free-radial" (or monodehydroascorbic acid), resulting from catalytic oxidation of the vitamin, for ascorbic acid itself is not mutagenic. This mutagenic action of ascorbate free radical, together with the hypertensive effects of dehydroascorbic acid reported by Patterson and Mastin (1951) and the diabetogenic effects of dehydroascorbic acid observed by Patterson (1950), make it more important than ever to utilize the plant polyphenols of natural foods to stabilize ascorbic acid in solution.

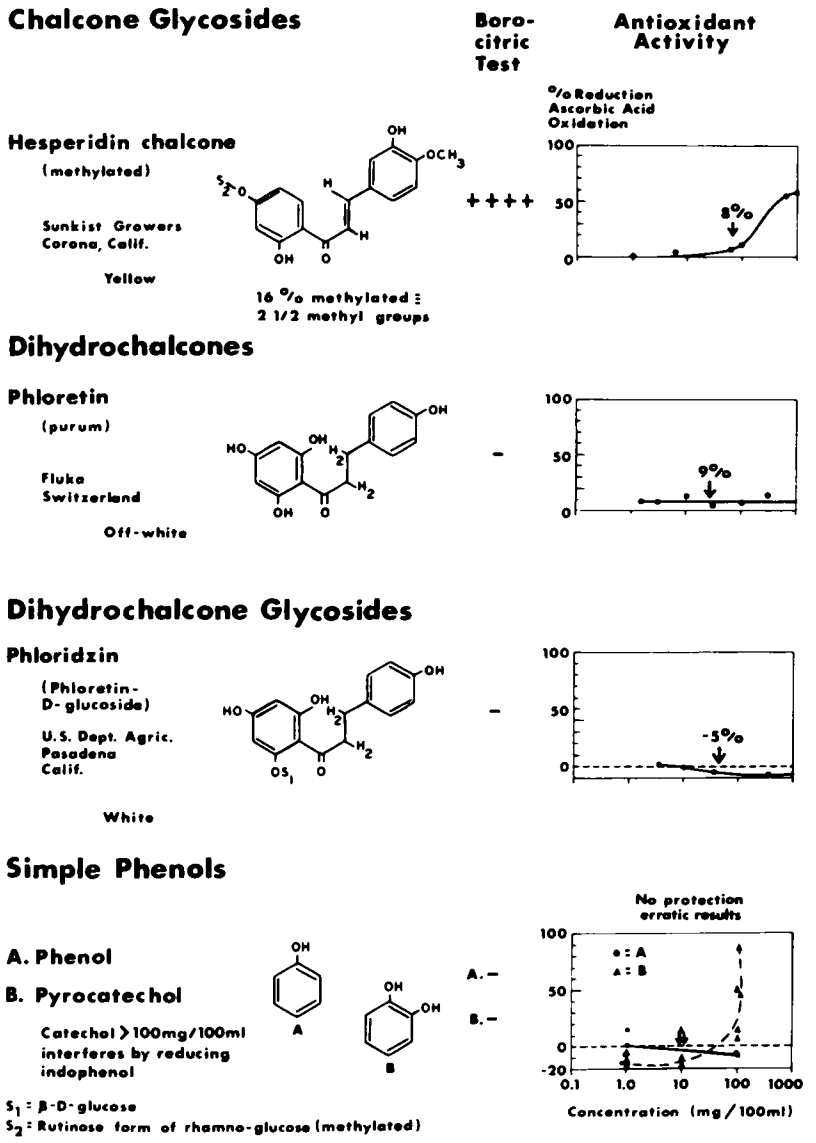


FIGURE 1G

Unfortunately, many flavonols (having the acidic 3 hydroxyl group adjacent to the 4 carbonyl group of the gamma pyrone ring), including fisetin, galangin, kaempferol, kaempferide, morin, myricetin, quercetin, rhamnetin, and robinetin, have also been found to be mutagenic in the "Salmonella/Microsome" test (Brown et al., 1977, Brown and Dietrich, 1979a, b, Ames, 1979, Brown, 1980) (Table 1). Also, Pamukcu et al.(1980a, b) have shown quercetin and the tannin of bracken fern to be carcinogenic in rats. Moreover, Tamura et al.(1980) have pointed out that many substances in the plant kingdom and in man's diet occur as nonmutagenic flavonol glycosides, but can become mutagens on hydrolysis of the glycosidic linkage by enzymes of the bacterial flora in the intestine. They point out that many beverages, such as red grape juice, red wine, and tea (but apparently not white wine) contain glycosides of quercetin, which is mutagenic. Thus, red wine, red grape juice and tea were all found to be mutagenic after incubation with feces. So rutin from buckwheat and, therefore, even pancakes are potential mutagens! What ever shall we eat? No more tea and pancakes?

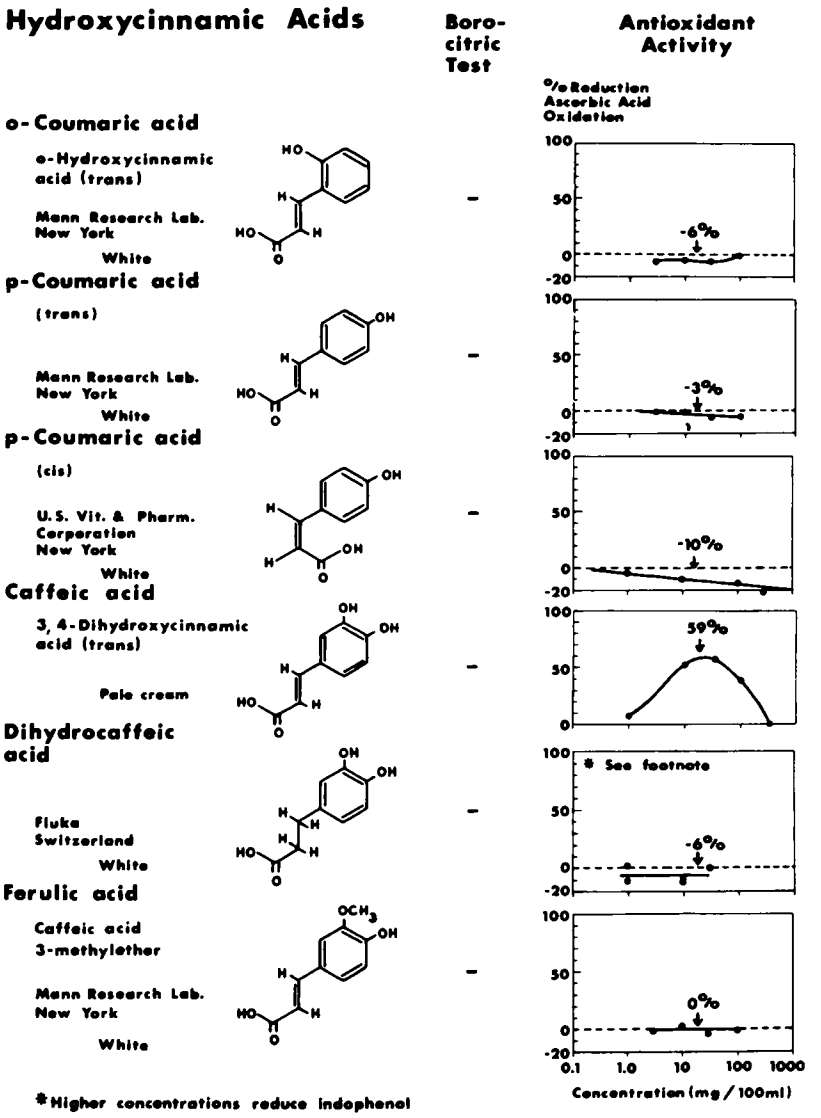
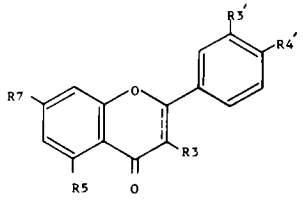


FIGURE 1H

Potentially mutagenic flavonol glycosides are so widespread among plant foods that they must have been present throughout our evolution; indeed they may have caused some of the mutations that made evolution possible. Brown (1980) estimates that the average American diet contains about 1 g of flavonoids and about 50 mg (quercetin equivalents) of flavonol glycosides a day; flavonol glycosides, mostly of quercetin and kaempferol, are listed by Brown as present in grapefruit, lemon, apple, apricot, banana, cherry, mango, quince, peach, red pepper, yellow plum, tomato, blackberry, blueberry, boysenberry, cranberry, currants, purple grape, raspberry, strawberry, asparagus, broccoli, kale, kohlrabi, brussel sprouts, endive, leek, lettuce, parsley, rhubarb, spinach, celery, horseradish, onion, potato, radish, sweet potato, caraway, coriander, dill, hops, mustard, oregano, broad bean, lima bean, mung bean, kidney bean, pea, barley, buckwheat, millet, maize, tea, cocoa, and honey.

The list of plants devoid of flavonols includes eggplant, fig, olive, red cabbage, carrot, yam, oats, rye, rice, and wheat; all of these contain flavone, flavanone, catechin, and anthocyanin glycosides, but not the promutagen flavonol glycosides.

Table 1
PERCENTAGE REDUCTION OF THE OXIDATION OF ASCORBIC ACID BY 10^{-3}-*M* CONCENTRATIONS OF VARIOUS BIOFLAVONOIDS



							Percentage reduction in rate of oxidation	Mutagenicity
Flavones								
Positions of chemical groups	7	5	4	3	3^1	4^1		
Apigenin	OH	OH	O	H	H	OH	7	0[a]
Acacetin	OH	OH	O	H	H	OCH_3	6	—[b]
Luteolin	OH	OH	O	H	OH	OH	36	0
Chrysoeriol	OH	OH	O	H	OCH_3	OH	0	—
Cosmetin	O-glucose	OH	O	H	H	OH	22	—
Flavanones								
Eriodictyol	OH	OH	O	H_2	OH	OH	26	0
Hesperetin	OH	OH	O	H_2	OH	OCH_3	7	0
Naringenin	OH	OH	O	H_2	H	OH	3	0
Hesperidin (puriss)	O-rutinose	OH	O	H_2	OH	OCH_3	−2	0
Hesperidin (commercial)							35	—
Naringin	O-neohesperidose	OH	O	H_2	H	OH	14	0
Neohesperidin	O-neohesperidose	OH	O	H_2	OH	OCH_3	0	—

Table 1 (continued)
PERCENTAGE REDUCTION OF THE OXIDATION OF ASCORBIC ACID BY 10^{-3}-*M* CONCENTRATIONS OF VARIOUS BIOFLAVONOIDS

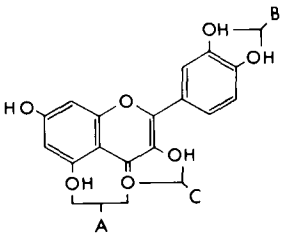
							Percentage reduction in rate of oxidation	Mutagenicity
Flavonols								
Quercetin	OH	OH	O	OH	OH	OH	96	***[c]
Rhamnetin	OCH_3	OH	O	OH	OH	OH	83	***
Fisetin	OH	H	O	OH	OH	OH	54	***
Kaempferol	OH	OH	O	OH	H	OH	82	***
3-Hydroxyflavone	H	H	O	OH	H	H	15	0
Rutin	OH	OH	O	O-rutinose	OH	OH	95	**[d]
Quercitrin	OH	OH	O	O-rhamnose	OH	OH	80	**
Hyperosid	OH	OH	O	O-galactose	OH	OH	84	*[e]
Robinin	O-rhamnose	OH	O	O-robinobiose	H	OH	23	**
Flavanonols								
Dihydroquercetin	OH	OH	O	H · OH	OH	OH	66	—
Dihydrofisetin	OH	H	O	OH · H	OH	OH	46	—
Flavanes								
d-Catechin	OH	OH	H_2	H · OH	OH	OH	51	0
l-Epicatechin	OH	OH	H_2	OH · H	OH	OH	36	—

Note: The percentage antioxidant activity of 10^{-3} *M* suspensions of different flavonoids in a 10^{-1}-*m* sodium phosphate buffer at pH 7.40 containing traces of iron, copper, and tin at 37°C in the presence of oxygen. The antioxidant activity is expressed as $\frac{(a - b) \times 100}{a}$, where a is ascorbic acid lost in a given time in buffer alone and b is ascorbic acid lost in the same time in the same buffer in the presence of a suspension of the flavonoid. Initial ascorbic acid concentration 19.6 μg/ml Analyses by dichloroindophenol photometric method. The couplets underlined are those believed to be responsible for chelation of the heavy metals. For details of method see Clemetson and Andersen (1966). It is evident

from this table that the compounds with a 3 hydroxyl, 4 carbonyl couplet in the gamma pyrone ring and those with a 3′,4′,catechol couplet in the B-ring have the best antioxidant activity. However, the choice of flavonoids for use in human nutrition is limited by the observations of Brown et al. (1977), Brown and Dietrich (1979), and Brown (1980), who showed that most flavonols (having the 3 hydroxyl, 4 carbonyl couplet) are mutagenic in the Ames test (Ames et al., 1973); moreover, Tamura et al. (1980) have shown that flavonol glycosides may become mutagenic after incubation with feces. It may be noted that D-catechin is a suitable indirect antioxidant for ascorbic acid which lacks mutagenic activity.

[a] 0 Indicates that the compound is nonmutagenic.
[b] — Indicates that no data are available concerning the mutagenicity or nonmutagenicity of this compound.
[c] *** Indicates that the compound is mutagenic.
[d] ** Indicates that the compound is a promutagen (can be converted into a mutagen in the bowel).
[e] * Indicates that the compound is probably a promutagen.

Table 2



A	B	C		Antioxidant activity	
+			Apigenin	7	
+			Acacetin	6	
+			Chrysoeriol	0	
+			Cosmetin	22	
+			Hesperetin	7	Mean 8%
+			Naringenin	3	
+			Hesperidin	2	
+			Naringin	14	
+			Neohesperidin	0	
+			Robinin	23	
+	+		Luteolin	36	
+	+		Eriodictyol	26	
+	+		Rutin	95	Mean 64%
+	+		Quercitrin	80	
+	+		Hyperosid	84	
+	+	+	Quercetin	96	Mean 89%
+	+	+	Rhamnetin	83	
+		+	Kaempferol	82	
	+	+	Fisetin	54	
		+	3-Hydroxyflavone	15	

Note: Here the flavones, flavanones, and flavonols are arranged according to the possible chelating couplets which they possess, and their antioxidant activities are shown. It seems that both the 3 hydroxyl, 4 carbonyl couplet of the gamma pyrone ring and the 3′,4′catechol couplet of the B-ring contribute antioxidant activity for ascorbic acid under the conditions of the experiment; (see Table 1) when other acidic hydroxyl groups are present to decrease the pK of the molecule, but the 4 carbonyl, 6 hydroxyl couplet seems to contribute little if any antioxidant activity.

From Clemetson, C. A. B. (1967), in *Symp. sui Bioflavonoidi*, Zambotti, V., Ed., Scuolo Grafiche Artigianelli Pavoniani, Milano, 584. With permission.

Clearly, the flavonols are very weak mutagens, perhaps because they are so insoluble, or else we have strong resistance to them, for we all eat them all the time. Indeed, it is not necessary and it would be fruitless to attempt to devise a diet without them.

Fortunately, as regards the subject at hand, we are left with D-catechin, the vitamin P or vitamin C_2 of Parrot et al.(1953), which is entirely nonmutagenic.

In future, therefore, we may predict that ascorbic acid will be available with a coating of D-catechin to chelate any cupric ions in the drinking water and prevent them from gaining access to the ascorbic acid core (Figure 9).

Although rutin, quercetin, and catechin are potent indirect antioxidants for ascorbic acid,

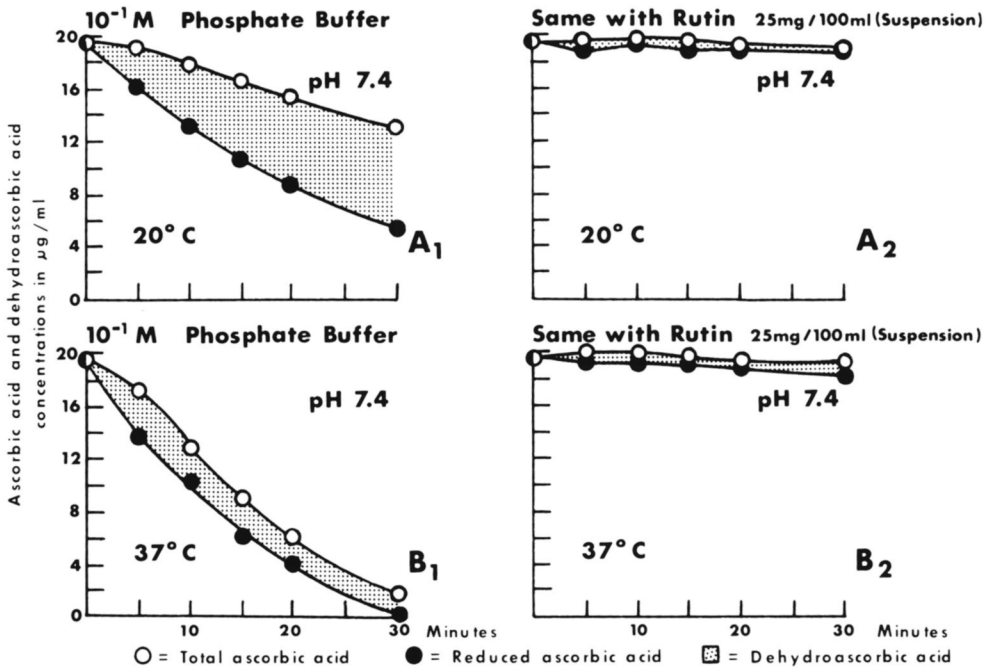


FIGURE 2. Showing the rapid oxidation and hydrolysis of ascorbic acid in 10^{-1}-*M* phosphate buffer containing traces of iron, tin, and copper at pH 7.40 in the presence of oxygen. A_1 at 20°C. B_1 at 37°C. It may be noted that dehydroascrobic acid is hydrolyzed (and its vitamin activity is lost) almost as fast as it is formed at 37°C. A_2 and B_2 show the marked antioxidant activity exerted by a suspension of rutin (25 mg/100 ml) in the same phosphate buffer under the same conditions. Analyses by the Hughes (1956) homocystein method. (From Clemetson, C. A. B. and Andersen, L. [1966], *Ann. N.Y. Acad. Sci.*, 136, 339. With permission.)

by virtue of chelating heavy metal catalysts, rutin and quercetin are also weak direct prooxidants for ascorbic acid, as shown by the decreased antioxidant activity which is observed when very high concentrations of these flavonoids are studied, and also when heavy metals are already chelated by proteins and amino acids, as shown in Table 3. This weak direct prooxidant acitivity is probably due to the catechol couplet of the B-ring, for it is evident in the decreased antioxidant activity shown by high concentrations of caffeic acid.

Thus, although we have not observed it, D-catechin may also exhibit a weak prooxidant activity in addition to its potent indirect antioxidant activity. It will therefore be wise to keep catechin and ascorbic acid apart until they are consumed; they can be separated by a layer of gelatin.

Parrot and Cotereau (1945) reported that a solution of epimers of D-catechin endowed with "vitamin P" activity acquires the property of an antivitamin after several days of exposure to air at room temperature. Moreover, this "anti-vitamin P" could be prepared by heating a solution of D-catechin, 2.5 parts/1000 for 1 h on a water bath and replacing the water lost with distilled water. It is therefore evident that heating and oxidation of some of these substances can result in loss of biological activity. Therefore, it will be necessary to keep catechin-coated ascorbic acid tablets or pills cool, dry, and sealed as long as possible.

Studying the reduction of dehydroascorbic acid to ascorbic acid by thiols in an anaerobic environment, Regnault-Roger et al. (1982) reached the conclusion that the flavan-3-ol configuration of factor C_2 compounds like *l*-epicatechin plays a specific role as a biological catalyst in this reaction, which was found not to be dependent on the redox potential alone. Rutin and quercetin were inactive in these studies.

It is not possible to include all the important papers on bioflavonoids in such a brief outline as this; a book on rutin alone, by Griffith et al. (1955) mentioned 803 works and

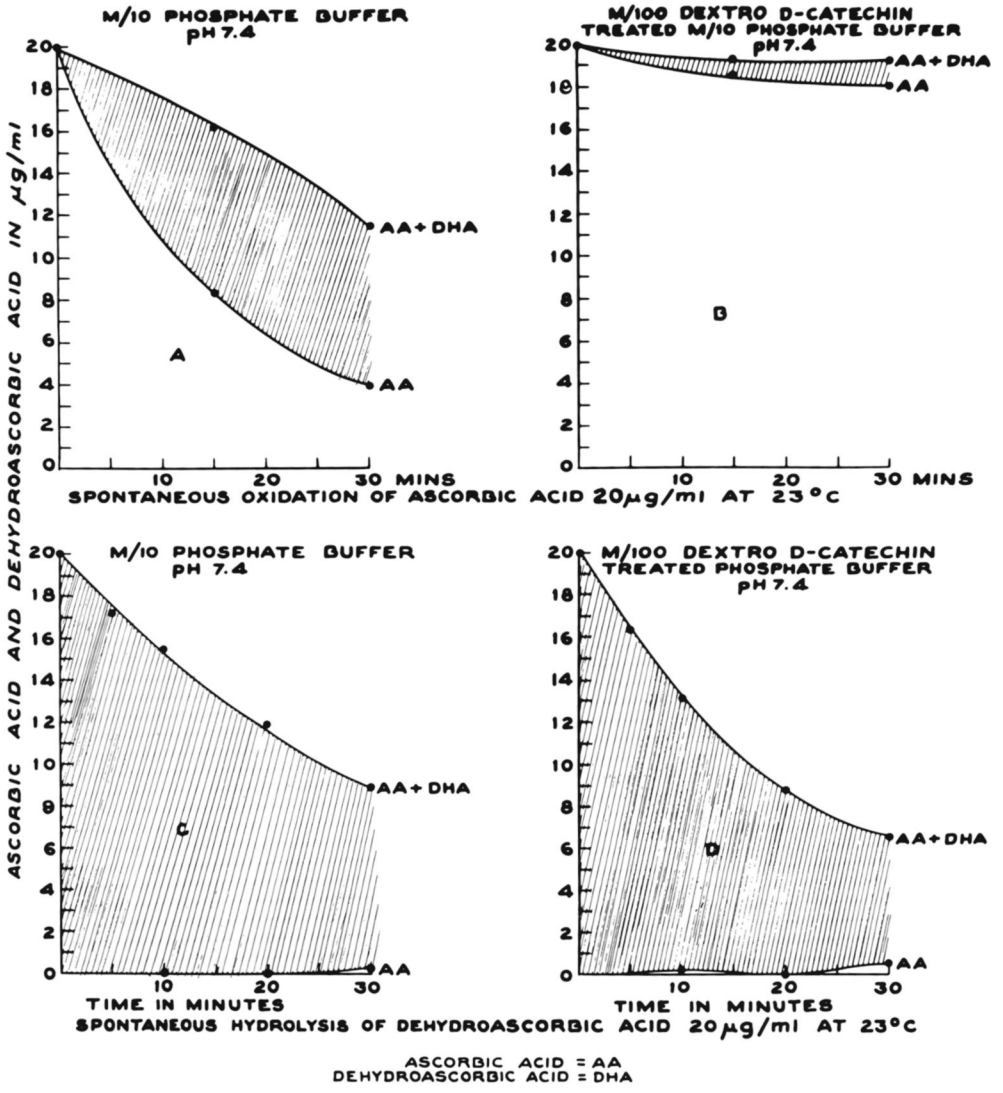


FIGURE 3 (A). Oxidation of ascorbic acid in *M*/10 sodium phosphate buffer at pH 7.4 and 23°C. Analyses by Hughes (1956) homocysteinemethod. Shaded area represents dehydroascorbic acid. (B) Same in phosphate buffer which had been preteated with (+)-catechin; a 10^{-2}-*M* suspension of catechin in phosphate buffer was shaken and filtered to remove most of the catechin before adding ascorbic acid at the beginning of the experiment. The antioxidant activity was just as good as if all the catechin were still present. This is undoubtedly because catechin acts by solid chelation of heavy metal catalysts which are inactivated just as well if they are chelated and precipitated as if they are chelated, filtered, and removed. (C and D) Pretreatment with (+)-catechin had no effect on the rate of hydrolysis of dehydroascorbic acid in phosphate buffer at pH 7.4 and 23°C. (From Clemetson, C. A. B. and Andersen, L. [1966], *Ann. N.Y. Acad. Sci.*, 136, 339. With permission.)

Böhm (1968) in a small book, *The Bioflavonoids*, cited 860 references. Extensive symposia on the physiology, pharmacology, and therapeutics of bioflavonoids have been edited by Miner (1955), Fairbairn (1959), Kursanov (1959), Pridham (1960), Zambotti (1967), and Parrot (1969) in the U.S., the U.K., Russia, the U.K., Italy, and France, respectively.

For the chemistry of bioflavonoids, the reader is referred to the book by Geissman (1962). For work on the estrogenic activity of the isoflavones, the reader may see Bradbury and White (1954), Bickoff (1968), a review by the Commonwealth Bureau of Pastures and Field Crops (1975), and an article by Clemetson et al.(1978).

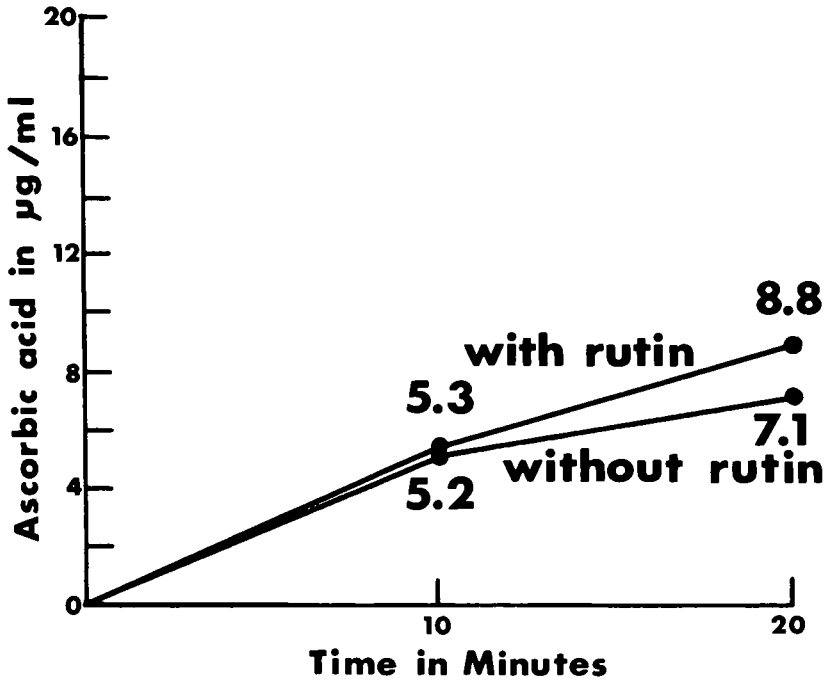


FIGURE 4. This study of the reduction of dehydroascorbic acid to ascorbic acid by reduced glutathione in phosphate buffer at pH 7.4 and 20°C is taken from Clemetson and Andersen (1966). There appears to be slight acceleration of the reduction in the presence of rutin, but the initial similarity of the rates of reduction, with and without rutin in the first 10 min, suggests that rutin may exert its effect by preserving ascorbic acid once formed, rather than potentiating its reduction. Initial concentration of dehydroascorbic acid, 19.6 µg/ml; reduced glutathione, 1 mg/ml; rutin, 0.25 mg/ml in suspension. (From Clemetson, C. A. B. and Andersen, L. [1966], *Ann. N.Y. Acad. Sci.*, 136, 339. With permission.)

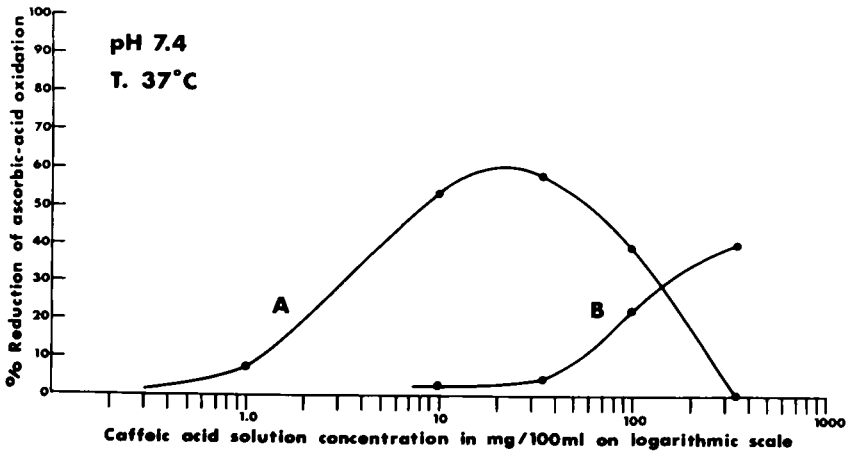


FIGURE 5. Percentage reduction of ascorbic acid oxidation by various concentrations of caffeic acid (A) in phosphate buffer with such traces of heavy metals, as were present in the salts from which the buffer was prepared, and (B) in the same buffer with 0.2 mg/100 ml of copper added as cupric sulfate. The added copper resulted in a 50-fold increase in the amount of caffeic acid needed to give the same antioxidant activity. (From Clemetson, C. A. B. and Anderson, L. [1966], *Ann. N.Y. Acad. Sci.*, 136, 339. With permission.)

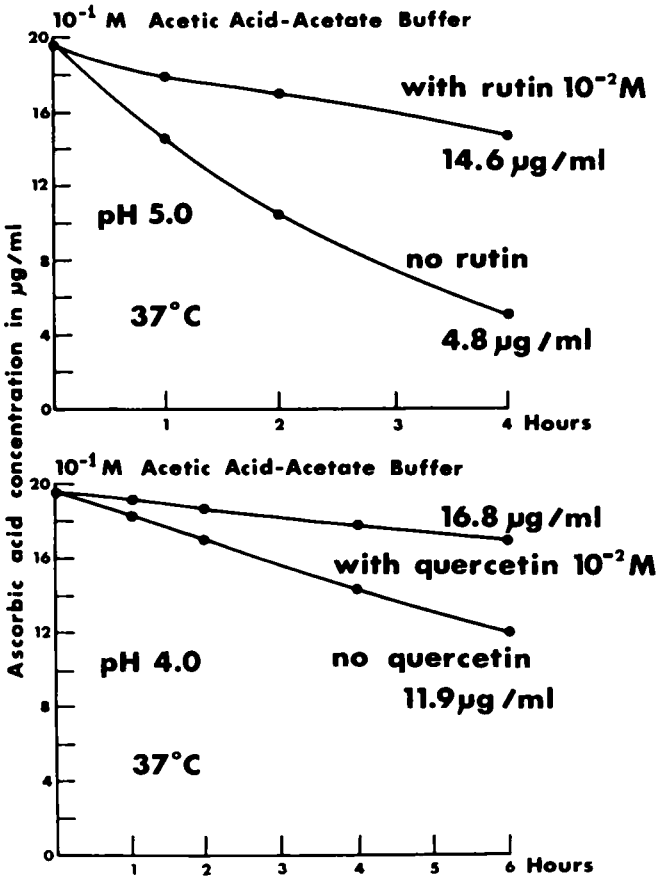


FIGURE 6. Rutin and quercetin in suspension also retard the slower oxidation of ascorbic acid in acetate buffers at pH 5.0 and 4.0, respectively. (From Clemetson, C. A. B. and Andersen, L. [1966], *Ann. N.Y. Acad. Sci.*, 136, 339. With permission.)

Observations concerning the structural requirements for certain biological activities of the flavonoids are shown in Figure 10.

Theories of the mechanism of action of flavonoids have included potentiation of epinephrine (*in vitro*) Clark and Geissman (1949), stimulation of adrenocorticotrophic hormone secretion by flavonoids with a catechol configuration in the B-ring, as judged by thymus involution in rats (? stress effect) Masri et al. (1959), and polymerization of mucopolysaccharides in the capillary sheath (hypothetical) Clemetson and Blair (1962). But it now seems clear that "indirect antioxidant activity for ascorbic acid" is the answer to the original conundrum, which was, "Why a crude yellow powder, containing ascorbic acid, was a more potent antiscorbutic than pure white crystalline ascorbic acid."

Indeed, the original observation of Cotereau et al.(1948) demonstrated that catechin caused a very marked increase in the storage of ascorbic acid in the tissues of the guinea pig (Table 4). This beneficial effect could be due to the antioxidant effect of catechin for ascorbic acid within the lumen of the gastrointestinal tract, preventing losses of ascorbic acid before absorption; or it could result from the removal of heavy metal catalysts, like copper, from the blood into the lumen of the bowel for excretion; or the two mechanisms may combine to account for the beneficial effects of catechin.

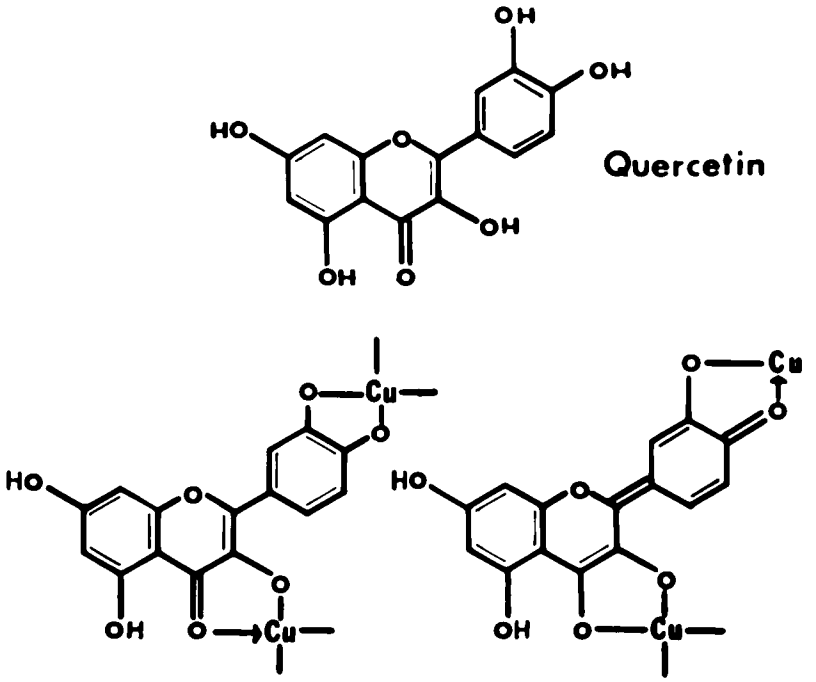


FIGURE 7. Chelation of copper by the 3′,4′catechol couplet of the B-ring and by the 3-hydroxyl, 4-carbonyl couplet of the gamma pyrone ring of quercetin, as envisaged by Clark and Geissman (1949). The participation of other chelatogenic groups in further combination with the copper atom is indicated but not shown.

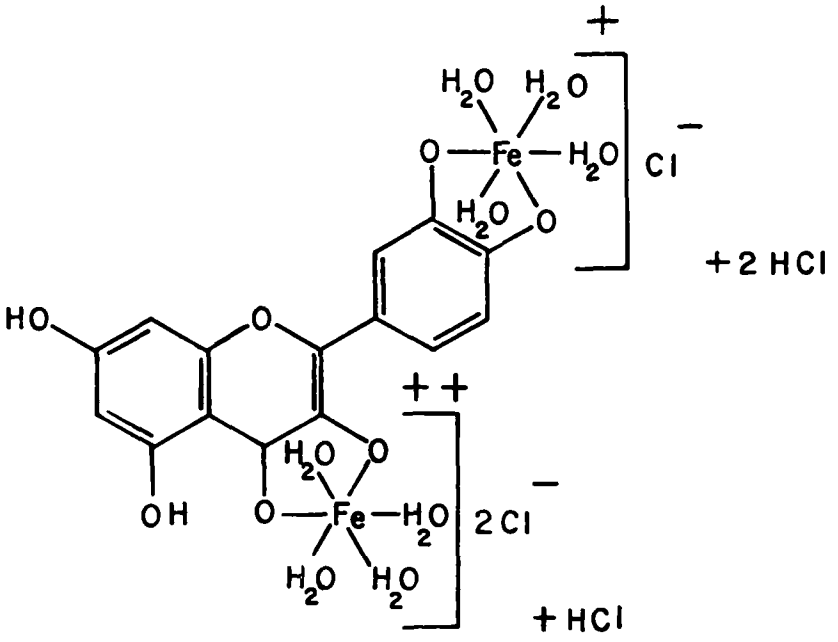


FIGURE 8. Removal of iron from solution by quercetin, catechin, rutin, or other chelating flavonoids can be demonstrated as follows. Prepare two flasks, each containing 4 mg of ferric chloride $6H_2O$ in 100 ml of distilled water. These are pale brown solutions. Add 500 mg of quercetin to one flask, shake for 3 min and filter the contents of both flasks. The quercetin-treated solution is now colorless and remains colorless on treatment with HCl and ammonium thiocyanate, whereas the untreated flask slowly develops a pink color due to iron. The removal of iron as a result of chelation by undissolved quercetin is envisaged as shown here.

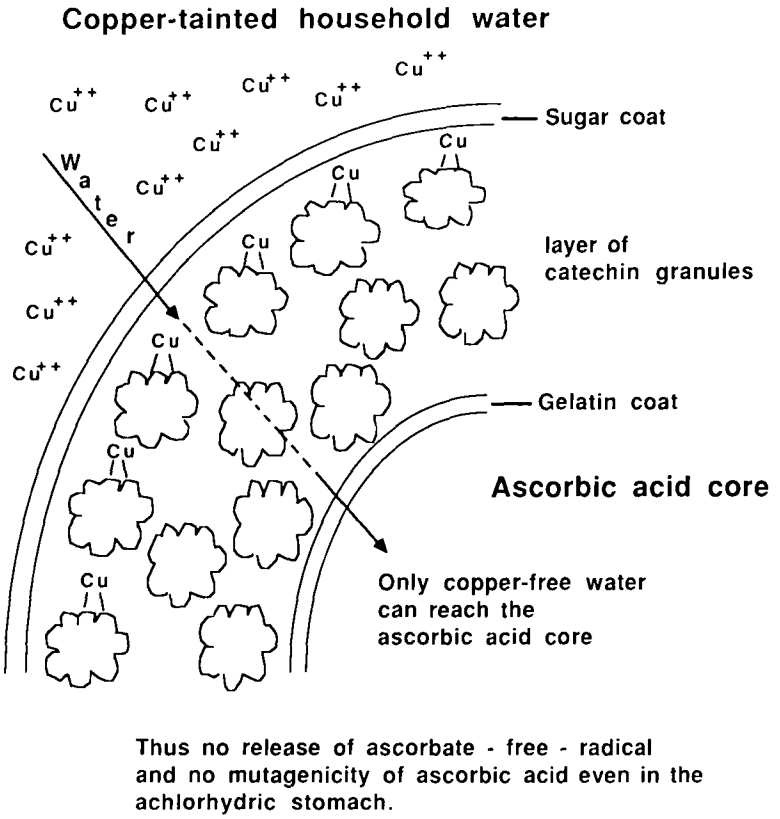


FIGURE 9. Proposal for catechin coating of ascorbic acid, so that cupric ions will be removed by surface chelation and will not be present in drinking water as it reaches the ascorbic acid core (U.S. and foreign patents pending).

Table 3
EFFECT OF RUTIN ON OXIDATION OF ASCORBIC ACID IN BLOOD-PLASMA EQUIVALENT

Blood plasma equivalent	Reduced ascorbic acid found when incubation arrested: 1 min	3 h
Phosphate buffer with albumin, globulin, and glycine	19.92 μg/ml	2.00
		1.58
		1.67
		Mean 1.75 μg/ml
Phosphate buffer with albumin, globulin, glycine, and rutin 10 mg/100 ml	19.25 μg/ml	1.08
		0.66
		0.50
		Mean 0.75 μg/ml

Note: Study of the effect of rutin 10 mg/100 ml on the oxidation of ascorbic acid in approximate protein and alpha-amino acid nitrogen equivalent of blood plasma, composed of crystalline bovine albumin (4 g), bovine globulins (2.5 g), and glycine (25 mg) in 100 ml of 10^{-1} *M* sodium phosphate buffer of pH 7.4 at 37°C. Initial ascorbic acid concentration 19.6 μg/ml in all eight tubes. Triplicate analysis of each of three samples with and three samples without rutin after 3h incubation showed no antioxidant activity by rutin and suggests that rutin is unlikely to possess antioxidant activity in concentrated protein solutions such as blood plasma where heavy metals are already bound. In fact, rutin may have had a very slight prooxidant effect under these conditions.

From Clemetson, C. A. B. and Andersen, L. (1966), *Ann. N.Y. Acad. Sci.*, 136, 339. With permission.

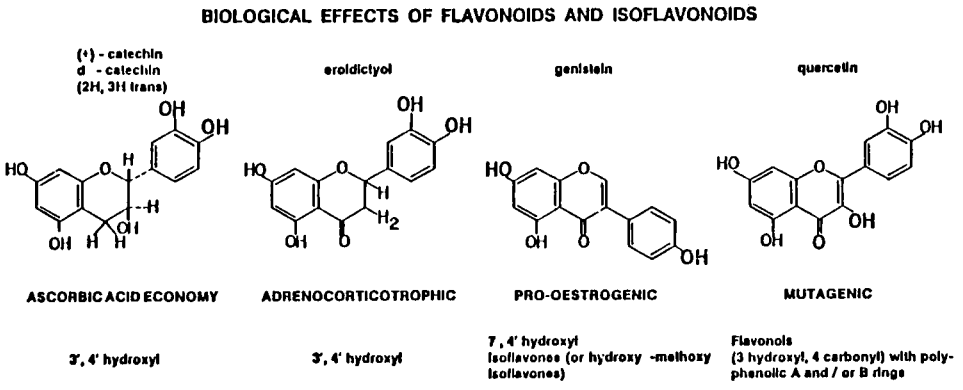


FIGURE 10. The compounds selected to illustrate the biological activities of the flavonoids are not mutually exclusive, as catechin, eriodictyol, and quercetin all possess adrenocorticotrophic activity and indirect antioxidant activity for ascorbic acid by virtue of having a 3′,4′ catechol couplet in the B ring. Moreover, quercetin has an even greater antioxidant activity because the acidic hydroxyl group at position 3, adjacent to the carbonyl group of the gamma pyrone ring, forms another chelatogenic couplet; however, quercetin is not a suitable nutritional supplement because of its mutagenicity.

Table 4
ASCORBIC ACID LEVELS IN GUINEA PIG TISSUES (mg/100 g)[a]

		Liver (n)	Spleen (n)	Kidneys (n)	Adrenals (n)
(a)	Basic vitamin C-deficient diet	1.9 (5)	3.4 (3)	3.8 (5)	6.7 (3)
(b)	Basic diet plus *l*-ascorbic acid 10 mg/animal/day	3.4 (3)	6.2 (2)	2.0 (5)	<5.3 (3)
(c)	Basic diet plus *d*-catechin 1 mg/animal/day	2.9 (1)	3.8 (5)	1.4 (5)	10.2 (5)
(d)	Basic diet plus ascorbic acid 10 mg and *d*-catechin 1 mg/animal/day	20.0 (5)	18.2 (4)	20.3 (5)	33.5 (5)

Note: The table shows the results of analysis of tissues from guinea pigs which had been fed for 3 weeks (a) on a scorbutogenic diet, (b) with a supplment of *l*-ascorbic acid, 10 mg daily, (c) with a supplement of *d*-catechin, 1 mg daily, or (d) with supplements of both *l*-ascorbic acid 10 mg and *d*-catechin, 1 mg daily. The ascorbic acid analyses were performed by the dichloroindophenol method of Tillmans. The catechin was described as being a mixture of epimers of *d*-catechin; alone it had little effect, but when given with ascorbic acid, it caused a three- to six-fold increase in the storage of ascorbic acid in the tissues of the guinea pigs.

[a] Data from the work of Cotereau et al. (1948).

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Chapter 12

DIETARY PROTEIN

I. QUANTITY OF PROTEIN

Chatterjee et al.(1971, 1975a) have found that the tissue ascorbic acid levels of guinea pigs fed on a high-cereal diet are markedly increased by supplementation with protein. They fed diets containing 0, 9, 25, and 60% of casein. Increasing amounts of casein, added as supplements to the cereal diet, caused increased body and organ weight and also increased storage of ascorbic acid in the liver, kidneys, brain, spleen, and adrenals, until a maximum was reached with 25% casein. This diet caused a more than twofold increase in the liver ascorbic acid stores over those found in guinea pigs fed the high-cereal diet without casein, as shown in Table 1.

Chatterjee et al. (1971) also studied the catabolism of L-ascorbic acid by the livers of guinea pigs fed on these various diets, as shown in Table 2. They found that there was a slight but statistically significant reduction in the activity of the enzyme dehydroascorbatase, catalyzing the conversion of dehydroascorbate to 2,3-dioxo-L-gulonate in guinea pigs receiving high-casein diets. They therefore suggested that the increased storage of ascorbic acid might be partly due to decreased losses by catabolism; this is quite conceivable. A second possibility is that the increased storage of ascorbic acid may be due to protein binding of heavy metal catalysts which may be present in the drinking water or in the metal of the animal cages. Proteins and alpha-amino acids form unionized copper complexes and thereby retard the copper-catalyzed oxidation of ascorbic acid, as first reported by Barron et al. (1936), who studied the effects of ovalbumin and glycine on the oxygen consumption of ascorbic acid in the presence of copper, as shown in Figure 1. Mystkowski (1939, 1942) confirmed the antioxidant effects of serum globulin, casein, and amino acids for ascorbic acid, as measured by indophenol titration of residual ascorbic acid *in vitro*. Nevertheless, the presence of casein in milk does not prevent the copper-catalyzed loss of antiscorbutic activity from milk during pasteurization in copper vessels, which was first observed by Hess in 1922. This is explained by the work of Stotz et al. (1937). These workers observed that although albumin and gelatin both retard the copper-catalyzed oxidation of ascorbic acid, as measured by oxygen uptake in a Warburg apparatus, the copper-protein complexes themselves assume the properties of "enzymes", each developing an optimum activity pH, as shown in Figure 2, and being inactivated either by acid or by boiling for 5 min. Thus, protein binding of copper retards the oxidation of ascorbic acid, but does not arrest it.

II. QUALITY OF PROTEIN

Aldashev (1977), at the Institute of Nutrition at Alma-Ata in the U.S.S.R., studied the effects of the quality of dietary protein on the tissue ascorbic acid levels of guinea pigs. Comparing diets of equal protein content fed to guinea pigs receiving 25 mg of ascorbic acid daily, he found that a casein-fed group had higher tissue ascorbic acid levels than a group of wheat gluten-fed animals. The liver ascorbic acid concentration of the gluten-fed group was only 60% as high as that of the casein-fed animals. The kidney and adrenal ascorbic acid levels were also lower in the gluten-fed group. Lysine and tryptophan supplementation of the gluten diet did not improve the liver ascorbic acid storage, but supplements of methionine and tryptophan or methionine and lysine increased ascorbic acid storage to 70 and 79% of that in the casein group, respectively. Clearly, dietary methionine is important for ascorbic acid storage.

Table 1
EFFECT OF VARYING CONCENTRATIONS OF DIETARY CASEIN ON TISSUE ASCORBIC ACID CONTENT IN GUINEA PIGS[a]

Casein in diet (%)	Tissue ascorbic acid levels (mg/100 g wet tissue)				
	Liver	Kidney	Brain	Spleen	Adrenal gland
0	8.6 ± 0.70	5.4 ± 0.54	14.4 ± 1.78	19.4 ± 2.27	32.5 ± 4.65
	(3.2 ± 0.42)	(2.0 ± 0.21)	(5.0 ± 0.97)	(3.8 ± 0.62)	(7.3 ± 0.66)
9	12.7 ± 0.61[b]	8.5 ± 0.32[b]	19.2 ± 1.05[c]	25.6 ± 1.78[c]	53.5 ± 11.80[d]
	(8.7 ± 0.65)	(4.6 ± 0.53)	(8.6 ± 0.78)	(11.0 ± 0.83)	(19.0 ± 0.78)
25	21.0 ± 6.58[c]	12.2 ± 2.90[c]	26.5 ± 2.32[b]	41.3 ± 9.38[c]	74.2 ± 12.20[b]
	(3.9 ± 0.56)	(2.6 ± 0.61)	(10.3 ± 0.66)	(5.5 ± 0.61)	(14.9 ± 2.67)
60	15.5 ± 4.27[d]	9.3 ± 1.40[b]	26.1 ± 0.72[b]	37.5 ± 9.06[c]	69.2 ± 13.32[c]
	(5.1 ± 0.36)	(2.9 ± 0.08)	(13.2 ± 0.48)	(9.9 ± 0.59)	(17.9 ± 2.13)

[a] Values are the mean ± SD of experiments on four animals taken in each group. The values in parentheses denote data obtained from ascorbic acid-deficient guinea pigs under the same experimental conditions.
[b] Highly significant ($t > 5.96$ for 6 degrees of freedom).
[c] Significant ($0.01 < p < 0.001$).
[d] Mean values significantly different from the 0% casein-fed group ($0.02 < p < 0.01$).

From Chatterjee, G. C., Sasmal, D., Kar, N. C., Sasmal, N., Mazumder, P. K., Roy, R. K., and Banerjee, S. K. (1971), *Indian J. Biochem. Biophys.*, 8, 94. With permission.

Table 2
THE INFLUENCE OF VARYING LEVELS OF DIETARY CASEIN ON CATABOLISM OF L-ASCORBIC ACID BY LIVER DEHYDROASCORBATASE AND SYNTHESIS OF L-XYLULOSE BY KIDNEY L-GULONATE DEHYDROGENASE AND 3-OXO-L-GULONATE DECARBOXYLASE OF GUINEA PIGS[a]

Casein in diet (%)	2,3-Dioxo-gulonate formed from dehydroascorbate (μmol/mg protein)		L-Xylulose formed from sodium L-gulonate (μmol/mg protein)	
	Control	Ascorbic acid deficient	Control	Ascorbic acid deficient
0	0.44 ± 0.042	0.26 ± 0.042[b]	0.25 ± 0.012	0.34 ± 0.037
9	0.49 ± 0.029[c]	0.33 ± 0.039[b]	0.43 ± 0.095[d]	0.43 ± 0.044[d]
25	0.38 ± 0.086[e]	0.27 ± 0.015[b]	0.57 ± 0.094[e]	0.54 ± 0.036[d]
60	0.37 ± 0.014[e]	0.20 ± 0.012[b]	0.55 ± 0.030[e]	0.54 ± 0.044[d]

Note: The activity of liver dehydroascorbatase catalyzing the conversion of dehydroascorbate to 2,3-dioxo-L-gulonate was found to be significantly decreased in guinea pigs on high-protein diets.

[a] Values are the mean ± SD of experiments on four animals taken in each group.
[b] Significantly different from the corresponding control group ($p < 0.001$, < 0.001, < 0.02, and < 0.001, respectively).
[c] Mean values significantly different from the 0% casein-fed group ($p < 0.05$ and < 0.01, respectively).
[d] Significantly different from the 0% casein-fed group ($p < 0.001$).
[e] Mean values significantly different from the 9% casein-fed group ($p < 0.001$ and < 0.05, respectively).

From Chatterjee, G. C., Sasmal, D., Kar, N. C., Sasmal, N., Mazumder, P. K., Roy, R. K., and Banerjee, S. K. (1971), *Indian J. Biochem. Biophys.*, 8, 94. With permission.

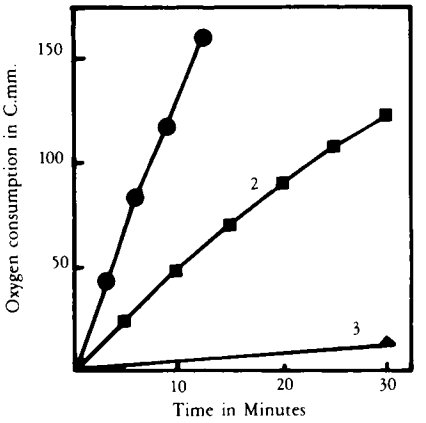

FIGURE 1. The effect of proteins and amino acids on the oxidation of ascorbic acid by $CuCl_2$ (0.0002 m*M*). Amount of ascorbic acid, 0.02 m*M*; pH, 6.34; temperature, 25°C. Curve 1 represents phosphate buffer + $CuCl_2$; Curve 2, phosphate buffer + $CuCl_2$ + glycine (0.1 *M*/l); curve 3, phosphate buffer + $CuCl_2$ + ovalbumin (6%). Both glycine and ovalbumin retard the rate of oxygen uptake by ascorbic acid in the presence of copper, as measured using Warburg vessels attached to Barcroft manometers. (From Barron, E. S. G., Barron, A. G., and Klemperer, F. [1936], *J. Biol. Chem.*, 116, 563. With permission.)

Not only were the tissue ascorbic acid levels lower in the guinea pigs on the gluten than on the casein-containing diet, but also Aldashev detected dehydroascorbic + diketogulonic acids much more frequently (74 to 80%) in the animals receiving the unsupplemented wheat gluten diet than in those on the casein diet (10 to 25%), indicating an oxidative disturbance of ascorbic acid metabolism in the gluten-fed animals.

However, similar experiments on guinea pigs receiving only 2 mg of ascorbic acid a day showed all groups to have low tissue ascorbate levels and did not demonstrate the same effect of protein quality. Aldashev concluded that when vitamin C intake is marginal, deficiency is less likely to appear if the diet contains high-quality protein.

In this context, it is interesting to note that the *in vitro* experiments of Stotz et al. (1937) led them to observe that, "albumin and gelatin in equal weights possessed considerably different inhibitory powers on copper catalysis of ascorbic acid oxidation. The lesser inhibitory power of gelatin is of interest in connection with its low sulfur content and non-coagulability."

The writer has compared the antioxidant effects of several proteins and amino acids in studies of the rate of loss of reduced ascorbic acid (AA)* by oxidation in phosphate buffer at pH 7.4, using albumin, globulin, casein, and certain individual amino acids, as shown in Figures 3 and 4.

Barron et al. (1936) confirmed the finding of others that the sulfhydryl tripeptide, reduced glutathione, is an even more potent inhibiting agent for the copper-catalyzed oxidation of ascorbic acid than ovalbumin or glycine, but they did not accept the explanation that this might be due to the reducing power of glutathione. They found complete inhibition of copper-catalyzed oxidation of ascorbic acid by glutathione when the glutathione/copper ratio exceeded 1 molecule of glutathione per atom of copper. They attributed the inhibitory effect entirely to the formation of the copper-glutathione complex described by Hopkins (1929). In fact, glutathione, like cysteine and other sulfhydryl amino acids, has both effects, being

* AA — ascorbic acid, reduced form.

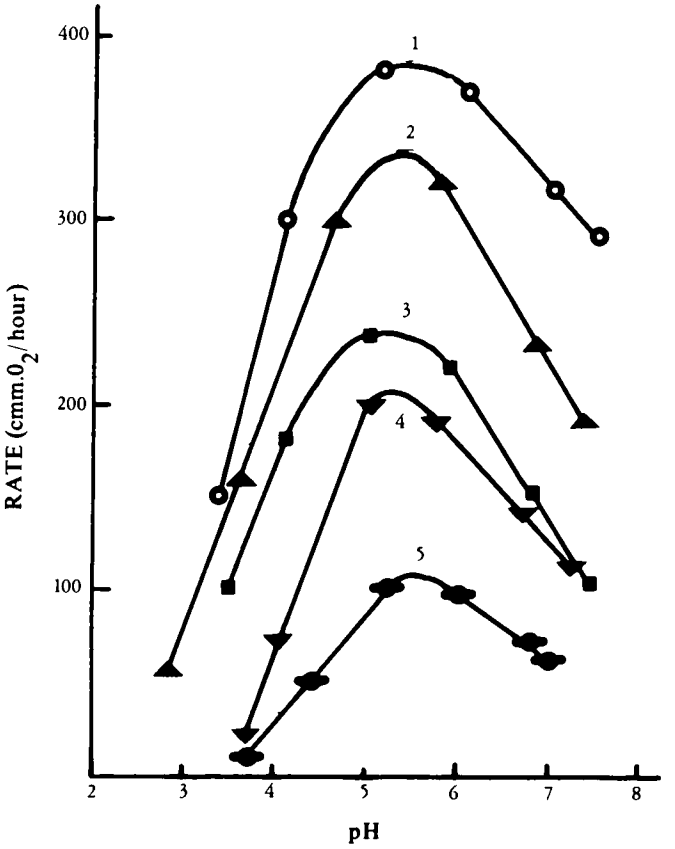

FIGURE 2. Optimum pH of ascorbate oxidase activities. Ascorbic acid, 0.01 *M*; temperature, 37°C; PO_4-citrate buffer; Cu in Cu-albumin, 3×10^{-4} m*M* (total); in Cu-gelatin, 6×10^{-5} m*M*; albumin and gelatin, 10 mg total. Curve 1, copper-albumin; Curve 2, squash oxidase; Curve 3, copper-gelatin; Curve 4, cauliflower juice; Curve 5, cabbage juice. (From Stotz, E., Harrer, C. J., and King, C. G. [1937], *J. Biol. Chem.*, 119, 511. With permission.)

able to bind and inactivate copper and in higher concentrations to reduce dehydroascorbic acid (DHAA) to AA as shown by Borsook et al. (1937). In this way a high-casein diet could act by preventing losses of ascorbic acid by copper-catalyzed oxidation and subsequent hydrolysis in food before ingestion, or in the gastrointestinal tract, just as do chelating flavonoids when they are present in the diet. Another possibility is that by supplying the essential amino acid methionine and other nonessential sulfur-containing amino acids, the high-casein diet may have increased the tissue concentrations of sulfhydryls like homocysteine, cysteine, and reduced glutathione, thereby aiding tissue storage of ascorbic acid in the reduced form. Hughes and Maton (1968) report that storage of ascorbic acid in red cells and other tissues seems to be achieved by a dehydroascorbate reductase and that glutathione appears to be the hydrogen donor in this system. Boscott and Cooke (1954) reported a beneficial effect of methionine on ascorbic acid metabolism in one patient, as evidenced by a marked reduction of parahydroxyphenylacetic acid excretion in the urine after methionine administration. Moreover, Localio et al. (1948) observed a pronounced acceleration of wound healing in protein-depleted rats which received a supplement of methionine and suggested that this was due to correction of a sulfhydryl deficiency. However, a 25% protein diet is

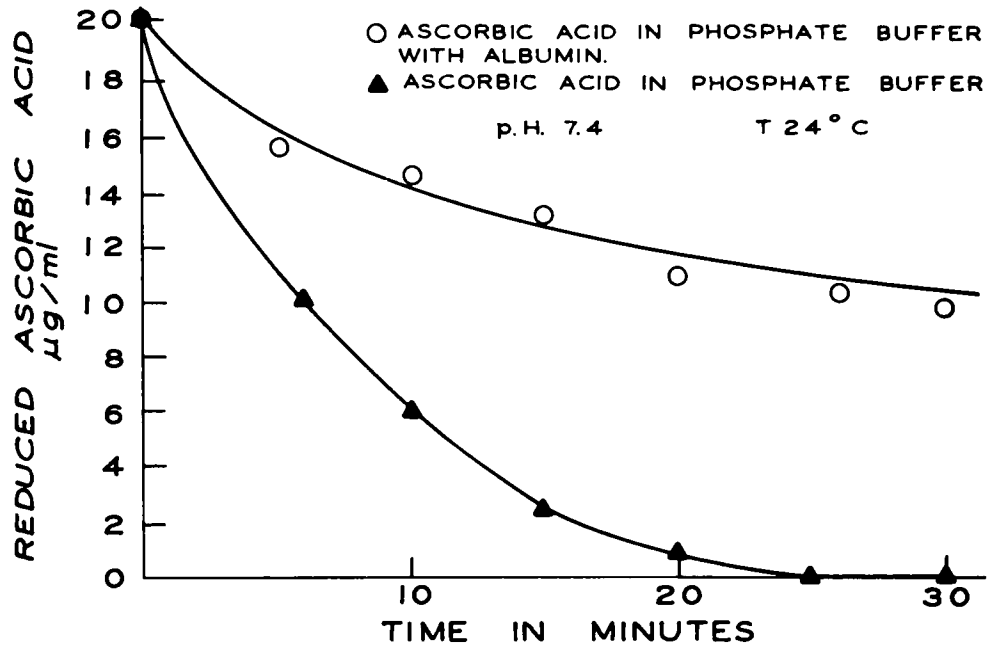

FIGURE 3. Study of the rate of loss of ascorbic acid (AA) by oxidation in 10^{-1} *M* sodium phosphate buffer, in the presence of oxygen, at pH 7.4, with and without albumin (5 mg/100 ml). Demineralized distilled water and acid-washed glassware were used throughout, so the only heavy metal catalysts were those present as contaminants in the reagent-grade monobasic and dibasic sodium phosphate salts from which the buffer was made. 2 ml of a fresh solution of ascorbic acid (1 mg/ml) at 5°C was added to 100 ml of phosphate buffer at 0 time, giving an initial ascorbic acid concentration of 1.96 mg/100 ml. Samples, 4 ml each, were removed and added to 6 ml of cold 3% HPO_3 at 5-min intervals, for 30 min, to arrest oxidation. Analysis of samples, with and without albumin, obtained immediately after addition of ascorbic acid, gave similar results, showing that no ascorbic acid had been adsorbed by the acid-protein precipitate. The highest concentration of albumin tested (1 g/100 ml) did show evidence of some slight adsorption of ascorbic acid by the protein precipitate, as noted under the albumin chart of Figure 4. Analyses were carried out by a modification of the 2,6-dichloroindophenol method of Roe (1954). The results shown in Figure 4 were built up from a series of such experiments, using different concentrations of proteins and amino acids.

abnormally high for a herbivore like the guinea pig, presumably supplying many times the daily requirement of all essential amino acids; so it would seem more likely that the casein was simply acting as an indirect antioxidant for ascorbic acid in the gastrointestinal tract, and that bioflavonoids would have performed the same function in a guinea pig left to graze in the wild.

III. RELATIVE DEFICIENCY OF PROTEIN

Nandi et al. (1973) and Chatterjee et al. (1975b) have found that large doses of ascorbic acid (AA) are toxic to guinea pigs on a high-cereal diet (composition in g per 100 g: wheat flour, 78; cane sugar, 10; peanut oil, 5; shark liver oil, 2; USP XVII salt mixture, 4; AOAC vitamin mixture, 1). At a dose of 60 mg AA per 100 grams of body weight per day, all of 114 guinea pigs died within 16 days, and at a dose of 30 mg AA per 100 grams, 54 guinea pigs died within 25 days; but, when the diet was fortified by replacement of 15 g of wheat flour with casein, the diet was no longer toxic.

Studying the cause of the toxicity of large doses of ascorbic acid in their guinea pig experiments, Chatterjee et al. (1975b) found that DHAA was markedly increased in the blood, urine, and liver of the animals on the high-cereal diet following high doses of ascorbic

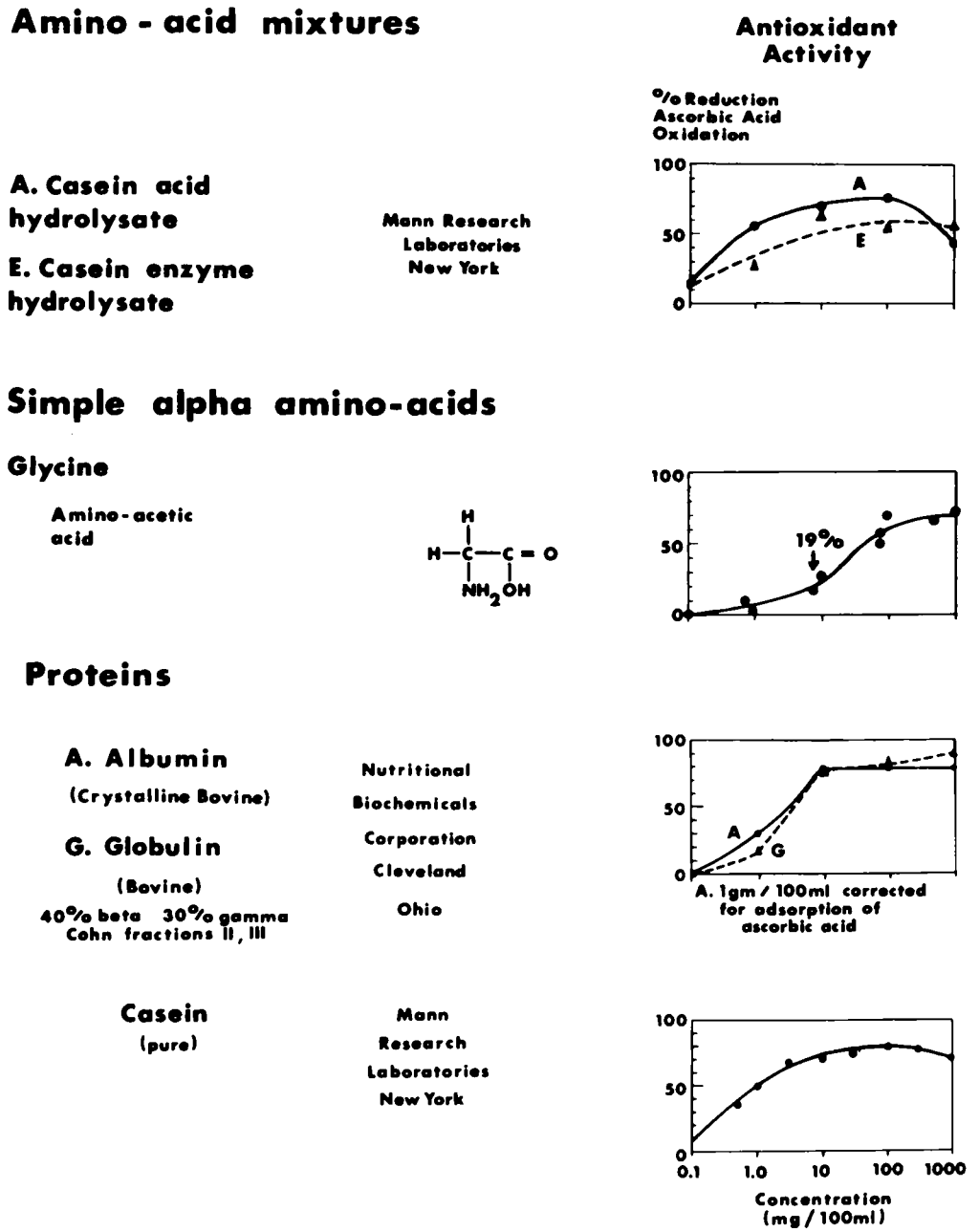

FIGURE 4. Proteins and simple alpha-amino acids, like glycine, act as indirect antioxidants for ascorbic acid in phosphate buffer at pH 7.4 and 37°C *in vitro* by chelating heavy metal catalysts, like copper, which are present as traces in the salts from which the buffer is made. Sulfhydryl amino acids, like cysteine, homocysteine, dimethyl cysteine, and the sulfhydryl tripeptide-reduced glutathione, also act as reducing agents which are capable of reversing the oxidation process when present in sufficient concentration. (From Clemetson, C. A. B. and Andersen, L. [1966], *Ann. N.Y. Acad. Sci.*, 136, 339. With permission.)

acid, as shown in Table 3. In this context, it is interesting to note, that Nandi et al. (1973) found that high doses of ascorbic acid, which are toxic to guinea pigs on high-cereal diets, are not toxic to guinea pigs fed on a diet of germinated Bengal gram and green grass. We may conjecture that such a natural diet includes sufficient quantities of ascorbic acid an-

Table 3
EFFECT OF A LARGE DOSE OF ASCORBIC ACID (AA), 100 mg/100 g BODY WEIGHT PER DAY, ON AA AND DEHYDROASCORBIC ACID (DHAA) LEVELS IN GUINEA PIGS FED A WHEAT DIET

	Ascorbic acid days		Dehydroascorbic acid days	
	Initial	**10th**	**Initial**	**10th**
Blood (mg%)	0.80±0.1	1.1±0.1	0.02±0.01	1.2±0.05
Urine (mg/24 h)	0.22±0.02	4.0±0.20	0.03±0.01	2.6±0.20
Liver (μg/g)	0.15±0.02	0.33±0.02	0.02±0.01	0.34±0.02

Note: Results are given from 12 guinea pigs, male 220 ± 10 g body weight. AA was estimated by dye titration and total ascorbic acid, reduced and oxidized forms (TAA), by the method of Bessey (1947). The values for DHAA were obtained by subtraction of AA from TAA. DHAA was identified by thin-layer chromatography and spectrophotometrically, as described by Dutta Gupta et al. (1972).

From Chatterjee, I. B., Majumder, A. K., Nandi, B. K., and Subramanian, N. (1975b), *Ann. N.Y. Acad. Sci.*, 258, 24. With permission.

tioxidants in the form of plant polyphenol bioflavonoids, catechins, and tannins to prevent the formation of DHAA. It would seem that plenty of proteins, bioflavonoids, or other chelating fibers, are necessary in the diet to prevent oxidation of ascorbic acid to its toxic dehydro form, and that this is especially important when large doses of ascorbic acid are consumed.

Clemetson (1962) found an increased plasma DHAA or ascorbone level, as shown in Figure 5, after oral administration of 1 g of ascorbic acid to a 44-year-old woman smoker, who had been on a low-ascorbic acid (and thus low-flavonoid) experimental diet (of 5 to 15 mg daily) for 1 month. This woman developed dermatitis of the face and swollen eyelids on the following day, and had such severe burning and itching of the face for the next 10 weeks, that she was nearly driven to distraction. She was not relieved by B-complex and other vitamins, nor by cortisone cream or antihistamines. There is no way of knowing whether the dermatitis had anything to do with the ascorbic acid loading, but it does remind one of the atrophic changes in the fur of rats observed following intravenous injection of DHAA, as described by Patterson and Mastin (1951) (see Table 4).

Patterson (1949, 1950) and Patterson and Mastin (1951) discovered that intravenous injection of DHAA, 100 mg/kg, in rats caused temporary diabetes mellitus, and large repeated injections caused permanent atrophy of the beta cells of the islets of Langerhans and, thus, permanent diabetes mellitus. It is thus of particular interest that Chatterjee et al. (1975b) found that as the blood DHAA levels increased in guinea pigs fed high doses of ascorbic acid, there was a concomitant increase in the blood sugar levels; the higher the doses of ascorbic acid, the higher were the blood DHAA levels and the higher were the blood sugar levels, as shown in Figure 6. When the administration of AA was discontinued, as the DHAA levels of the guinea pigs decreased, there was a fall in their blood sugar levels, as seen in Figure 7. When a large dose of ascorbic acid, 100 mg/100 g of body weight per day, was administered to guinea pigs on the casein-fortified wheat diet, neither the DHAA nor the blood sugar became elevated.

Chatterjee et al. (1975b) also found that feeding large doses of ascorbic acid to normal human volunteers on high-cereal diets led to high blood DHAA levels (Table 5). After giving AA, 4 g/man per day for 15 d, all of ten volunteers had markedly increased blood

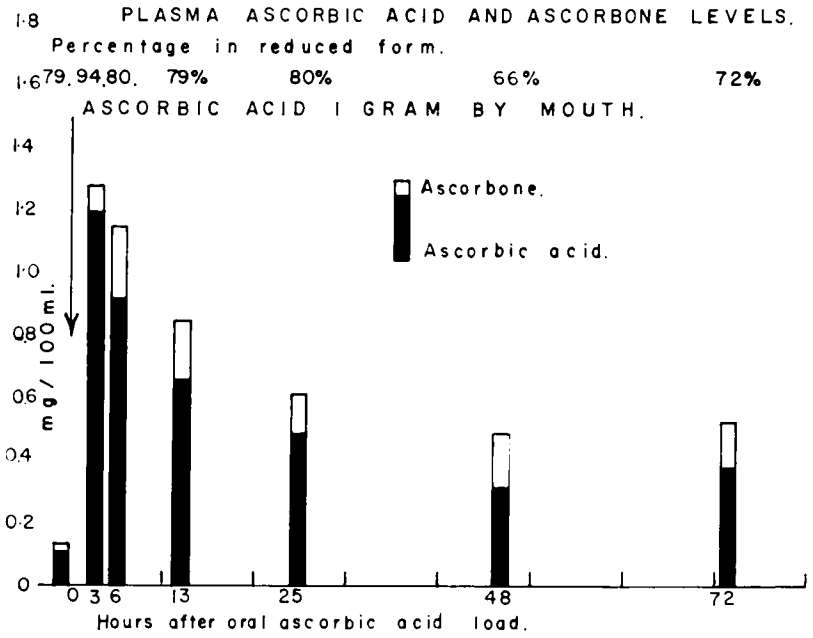

FIGURE 5. Unpublished work by Clemetson (1962) showing the results of analysis of blood plasma samples, before and after administration of 1 g of ascorbic acid by mouth. The subject was a 44-year-old woman who had been on a low-ascorbic acid diet, containing 5 to 15 mg of ascorbic acid a day, for 1 month. Analyses were carried out by the method of Stewart et al. (1953). The black bars represent ascorbic acid (AA); the white portions of the bars represent dehydroascorbic acid (DHAA) or ascorbone. It may be noted that there was a pronounced increase in the DHAA concentration in this woman's blood plasma which started 6 h after she had taken the ascorbic acid.

Table 4
EFFECTS OF INTRAVENOUS INJECTION OF DEHYDRO L-ASCORBIC ACID INTO RATS

5 mg/kg	Hypertension
200 mg/kg	Hyperactivity, salivation, lacrimation, collapse, dyspnea
Moderate doses	Temporary diabetes mellitus
Repeated large doses	Permanent atrophy of the islets of Langerhans
	Atrophic changes in fur
	Fatty liver
320 mg/kg (Single injection)	Death 50%

After Patterson, J. W. and Mastin, D. W. (1951), *Am. J. Physiol.*, 167, 119. With permission.

DHAA levels. The blood DHAA level also increased when the dosage of AA was 2 g/man per day for 20 d. A few of the volunteers had increased 2-h postprandial blood sugar levels, but both the blood DHAA and the blood sugar level returned to normal 10 d after discontinuing AA administration.

These authors also discovered markedly elevated DHAA levels in patients with diabetes mellitus. Their results are of great interest as regards the possible etiology of human diabetes mellitus and will be discussed in a later section of this book.

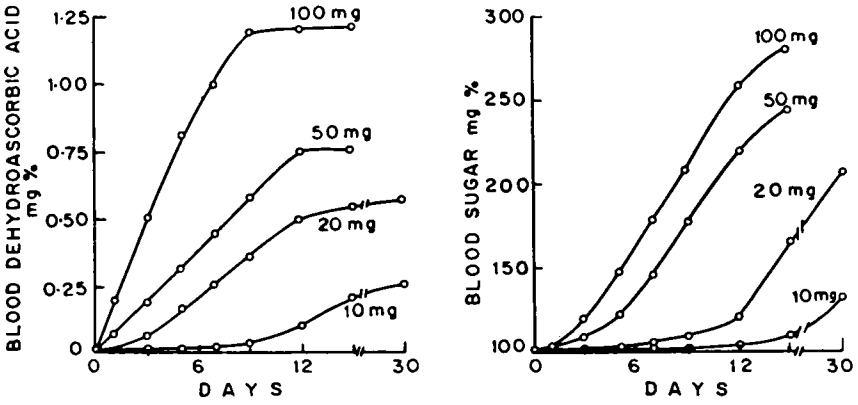

FIGURE 6. Effects of different doses of ascorbic acid on blood DHAA and sugar levels in guinea pigs fed high-cereal diets. The number on each represents the amount of ascorbic acid administered per 100 g body weight of guinea pig per day. Methods of analysis as in Table 3. (From Chatterjee, I. B., Majumder, A. K., Nandi, B. K., and Subramanian, N. [1975b], *Ann. N.Y. Acad. Sci.*, 258, 24. With permission.)

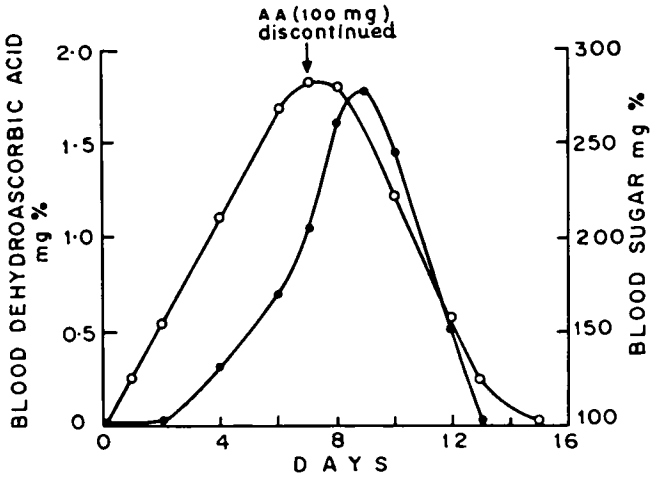

FIGURE 7. Blood DHAA and sugar levels in guinea pigs on high-cereal diet fed AA 100 mg/100 g body weight per day for 7 d. Arrow indicates discontinuation of AA administration. o–o DHAA; ●–● sugar. (From Chatterjee, I. B., Majumder, A. K., Nandi, B. K., and Subramanian, N. [1975b], *Ann. N.Y. Acad. Sci.*, 258, 24. With permission.)

Confirmation of the influence of dietary protein intake on ascorbic acid metabolism was provided by Enwonwu and Okolie (1983) in a study of infant monkeys (*M. nemestrina*). Comparing 20 and 2% casein diets, they found that protein deficiency caused poor brain retention of ascorbic acid (Table 6) and also an increase in the brain histamine level (Table 7).

Table 5
EFFECT OF LARGE DOSES OF ASCORBIC ACID (AA) ON BLOOD DEHYDROASCORBIC ACID (DHAA) LEVELS IN HUMAN VOLUNTEERS

Number[a] of volunteers	Dosage of AA g/man/day[b]	Period of intake (days)	AA[c] mg/100 ml 16th day	DHAA[d] mg/100 ml 16th day	Sugar[e] mg/100 ml 16th day
4	4	15	1.1±0.1	2.1±0.2	140±5
6	4	15	1.1±0.1	2.1±0.2	100±10
3	2	20	1.0±0.1	1.5±0.3	125±5
9	2	20	1.0±0.1	1.2±0.2	100±5

[a] The volunteers were men aged 22 to 40 years with no history of organic disease. The diet was 80 to 85% cereal (wheat and rice), 8 to 10% legumes, some vegetables, 4 to 5% fish, no milk; average caloric intake was 1700.

[b] 500 mg Redoxon® tablets (Roche India, Ltd.) 2 g b.i.d. or as a single dose, taken after meals.

[c] Initial blood AA values were in the rage of 0.55 to 0.90 mg/100 ml.

[d] Initial blood DHAA values were in the range of 0.01 to 0.03 mg/100 ml.

[e] Estimated 2 h after taking 75 g glucose by the method of Nelson and Somogyi. Initial postprandial sugar levels were in the range of 80 to 100 mg/100 ml.

From Chatterjee, I. B., Majumder, A. K., Nandi, B. K., and Subramanian, N. (1975b), *Ann. N.Y. Acad. Sci.*, 258, 24. With permission.

Table 6
THE EFFECTS OF PROTEIN OR ASCORBIC ACID DEFICIENCY ON THE BRAIN, LIVER, AND BLOOD ASCORBIC ACID LEVELS OF INFANT NONHUMAN PRIMATES (*M. NEMESTRINA*)

Dietary group	Initial body weight (kg)	Final body weight (kg)	Total ascorbic acid		
			Brain (mg/organ)	Liver (mg/organ)	Blood (mg/100 ml)
Control (3)	1.53 ± 0.54	2.31 ± 0.32	34.41 ± 0.81	24.55 ± 1.05	1.65 ± 0.06
Low protein (6)	1.74 ± 0.12	1.39 ± 0.09	23.86 ± 0.79 (69)	N.A.	0.93 ± 0.42 (56)
Low ascorbic acid (4)	1.96 ± 0.87	2.74 ± 0.16	14.78 ± 2.40 (43)	3.02 ± 0.68 (12)	0.48 ± 0.04 (29)

Note: There was a significant reduction in the brain ascorbic acid concentration ($p < 0.005$) in the monkeys fed a 2% casein diet when compared with those receiving 20% casein.

From Enwonwu, C. O. and Okolie, E. E. (1983), *J. Neurochem.*, 41, 230. With permission.

Table 7
EFFECT OF PROLONGED DIETARY PROTEIN OR ASCORBIC ACID DEFICIENCY ON LEVEL OF HISTAMINE IN MONKEY BRAIN

Dietary group	No. of animals	Brain histamine (ng/g)	% of control
Control	3	40.01 ± 2.47	—
Low protein	6	52.37 ± 2.64	131[a]
Low ascorbic acid	4	54.25 ± 3.79	136[a]

Note: Brain histamine levels were significantly elevated ($p < 0.01$)[a], not only in the infant monkeys on a low-ascorbic acid diet, but also in those on a low-protein diet.

From Enwonwu, C. O. and Okolie, E. E. (1983), *J. Neurochem.*, 41, 230. With permission.

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Chapter 13

HORMONE ADMINISTRATION: BIRTH CONTROL PILLS

I. INTRODUCTION

There are many ways in which hormones have been shown to affect ascorbic acid metabolism. The most notable and definite are the changes occurring in the adrenals and ovaries when stimulated by their respective pituitary hormones; the testes almost certainly respond in a similar manner, but have not been studied so extensively. These steroid hormone-producing glands are normally rich in ascorbic acid, but can lose 40 to 50% of their ascorbate stores within 1 h after stimulation.

The relationships of ACTH and cortisone to ascorbic acid metabolism are discussed more fully in the section on stress (Chapter 16 of this volume), and the effects of luteinizing hormone (LH) and human chorionic gonadotrophin (HCG) on ovarian ascorbic acid metabolism are to be found in more detail in the section on the menstrual cycle and ovulation (Chapter 7 of this volume). Likewise, the relationship between ascorbic acid metabolism and insulin is discussed in the section on diabetes mellitus (Chapter 2, Volume III); these hormones are discussed here insofar as they may affect ascorbic acid metabolism when they are used in the treatment of patients. This section is mainly devoted to the effects of ovarian hormones, particularly estrogens, on ascorbic acid metabolism, as these hormones are so widely used both for birth control and for the treatment of menopausal symptoms.

II. ADRENOCORTICOTROPHIC HORMONE (ACTH)

ACTH causes a rapid and profound fall in the ascorbic acid content of the adrenal glands, as shown by Sayers et al. (1944, 1949) in the rat. This effect is so well established that adrenal ascorbic acid depletion has been used in the assay of ACTH, by Munson and Koch (1946) and by many others; it is still considered as an indicator of stress.

Stewart et al. (1953a, b) found that ACTH causes a modest rise in the plasma levels of total and reduced ascorbic acid and a diminution of plasma dehydroascorbic acid (DHAA) in human subjects. Kark (1953) confirmed that the plasma reduced ascorbic acid increased by 11 to 28% after the injection of ACTH. He also observed a temporary increase in the urinary excretion of ascorbic acid while ACTH was given by intramuscular injection (100 mg daily) for 3 d, in three healthy young men. However, his results suggested that this urinary ascorbic acid loss was not sufficient to cause a negative ascorbic acid balance in patients on a dietary ascorbic acid intake of 160 mg daily.

Several writers in the past wondered whether repeated ACTH stimulation would not deplete the adrenal glands of ascorbic acid and if this might not cause adrenal exhaustion, or acute adrenal insufficiency, after prolonged overstimulation with ACTH. There were five reports of scurvy occurring in patients with rheumatoid arthritis after prolonged treatment with ACTH by Stefanini and Rosenthal (1950) and by Holley and McLester (1951); administration of large doses of ascorbic acid led to prompt regression of the lesions, and it was possible to continue ACTH therapy without further hemorrhagic manifestations, provided the ascorbic acid supplement was continued. Kark (1953), who studied this problem in depth, reported that the increase in urinary excretion of ascorbic acid after ACTH is only temporary; he expressed the belief that ACTH administration could not have been the sole cause of scurvy in these patients. Rheumatoid arthritis itself may be associated with a disturbance of ascorbic acid metabolism even before ACTH treatment is commenced.

Moreover, many studies in the past 30 years (Chapter 16 of this volume) have shown

that the adrenal glands of human beings, monkeys, and guinea pigs function normally and secrete normal amounts of cortisone and other steroids until there is loss of body weight from scurvy; they then secrete increased amounts of cortisone. Furthermore, vitamin C-deficient animals respond normally to ACTH until corticosteroid secretion becomes maximal due to the stress of scurvy and the animals are terminal, so it seems unlikely that the "adrenal exhaustion syndrome" is due to adrenal ascorbic acid deficiency. Nevertheless, the studies of Chretien and Garagusi (1973) have shown that oral ascorbic acid supplements were able to correct the abnormal leukocyte metabolism of six patients receiving long-term corticosteroid therapy, so it would seem wise to give ascorbic acid supplements to all patients being treated with ACTH.

III. GONADOTROPHINS

It is almost certain that gonadotrophins are responsible for the pronounced fall in the ascorbic acid content of the testes preceding puberty in rats, which was shown by Coste et al. (1953a).

Ovarian ascorbic acid is markedly reduced at the time of ovulation in the rat, as shown by Coste et al. (1953b) and by Foreman (1963), as discussed in Chapter 7 of this volume.

Injection of LH into young female rats pretreated with chorionic gonadotrophins causes a rapid, dose-related depression of ovarian ascorbic acid, as shown by Parlow (1958, 1961). Follicle-stimulating hormone (FSH) and ACTH had little or no effect on the ovarian ascorbic acid of pretreated rats according to McCann and Taliesnik (1960), but they found that vasopressin depletes ovarian ascorbic acid just like LH.

Human pituitary gonadotrophins (pergonal) and HCG are still used occasionally to induce ovulation in women who are infertile as a result of nonovulation, although chlomiphene (clomid) is much cheaper and gentler and often serves the purpose. These hormones, pergonal and HCG, have on occasion led to extensive ovarian hemorrhage requiring surgical intervention. This has always been attributed to overstimulation, as the hemorrhagic ovaries are markedly enlarged. The proper dose seems to vary widely from one woman to another and is therefore hard to predict, even with blood or urine estrogen monitoring. It is, however, conceivable that ovarian ascorbic acid depletion could play a role in these ovarian hemorrhages, so modest ascorbic acid supplementation would seem to be wise during artificial induction of ovulation. High dosage ascorbic acid would not be desirable, as Sharma et al. (1976) have shown that large doses of ascorbic acid inhibit copper acetate-induced ovulation in the rabbit (see Chapter 7 of this volume).

IV. GROWTH HORMONE

Growth hormone causes an increased excretion of ascorbic acid in rats, according to the work of Salomon and Stubbs (1951), but rats can synthesize ascorbic acid, so the meaning of this observation is not certain.

V. CORTISONE

The effects of cortisone on ascorbic acid metabolism were studied by Stewart et al. (1953a). These authors demonstrated that human blood plasma normally contains small amounts of DHAA, varying from less than 0.1 to 0.36 mg/100 ml, as well as larger amounts of ascorbic acid, and that DHAA completely disappears from the plasma, being reduced to ascorbic acid, within 30 min of the administration of cortisone, as shown in Figure 1. ACTH injection caused a reduction of the DHAA, associated with a slight increase in the plasma total ascorbic acid, as shown in Figure 2, presumably by releasing cortisone and ascorbic acid from the adrenals.

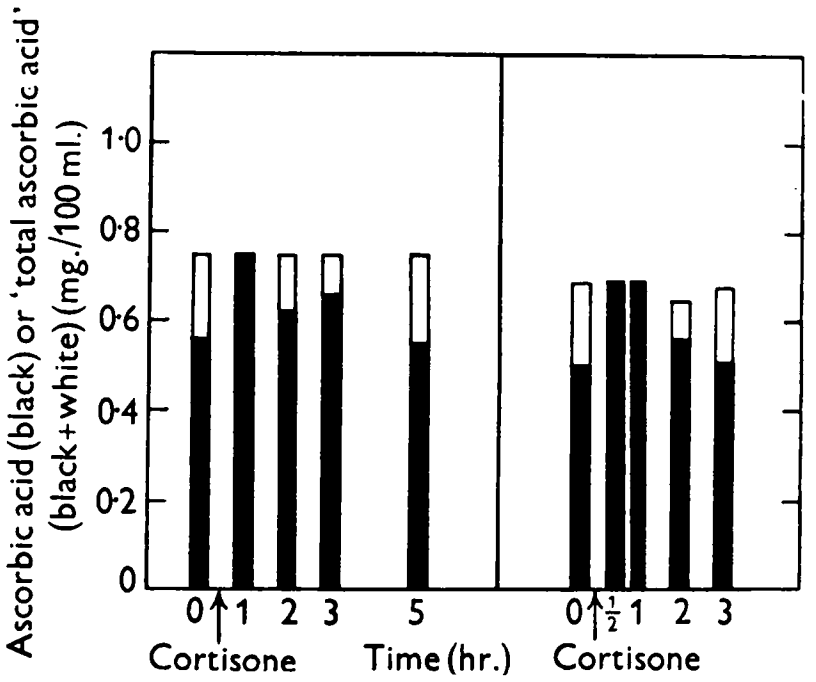

FIGURE 1. The effect on ascorbic acid (black columns) and dehydroascorbic acid (white columns) of human plasma, of oral administration of 350 mg of cortisone acetate, given immediately after withdrawal of the control sample. Two cases. (From Stewart, C. P., Horn, D. B., and Robson, J. S. [1953a], *Biochem. J.*, 53, 254. © 1953 The Biochemical Society, London. With permission.)

The disappearance of DHAA resulting from cortisone administration is consistent with the findings of Loxton and Le Vay (1953) who found a fall in the oxidation/reduction or redox potential of the peripheral tissues 1 to 2 h after giving cortisone orally.

In contrast to this effect on the blood plasma, the work of Crandon et al. (1961) showed a reduction of the buffy coat (leukocyte and platelet) ascorbic acid levels of patients following cortisone administration. This is particularly interesting because Chretien and Garagusi (1972) reported that corticosteroids cause several defects in polymorphonuclear neutrophil leukocyte function, including impaired reduction of nitroblue tetrazolium. Furthermore, these same workers (1973) observed that oral ascorbic acid supplements rapidly corrected this defect of leukocyte function in all of six patients under their care. They have therefore recommended that ascorbic acid supplements should be given to all patients receiving long-term steroid therapy.

VI. THYROXIN, THYROTROPHIC HORMONE

Mesonyi (1936) reported that the administration of thyroxin decreased the ascorbic acid content of the liver of guinea pigs and quoted Plaut and Bulow, and also Demole and Ippen, as having made similar observations in guinea pigs and rabbits.

Sure and Theis (1937, 1938) observed that rats develop a need for vitamin C when they are deficient in vitamin B_1. They also observed that a daily dose of thyroxin (0.1 mg) caused marked reductions in the ascorbic acid contents of the adrenals, thymus, liver, and kidneys of rats. Moreover, B vitamins provided only a partial protection against the ascorbate depletion in the thymus, liver, and kidneys.

Ray (1938) observed that the amount of ascorbic acid normally necessary to prevent scurvy

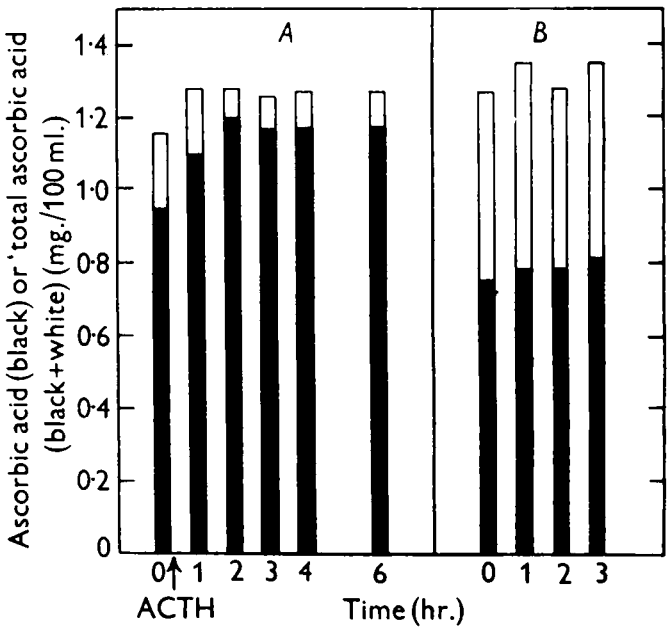

FIGURE 2. The ascorbic acid (black columns) and dehydroascorbic acid (white columns) of human plasma. (A) After intramuscular injection of 100 mg of ACTH (time measured from the time of ACTH injection), and (B) in successive hours without ACTH administration. (From Stewart, C. P., Horn, D. B., and Robson, J. S. [1953a], *Biochem. J.*, 53, 254. © 1953 The Biochemical Society, London. With permission.)

in guinea pigs was insufficient when administered with dessicated thyroid. Thaddea and Runne (1938) observed that the injection of thyroxin or thyrotrophic hormone decreased the ascorbic acid content of the liver and adrenals of rabbits, while removal of the thyroid gland increased the ascorbic acid concentration of these organs.

Ghosh (1948) observed a decrease in the ascorbic acid concentration in the liver, kidneys, and adrenals of guinea pigs following the administration of large doses of dessicated thyroid (65 mg/d) and quoted Eitel as having made similar observations.

However, in clinical use, thyroid preparations are usually given only in deficit replacement dosage, and any small inadvertent extra dosage causes an equivalent reduction of endogenous thyroxine production by suppressing thyrotrophic hormone release, so it is unlikely that thyroid administration in proper dosage will cause any interference with ascorbic acid metabolism.

Gemmill (1951) and Frieden (1952) reported that thyroxine accelerates oxygen uptake by ascorbic acid oxidase (a plant enzyme). Gemmill postulated that thyroxin may act in a similar fashion, as an accelerator of an ascorbate oxidase, in animal tissues.

Long and Miles (1950), studying tuberculin hypersensitivity in guinea pigs, noted that thyroxine and cortisone administration have opposite effects. Administration of thyroxine in moderate dosage, for 2 weeks, increased hypersensitivity, while daily injections of cortisone or ACTH diminished it. Reviewing the work of others, they noted that adrenalectomy increases sensitivity to anaphylactic shock, while ACTH or adrenal cortical extracts temporarily diminish the hypersensitivity. They pointed out that this hypersensitivity by deprivation and its decrease by administration of adrenal cortical hormones is matched, in the opposite sense, by the effect of thyroid secretions. Both tuberculin and anaphylactic hypersensitivity are decreased in thyroidectomized animals and are increased by thyroid hormone administration.

It is therefore interesting to note that cortisone and thyroxine seem to have opposite effects on ascorbic acid metabolism, the former causing reduction of DHAA to AA* and increasing blood AA levels, while the latter causes ascorbic acid depletion. This becomes especially pertinent in the light of recent studies which have shown that ascorbic acid plays a major role in histamine metabolism, both in guinea pigs and in human subjects, as discussed in Chapter 1, Volume III. Ascorbic acid aids the conversion of histamine to hydantoin-5-acetic acid. Moreover, it has been found in healthy human subjects that histamine begins to accumulate in human blood when the plasma ascorbic acid level falls below 1.0 mg/100ml; the blood histamine level is significantly elevated ($p < 0.001$) when the plasma AA level is below 0.7 mg/100 ml. Moreover, ascorbic acid has been found to be beneficial in various allergic phenomena and to decrease histamine-induced bronchospasm, etc. (Chapter 18 of this volume).

DesMarais (1956) observed that thyroidectomized rats were protected against cold by ascorbic acid only if thyroxine was also administered. It was therefore concluded that ascorbic acid is in some way involved with the activity of thyroxin.

Thaddea and Runne also conducted clinical studies of urinary ascorbic acid excretion by human subjects, which complemented their animal studies. They found that patients with hyperthyroidism demonstrated increased ascorbic acid utilization, and conversely patients with myxoedema had decreased ascorbate utilization. These writers therefore recommend that all patients with Graves' disease should be given ascorbic acid supplements.

VII. ADRENALINE (EPINEPHRINE)

Göbell and Krause (1941) observed that the injection of adrenaline lowered the content of reduced ascorbic acid (AA) in the blood of human volunteers (Table 1).

Long and Fry (1945) injected epinephrine subcutaneously into rats and observed a decrease in adrenal ascorbic acid and cholesterol levels; this effect was inhibited by hypophysectomy.

Bacchus and Toompas (1951) observed that an intraperitoneal injection of ascorbic acid prior to a subcutaneous injection of epinephrine in female rats prevented the histological reaction of the adrenal and the eosinopenia due to this stress.

Thus, ascorbic acid seemed to act as an inhibitor of epinephrine stress.

There were reports that epinephrine causes release of ascorbic acid from cellular elements, but Golden and Sargent (1952) could not confirm or deny this. They reported that adrenaline had no effect on the movement of ascorbic acid into or out of red blood cells *in vitro*.

VIII. INSULIN

There is a complex relationship between insulin and ascorbic; for ascorbic acid deficiency is known to decrease insulin output, and there is also evidence that injection of insulin causes a temporary decrease in the ascorbic acid level of the blood.

It was Sigal and King (1936) who first demonstrated that ascorbic acid deficiency causes a decrease in the glucose tolerance of guinea pigs; this has since been amply confirmed and is discussed in the section of this book which is devoted to diabetes mellitus (Chapter 2, Volume II). It is now well established that scurvy is associated with a decreased output of insulin by the beta cells of the islets of Langerhans of the pancreas.

It was Ralli and Sherry (1939) who first observed that injection of insulin caused a fall in the plasma level and in the urinary excretion of ascorbic acid in dogs.

Göbell and Krause (1941) observed that insulin injection caused a marked reduction in the blood ascorbic acid (AA) concentration of human volunteers, reaching the lowest levels 2 to $2^1/_2$ h later, depending on the dose. Even $^1/_2$ unit of insulin seemed to cause a reduction

* AA — ascorbic acid, reduced form.

Table 1
BLOOD ASCORBIC ACID (AA) LEVELS OF HUMAN SUBJECTS (mg/100ml) AFTER INJECTION OF (A) 1 ml AND (B) 0.3 ml OF ADRENALINE[a]

A.	0 time	1 h	2 h	3 h	4 h
	0.43	0.33	0.26	0.33	0.39
	0.59	0.36	0.33	0.43	0.49
	0.49	0.34	0.29	0.33	0.39
	0.82	0.73	0.49	0.73	
	0.73	0.66	0.39	0.66	
	0.66	0.63	0.43	0.66	
	0.43	0.33	0.23	0.39	
	0.66	0.49	0.26	0.66	
	0.49	0.33	0.29	0.59	

B.	0 time	1 h	2 h	3 h
	0.66	0.59	0.59	0.66
	0.49	0.43	0.33	0.53
	0.66	0.53	0.39	0.66
	0.76	0.66	0.43	0.73
	0.53	0.49	0.39	0.53
	0.66	0.53	0.39	0.59
	0.53	0.49	0.39	0.53

[a] One may presume that the adrenaline was 1:1000 dilution

From Göbell, O. and Krause, B. (1941), *Klin. Wochenschr.*, 20, 342. With permission.

of the blood ascorbic acid level; but intravenous injection of 4 units of insulin halved the blood ascorbic acid levels 2 h later, and 10 units reduced it even more, as shown in Table 2: after 3 h, the blood ascorbic acid level was returning towards its normal level, so these authors did not believe there was a loss of ascorbic acid; rather, they suggested that ascorbic acid moved from the blood into the tissues and later returned to the blood. Likewise, Haid (1941), studying the effects of high doses of insulin (70 to 90 units), given by subcutaneous injection as shock therapy to 11 schizophrenic patients, recorded a profound drop in the mean blood ascorbic acid level from 1.7 mg/100 ml to 0.7 mg/100 ml during insulin shock, returning to 1.3 mg/100 ml 6 h later. The reason for the high initial level was that these patients were all receiving vitamin supplements at a neurological institute. Haid also suggested that the ascorbic acid moves into the tissues under the influence of insulin and subsequently returns to the blood.

However, Ghosh (1942), studying guinea pigs, reported that insulin caused a reduction in the ascorbic acid concentration of the liver, kidneys and adrenals.

Sherry and Ralli (1948) made a detailed study of the effects of insulin on ascorbic acid metabolism. They found that there was a prompt fall in the plasma and urinary ascorbic acid levels of man, dog, rat, and guinea pig following injection of insulin; the whole blood ascorbic acid was also reduced. The red blood cell ascorbic acid was not changed, but there was an increase in the white blood cell and platelet (buffy coat) ascorbic acid. They concluded that insulin causes a transient shift of ascorbic acid from blood plasma to the tissues. The work of Cox et al. (1974) also suggested a redistribution of ascorbic acid following insulin administration in man.

Table 2
BLOOD ASCORBIC ACID (AA) LEVELS (mg/100 ml) AFTER INTRAVENOUS INJECTION OF INSULIN (A) 10 UNITS, (B) 4 UNITS, AND (C) 1/2 UNIT IN HUMAN VOLUNTEERS

A.	0 time	30 min	60 min	100 min	150 min	210 min
	0.99	0.83	0.83	0.49	0.33	0.66
	0.66	0.59	0.49	0.43	0.29	0.53

B.	0 time	1 h	2 h	3 h
	0.66	0.53	0.39	0.49
	0.66	0.49	0.33	0.49
	0.59	0.43	0.33	0.53
	0.69	0.43	0.36	0.56

C.	0 time	1 h	2 h	3 h
	0.66	0.63	0.59	0.66
	0.69	0.66	0.62	0.66
	0.83	0.79	0.69	0.79
	0.59	0.58	0.56	0.59

From Göbell, O. and Krause, B. (1941), *Klin. Wochenschr.*, 20, 342. With permission.

We may conjecture that insulin may accelerate the phosphorylation of NAD to NADP. In the presence of glutathione reductase this will provide the energy to convert oxidized glutathione (GSSG) to its reduced form (GSH); such thiolation would promote storage of ascorbic acid, entering the cells as DHAA, being reduced to AA by GSH inside the cells, and being stored there as such. Indeed, Mann (1974) has postulated that insulin is necessary for the transport of ascorbic acid into cells and tissues and that this transport mechanism becomes impaired with age. He drew an analogy between the endothelial damage of scurvy and the more localized microangiopathy of diabetes mellitus. This theory of Mann was expanded when Mann and Newton (1975) showed that a number of sugars, including D-glucose, D-mannose, and D-xylose strongly inhibited the uptake of DHAA, the transportable form of ascorbic acid, into human red blood cells. Moreover, Sarji et al. (1979) found low ascorbic acid levels in the blood platelets of patients with diabetes mellitus.

Subsequently, Verlangieri and Sestito (1981) demonstrated that insulin caused a highly significant ($p < 0.005$) 2.5-fold increase in the transport of ^{14}C-labeled ascorbic acid into cultured fetal bovine heart endothelial cells. These workers also found that D-glucose, 180 mg/100 ml, caused a fivefold decrease in ascorbate uptake by these cells. Further substantiation was provided by the findings of Bigley et al. (1982) and of Chen et al. (1983) who showed that glucose impairs ascorbic acid uptake by leukocytes (Chapter 2, Volume II). Moser and Weber (1984) found that glucose and DHAA enter human neutrophils by the same pathway; indeed, glucose impairs DHAA uptake by competitive inhibition and insulin facilitates DHAA uptake. Bigley has also suggested that the inhibition by glucose of DHAA uptake may contribute to the high plasma DHAA concentrations observed in diabetic subjects by Chatterjee et al. (1975) and by Som et al. (1981).

It seems highly probable that the microangiopathy of diabetes, the atherosclerosis of diabetes, the decreased resistance to infection by diabetics, and their predisposition to deep-vein thrombosis may all be related to ascorbic acid deficiency in the endothelium of the capillaries, in the endothelium of the arteries, in the leukocytes, and in the platelets, re-

spectively, and that insulin therapy, caloric restriction, and ascorbic acid supplements will all play important roles in the treatment of these patients

IX. ESTROGENS: BIRTH CONTROL PILLS

Several investigators, Mesonyi (1936), di Martini et al. (1950), Leathem (1959), Clemetson (1968), Saroja et al. (1971), and David and Kovaks (1967) have studied the effects of estrogen administration on the blood and tissue ascorbic acid levels of intact or ovariectomized female animals and have reported appreciable reductions in their liver, adrenal, uterine, plasma, and blood vessel ascorbic acid concentrations (see Table 3 and Figure 3). Clearly the adrenal ascorbic acid depletion could be a "stress" effect, mediated by ACTH, but other tissues also develop a reduction of ascorbate concentration after estrogen administration. Sutton et al. (1942) showed that injection of diethylstilbestrol into female rats produced an increased excretion of vitamin C. Selkurt et al. (1943) found that estradiol benzoate caused increased urinary excretion of ascorbic acid due to decreased tubular reabsorption in female dogs. Subsequent studies by Kalesh et al. (1971), Rivers and Devine (1972), Briggs and Briggs (1972), and McLeroy and Schendel (1973) have shown a disturbance of ascorbic acid metabolism in women taking the old high-dose combined estrogen and progestagen) oral contraceptive pills: these women were found to have depressed plasma, platelet, and white blood cell ascorbic acid levels. McLeroy and Schendel studied 126 women; 63 of them had taken oral contraceptives for at least 1 year, and 63 served as controls. The two groups were well matched for age and body weight and had comparable dietary ascorbic acid intakes, estimated to be 86 ± 45 mg and 84 ± 48 mg, respectively. The group taking oral contraceptives had significantly lower leukocyte ascorbic acid levels — 19.0 ± 6.6 vs. 25.7 ± 14.5 mg/100 g: there are good reasons for believing that this abnormality of ascorbic acid metabolism is due to the estrogenic component of the pills. di Martini et al. (1950), studying sexually mature female albino rats, observed that estradiol injections caused marked reductions of liver and adrenal ascorbic acid levels, while progesterone 0.5 mg daily for 5, 10, or 15 d did not affect the ascorbic acid levels of these tissues.

Leathem (1959), studying immature female mice, found that estradiol decreased the ascorbic acid concentration of the uterus from 54 to 12 mg/100 g, while progesterone injections had no significant effect (54 to 51 mg/100 g). Similarly, Briggs and Briggs (1972, 1973) studied the ascorbic acid levels in women using oral and depot progestins and also in women using combined estrogen-progestagen (E + P) contraceptives, as well as older women receiving estrogen (E) therapy. The women using the oral progestins (norethisterone, 0.35 mg daily) and depot progestin (medroxyprogesterone acetate, 150 mg every 3 months) had leukocyte and plasma ascorbic acid levels similar to those of controls, while women taking combined oral contraceptives (E + P) and those taking conjugated equine estrogens (E) (premarin®, 0.625 mg daily) had lower plasma, leukocyte, and platelet ascorbic acid levels than the controls.

The lower ascorbate levels of the older women taking Premarin®, a mild estrogen, may have been due to the older age of these women, and not to the estrogen, but the other groups were of comparable ages to one another and only those using E + P contraceptives had decreased ascorbic acid levels: those taking progestins alone did not.

Harris et al. (1973) reported decreased urinary excretion of ascorbic acid in women taking these oral contraceptive pills and suggested that ascorbic acid is metabolized more quickly in women taking oral contraceptives; but Rivers (1975) expressed his belief that the depression of plasma and leukocyte ascorbic acid levels in oral contraceptive users may be due to a redistribution of ascorbic acid.

Smith et al. (1975), Prasad et al. (1975), and Horwitt et al. (1975) did not observe any difference between the plasma ascorbic acid levels of "combined oral contraceptive" users

Table 3
THE PERCENTAGE CHANGES IN THE ASCORBIC ACID CONCENTRATIONS OF BLOOD VESSELS, PLASMA, AND ADRENALS AND PLASMA COPPER IN GUINEA PIGS FOLLOWING 10 TO 14 d OF TREATMENT BY DAILY INTRAMUSCULAR INJECTION OF ETHINYL ESTRADIOL-3-METHYL ETHER

Results Expressed as Percentages of Respective Control Means

		Blood vessel ascorbic acid		Plasma ascorbic acid		Plasma copper		Adrenal ascorbic acid	
		Control	Mestranol	Control	Mestranol	Control	Mestranol	Control	Mestranol
Experiment #I	Mean	100.0	55.20	100.0	69.9	100.0	101.50	100.0	86.7
(guinea pigs injected Monday	(n)	(4)[a]	(4)[a]	(8)	(7)	(12)	(12)	(13)	(13)
through Friday only; total 10	±SD	±35.7	±9.4	±25.7	±17.0	±34.4	±27.8	±10.7	±12.1
injections)									
Experiment #II	Mean	100.0	73.8	100.0	64.7	100.0	119.7	—	—
(guinea pigs injected 7 d of the	(n)	(4)[a]	(4)[a]	(9)	(7)	(7)	(5)	—	—
week; total 14 injections)	±SD	±39.9	±12.9	±32.1	±14.3	±14.5	±20.0	—	—
Experiment #III	Mean	100.0	58.0	100.0	89.3	100.0	150.7	—	—
(same as #II)	(n)	(5)[a]	(5)[a]	(12)	(11)	(10)	(10)	—	—
	±SD	±60.8	±23.9	±32.5	±32.5	±41.8	±37.5	—	—
Combined I + II + III	Mean	100.0	62.0	100.0	77.0	100.0	123.0	—	—
	(n)	(13)[a]	(13)[a]	(29)	(25)	(29)	(27)	—	—
	±SD	±43.91	±18.0	±29.6	±26.4	±32.73	±35.8	—	—
Combined statistical significance		$p<0.01$		$p<0.005$		$p<0.02$		$p<0.01$	

Note: The actual values of the respective combined control means were
Blood vessel ascorbic acid = 3.51 mg AA per 100 g tissue.
Blood plasma ascorbic acid = 2.15 mg AA per 100 ml plasma.
Blood plasma copper = 62.3 μg Cu per 100 ml plasma.
Adrenal ascorbic acid = 163.4 mg AA per 100 g tissue.

[a] (n) = Number of pooled samples for blood vessels.

Data from Saroja et al. (1971).

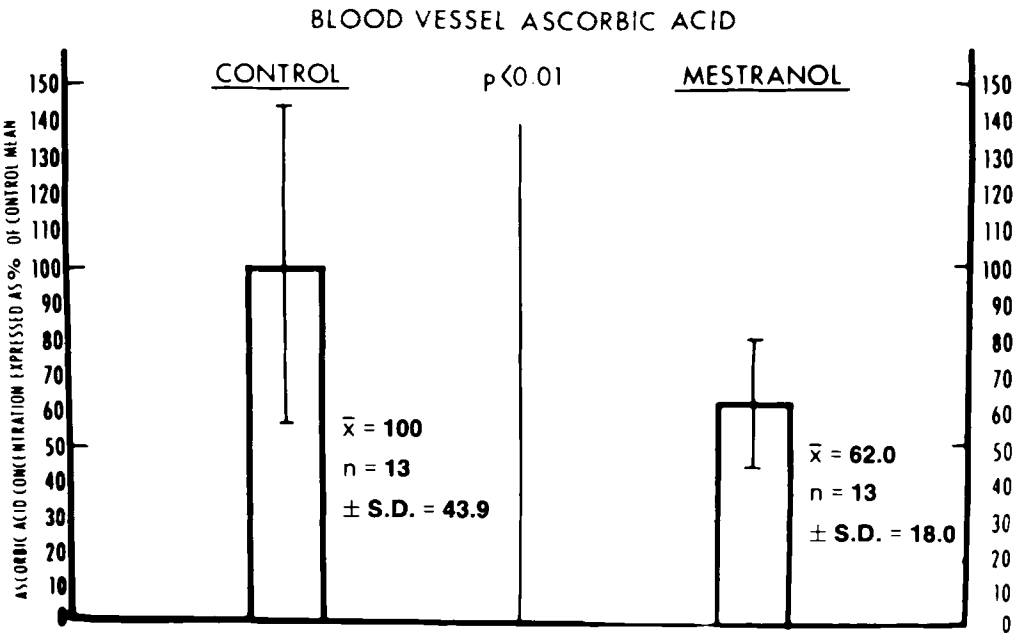

FIGURE 3. The marked reduction of the ascorbic acid content of the blood vessels of the guinea pigs which had been treated by 1 *M* injection of mestranol, 50 μg daily for 2 weeks is clearly evident ($p < 0.01$); the control animals received daily injections of the same volume (0.2 ml) of sesame oil base. (From Saroja, N., Mallikarjuneswara, V. R., and Clemetson, C. A. B. [1971], *Contraception*, 3, 269. With permission.)

and nonusers, but there was no statement, in any of these papers, as to how many of the control subjects were using the popular alternative, namely copper intrauterine devices (copper 7 or copper T). Such copper devices slowly dissolve, and the copper is carried as traces of "free copper" (cupric ions loosely bound to albumen) in the plasma and is deposited in the liver. Such traces of "free copper" would not cause any appreciable increase in the total plasma copper, but might well affect ascorbic acid metabolism, as Szöke et al. (1963) observed a reduction of the ascorbic acid levels of the livers and adrenals of guinea pigs which had received 15 mg of copper daily by mouth. The author was a lone voice when he spoke and voted, at the Medical Advisory Committee of Planned Parenthood of New York City, against the introduction of copper intrauterine devices. He could not explain in a few minutes the concepts contained in this book, so he simply requested that his opposition be recorded.

Weininger and King (1977) studied five young women, nonsmokers, using "combined" oral contraceptives, and five comparable nonusers at the Berkeley campus of the University of California both before and during 8 to 12 days of confinement in a metabolic ward. They found that the serum level of the copper-protein ceruloplasmin in the users was twice that in the nonusers, even though they were mostly taking the reduced dosage (50 μg mestranol) pills, but found no difference between the plasma and leukocyte ascorbic acid levels of the two groups. This is most probably due to the fact that these women had very high ascorbic acid intakes both before (100 to 500 mg daily) and during (155 mg daily) the study.

Special vitamin preparations containing extra vitamin C, B_6, and folic acid have since been marketed for women "on the pill", as Shojania et al. (1969) observed that these women have an abnormality of folate metabolism and Luhby et al.(1971) and Leklem et al. (1975) have shown pyridoxine-dependent abnormalities of tryptophan metabolism. Since altered tryptophan metabolism persisted in contraceptive users, even when other indices of B_6 nutrition were normal, Leklem et al. suggested that oral contraceptives may affect tryptophan

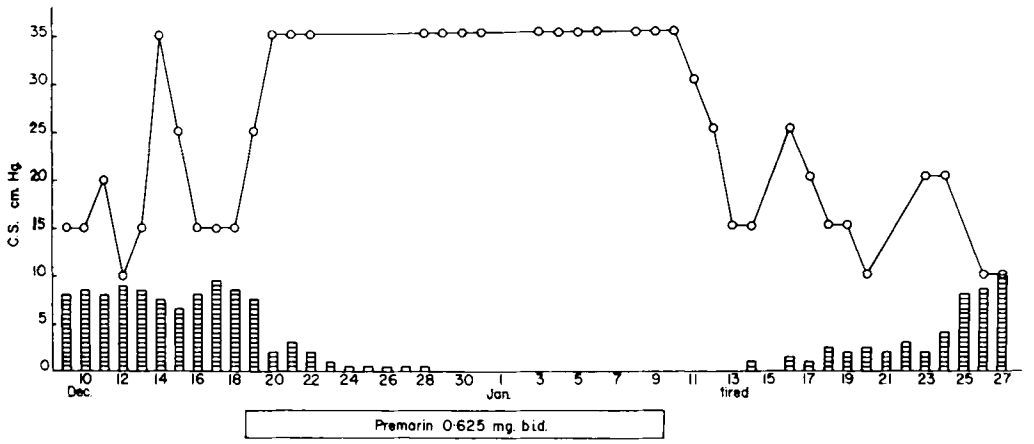

FIGURE 4. The effect of conjugated equine estrogens, given orally, in elevating the capillary strength of the skin of the arm of a menopausal woman suffering from hot flushes. The rungs of the ladders indicate the numbers of hot flushes reported each day. Capillary strength (the opposite of fragility) was measured by determining the negative pressure that was needed to be exerted, in a plastic cup 2 cm in diameter, to cause petechial hemorrages when applied to the thin skin on the inner aspect of the upper arm. Similar results have been obtained with small doses of many estrogens, including ethinyl estradiol, 10 to 20 μg daily, but only when given to estrogen-deficient menopausal women with hot flushes. (From Clemetson, C. A. B., Blair, L., and Reed, D. H. [1962], *Am. J. Obstet. Gynecol.*, 83, 1261. With permission.)

metabolism by some means other than through a B_6 deficiency. However, the fact that they found the tryptophan metabolism to be correctable by the administration of high-dosage pyridoxine hydrochloride (20 mg daily) alone would seem to prove that the abnormality is due to a disturbance of vitamin B_6 metabolism. The abnormality of folic acid metabolism is probably due to the abnormal ascorbic acid metabolism, as normal ascorbic acid levels are necessary for the conversion of folic to folinic acid and also to prevent destruction of folinic acid, as will be discussed in Chapter 4, Volume III. However, the abnormality of pyridoxine metabolism could, at least in theory, be the cause of an abnormality of ascorbic acid metabolism. Hepatic cystathione synthase (CSase) and cystathionase (CNase) are B_6-dependent enzymes which catalyze the synthesis and cleavage of cystathione, an intermediate in the metabolism of methionine to cysteine. Indeed, Shannon and Demos (1977) have reported a reduction of hepatic CNase in estrogen-treated rats. It would be interesting to know the cysteine levels in the tissues of estrogen-treated guinea pigs, for decreased cysteine levels could account for decreased tissue storage and increased excretion of ascorbic acid.

Fortunately, the estrogen content of the combined estrogen-progestagen oral contraceptive pills has been reduced over the last 15 years from 100 to 80 to 50 to 35 and now to 30 μg per pill, without any loss of effectiveness, and women are consuming more vitamin C, so the effect on ascorbic acid metabolism and the side effects are becoming harder and harder to detect.

Low-dose estrogens undoubtedly do have a direct beneficial effect on the endothelium of the blood vessels, as estrogen has been shown to increase the capillary strength of menopausal women (Clemetson et al., 1962, Clemetson et al., 1962), as shown in Figure 4. Moreover, endogenous hormones seem to protect women against atherosclerosis during the reproductive years.

However, the use of high-potency or high-dosage estrogens definitely has harmful effects on the blood vessels and accelerates the development of cardiovascular disease, as shown by the Veterans Urological Research Group (1967) study of men receiving stilbestrol (5 mg daily) or placebo for metastatic carcinoma of the prostate. Similarly, increased incidences

of cardiovascular disease, deep-vein thrombosis, and embolism have been observed in women taking the original 100-μg daily dose of mestranol (3 weeks out of 4) in the combined birth control pills (Inman and Vessey, 1968, Vessey and Doll, 1968, Sartwell et al., 1969, Mann and Inman, 1975, Mann et al., 1975, Beral and Kay, 1977). This is the reason that the dose of ethinyl estradiol 3-methyl ether or of ethinyl estradiol in the various combined pills was progressively reduced from 100 to 30 μg daily, and fortunately without any loss of efficacy. The dose still seems to be high enough to inhibit ovulation.

Much has been written about the possible causes of cardiovascular disease in women "on the pill". Some investigators have reported a slight increase in this or that blood coagulation factor and some even a slight reduction of the coagulation time of women taking oral contraceptives, 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 to clot?" So we must look for changes in the endothelium or inner lining of the blood vessels, as first suggested by Quick (1963). Moreover, we must remember that the endothelium of the larger arteries and veins is the same tissue as, and an extension of, the capillaries.

The benefits of low-dose estrogen, which are associated with an increase of capillary strength, may well be a direct effect due to polymerization of mucopolysaccharides in the capillary sheath. A similar increase in capillary strength results from cortisone administration, as shown by Kramar et al. (1957) in adrenalectomized rats. The adverse effects of estrogens on the endothelium may be an indirect effect, resulting from the disturbance of ascorbic acid metabolism, as suggested by Clemetson (1968, 1979), perhaps due to a reduced negative electrical ascorbate potential of platelets relative to plasma and of endothelium relative to plasma, as suggested by Kalesh et al. (1971).

Usually one thinks of low ascorbic acid levels as causing a tendency to hemorrhage rather than thrombosis, but it is quite conceivable that small subendothelial hemorrhages may act as the nidus for the formation of a thrombus. We must not forget that blood coagulation is the normal mechanism for the arrest of hemorrhage, so hemorrhage is the most common cause of thrombosis.

In human subjects, estrogen administration, pregnancy, liver disease, infection, and many other disorders cause a marked elevation of plasma copper levels. Even when elevated, 95% of plasma copper is bound to an alpha globulin as the blue copper protein ceruloplasmin (Gubler et al. 1952b, 1953, Russ and Raymunt, 1956). This enzyme has a molecular weight of 151,000 and has 8 atoms of copper per molecule; it has been shown by Humoller et al. (1960) and Osaki et al. (1963, 1964) to be a true ascorbate oxidase; it is also a polyphenol oxidase and a polyamine oxidase.

All women taking estrogen-containing oral contraceptives have high plasma total copper levels, as shown by Clemetson (1968) in a study of women taking combined oral contraceptives containing 100 μg of mestranol per pill (see Table 4 and Figures 5 and 6). In some women the increased level of copper in the blood was sufficient to give a green tinge to the plasma. The increased blood copper and ceruloplasmin levels of women "on the pill" have since been amply confirmed by many workers, including O'Leary and Feldman (1969) and Horwitt et al. (1975).

Weininger and King (1982) have reported increased ceruloplasmin levels and a significantly increased turnover rate of (1-^{14}C) L-ascorbic acid in female rhesus monkeys receiving a combined oral contraceptive pill containing 50 μg of mestranol and 1 mg of norethindrone. Basu (1986) studied guinea pigs on a low-ascorbic acid diet (5 mg daily) and observed significantly decreased plasma and tissue ascorbic acid levels following the oral administration of either ethinylestradiol (5 μg) or progestagen (250 μg) daily for 25 d, but these are very high doses for such small animals. In all probability, the modern low-dose (30 μg

Table 4
PLASMA TOTAL COPPER LEVELS IN VARIOUS GROUPS

	Group	No. of subjects	Mean plasma total copper level (μg/100 ml)	Standard deviation	Significance of differences
A	Normal men, aged 24—42	17	106.9	±12.5	—
B	Normal women in reproductive life, aged 20—38	14	124.2	±24.7	B vs. A $p < 0.02$
C	Women taking oral contraceptive tablets, aged 19—42	16	215.8	±27.1	C vs. B $p < 0.001$
D	Normal pregnant women, 2—10 weeks after L.M.P.	3	154.0	±14.8	—
E	11—20 weeks after L.M.P.	13	165.9	±35.5	E vs. B $p < 0.01$
F	21—30 weeks after L.M.P.	12	220.4	±37.2	F vs. B $p < 0.001$
G	31—40 weeks after L.M.P.	12	225.9	±47.5	G vs. B $p < 0.001$

Note: Plasma total copper levels of men, nonpregnant women, pregnant women, and women taking mestranol, 100 μg daily, 3 weeks out of 4, in combined oral contraceptive pills, were measured by the diethyldithiocarbamate method of Gubler (1952a).

From Clemetson, C. A. B. (1968), *Lancet*, 2, 1037. With permission.

mestranol) birth control pills have very little effect on the ascorbic acid metabolism of the young women who take them; such a minor change in ascorbic acid metabolism is not likely to harm any woman, unless she is on a markedly ascorbic acid-deficient diet. Moreover, the direct effect of estrogens on capillary strength may even have a beneficial effect on the endothelium of the blood vessels.

The progestational agents probably have no effect on the plasma copper, except insofar as some of them are slowly and in part converted to estrogens. For chlormadinone, which is not converted to an estrogen, given in a dose of 2 mg daily, for two 20-d courses, had absolutely no effect on the plasma copper levels of seven women studied by the author at the University of California Medical Center in San Francisco. In contrast, seven women who took mestranol (ethinyl estradiol-3-methyl ether), 100 μg daily, for 20 d, and seven others who took a combination of mestranol, 100 μg, and chlormadinone, 2 mg, daily for 20 d, all developed increased plasma total copper levels; the average increase was 70%. There is no certainty that the increased ascorbate oxidase activity, resulting from high levels of ceruloplasmin, is the cause of the disturbance of ascorbic acid metabolism in women taking the old high-dose pills, but ascorbic acid metabolism does seem to be disturbed. We can only conjecture as to its relationship to the increased incidence of thromboembolism, coronary thrombosis, and cerebrovascular disease. Clemetson (1979) has pointed out that the incidence of these cardiovascular problems is increased by estrogens, pregnancy, aging, smoking, infection, trauma, surgery, soft water, and winter season. Is it only a coincidence that these nine factors which predispose to cardiovascular disease are all associated with a tendency towards decreased plasma ascorbate levels?

X. ANDROGENS

Mesonyi (1936) reported that large doses of male sex hormones, from bull testis, caused a 30 to 40% decrease in the ascorbic acid content of the liver and adrenals of guinea pigs.

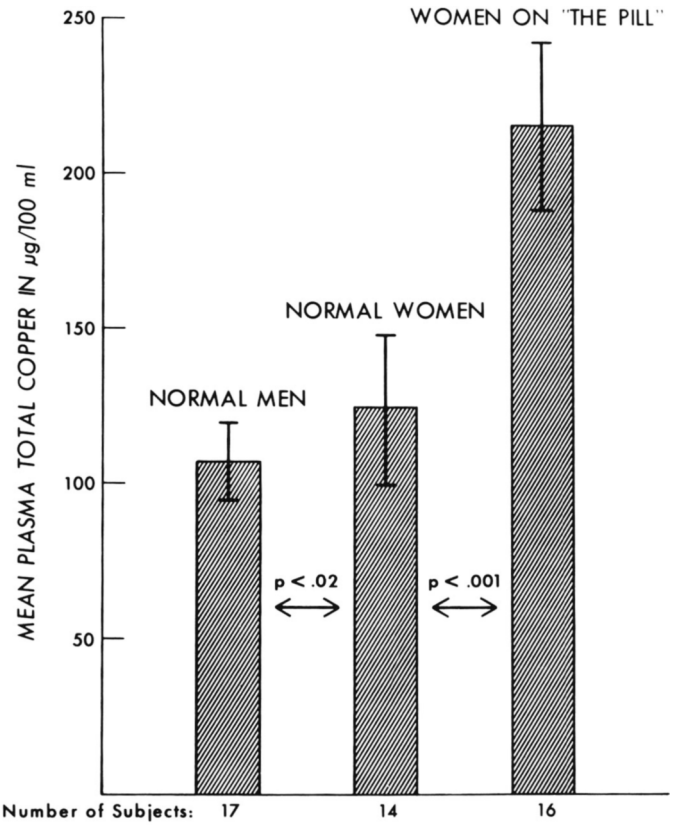

FIGURE 5. The plasma total copper levels of women taking the old (100 µg mestranol) combined birth control pills showed a highly significant elevation; this was due to the estrogenic component.

This could in part be due to the ascorbate oxidase activity of ceruloplasmin, for Johnson et al. (1959) observed that not only estradiol benzoate, but also testosterone proprionate caused a significant elevation of the plasma total copper level, both in men and in women.

However, there must be some other reason for the tendency of men to have lower ascorbate levels than women; for the plasma total copper level of men was found to be 107 µg/100 ml, while that of women was 124 µg/100 ml, as shown in Figure 5.

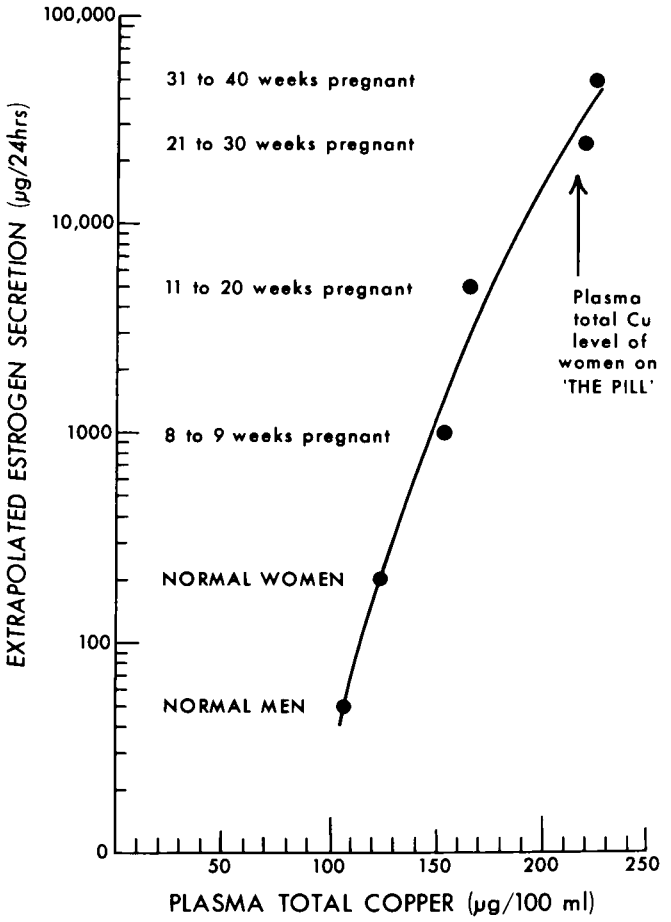

FIGURE 6. The plasma total copper levels of women taking the old 100-μg mestranol combined birth control pills were elevated to a level comparable to that found in late pregnancy, when the extrapolated endogenous estrogen secretion is probably increased 100-fold; 95% of this copper is in the form of ceruloplasmin, which is an ascorbate oxidase.

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Chapter 14

PREGNANCY

I. PLASMA AND LEUKOCYTE ASCORBIC ACID LEVELS IN PREGNANCY

Most authors are agreed that women need more ascorbic acid during pregnancy than in the nonpregnant state, if for no other reason than to supply the additional needs of the fetus; but there is no certainty as to how much ascorbic acid they need. Indeed, it now seems that some women may need much more than others; this will be discussed further in the sections dealing with recurrent abortion, premature rupture of the membranes, and abruptio placentae.

Certainly the plasma ascorbic acid levels of many women tend to fall during pregnancy, as reported by Elby and Christensen (1938), Teel et al. (1938), Javert and Stander (1943), Macy et al. (1954), Javert (1955) (Figure 1), Martin et al. (1957), and Mason and Rivers (1970, 1971); some fall to dangerously low levels. Camara and Concepcion (1940) reported mean plasma ascorbic acid levels of 0.83 mg/100 ml and found no significant change in this level as pregnancy advanced. This certainly suggests that the Philippine women whom they studied live on a diet well provided with ascorbic acid, and that they do not destroy it with cooking. In contrast, Vobecky et al. (1974), working at the University of Sherbrooke, Quebec, found that the plasma vitamin C levels of pregnant women tended to fall as pregnancy advanced, even though most of them were taking multivitamin supplements. The percentage of women with ascorbic acid levels below 0.5 mg/100 ml rose from 13.4% in the first trimester, to 22.5% in the second, and to 45.6% in the third trimester of normal uncomplicated pregnancies.

Ganich (1973) found no fall in the serum ascorbic acid levels of the pregnant women he studied at Uchgorod University in the Ukraine, but Solomento (1975) found dangerously low maternal plasma ascorbate levels (mean 0.12 ± 0.01 mg/100 ml) in late pregnancy in Vladivostok, U.S.S.R., in the spring, and recommended ascorbic acid, 200 mg daily, as a dietary supplement for pregnant women in that region.

Barton and Roath (1976) of Southampton University, studying leukocyte ascorbic acid (TAA)* levels at a hospital in the south of England, reported that 30 out of 39 pregnant women tested had subnormal levels (below 15 $\mu g/10^8$ cells).

Schorah et al. (1978) at the University of Leeds (U.K.) studied leukocyte ascorbic acid levels in early pregnancy and found them to be affected by season, by social class, and by smoking. Thus, a small subgroup of 11 smokers of social classes IV and V had a mean ascorbic acid (TAA) level of 21.7 $\mu g/10^8$ leukocytes in April and May, while 33 nonsmokers of social classes I and II had a mean level of 45.1 $\mu g/10^8$ cells in July, August, and September. There was no difference between the ascorbic acid levels of the women who aborted spontaneously and those who did not, but the women with lower leukocyte ascorbic acid levels in early pregnancy did tend to give birth to smaller babies (<3250 g), possibly due to smoking or indirectly to the low ascorbate levels resulting from that habit.

Moore et al. (1979) studied women in late pregnancy, at the prenatal clinic of Robroyston Hospital in Glasgow; they found that 40% of the women had leukocyte total ascorbic acid levels less than the lower limit of normal for nonpregnant women (100 $nmol/10^8$ leukocytes). Moreover, they confirmed the association between low social class and low ascorbic acid levels.

Clearly pregnancy constitutes a drain on the ascorbic acid reserves; this is potentially dangerous, especially in the spring and early summer in those regions where the poor may still depend on the potatoes of last year, which have lost much of their vitamin C content.

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

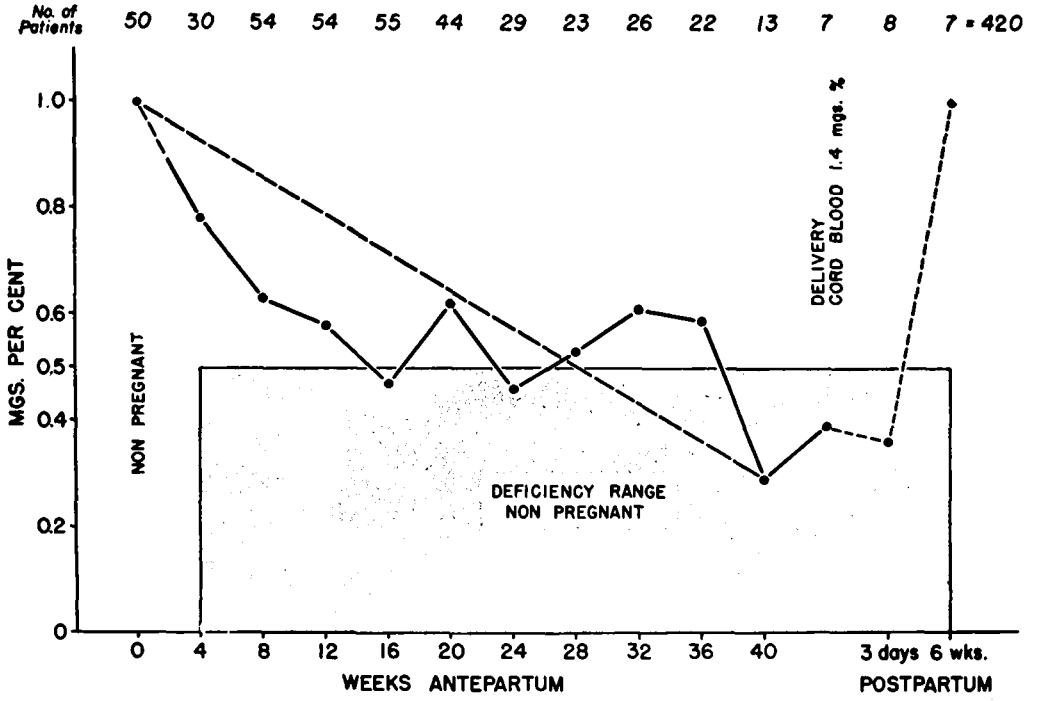

FIGURE 1. Average maternal blood plasma vitamin C concentration values, by week of pregnancy, in normal untreated women. (From Javert, C. T. [1955], *Ann. N.Y. Acad. Sci.*, 61, 700. With permission.)

II. BLOOD HISTAMINE LEVELS

Clemetson (1980, 1981) has observed that the whole blood histamine levels of pregnant women rise when their plasma ascorbic acid levels fall (see Figure 2). This is particularly important because Hofbauer (1926) showed that injection of histamine into guinea pigs and cats caused abruptio placentae. Fortunately, blood histamine levels usually return rapidly to normal following oral administration of ascorbic acid, as shown in Figure 6 of Chapter 1, Volume III.

III. THE QUESTION OF ASCORBIC ACID SYNTHESIS

Ingier (1915) observed a high percentage of premature births, stillbirths, and maternal deaths in guinea pigs fed a scorbutogenic diet during pregnancy. She observed that the pregnant animals showed a more advanced state of the disease in their bones, at an earlier period of defective diet, than did nonpregnant animals. Moreover, some of the stillborn fetuses showed multiple intrauterine fractures. It is possible that the diet used by Ingier may have lacked other vitamins besides vitamin C, but similar observations by subsequent workers have confirmed that ascorbic acid deficiency can cause serious problems in pregnancy, both in guinea pigs and in human beings.

However, there have been reports that pregnant guinea pigs survive longer on a vitamin C-deficient diet than do nonpregnant females. It was suggested by Mouriquand and Schoen (1933) that guinea pig foetal tissues might be capable of synthesizing ascorbic acid. However, Kramer et al (1933) reported definite ill effects in guinea pigs which became pregnant while receiving an inadequate supply of vitamin C (3 to 5 ml of orange juice or tomato juice per 300 g body weight per day); they suffered ovarian degeneration, embryo death, and abortion. No guinea pigs became pregnant while on a diet containing less than 3 ml of orange juice a day.

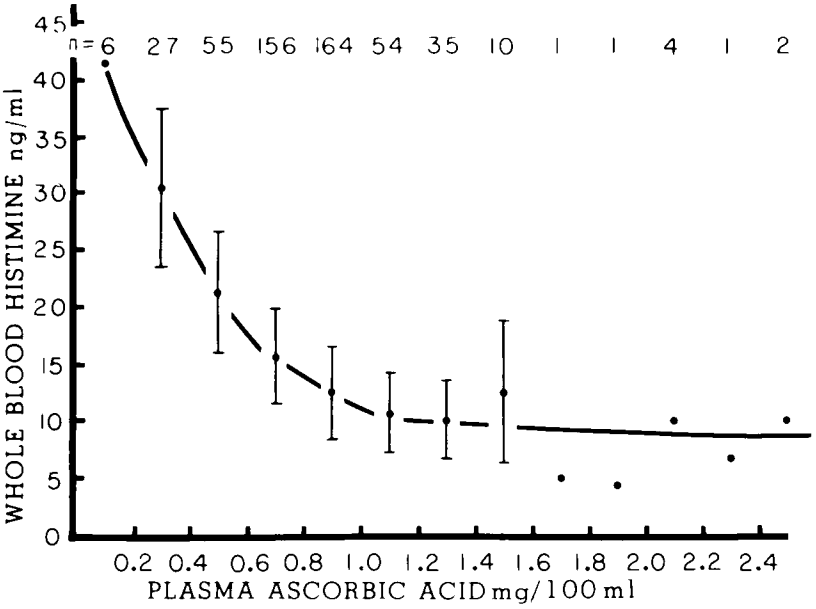

FIGURE 2. Analysis of 516 blood samples from pregnant women, both for ascorbic acid and for histamine, shows a well-defined relationship between these two substances. The mean histamine level (± SD) is given for each 0.2-mg/100 ml ascorbic acid group; the numbers along the top of the graph indicate the number of blood samples in each group. It is evident that the whole blood histamine level rises exponentially as the plasma ascorbic acid level falls. (From Clemetson, C. A. B. and Cafaro, V. [1981], *Int. J. Gynaecol. Obstet.*, 19, 453. With permission.)

However, Bourne (1935) observed prolonged survival on a vitamin C-deficient diet, both in pregnant guinea pigs and in nonpregnant female guinea pigs treated with luteinizing hormone. He suggested the possibility that the corpus luteum of pregnancy might be able to synthesize ascorbic acid in the guinea pig, but this does not seem to have been confirmed.

In vitro experiments with placental slices by Barnes (1947) provided no evidence that the human placenta can synthesize ascorbic acid. Likewise, King (1953) demonstrated in radioisotope studies, using glucose ^{14}C, the failure of guinea pig embryonal tissues to form any detectable quantity of ascorbic acid ^{14}C. Moreover, Pye et al. (1961) studied the reproductive performance of guinea pigs fed 2, 4, 6, or 8 mg/d of ascorbic acid and found that not only was fertility, as measured by the number of living young produced per female, three times greater in the 8-mg than in the 2-mg group, but also the litter sizes were 2.0, 2.4, 2.3, and 2.9 and the mean birth weights were 182, 287, 222, and 415 g, respectively, in the four ascorbic acid intake groups, showing a distinct advantage for the 8-mg/d ascorbate group. The maternal mortality rate per pregnancy in the 8-mg group was half that in the 2-mg group, but the overall death rate was higher in the 8-mg group due to the greater number of pregnancies.

However, Rajalakshmi et al. (1967) reported synthesis of ascorbic acid from D-glucuronolactone in 27 out of 40 human placentas which they studied, and de Fabro (1968) reported the existence of an ascorbic acid-synthesizing enzyme-gulonolactone-oxidase in the liver microsomes of guinea pig embryos, which disappeared as they matured, so this matter still remains *sub judice*. If indeed the guinea pig embryo can synthesize ascorbic acid at one stage of its development, as it climbs its own ancestral tree, it is clear that it does not produce enough for its own sustenance. However, the matter is of considerable academic interest as it does suggest that the essential gene may be suppressed and not completely absent in such species as ourselves.

Table 1
MEAN MATERNAL AND FETAL PLASMA ANALYSES REPORTED FROM GUINEA PIG STUDIES[a]

Blood plasma	Ascorbic acid (AA) (mg/100 ml)	Dehydroascorbic acid (DHAA) (mg/100 ml)	Total ascorbic acid (TAA) (mg/100 ml)
Mean maternal level	0.40	0.38	0.78
Mean fetal level	1.06	0.40	1.46

[a] Data of Räihä (1958)

Certainly rats can synthesize ascorbic acid; Morehouse and Guerrant (1952) have reported that the ascorbic acid level in the liver of the pregnant rat increases progressively as pregnancy advances. On the other hand, Kratzing and Kelly (1982), expressing rat tissue ascorbate levels relative to cellular DNA content, observed a fall in the liver, lung, and kidney ascorbic acid content to a low level on day 15 of pregnancy, followed by a significant rise on day 20. They also reported a similar fall, followed by a late rise in the ascorbic acid content of the rat fetal liver and lung tissues. However, it is not known whether this rise in the rat fetal tissue ascorbate level in late pregnancy is due to increased placental transfer, increased maternal synthesis, or to maturation of the fetal synthetic capacity for ascorbic acid in late pregnancy.

IV. PLACENTAL HISTAMINASE ACTIVITY

In view of the studies by Chatterjee et al. (1975) and Clemetson (1980) showing elevated blood histamine levels in ascorbic acid deficient guinea pigs and human beings, respectively, it is possible that the endothelial damage and the bleeding of scurvy may be largely due to histamine intoxication. If this is so, then the apparent protective effect of pregnancy against vitamin C deficiency in the guinea pig might be due to the histaminase production by the placenta, which was so extensively studied by Kapeller-Adler. Perhaps women are partially protected against the histamine intoxication of vitamin C deficiency when they are pregnant. However, they may lose this protection when the placental histaminase level falls, as Kapeller-Adler (1949) has shown that it does in preeclampsia or toxemia of late pregnancy. There is an inadequate blood supply to the uterus in preeclampsia, which would account for the decreased histaminase production; this in turn could account for the increased incidence of retroplacental hemorrhage, which is known to exist in preeclampsia.

V. THE PLACENTAL TRANSFER OF ASCORBIC ACID

The ascorbic acid concentration of human umbilical cord blood was found by Wahren and Rundquist (1937) to be greater than that of maternal blood; this has since been confirmed by many workers. Räihä (1953) conducted extensive studies of the placental transfer of ascorbic acid in guinea pigs. He confirmed that the fetal plasma always has a higher concentration of total ascorbic acid (TAA) than maternal plasma, but reported that the dehydroascorbic acid (DHAA) levels were about the same; the mean figures obtained by Räihä from guinea pig studies are shown in Table 1.

Moreover, Räihä conducted *in vivo* experiments in guinea pigs, in which L-ascorbic acid or DHAA was infused into the maternal circulation. He found that infused L-ascorbic acid only slightly increased the values of total ascorbic acid in the fetal blood, whereas infusion of DHAA produced a marked increase of total ascorbic acid, especially in the fetal erythrocytes.

Table 2
COMPARISON OF THE PROPERTIES OF ASCORBIC ACID AND DEHYDROASCORBIC ACID (OR ASCORBONE), ITS REVERSIBLY OXIDIZED FORM

L-Ascorbic acid	Dehydro L-ascorbic acid
Strong reducing agent	Insignificant reducing agent
Acid	Neutral
Electrolyte	Nonelectrolyte
Poorly fat soluble	Ten times as fat soluble
Slow passage through cell membranes	Rapid passage through cell membranes
Antiscorbutic by mouth	Antiscorbutic by mouth
Structure not related to alloxan	Structure related to alloxan
Innocuous by i.v. injection	Toxic by i.v. injection

Note: The greater lipid solubility of ascorbone is believed to account for its passage across cell membranes and across the placenta mainly in this form.

Thus, Räihä found that ascorbic acid crosses the placenta from mother to fetus as DHAA, just as it had been shown, by Panteleeva (1950), Lloyd (1951), and Lloyd and Parry (1954), to cross the cell membrane from blood plasma into red blood cells.

Räihä's conclusion that ascorbic acid crosses the placenta in the dehydro form can be readily accepted, as his experiment seems to be conclusive and DHAA or ascorbone as it is sometimes called, is a lipid-soluble nonelectrolyte, while ascorbic acid is a polar electrolyte and is relatively insoluble in lipids, as shown in Table 2. However, the DHAA levels reported by Räihä are much higher than the writer has ever found in any human or guinea pig plasma samples. One can only conclude that Räihä's blood samples may have been saved for several hours until it was convenient to analyze them, or else they had undergone some hemolysis and that, as a result, they had oxidized to some extent before analysis. Some of his specimens showed 100% oxidation. The findings in two pairs of human maternal and fetal blood samples, analyzed by a method similar to that used by Stewart et al. (1953), in the writer's laboratory at the University of California Medical Center in San Francisco in 1965, are shown in Figure 3.

The plasma DHAA levels in maternal and fetal blood are almost certainly much lower than were reported by Räihä, but they do seem to be about the same on the two sides of the placenta, as he suggested. Thus, we can envisage the DHAA as possibly crossing the placenta to the fetus by free diffusion. If so, the higher concentration of ascorbic acid in the fetal blood plasma is most likely due to the greater ability of fetal blood to reduce DHAA to ascorbic acid. In fact, it is probably the ability of any tissue to reduce DHAA to ascorbic acid that determines its ability to store ascorbic acid, as was suggested by Clemetson (1966).

The chemical mechanism for the transfer of ascorbic acid from maternal to fetal plasma may be considered as shown in Figure 4, where GSH represents reduced glutathione and GSSG its oxidized form. The high plasma total copper level in human maternal plasma (mean 222 μg/100 ml) and the low total copper in the plasma of newborn infants (mean 75 μg/100 ml), as reported by Gubler (1956), are indicative of high and low ceruloplasmin, ascorbate oxidase activities which encourage transfer of ascorbic acid to the fetus. Moreover, the higher level of amino acids in fetal blood, resulting from active transfer of amino acids by the placenta, as shown by Christensen and Streicher (1948), Crumpler et al. (1950), Clemetson and Churchman (1954), and Ghadimi and Pecora (1964), may also aid fetal storage of ascorbic acid. Norkus et al. (1979), studying guinea pigs, favor the concept of a carrier-mediated transport mechanism involved in conveying ascorbic acid to the fetus; so also do Streeter and Rosso (1981), studying human placentas.

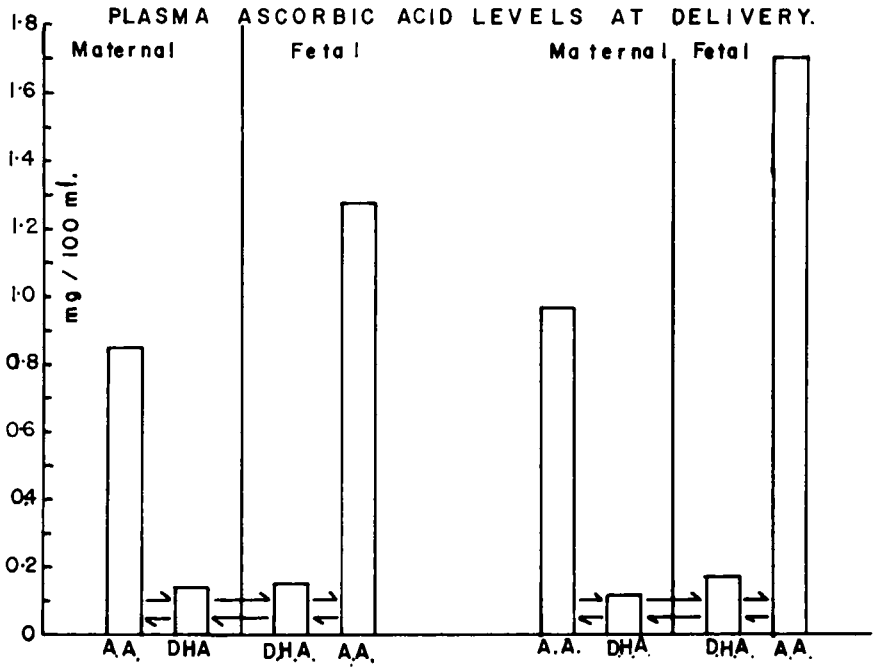

FIGURE 3. Bar graphs illustrating the plasma levels of ascorbic acid and dehydroascorbic acid (DHAA) found in two pairs of maternal and fetal blood samples, obtained at the time of human birth. Total ascorbic acid (TAA) and reduced ascorbic acid (AA) were determined by analysis; (DHAA) was obtained by difference. It may be seen that the fetal AA level exceeds the maternal level, but DHAA differs little, if at all, between maternal and fetal blood.

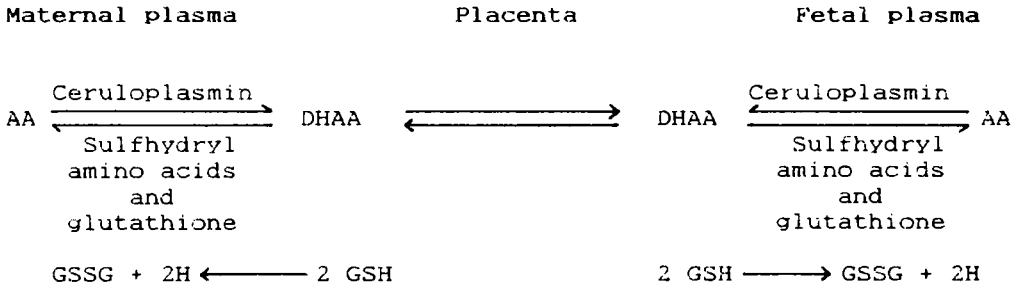

FIGURE 4

Not only the plasma, but also the leukocyte ascorbic acid level is consistently higher in the cord blood of newborn infants than in maternal blood, as shown by Dawson and Osborn (1979).

However, there is reason to believe that the transport of ascorbic acid from the mother to the fetus may be impaired in women with diabetes mellitus; for Norkus et al. (1982) have shown in guinea pig experiments that the rate of transfer of ascorbic acid across the placenta is significantly impaired when the maternal plasma glucose level exceeds 200 mg/dl; this is consistent with the findings of others concerning the effect of glucose on the transfer of ascorbic acid into human mononuclear leukocytes (Chapter 2, Volume II).

VI. ASCORBIC ACID METABOLISM IN PREGNANCY

In addition to the transfer of ascorbic acid to the fetus, many factors, including corysa,

pyelonephritis, trauma, surgery, or sickle-cell crisis, may interfere with ascorbic acid metabolism during or after pregnancy, but they are not peculiar to pregnancy. However, hyperemesis gravidarum is peculiar to early pregnancy and does sometimes have profound effects on vitamin B_1 and vitamin C metabolism.

Swanson (1927) observed a case of scurvy in pregnancy associated with hyperemesis gravidarum. Teel et al. (1938) reported very low vitamin C levels in the blood plasma of three patients with hyperemesis gravidarum; in two instances they found ascorbic acid (AA)* levels less than 0.1 mg/100 ml, and in the other the value was 0.2 mg/100 ml.

Lund and Kimble (1943), writing from Madison, WI, reported as follows: "Hyperemesis gravidarum may lead to dangerously low levels of vitamin C. Clinical scurvy may appear. The retinal hemorrhages of severe hyperemesis gravidarum are a manifestation of vitamin C deficiency and are similar to petechial hemorrhages seen elsewhere. These hemorrhages cease after adequate therapy with vitamin C, henceforth they are not necessarily an indication for the use of therapeutic abortion."

Similarly, severe pelvic infections following pregnancy, like septic abortion, chorioamnionitis, and puerperal sepsis, including endometritis, parametritis, pelvic abscess, bacteremia, septicemia, and septic shock with disseminated intravascular coagulation, are associated with profound disturbances of ascorbic acid metabolism, which need to be recognized and treated as part of the greater problem.

Another problem peculiar to pregnancy is preeclampsia, and this, too, seems to be associated with a disturbance of ascorbic acid metabolism. Mukherji and Banerjee (1958) found a significant reduction of blood glutathione and a slight but significant reduction of the blood ascorbic acid level in women at term with preeclamptic toxemia. They also reported that DHAA, which was absent from the blood of normal pregnant women at term, was detectable in the blood of a few of the preeclamptic patients. Similarly Clemetson and Andersen (1964) reported a significant reduction in the ratio of reduced to oxidized ascorbic acid in the blood plasma of women with preeclamptic toxemia, as shown in Figure 5. The percentage of the plasma ascorbic acid in the oxidized forms was found to be higher in the toxemic (20%) than in the normal pregnant women (13%), and this difference was highly significant ($p < 0.01$). However, present techniques are only just capable of measuring such small amounts of DHAA. For this reason one cannot place much reliance on individual analyses; only a statistical analysis of grouped figures can give reliable data.

Much better methods are needed for measuring the small quantities of DHAA in plasma. Until such methods are developed, we may have to be content to study whole blood histamine levels in pregnancy, as these rise when ascorbic acid metabolism is disturbed, and reflect even the slightest tendency to ascorbic acid deficiency.

The condition which we know as preeclamptic toxemia has many causes, but all seem to involve relative uteroplacental ischemia. This results when the uterine arteries do not enlarge sufficiently to provide an adequate oxygen supply to the fetus or fetuses near term. The cause of an inadequate blood flow to the uterus may be first pregnancy, extremes of youth or age, multiple pregnancy, uterine trauma, placental infarction, diabetes mellitus, essential hypertension, arteriosclerosis, chronic nephritis, lupus nephritis, aortic hypoplasia, or any other vascular disorder affecting the blood flow through the uterine arteries. The association between a disturbance of ascorbate metabolism and preeclampsia may simply be that ascorbic acid deficiency and histamine excess cause endothelial damage, which predisposes to vascular disease, even before pregnancy. Pregnancy then shows up even the mildest degrees of vascular disease.

* AA — ascorbic acid, reduced form.

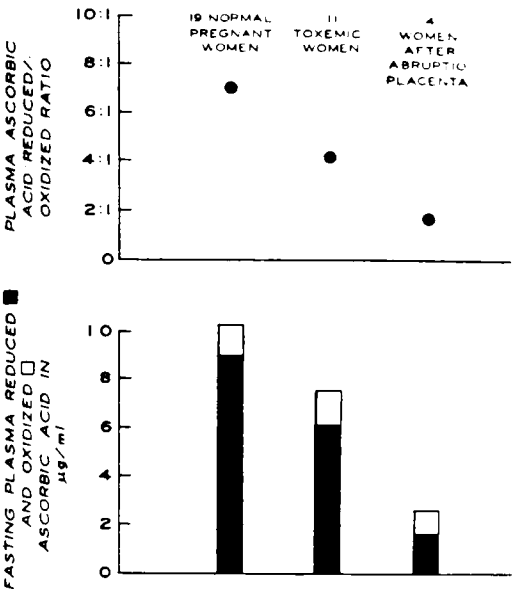

FIGURE 5. Mean reduced and oxidized ascorbic acid levels and reduced/oxidized ratios in fasting plasma samples obtained from pregnant women (analyzed 1 h after collection). (From Clemetson, C. A. B. and Andersen, L. [1964], *Obstet. Gynecol.*, 24, 774. ©The American College of Obstetricians and Gynceologists. With permission.)

VII. CAPILLARY FRAGILITY AND THE PREGNANCY CYCLE

It is generally believed that the menstrual cycle is nonexistent during pregnancy; some women have a little vaginal bleeding at the time of their first missed period, and this, for some reason, is known as the placental sign. Occasionally one sees a woman who has bled several times at monthly intervals during early pregnancy, causing confusion over the expected date of her delivery. On one occasion the author saw a woman who said she had bled regularly, once a month, throughout the whole of pregnancy.

One might be inclined to dismiss the monthly timing of these episodes of bleeding as simple chance or happenstance; for the bleeding of threatened abortion or antepartum hemorrhage can occur at any time. However, Bieber (1953), reviewing the charts of 353 women who had suffered premature separation of the placenta at the Charity Hospital in New Orleans, reported that the incidence of retroplacental bleeding, or abruptio placentae, was greater at 24, 28, 32, 36, and 40 weeks than at the intervening weeks of pregnancy, as shown in Figure 6.

Page et al. (1954) confessed that they suspected that Bieber's results might have been due to a natural tendency to "round out" the months in the individual records. They therefore studied the charts of 225 patients with abruptio placentae at the University of California Medical Center in San Francisco, taking special care to count the number of weeks from the first day of the last menstrual period, as recorded at the time of each patient's first prenatal visit. Their results confirmed Bieber's observations; for all three grades of abruptio placentae — mild, moderate, and severe (1, 2, and 3) — they found a definite tendency to a 4-week cyclic incidence, as may be seen in Figure 7.

So it seems that some women have incomplete suppression of the menstrual cycle; this is difficult to understand, for we have always assumed that the ovarian cycle is arrested in the luteal phase by chorionic gonadotropin. Could it be that estrogen and progesterone from the placenta in the remainder of pregnancy, fail to suppress completely the follicle-stimulating

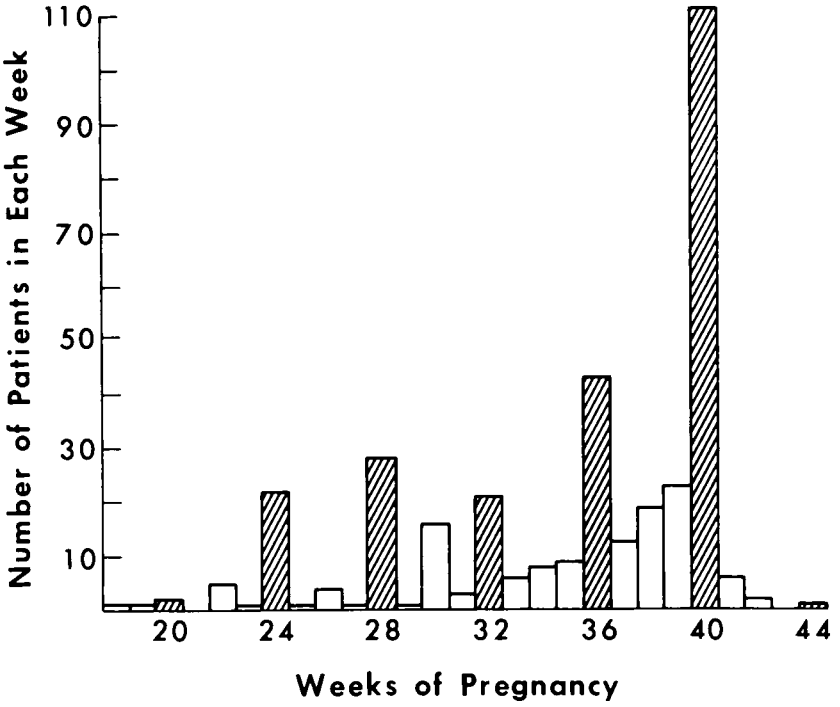

FIGURE 6. Bar graph constructed from the data of Bieber (1953) showing the number of patients in whom abruptio placentae occurred in each week of pregnancy. In his total group of 353 patients with this condition in New Orleans 5 patients were listed as maturity unknown and had to be omitted from this graph. The bars for 20, 24, 28, 32, 36, 40, and 44 weeks have been cross-hatched so that the tendency to a 4-week cyclic incidence of abruptio placentae may be clearly seen.

hormone and/or luteinizing hormone production by the pituitary? Could it be that a chemical clock continues to operate, either in the hypothalamus or in the pituitary, throughout pregnancy?

If such a chemical clock does continue to operate in pregnancy, does it only operate in women with a predisposition to abruptio placentae, or is the clock operating in all pregnant women, and only manifesting itself through bleeding in those with capillary fragility?

Having studied capillary strength and the menstrual cycle (Clemetson et al., 1962) and knowing capillary strength to be related both to steroid hormone levels and to ascorbic acid status, it was natural to extend such studies to include pregnancy. To do this, it was necessary to study the capillary strength of women daily, and to be already studying them as they became pregnant.

Capillary strength was measured daily by applying a 2-cm diameter cup to the thin skin on the inner aspect of the upper arm; a negative pressure of 10, 20, or 30 cmHg was applied for 60 sec. Subsequent inspection for petechial hemorrhages was made 30 sec later in bright light. If there were one or two petechiae after suction at 30 cmHg, that would be recorded as the capillary strength. On the other hand, if there were many petechiae at 30 cmHg and none at 20 cmHg, then the capillary strength would be recorded as 25 cmHg.

Fortunately, two women, A and B, became pregnant during studies of excessive menstrual bleeding due to capillary fragility, and two more, C and D, during studies of volunteers; they are all recorded in Figure 8. It may be noted that all four women showed a pronounced drop in capillary strength at about the time of their first missed period, but only one had any vaginal bleeding in early pregnancy: that was subject A, who reported vaginal bleeding on day 34, a few days after a pronounced fall in capillary strength.

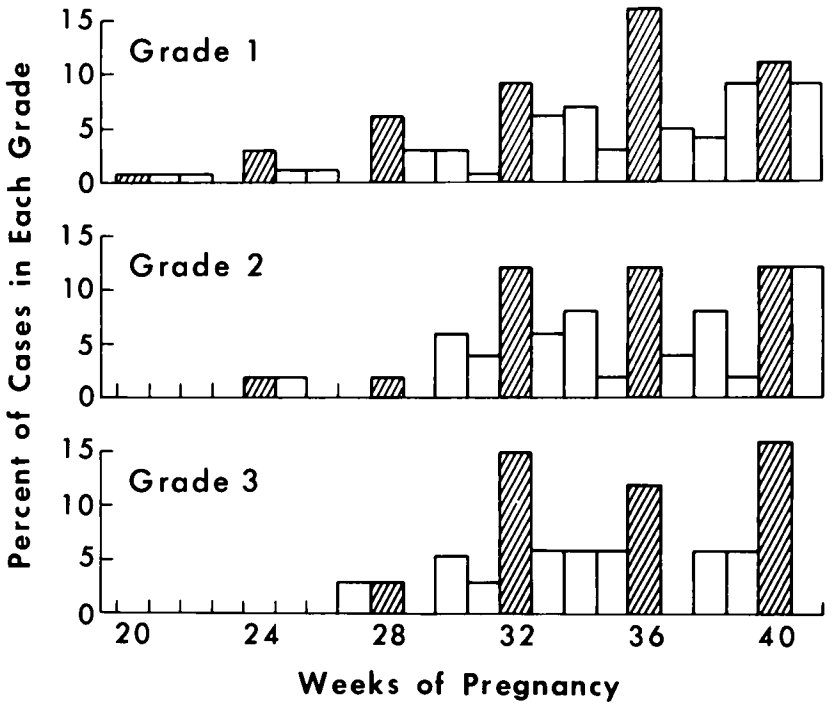

FIGURE 7. Bar graph constructed from the data of Page et al. (1954) showing the frequency distribution of abruptio placentae, grades 1, 2, and 3 according to the week of gestation, in a group of 225 patients in San Francisco. Like Bieber's figures, it shows a 4-week cyclic tendency to abruptio placentae, which is evident for all three grades of severity.

Subject B had nose bleeds and spontaneous petechiae of the arms associated with episodes of low capillary strength in early pregnancy. She also experienced "menstrual" cramps at the onset of a period of continually low capillary strength (days 103 to 107) which was accompanied by spontaneous petechiae and nose bleeds. This woman showed a cyclic fluctuation of capillary strength in the latter half of pregnancy and had a minor revealed accidental hemorrhage or grade 1 abruptio on day 259 at 37 weeks, just preceding a fall of capillary strength.

Subject C reported no untoward symptoms during pregnancy beyond the usual nausea from days 63 to 92.

Subject D reported frequent nose bleeds and bleeding gums between days 62 and 74, at a time when her capillary strength was low. These symptoms disappeared and her capillary strength improved when her low dietary ascorbic acid intake was increased.

Both subjects A and B were receiving dietary supplements of vitamin C and bioflavonoids (Duo C.V.P.), 200 mg of each, three times a day, for some time before and throughout pregnancy, while subjects C and D received only dietary advice in early pregnancy and multivitamins later.

No comparison of capillary strengths with and without the ascorbic acid and bioflavonoid supplement can be made from these charts, as subjects A and B were patients who were being treated for menorrhagia associated with capillary fragility before pregnancy, while C and D were random volunteers. The dietary supplement did not prevent the occurrence of a grade 1 abruptio placentae in subject B. However, it should be noted that this patient had had a severe capillary fragility problem associated with menorrhagia and infertility before pregnancy, and that several of the manifestations, such as the epistaxes, the spontaneous petechiae, and the capillary fragility, had improved with treatment before pregnancy. Thus, one does not know how much worse her situation might have been without treatment.

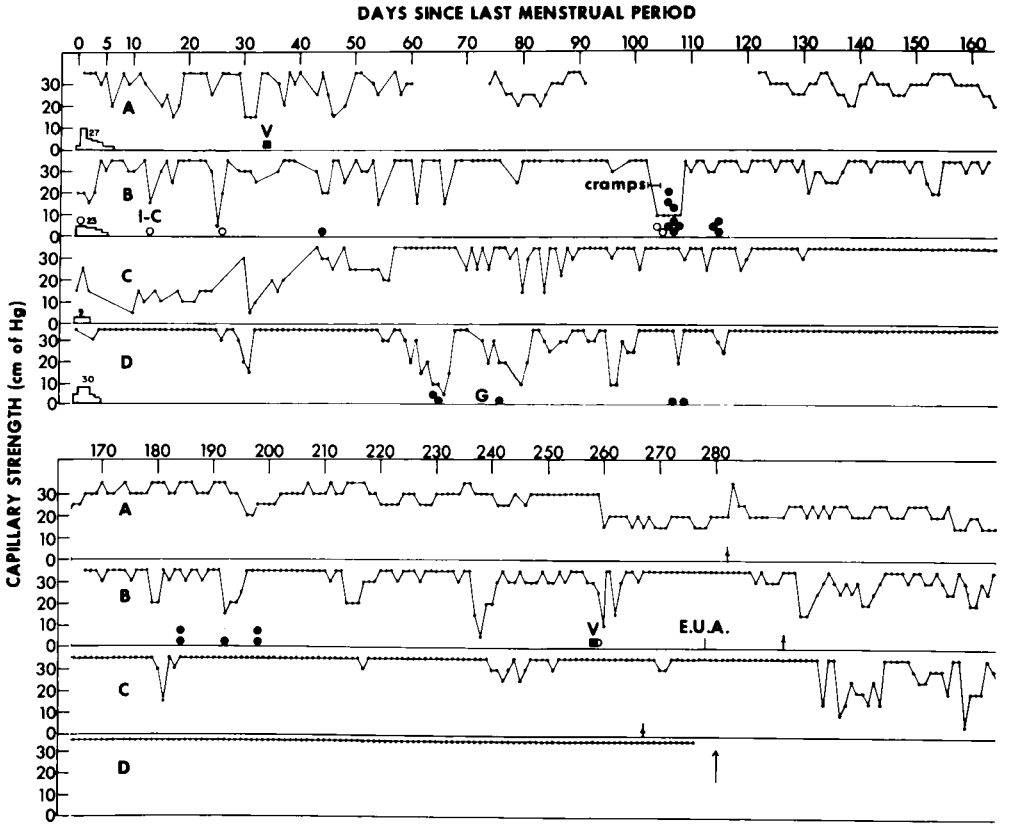

FIGURE 8. Previously unpublished records of daily skin capillary strength observations throughout pregnancy in four women. The upper four charts, A, B, C, and D, show the results obtained from the onset of the last menstrual period up to the 165th day and the lower four charts show observation of the same women from the 166th day until the day of delivery, which is marked with a vertical arrow. Subjects A and B were patients under treatment for menorrhagia associated with capillary fragility before pregnancy, while C and D were volunteers who became pregnant while being studied. Subject B had a minor revealed hemorhage on the 259th day at 37 weeks, but all four women gave birth to healthy infants which thrived. Each pregnancy is discussed in the text. The numbers adjacent to the castles drawn under day 0 represent the number of pads saturated by each subject during the last menstrual period; it may be noted that subjects A, B, and D all had heavy periods, using 27, 23, and 30 pads, respectively; so only subject C, who had a light period, using 9 pads, could be called a normal volunteer.

I-C — Sexual intercourse
■ — Vaginal bleeding
○ — Spontaneous petechiae
G — Gums bleeding
E.U.A. — Examination under anesthesia
● — Epistaxis

All four women gave birth to healthy infants which thrived.

Episodes of decidual bleeding in pregnancy are frequently associated with ascorbic acid deficiency and have been successfully treated with ascorbic acid and bioflavonoids, as shown by Javert (1955) and Greenblatt (1955), studying recurrent abortion, by Ainslie (1959), studying threatened abortion, and by Jacobs (1965), studying Rh isoimmunization.

Moreover, Martin et al. (1957) found that nine out of ten cases of abruptio placentae occurred in women with consistently low serum ascorbate levels in all three trimesters of

pregnancy. Clemetson and Cafaro (1981) have confirmed this finding, that abruptio placentae is much commoner in ascorbic acid-deficient women, and have also shown that these women have higher than normal blood histamine levels. So it seems that this cyclic tendency to decreased capillary strength may become apparent in those women who have a capillary fragility state resulting from a disturbance of ascorbic acid metabolism.

It remains for future research to determine whether there are monthly fluctuations in hormone levels, or in the plasma ascorbate oxidation-reduction status and blood histamine levels.

Unfortunately, capillary strength or fragility studies are somewhat unreliable, because they can be affected by the emotional state of the patient, as shown by Kramár (1954). On occasions, one has seen a patient with nonthrombocytopenic purpura, a true capillary fragility state, as evidenced by easy bruising, bleeding gums, epistaxes, and menorrhagia, who shows a low capillary strength of 5 cmHg one minute, then no petechiae at 30 cmHg a few minutes later. This is presumably due to vasospasm, most probably arteriolar vasoconstriction. It seems that the capillaries or venules are less sensitive to negative pressure when the skin is blanched. Some workers have tried to overcome this problem by warming the arm with hot moist towels, but no truly satisfactory method of measuring capillary strength has been found. The author believes that a low capillary strength result is usually reliable, but a high test result may not be.

Dieckmann et al. (1949) studied capillary fragility in pregnant women with essential hypertension, preeclampsia, and abruptio placentae. They found that pregnancy accentuated capillary fragility and was present in 33% of pregnant women with essential hypertension. Treatment with rutin, or rutin and ascorbic acid, was found to be effective in reducing the capillary fragility in 12 out of 13 of their patients. These authors expressed their belief that the capillary fragility state associated with essential hypertension might predispose to abruptio placentae. However, they found no evidence of capillary fragility in patients with preeclampsia or abruptio placentae. both of these conditions are associated with vasospasm as well as endothelial damage, and the capillary consists of endothelium; it is therefore possible that a capillary fragility state could exist in both of these conditions, but be concealed by vasospasm.

For this reason, current research by the writer includes blood histamine levels, which can now be measured accurately, and which seem to reflect even minor changes in ascorbic acid metabolism; these studies are reported in the section on abruptio placentae (Chapter 14, Volume III).

We still do not know whether the "pregnancy cycle" is a factor affecting the economy of ascorbic acid or not. However, some women do seem to have a cyclic tendency to capillary fragility in pregnancy, and this does seem to compound any preexistent capillary fragility state.

VIII. LABOR

Banerjee and Deb (1952) have reported markedly decreased rhythmic contractility of the uterine muscle from scorbutic guinea pigs *in vitro*. This is of interest because certain Russian writers have recommended ascorbic acid for the treatment of hypotonic labor, and Spitzer (1947) states that he uses it instead of oxytocin to stimulate labor.

Tash (1951) supplemented 30 primigravidas and 30 multigravidas with 300 mg of vitamin C daily for 8 d, and then 100 mg/d until delivery. Observing an average duration of labor of 6.5 h, and only one case of post-partum uterine atony, he concluded that this regimen was beneficial in shortening labor.

Umanskii (1970) observed a sharp increase in the urinary excretion of ascorbic acid during labor, which was replaced by a significantly decreased rate of excretion during a period of 1 to 3 h post partum.

IX. PUERPERIUM

Snelling and Jackson (1939), working in Toronto, reported a relatively large fall in the ascorbic acid (AA) level from 0.6 mg/100 ml in the first 5 months of pregnancy to 0.42 at term and to 0.23 mg/100 ml in the immediate puerperium. Javert and Stander (1943) working at Cornell University Medical Center in New York City, found the average plasma ascorbic acid (AA) level of nonpregnant women to be 1.1 mg/100 ml. It fell to 0.54 in pregnancy and to 0.40 at the time of delivery; on the third postpartum day, they recorded an average maternal plasma ascorbic acid level of 0.35, slightly lower than the value at delivery. They felt that the onset of lactation might be responsible for the low value, while wound healing (episiotomies and lacerations), uterine involution, and impaired intake might also be contributing factors. They cited the findings of Sadovsky et al. (1939) that breast milk during the first days contained an average of 4.6 mg of ascorbic acid per 100 ml, which is considerably higher than the amount present in maternal plasma and must constitute a drain on maternal stores. This high ascorbic acid level in milk is no doubt due to the fact that milk is loaded with fat-laden leukocytes. Nevertheless, at 6 weeks post partum Javert and Stander found that the maternal plasma ascorbic acid level had returned to normal.

Young et al. (1946) reported work they had done at the Hammersmith Postgraduate Hospital in London during the "buzz-bomb" raids of World War II, when food at the "British Restaurants" was available to all. They found mean plasma ascorbic acid (AA) levels of 0.59 before the 24th week of pregnancy, 0.57 after the 24th week, and 0.49 mg/100 ml during the first 10 d of the puerperium.

Moore et al. (1979), in Glasgow, found the mean leukocyte ascorbic acid (TAA) level in late pregnancy to be 120 nmol/10^8 cells and observed a highly significant fall in this level to 84 nmol on the first day post partum ($p < 0.001$). The mean ascorbic acid level rose again to 112 on the third day and to 132 on the fourth or fifth day post partum. However, this post-partum recovery of the ascorbic acid level varied from person to person and was dependent not only on adequate nutrition, but also on the absence of other complicating factors such as puerperal infection.

Clearly, all further studies along these lines will be complicated by our obligation to advise patients concerning their nutritional needs.

X. THE NEWBORN

Findlay (1921) suggested that not only human beings, monkeys, and guinea pigs, but also rabbits have a requirement for ascorbic acid under certain circumstances. It seems that they can synthesize ascorbic acid, but not always enough. Findlay suggested that, "the non-susceptibility of the rabbit to scurvy must therefore be looked upon as relative rather than absolute." This was confirmed more than half a century later by the present writer when he observed ascorbate-responsive histaminemia in rabbits. It is therefore pertinent to recall Findlay's report that female rabbits can become pregnant and carry their fetuses almost to term on a diet of oats and wheaten bran, but the young are born dead, with the typical hemorrhages and bone and joint changes of scurvy. Addition of 10 ml of swede-juice daily made the diet quite adequate for rabbits.

Toverud (1936), at the University of Norway, reviewed the post-mortem findings in stillbirths and neonatal deaths at a hospital in Oslo during the 10-year period from 1924 to 1933. More than half were premature infants. Cerebral hemorrhage was found to be the cause of death in 39% and subserous hemorrhages in the heart, lungs, and thymus due to asphyxia were recorded in 52% of the infants. Toverud noted that some of the cerebral hemorrhages were associated with spinal hemorrhage and many were associated with other minor hemorrhages in and around the tooth follicles in the jaws. Often the cerebral hemorrhages showed evidence of having occurred before labor and were associated with pree-

clampsia or abruptio placentae; he did not think that many were of traumatic origin. Many of the women were known to have received insufficient fresh fruits and vegetables in their diets, so Toverud suspected vitamin C deficiency which may indeed have predisposed to abruptio placentae (Chapter 14, Volume III). There was no significant difference between the liver ascorbic acid levels of infants dying in the neonatal period with or without cerebral hemorrhage, but the mean liver ascorbic acid levels of 6.05 mg/g found in premature and 7.01 mg/g in mature infants were very low compared with values reported by other workers. Seven of the dead infants showed values so low that Toverud considered them as suffering from latent scurvy.

Studying 23 newborn infants at the State University of Iowa, Braestrup (1936) found a fall in the mean plasma ascorbic acid (AA) level from 1.07 mg to 0.27 mg/100 ml in the first 10 days of life; nearly 50% of this fall occurred in the first 24 h of life. Moreover, Javert and Stander (1943) found plasma AA levels ranging from 0.0 to 0.3 mg/100 ml in several of their infants who developed hemorrhagic disease of the newborn, which Javert believed due to a combined deficiency of vitamin C and K. More often it represents a deficiency of vitamin C and a deficiency of prothrombin due to immaturity of the liver. The livers of some immature infants cannot manufacture enough prothrombin even when there is a plentiful supply of vitamin K, as the necessary enzymes have not yet developed.

Sandford et al. (1942) found that infants with cerebral hemorrhage had low blood values for vitamin C, so the need for treatment may be urgent.

Arad et al. (1982) at Hadassah University of Israel, noted that premature infants, especially those with respiratory distress syndrome, seem to have higher ascorbic acid requirements than normal-term infants. They recommend the parenteral administration of ascorbic acid, 50 mg daily, to premature infants, especially when they are breathing higher than normal concentrations of oxygen, as this vitamin has been shown to protect the lungs against oxygen toxicity (Chapter 24 of this volume). Arad et al. (1985) found low plasma vitamin C levels in bottle-fed premature infants receiving a "humanized" milk formula containing 61 mg of ascorbic acid per liter. The plasma ascorbic acid level of these premature infants was increased from 0.39 to 0.94 mg/100 ml by the provision of an additional oral ascorbic acid supplement of 50 mg/d. Moreover, these workers noted that the ascorbic acid supplement seemed to have a sparing effect on vitamin E metabolism in these premature infants.

XI. CONGENITAL ABNORMALITIES

Record (1961) observed that the incidence of anencephaly in Scotland is four times higher for social class V than for social class I, and is also higher for fetuses born in the late autumn and winter months than for those born in the summer. Assuming that a causal factor acts when the embryo is 3 weeks old, and allowing for the fact that anencephalics are born on the average a month early, Record calculated that the causal factor operates most strongly from March to July and least strongly from September to December. While these data may be subject to several interpretations, it is worthy of note that these are the seasons when plasma and leukocyte ascorbic acid levels are lowest and greatest respectively, in the U.K. (Chapter 19 of this volume). Moreover, ascorbic acid deficiency will affect the poor more often than the well-to-do.

Nelson and Forfar (1971) conducted an extensive study of drugs taken by women during pregnancy and observed a highly significant negative correlation between the taking of vitamin C supplements and congenital abnormalities in the offspring; 23.1% of the mothers of normal infants took vitamin C supplements during pregnancy; significantly fewer mothers of infants with all (12.7%), major (13.2%), and minor abnormalities (12.4%) took vitamin C supplements during pregnancy: $p < 0.0001$, $p < 0.001$, and $p < 0.0001$, respectively. So plentiful vitamin C supplies during pregnancy would seem to be essential for normal fetal development.

Folic acid supplements showed a much less significant negative correlation for all abnormalities ($p < 0.05$), and no significance for either major or minor congential abnormalities alone.

The findings in the "Leeds Pregnancy Nutrition Study", reported by Smithells et al. (1976), revealed significantly lower than normal red cell folate (141 vs. 228 ng/ml, $p < 0.001$) and leukocyte ascorbate levels (23.9 vs. 34.5 $\mu g/10^8$ cells, $p < 0.05$) in blood samples drawn in the first trimester of pregnancy from women who subsequently gave birth to infants with neural tube defects such as anencephaly, meningomyelocele, or hydrocephalus (Table 3). In contrast, mothers whose infants had malformations of the cardiovascular system had mean values close to the control means.

Neural tube defects occur more commonly in female fetuses (1.9:1) according to Stocks (1970), but show a low concordance rate of 5.8% in identical twins according to Renwick (1972); so it seems unlikely that any single genetic or environmental factor could be responsible. Nevertheless, it is clear that nutrition in early pregnancy plays a very important role; special attention must therefore be paid to the provision of parenteral vitamin supplements for those who cannot take nourishment because of excessive vomiting in early pregnancy.

Many different theories have been proposed concerning the etiology of neural tube defects, which are so common in the U.K. and in Ireland, but there may be a common final pathway relating these apparently unrelated theories. The theory of "infection" proposed by Record (1961), of "soft water" proposed by Stocks (1970), and the "potato blight" theory of Renwick (1972) may all be related, as infection can cause ascorbic acid deficiency (Chapter 8 of this volume), as also can the heavy metals in soft water (Chapter 10 of this volume), and, of course, good potatoes are a major source of vitamin C in the U.K. and in Ireland (Chapter 19 of this volume). Moreover, ascorbic acid deficiency causes a disturbance of folic acid metabolism (Chapter 4, Volume III), which no doubt accounts for the association of these two deficiencies. Indeed, it may be a secondary folinic acid deficiency, induced by ascorbic acid deficiency, or by a disturbance of ascorbic acid metabolism that actually causes the neural tube defects.

Confirmation of the role played by vitamin deficiency in the etiology of neural tube defects has been provided by Smithells et al. (1980, 1981a, b) and by Schorah et al. (1983). Usually the risk of recurrence of a neural tube defect in a subsequent pregnancy is about ten times that in the general population. But these workers, by providing a multivitamin supplement to be taken before and during early pregnancy for those at risk, found the incidence of neural tube defects to be reduced eight times in the supplemented group, compared with that in others who had not received a supplement. There was a neural tube defect in 13 of 308 infants or fetuses examined in an unsupplemented high-risk group, but only 1 recurrence among 196 in the high risk group receiving the multivitamin supplement. The supplement provided 0.36 mg of folic acid and only 40 mg of ascorbic acid a day, which is a very meager allowance. The mean erythrocyte folic acid level rose from 250 to 478 ng/ml and the mean leukocyte ascorbic acid level rose from 1.82 to 3.21 $\mu g/ml$ of blood as a result of the supplement; but 23% of the women still had leukocyte ascorbate levels below the "neural tube defect threshhold" at 8 weeks of pregnancy. Hopefully even better results may be achieved with ascorbic acid, 200 mg a day, preferably with D-catechin to chelate heavy metals or, better still, as catechin-coated ascorbic acid tablets.

The association between maternal and cord blood mercury levels and a history of stillbirth ($p < 0.05$) or earlier deliveries of malformed infants ($p < 0.05$), as reported by Kuntz et al. (1982), makes the need for chelating food fiber even more evident. Moreover, it is known that mercury is a potent cause of ascorbic acid deficiency (Chapter 10 of this volume).

Table 3
VITAMIN ASSAYS: MEAN VALUES FOR CONTROL POPULATION, SOCIAL CLASS SUBDIVISIONS, AND THE CNS DEFECT INFANTS

Numbers in Each Group Shown In Parentheses

		Social class					
	Controls Mean 95% range	I + II	III nonmanual	III manual	IV + V	CNS defect	Significant differences
Red cell folate (ng/ml)	228 (959) 86—460	249 (245)	218 (148)	221 (420)	220 (146)	141(6)	I + II vs. IIINM p <0.005 I + II vs. IIIM p <0.001 I + II vs. IV + V p <0.01 CNS defect vs. controls p <0.001
Serum folate (ng/ml)	6.3 (953) 1.9—15.8	6.7 (245)	5.8 (148)	6.3 (412)	6.0 (148)	4.9 (5)	(No significant differences)
Vitamin C ($\mu g/10^8$ WBC)	34.5 (1098) 15—66	36.9 (229)	35.3 (177)	33.4 (441)	32.5 (181)	23.9 (4)	I + II vs. IIIM p <0.001 I + II vs. IV + V p <0.001 IIINM vs. IV + V p <0.05 CNS defect vs. controls p <0.05
Riboflavin (saturation index)	1.23 (1284) 1.03—1.53	1.20 (357)	1.24 (204)	1.24 (519)	1.26 (204)	1.28 (6)	I + II vs. IIINM p <0.001 I + II vs. IIIM p <0.001 I + II vs. IV + V p <0.001
Serum vitamin A (μg/100 ml)	68.2 (971) 43—108	71.7 (288)	67.3 (150)	66.4 (377)	66.6 (156)	75.7 (3)	I + II vs. IIINM p <0.05 I + II vs. IIIM p <0.001 I + II vs. IV + V p <0.01

Note: This table shows the mean first trimester vitamin assay results obtained in the mothers of six infants born with neural tube defects, for comparison, with the first trimester results observed in mothers of normal infants, divided by social class. Three were anencephalics, one of which aborted spontaneously at 13 weeks; one had a meningocele, a lesion which does not involve neural tissue, but closely resembles failure of closure of the neural tube; one had a meningomyelocele and hydrocephalus and the sixth infant had microcephaly.

From Smithells, R. W., Sheppard, S., and Schorah, C. J. (1976), *Arch. Dis. Child.*, 51, 944. With permission.

XII. VITAMIN C TOXICITY?

Crampton and Bell (1947) compared the effect of natural and synthetic sources of vitamin C on the outcome of pregnancy in guinea pigs and noted that aqueous ascorbic acid permitted more reproductive failures (abortions and hemorrhages) than did natural sources of ascorbic acid such as fresh greens, orange juice, or lemon juice. The natural juices tended to promote a higher proportion of successful litters and living young. They concluded that the poor performance of the ascorbic acid-supplemented animals suggested a deficiency of some other factor present in vegetable roughage. There were only 68% of successful litters in animals receiving no roughage, 83% with dry roughage and 92% of successes in animals receiving greenfeed. Today, this roughage is usually referred to as fiber, and the present writer would specify chelating fiber such as D-catechin to inactivate any heavy metal catalysts which can otherwise cause losses of ascorbic acid by oxidation and subsequent hydrolysis.

Neuweiler (1951) recorded observations suggesting that a dose of vitamin C which is perfectly harmless to nonpregnant guinea pigs (25 mg/d) might be harmful to pregnant guinea pigs, as it caused premature delivery, at 65 d instead of 72, and a 10% stillbirth rate in one group of animals. However, the dose he found toxic is no more than his control guinea pigs may have consumed every day, as they were allowed lots of green food, and each lettuce leaf contains about 1 mg of vitamin C. Neuweiler recognized this, for he pointed out that "hypervitaminosis" is not so much the result of wrong diet, but more the result of high doses of pharmaceutical products. Moreover, he discussed the possible synergisms and antagonisms of various vitamins and hormones.

In retrospect, the toxicity could have been due to trace quantities of free cupric ions in the drinking water acting on the ascorbic acid supplement to form "ascorbate-free-radical". Ascorbic acid as it occurs in green vegetables is already protected from heavy metals by chelating fiber; thus, it seems likely that the 25 mg of ascorbic acid would not have been toxic if it had been given with protein (Chapter 12 of this volume) or with a natural plant chelating agent such as D-catechin (Chapter 11 of this volume).

Mouriquand and Edel (1953), also studying guinea pigs, reported that very high doses of vitamin C (250 mg daily) by mouth or by injection were harmless in male and in nonpregnant female animals, but seemed to be toxic in pregnant females, sometimes causing the death of one or more fetuses and subsequent infertility.

Cochrane (1965) reported that 42 cases of infantile scurvy were seen at the Children's Hospital in Nova Scotia between October 1959 and January 1961. Noting that two of the infants had supposedly been receiving a total of 60 mg of vitamin C daily by diet and supplement, it was suggested that they might have developed "conditioned" scurvy due to withdrawal of excessive maternal vitamin C supplements taken by their mothers during pregnancy, but there was little evidence of this, as the total daily maternal vitamin C intake of each woman was only 400 mg, which is less per pound of body weight than the supposed infant intake. Preliminary guinea pig studies were reported, but these were by no means conclusive.

Samborskaya and Ferdman (1966) reported that massive doses of ascorbic acid (6 g daily for 3 d) may have a paradoxical effect and actually cause abortion when given to women in early pregnancy (10 to 15 d overdue).

Norkus and Rosso (1975) reported that feeding high levels of ascorbic acid during the last 30 d of pregnancy in the guinea pig caused the young to develop scurvy after 18 instead of 22 d when fed a vitamin C-deficient diet. Norkus et al. (1979) suggested that the high levels of ascorbic acid experienced by the fetuses in utero may have conditioned them to need more vitamin C after birth.

However, Ginter et al. (1982) found no evidence of an increased rate of tissue ascorbate depletion in adult guinea pigs following 7 months of high ascorbate intake.

There does not seem to be any conclusive evidence that high blood ascorbic acid levels in the mother can cause a "conditioned" vitamin C deficiency in the infant. However, it does seem that massive doses of ascorbic acid (6 g daily) can be toxic to the fetus in utero; possibly even low doses could be toxic if consumed with water containing cupric ions.

XIII. CONCLUSIONS

1. Pregnancy constitutes a considerable drain on maternal vitamin C reserves.
2. Vitamin C and folic acid deficiency in early pregnancy may well be responsible for neural tube defects in the fetus.
3. Massive doses of ascorbic acid may also be toxic to the fetus and may cause abortion.
4. Ascorbic acid supplements are needed during pregnancy in many parts of the world, especially in northerly regions in late spring and early summer.
5. Ascorbic acid, 200 mg daily, can be given safely in orange juice throughout pregnancy.
6. Women with special problems, such as capillary fragility, habitual abortion, previous abruptio placentae, or bleeding in pregnancy, may need higher doses, such as 200 mg three times a day, which can best be given along with a nonmutagenic chelating flavonoid, such as D-catechin, to prevent the formation of ascorbate free radical (Chapter 11 of this volume).

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Chapter 15

HEMOLYSIS

The work of Lloyd (1950), Panteleeva (1950), Patterson and Mastin (1951), and Lloyd and Parry (1954) led to the demonstration by Christine et al. (1956) and Räihä (1958) that ascorbic acid (AA)* enters red blood cells mainly in the form of dehydroascorbic acid (DHAA) and is reduced to ascorbic acid in the erythrocytes. DHAA, or ascorbone as it is sometimes called, is not an acid, but a trione or triketone which is nonionic and lipid soluble. As such, it is the form in which ascorbic acid most readily crosses cell membranes (Martin, 1961), but being an unstable compound with a short half-life, it must be reduced to ascorbic acid inside the cell. Indeed, the storage of ascorbic acid in any tissue is probably dependent on the ability of that tissue to reduce DHAA to AA.

Some workers, like Kellie and Zilva (1935) and Borsook et al. (1937), observed that the presence of intact red blood cells appears to exert a protective effect on ascorbic acid in plasma, although Barron et al. (1937) found no such protective effect in one experiment on dog blood. Greenberg and Rinehart (1940) confirmed the protective effect of red blood cells in some human blood samples, but found this property to be somewhat variable; it probably depends on the concentration of reduced glutathione in the red blood cells.

However, when oxyhemoglobin is present in blood plasma, as a result of hemolysis, or is released by addition of trichloracetic acid to whole blood, ascorbic acid loss is accelerated, as shown by Kellie and Zilva (1935), Table 1, and by van Eekelen (1936), Klodt (1936), Emmerie and van Eekelen (1937), Berend and Fischer (1937), and Greenberg and Rinehart (1940). Various explanations have been offered for the mechanism of action of hemoglobin on ascorbic acid. Van Eekelen and Kellie and Zilva attributed the loss to oxidation of the latter by oxyhemoglobin. Klodt supported this view, as he found that the loss of ascorbic acid paralleled the oxygen saturation of the hemoglobin. On the other hand, Fischer and also Gabbe (1937) did not agree with this opinion. They expressed the belief that ascorbic acid is bound adsorptively by hemoglobin and does not disappear as a result of oxidation. However, Emmerie and van Eekelen showed that AA is reversibly oxidized to DHAA by oxyhemoglobin from laked red blood cells and can be recovered completely by reduction with hydrogen sulfide, as shown in Table 2.

Lemberg et al. (1939) demonstrated conclusively that oxyhemoglobin released by the lysis of erythrocytes reacts directly with ascorbic acid to form choleglobin. Two hydrogen atoms are transferred from ascorbic acid to oxyhemoglobin, giving rise to an unstable hemoglobin-hydrogen peroxide compound which breaks down with the formation of choleglobin. They showed that diethyldithiocarbamate, a copper inhibitor, blocks the so-called autoxidation of ascorbic acid, but does not block the action of oxyhemoglobin, while carbon monoxide could be used to block the formation of choleglobin without considerably diminishing the rate of autoxidation.

Borsook et al. demonstrated that AA undergoes oxidation in three stages. First the reversible oxidation to DHAA; they showed that DHAA is unstable above pH 4, undergoing an irreversible change to diketogulonic acid with loss of vitamin activity; then a second oxidation to *l*-threonic acid and oxalic acid, and a third unknown oxidation stage. They knew that protein slowed the first oxidation stage and, thus, ascorbic acid oxidation is slower in blood plasma than in tap water. They demonstrated that the half-life of ascorbic acid in whole blood is considerably greater than in plasma and suggested that erythrocyte glutathione exerts this action by reducing DHAA to AA. Ascorbic acid is lost more slowly *in vivo* than

* AA — ascorbic acid, reduced form.

Table 1

Milligrams of Ascorbic Acid per 20 ml of Solution Determined Immediately After Addition

	Control	1 ml	2 ml	4 ml
Intact red corpuscles	7.0	7.1	7.5	7.8
Laked red corpuscles	6.7	5.8	4.9	3.0
Laked red corpuscles treated with carbon monoxide	6.8	6.8	7.0	7.5

Note: "Laked red corpuscles were also found to be capable of protecting ascorbic acid from oxidation but in this case an initial disappearance of the vitamin was noted. It was possible to show that the quantity of ascorbic acid which disappeared was proportional to the amount of haemolysed blood added and further that when the red corpuscles were previously treated with CO the destructive action of the haemolysed erythrocytes was hardly appreciable. It would appear that on haemolysing a substance, most probably oxyhaemoglobin, is set free which is capable of interacting with ascorbic acid."

From Kellie, A. E. and Zilva, S. S. (1935), *Biochem. J.*, 29, 1028. ©1935 The Biochemical Society, London. With permission.

Table 2

	Milligrams ascorbic acid per 10 ml trichloroacetic acid extract	
	After precipitation with CCl_3COOH	After reduction with H_2S
Aerated blood	0.55	1.03
Blood saturated with CO_2	0.99	1.03

Note: When the proteins of whole blood are precipitated with trichloracetic acid, oxyhemoglobin is released from the red cells and this results in an apparent loss of titratable ascorbic acid (AA). These observations demonstrate that the apparent loss is due to oxidation, to DHAA, for all of the ascorbic acid can be recovered after reduction with hydrogen sulfide.

From Emmerie, A. and van Eekelen, M. (1937), *Biochem. J.*, 31, 2125. ©1937 The Biochemical Society, London. With permission.

in vitro; the work of Borsook et al. (1937) left little doubt that this results from the reducing effect of glutathione and other thiols in the tissues.

Mystkowski and Lasocka (1939) confirmed the observation that the addition of even small amounts of hemolysed blood to serum causes considerable acceleration of the oxidation of ascorbic acid. Thus, oxyhemoglobin released from hemolysed erythrocytes may cause permanent loss of vitamin activity unless there are enough thiols in the intact red cells and other body tissues to reduce DHAA to AA as fast as it is formed. It seems that oxyhemoglobin outside the erythrocyte behaves in an entirely different manner from oxyhemoglobin inside the cell. This could cause major losses of ascorbic acid in a patient with hemolytic anemia, especially during a hemolytic crisis.

Greenberg and Rinehart (1940) showed that neither sodium nor potassium cyanide inhibits the destruction of ascorbic acid by hemolysed red blood cells. On the other hand, Christine et al. (1956) showed that carbon monoxide treatment of hemolysed red cells completely

inhibits ascorbic acid oxidation; in fact, they showed that a cell-free filtrate of hemolysed red cells rapidly reduces DHAA to AA when all oxygen has been replaced by carbon monoxide; the site of the reduction was found to be within the erythrocyte and not on the wall of the cell, since the filtrate of hemolysed centrifuged erythrocytes in carbon monoxide gave substantially the same rate of reduction of DHAA to AA, as did intact red cells. They gave reasons for believing that the reducing capacity of the red cells is due to reduced glutathione (GSH), as it is blocked by –SH inhibitors. They also provided evidence of an enzyme system which reduces oxidized glutathione (GSSG) and replenishes GSH as fast as it is used, for heating impaired the replenishment of GSH in their system. They quoted the findings by Francoeur and Denstedt (1954) and by Collier and McRae (1955) of enzyme systems capable of forming GSH from GSSG in human erythrocytes. The electron donors in those systems were reduced triphosphopyridine nucleotide (TPNH) and reduced diphosphopyridine nucleotide (DPNH), of which the supplies are maintained by glucose-6-phosphate dehydrogenase of the cells. They felt that such systems would explain why no fall of GSH concentration occurred during the reduction of DHAA.

Grimble and Hughes (1967) reported the finding of a DHAA reductase factor in guinea pig tissues including stomach, brain, adrenals, small intestine, lung, and liver, but Hughes and Maton (1968) reported that DHAA uptake by erythrocytes does not seem to be enzyme dependent.

It seems that ascorbic acid is oxidized progressively more slowly, progressing from tap water to plasma containing hemolysed red cells, to nonhemolysed plasma, to whole blood *in vitro*, to whole blood *in vivo*, due to the protein, oxyhemoglobin, red cell –SH, and tissue –SH effects, respectively.

Vilter et al. (1946), observing an abnormally high serum bilirubin level and the occurrence of reticulocytosis, suggested that there may be a hemolytic process in scurvy. Bronte-Stewart (1953) reported increased urinary and fecal urobilinogen excretion in scurvy and gave evidence that this is probably due to the resolution of large intramuscular hematomata (i.e., extravascular hemolysis). However, Merskey (1953) has reported finding markedly reduced donor red cell survival times in scorbutic patients who did not have any major hemorrhages and suggests the possibility that the anemia, which is often found in scurvy, may be partly due to hemolysis.

Goldberg (1963) reported on 55 patients with scurvy who had been seen at the Western Infirmary in Glasgow over 22 years; most of them were anemic. He reported several kinds of scorbutic anemia, including normocytic, microcytic, and megaloblastic varieties and observed that blood loss, iron deficiency, folate disturbance, and hemolysis were major contributing factors in different patients. Extensive studies of six patients revealed evidence of hemolysis in four, serum bilirubin was elevated in four, two of these had clinical jaundice, the urinary urobilinogen excretion was elevated in four, reticulocytosis was present in all of them. Addition of ascorbic acid to the diet resulted in a rise in the hemoglobin level, an initial rise, then a fall, in the reticulocyte count, and a fall in the urinary urobilinogen level. Radiochromium studies revealed further evidence of hemolysis, with markedly reduced erythrocyte half-life survival times (10 to 16 d) in four of the six patients, which returned to normal (26 to 30 d) when they were saturated with ascorbic acid.

Erythrocytes from the four patients with scorbutic hemolytic anemia were found to have normal fragility when tested *in vitro* in hypotonic saline. However, erythrocytes from a normal subject had a reduced survival time ($t_{1/2}$ = 15 d) when injected into a patient with scurvy, and erythrocytes form that scorbutic patient had a normal survival time ($t_{1/2}$ = 27 d) when injected into a normal subject. Subsequent experiments involved the incubation of scorbutic red cells with normal plasma and normal red cells with scorbutic plasma; ascorbic acid diffused from the normal red cells to the scorbutic plasma and from the normal plasma to the scorbutic red cells. It was therefore concluded that it was not the scorbutic red cells

themselves that were fragile, but the scorbutic milieu that was unfavorable to the survival of erythrocytes. Clearly, part of the hemolysis is extravascular, taking place in the tissue ecchymoses, but Goldberg stated that this was not sufficient to account for the degree of anemia and hemolysis observed.

Traina (1950) demonstrated that normal citrated human erythrocytes were protected from *in vitro* hemolysis in hypotonic solutions by the addition of ascorbic acid. Moreover, glutathione depletion renders red cells vulnerable to hemolysis (Wintrobe, 1961), so we may consider that the whole chain, including glutathione reducing enzymes, glutathione itself, and ascorbic acid, is needed to provide a store of ascorbic acid (AA) within the red cell to protect it from lysis.

Thus, one can envisage the possibility of a vicious cycle. We have seen that hemolysis destroys ascorbic acid by oxidation and subsequent hydrolysis; now it is seen that severe ascorbic acid deficiency can cause hemolysis. One wonders whether this may not be the way in which various infections and other illnesses and drugs may precipitate hemolytic crises in patients with sickle cell disease and other hemolytic anemias.

A $2^1/_2$-year study of blood donors at Altay Medical School in Barnaul, Central Asia (U.S.S.R.), by Barkagan (1965) showed that blood from blood donors on a low-ascorbic acid diet, with a mean plasma ascorbic acid level of 0.35 mg/100 ml, had erythrocytes, whose acid-fast properties dropped in the fourth week of blood preservation. In donors supplied with optimal allowances of ascorbic acid, whose mean plasma ascorbic acid level was 0.70 mg/100 ml, the changes in acid fastness occurred in the sixth week of preservation. He found that the survival rate of transfused erythrocytes in the recipient's bloodstream is higher when optimal amounts of ascorbic acid have been supplied to the donors. It was concluded that the observed phenomena were not due to a direct effect of ascorbic acid on erythrocytes, but due to the fact that the presence in the organism of sufficient amounts of ascorbic acid is conducive to the production of more resistant and physiologically normal erythrocytes.

Schulman (1980) points out that, "Excessive erythrocyte destruction under circumstances of increased oxidative susceptibility is a well-recognized phenomenon in certain human disorders. The most important of these is glucose-6-phosphate dehydrogenase (G6PD) deficiency, in which the cellular capacity to regenerate adequate quantities of reduced glutathione is compromised by an enzymatic defect not directly interfering with glutathione synthesis." According to Boxer (1980), the glutathione peroxidase-glutathione reductase system disposes of H_2O_2 by using reduced glutathione (GSH) as a substrate for H_2O_2 in a reaction catalyzed by glutathione peroxidase ($2\ GSH + H_2O_2 \rightarrow GSSG + 2H_2O$). The oxidized glutathione is then reconverted to reduced glutathione by glutathione reductase catalysis in which nicotinamide-adenine dinucleotide phosphate (NADP) is generated: $GSSG + NADPH + H \rightarrow 2\ GSH + NADP$. Erythrocyte inability to adequately reduce or synthesize glutathione results in hemolytic anemia. Boxer also observed that human polymorphonuclear leukocytes unable to maintain adequate levels of reduced glutathione, are vulnerable to oxidant damage, resulting in impaired phagocytic function. This caused increased susceptibility to infection in one of his patients, a newborn child with 5-oxoprolinuria secondary to a generalized deficiency of cellular glutathione synthetase activity, but the child showed a good response to treatment when vitamin E was given as an antioxidant.

One would certainly like to have information concerning the leukocyte ascorbic acid-storing capacity and plasma ascorbate levels of such patients with this and six other known enzyme deficiencies listed by Spielberg (1980) as causing human inborn errors of glutathione metabolism. It would seem that some of these conditions might interfere with ascorbic acid metabolism in two ways: by impairing tissue ascorbate storage and by causing hemolysis. One would also like to know the ascorbic acid levels of patients with sickle cell disease in crisis and in remission.

Wapnick et al. (1969) reported leukocyte ascorbic acid levels in eight patients with thalassemia major and one with sickle cell thalassemia, all of whom had excessive iron stores due to multiple blood transfusions. The mean white cell ascorbic acid (TAA)* level of these nine patients was 9.6 $\mu g/10^8$ leukocytes in contrast with 31.8 $\mu g/10^8$ leukocytes in six normal control subjects. Similar low levels were found in patients with idiopathic hemochromatosis, and even lower levels in Bantu with dietary siderosis, so the low ascorbic acid levels were attributed to the excessive iron stores. They found that oral administration of ascorbic acid, 500 mg three times a day for 7 d, caused the desferrioxamine-induced urinary iron excretion to increase by 88% in patients with transfusional siderosis, by 60% in those with idiopathic hemochromatosis, and 350% in the Bantu patients with dietary siderosis. The white cell ascorbic acid level of the thalassemics rose to 26.8 $\mu g/10^8$ leukocytes after loading with ascorbic acid.

O'Brien (1974) confirmed that ascorbic acid enhances desferrioxamine-induced excretion of iron by thalassemics. Modell and Beck (1974) gave vitamin C, 200 mg daily, as a dietary supplement to children with thalassemia major who were undergoing desferrioxamine treatment for removal of excessive iron stores. They also confirmed that vitamin C increases urinary iron excretion due to desferrioxamine. These authors stated that untransfused patients were generally less severely iron loaded, although they can be as severely iron loaded as transfused patients of the same age. Low leukocyte ascorbic acid levels and also plasma vitamin E levels at the lower end of the normal range were reported in their studies of thalassemics. They suggested that iron overload causes oxidative damage, which is maximal in the pancreas and the adrenal, for they found decreased glucose tolerance and adrenal insufficiency, as well as cirrhosis of the liver and retarded growth in these children. They gave reasons for believing that vitamin C (a reducing agent) and vitamin E (an antioxidant) might help prevent this oxidative damage.

Nienhuis et al. (1976) studied both urinary and fecal iron excretion during desferrioxamine treatment of six patients with thalassemia major and two others with refractory anemias and transfusional siderosis: the increased urinary iron excretion following ascorbic acid administration was counterbalanced by a decrease in fecal iron excretion in the two refractory anemias, but in all six thalassemics, ascorbic acid resulted in a net increase in iron excretion sufficient to exceed iron gain from absorption by the alimentary tract and from frequent blood transfusions, as shown in Figure 1. They found that it was essential to continue ascorbic acid supplementation during desferrioxamine treatment, as the white cell ascorbic acid levels fell and urinary iron excretion decreased, as shown in Figure 2, when ascorbic acid was discontinued.

Chatterjea et al. (1980) reported a highly significant reduction in the platelet ascorbate levels of patients with beta thalassemia and HbE thalassemia. Mean leukocyte ascorbic acid levels were also found to be lower in their studies of thalassemics, but the difference from normal was not statistically significant, perhaps because their "normals" had unusually low leukocyte ascorbic acid levels. These authors believe that the reduced platelet ascorbic acid levels of thalassemics are due to iron overload resulting from repeated blood transfusions. In support of this thesis they report that patients with iron deficiency anemia have abnormally high platelet ascorbic acid levels, as shown in Table 3.

Severe ascorbic acid deficiency has been reported by Cohen et al. (1981) in a 26-year-old woman with thalassemia major who had received repeated blood transfusions. She had two episodes of scurvy despite a normal intake of vitamin C; her derangement of ascorbic acid metabolism was probably due to her excessive iron stores, but the hemolysis of Cooley's anemia played a central role. Graziano and Piomelli (1980, 1981) agreed that ascorbic acid is needed during desferoxamine B (desferal) treatment of patients with thalassemia to aid

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

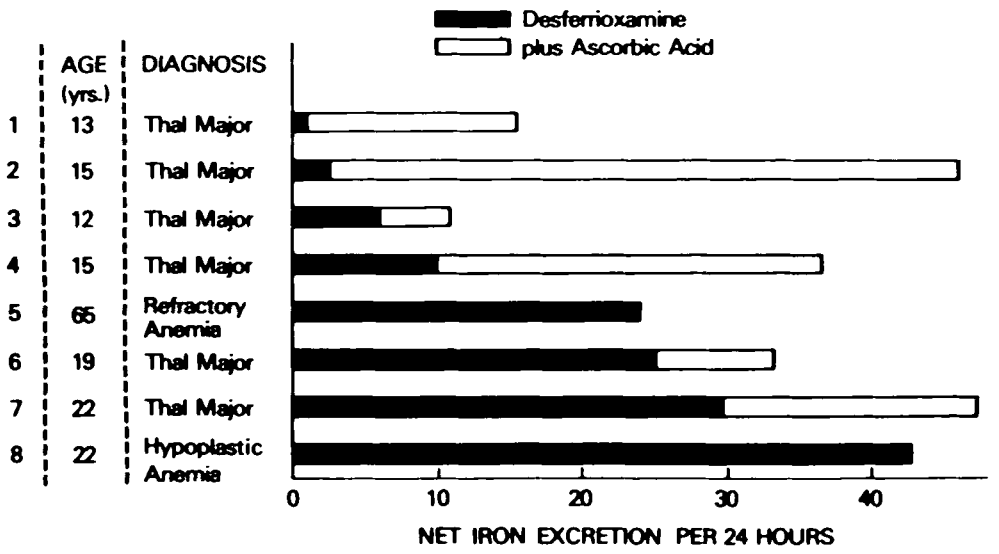

FIGURE 1. Summary of iron balance studies of patients with hemosiderosis undergoing chelation therapy for removal of excess iron, first with desferrioxamine alone, and then with ascorbic acid as well. Ascorbic acid caused a net increase in iron excretion (fecal + urinary loss) in all six patients with thalassemia major. (From Nienhuis, A. W., Delea, C., Aamodt, R., Bartter, F., and Anderson, W. F. [1976], in *Iron Metabolism and Thalassemia*, Bergsma, D., Cerami, A., Peterson, C. M., and Graziano, J. H., Eds., Alan R. Liss, New York, 177. ©Alan R. Liss for the National Foundation-March of Dimes. With permission.)

in the release of iron from storage by the reticuloendothelial system. These authors observed that ascorbic acid is oxidized at an accelerated rate in the presence of excessive iron stores, and gave this as the reason for increased ascorbic acid requirements in patients with thalassemia major. Nienhuis (1981) pointed out that while ascorbic acid supplementation will increase the excretion of iron in response to desferal, ascorbic acid administration is potentially cardiotoxic in these patients. Graziano and Piomelli reported the occurrence of deaths from cardiac dysfunction and also diabetes mellitus in patients with thalassemia who began chelation therapy in the teenage or later years of life.

Clearly, it is DHAA, resulting from rapid oxidation of ascorbic acid and not ascorbic acid itself that is toxic. Intravenous injection of DHAA is known to damage the beta cells of the islets of Langerhans and to cause temporary or permanent diabetes mellitus, as shown by Patterson (1950) in rats. Thus there is a real problem in these patients who need ascorbic acid, but cannot keep it in the reduced form. The writer suggests that the diabetes of hemochromatosis (bronzed diabetes) may be a direct result of DHAA intoxication. Cohen et al. have suggested that tissue damage due to iron overload may be reduced by low vitamin C stores. If this be so, it could indeed be harmful to give ascorbic acid alone as a nutritional supplement to patients with hemolytic anemias, glutathione deficiencies, or iron storage disease. We must give antioxidants like vitamin E, chelating antioxidants like rutin or D-catechin, chelating and reducing agents like penicillamine (dimethyl cysteine), reducing agents like cysteine or homocysteine, or cortisone, along with moderate doses of vitamin C in an attempt to keep the ascorbic acid in the reduced form. Corash (1980) made the following observations: "Erythrocytes are subjected to various oxidant stresses throughout their 120-day life span in the peripheral circulation. A major defense against this stress is derived from the production of reduced nucleotides and sulfhydril containing molecules via the hexose monophosphate shunt and glutathione-related pathways. Inherited defects in these metabolic routes, depending on their severity, may compromise the survival ability of the erythrocyte. Such disorders may result only in episodic hemolysis during periods of acute

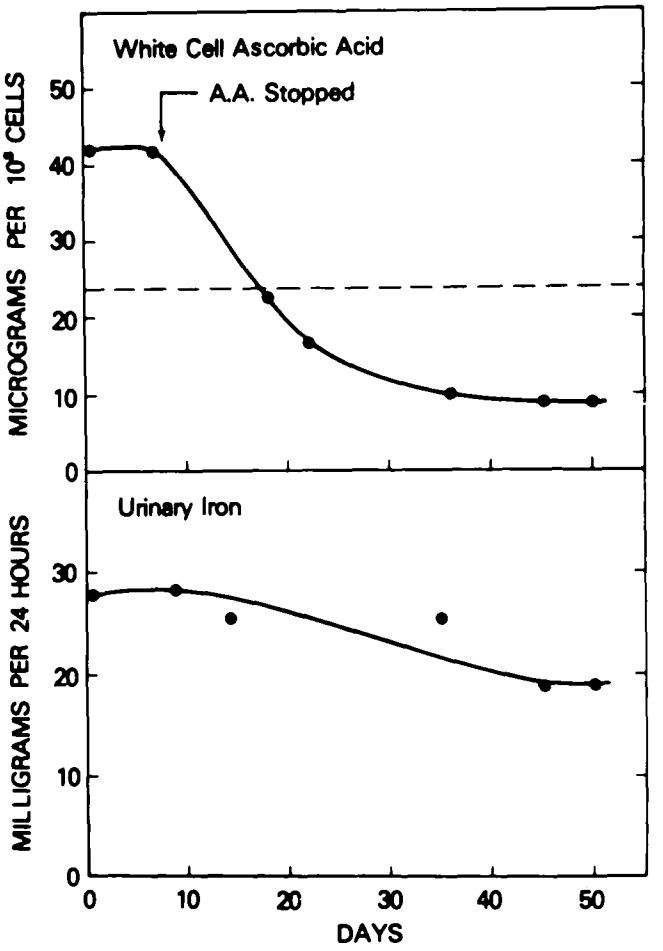

FIGURE 2. During treatment with desferrioxamine and ascorbic acid, 500 mg daily, the white cell ascorbic acid (TAA) level of thalassemics was within the normal range, but within 4 weeks of discontinuing the ascorbic acid supplement (and continuing the desferrioxamine), the leukocyte ascorbate values became subnormal. Urinary iron excretion was also reduced when the ascorbic acid was discontinued. (From Nienhuis, A. W., Delea, C., Aamodt, R., Bartter, F., and Anderson, W. F. [1976], in *Iron Metabolism and Thalassemia*, Bergsma, D., Cerami, A., Peterson, C. M., and Graziano, J. H., Eds., Alan R. Liss, New York, 177. ©Alan R. Liss for the National Foundation-March of Dimes. With permission.)

stress or may be associated with chronic severe hemolysis on a continuous basis." He quoted the work of others who have shown that oxidant-induced pathologic changes due to glutathione depletion can be partially prevented by treatment with vitamin E in animals, and he reported considerable success in treatment of three patients with vitamin E (D,L-alpha tocopherol). One had glutathione synthetase deficiency without oxyprolinemia and one had chronic nonspherocytic hemolytic anemia due to glucose-6-phosphate deficiency. All three had prolonged red cell survival times following treatment with vitamin E. One wonders whether it is a high DHAA level, an abnormally low AA/DHAA ratio, or a grossly abnormal blood and tissue redox potential that should be considered as the cause of the cell damage, for all tend to occur together.

Hemolytic anemia is not a subject which the writer has investigated as such, but he did

Table 3
MEAN VALUES OF PLATELET AND LEUKOCYTE ASCORBIC ACID IN DIFFERENT GROUPS OF SUBJECTS

Subjects	Number of cases	Platelet ascorbic acid μg/10^{10} cells		Leukocyte ascorbic acid μg/10^{8} cells	
		Range	Mean ± SE	Range	Mean ± SE
Normal	18	28—80	49.3 ± 3.5	2—25	10.78 ± 1.6
Iron deficiency anemia	32	25—270	102.3 ± 11.8 t = 3.5 p <0.01 HS[a]	5—25	14.0 ± 1.19 t = 1.74 p <0.1 NS[b]
HbE thalassemia	28	10—77	33.7 ± 3.7 t = 2.85 p <0.01 HS	2—24	7.5 ± 1.0
β-Thalassemia	7	9—47	23.7 ± 5.3 t = 3.84 p <0.001 HS	4—19	7.1 ± 2.1

Note: Platelet ascorbic acid levels were significantly elevated in severe iron deficiency anemia and significantly reduced in thalassemia.

[a] HS — highly significant.
[b] NS — not significant.

From Chatterjea, B., Maitra, A., Banerjee, D. K., and Basu, A. K. (1980), *Acta Haematol.*, 64, 271. S. Karger AG, Basel. With permission.

obtain blood samples from a 26-year-old Italian woman with thalassemia minor whom he saw in consultation because of dysfunctional uterine bleeding. She also had bleeding gums and easy bruising and was already under treatment with propranolol hydrochloride and hydrochlorothiazide in hospital when first seen, with diagnoses of thalassemia minor and prolapsed mitral valve; her differential white blood cell count, platelet count, coagulation time, and partial thromboplastin time were all normal, so her bruising and bleeding tendency was diagnosed as a form of nonthrombocytopenic purpura, or purpura simplex. Her hemoglobin level was 11.0 g/100 ml. No abnormality was detected on bimanual pelvic, speculum vaginal, or rectal examination, so the heavy irregular vaginal bleeding was assessed as being part of the general purpuric condition, as evidenced by the bruises on her legs and the blood on her pillow from gingival bleeding. The initial blood sample showed a plasma reduced ascorbic acid (AA) level of 0.30 mg/100 ml, as shown in Table 4. This is below the normal range, but not low enough, one would have thought, on its own to cause such purpura. Unfortunately, we do not know her leukocyte ascorbic acid level nor the AA/DHAA ratio of her blood plasma, which might have provided evidence of a disturbed ascorbate metabolism; the whole blood histamine level was slightly elevated. She received ascorbic acid, 1 g daily by mouth for 3 days, and had blood drawn again on the morning of the fourth day. This supplement would normally have brought her plasma ascorbic acid level up to 1.3 mg/100 ml, but it rose only slightly to 0.42 mg/100 ml. Subsequent treatment with ascorbic acid, rutin, and cortisone for 6 weeks, as shown in Table 4, did increase her plasma ascorbic acid level to 0.84 mg/100 ml; at the end of that time, her vaginal and gingival bleeding had ceased, but she was still bruising easily. One expects full saturation of the body of an adult after 3 or 4 g of ascorbic acid, so this blood level of 0.84 mg/100 ml was a very poor response following 55 g of ascorbic acid and suggests the existence of a profound disturbance of ascorbic acid metabolism in this woman with thalassemia minor; she had not received any blood transfusions, but it is possible that she may have had excessive iron stores.

Table 4
NONTHROMBOCYTOPENIC PURPURA IN A 26-YEAR-OLD ITALIAN WOMAN WITH THALASSEMIA MINOR, PROLAPSED MITRAL VALVE, AND DYSFUNCTIONAL UTERINE BLEEDING, SHOWING GREAT RESISTANCE TO REPLENISHMENT WITH ASCORBIC ACID

Date	Treatment	Clinical condition	Plasma ascorbic acid (mg/100 ml) (normal 0.4 to 1.5)	Whole blood histamine (ng/ml) (normal 10 to 20)
6/6/80 in hospital	On propranolol hydrochloride 200 mg daily and hydrochlorothiazide 50 mg daily	Gingival and irregular uterine bleeding with purpura	0.30	32
6/11/80	After ascorbic acid, 1 g daily for 3 days; to commence treatment with cortisone 40 mg daily, as well as rutin and ascorbic acid	She still has blood on her pillow	0.42	25
7/24/80	After tablets of sodium ascorbate (333 mg) with rutin (20 mg) four times a day for 6 weeks; now on cortisone, 20 mg daily, and reducing 5 mg every week; propranolol reduced to 120 mg daily; hydrochlorothiazide 50 mg daily	No more vaginal bleeding from June 10 until normal menses on July 10; 4 pads; only one episode of gingival bleeding in the last month; she still bruises easily	0.84	16

Note: Both thrombocytopenic and nonthrombocytopenic purpura have been reported as adverse reactions to propranolol, but this woman's bleeding problems preceded the prescription of propranolol; her purpura is believed to have been due to a disturbance of ascorbic acid metabolism resulting from thalassemia minor.

De Alarcon et al. (1979) reported increased iron absorption in thalassemia major and intermedia and found that iron absorption increased as the hemoglobin level fell (Table 5). They stated that iron overload has been documented in thalassemics who have never received a blood transfusion. These workers have studied the effects of tea on iron absorption and have found that this beverage decreased iron absorption in all of seven patients with thalassemia major and in one with thalassemia intermedia. The inhibition of iron absorption varied from 41 to 95% when a 240-ml infusion from one tea bag containing 275 mg of tannins was substituted for 240 ml of water, given to thalassemics with a meal containing a trace of ^{59}Fe in 2.8 mg of nonheme iron, as shown in Table 6.

Likewise, Hochstein et al. (1980) studied the hemolytic effects of copper toxicity, which occurs when copper is present in excess of the capacity of specific intracellular and extracellular proteins to sequester the metal. They observed that copper has an inhibitory effect on glycolytic enzymes, such as hexokinase, in human erythrocytes. They also observed the formation of superoxide anions by erythrocyte membranes in the presence of copper and suggested that the hemolysis resulting from copper toxicity is most likely due to lipid peroxidation in the cell membranes.

Table 5
RELATION OF IRON ABSORPTION, HEMOGLOBIN, AND NUCLEATED RED CELL COUNT[a]

Subject no.	Hemoglobin (g/dl)	Iron absorption %	Nucleated red cell count (per nl)
1	14.6	0.5	0
	12.5	3.6	0.2
	11.4	4.6	2.2
	10.9	7.9	18.4
	9.8	9.8	23.2
2	13.8	0.7	0
	11.0	2.0	1.4
	10.0	3.2	7.7
	9.7	7.2	15.1
	9.1	8.9	25.9
3	12.1	1.1	0.6
	10.4	1.7	1.2
	9.8	4.1	7.5
	9.5	5.4	7.5
	8.3	7.5	27.7
4	10.5	7.0	0.7
	10.1	10.2	1.3
	9.8	16.5	15.1
	9.4	18.0	—
5	11.6	4.3	2.5
6	10.8	10.2	13.1
	10.4	24.0	23.8
	10.2	28.2	36.3
	10.0	33.3	75.8
	9.0	41.1	79.8

Note: Radioactive iron studies of six patients with thalassemia at two weekly intervals following each blood transfusion revealed that the percentage absorption of nonheme iron increased as the hemoglobin level fell. There was a close relationship between the nucleated red cell counts and the percentage iron absorption.

[a] Subject 6 has thalassemia intermedia. All others have thalassemia major.

From De Alarcon, P. A., Donovan, M. E., Forbes, G. B., Landaw, S. A., and Stockman, J. A. (1979), *N. Engl. J. Med.*, 300, 5. With permission.

Here we have a direct linkage to the section of this book on bioflavonoids, tannins, and catechins, where it is suggested that these insoluble compounds may have a beneficial effect on ascorbic acid metabolism by removing heavy metals or by reducing their absorption. More work needs to be done to find out whether these substances, the "chelating fiber" of vegetable foods, can actually remove heavy metals from the blood into the lumen of the bowel for excretion.

Table 6
EFFECT OF TEA ON IRON ABSORPTION

Subject no.[a]	Meal with water		Meal with tea		Inhibition (%)
	Hemoglobin (g/dl)	Absorption (%)	Hemoglobin (g/dl)	Absorption (%)	
1	10.9	7.9	10.8	0.4	95
6	10.2	28.2	10.2	12.6	55
1	9.8	9.8	9.5	1.7	83
2	9.7	7.2	9.6	0.5	93
3	9.5	5.4	9.6	0.5	91
4	9.4	17.6	9.5	10.3	41
6	9.0	41.1	8.6	11.9	71
3	8.3	7.5	8.5	3.4	55

Note: Studies of iron absorption by patients with thalassemia revealed that substitution of an infusion of tea for an equal volume of water, with an iron-containing standard meal, caused a marked reduction of iron absorption. Clearly, tea tannins chelate iron and retain it within the lumen of the bowel.

[a] Subject 6 has thalassemia intermedia; all other subjects have thalassemia major.

From De Alarcon, P. A., Donovan, M. E., Forbes, G. B., Landaw, S. A., and Stockman, J. A. (1979), *N. Engl. J. Med.*, 300, 5. With permission.

In any event we may in future choose to use catechin with ascorbic acid or, better, catechin-coated ascorbic acid tablets, so as to prevent the development of mutagenicity by ascorbic acid in the presence of the cupric ions of tap water. This may provide an added bonus, for we may expect that any unabsorbed catechin will act like tea and will remove iron by drawing it into the lumen of the bowel for evacuation. Barclay et al. (1983) have demonstrated a remarkable synergistic effect between the antioxidant activities of alpha tocopherol and vitamin C *in vitro*, so vitamin E will also be of benefit, along with vitamin C and catechin, during desferrioxamine treatment of iron overload in patients with thalassemia.

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Chapter 16

STRESS AND THE PITUITARY-ADRENAL SYSTEM

I. STRESS

Even James Lind, who conducted his classical controlled experiment in the year 1747, proving that oranges and lemons are a complete cure for scurvy (Chapter 1 of this volume), nevertheless, writing in his *Treatise of the Scurvy* (1753), expressed his belief that stresses such as exposure to cold, damp weather and long hours of arduous duty played a part in the onset of this disease. This clinical impression has been shared by many physicians over the ages, but has received little support in modern times from the analysis of human blood ascorbic acid levels.

The first observation that stress depletes adrenal ascorbic acid seems to have been by Van Eekelen and Kooy (1933) who reported that when rats were fatigued by long exercise on a treadmill, the ascorbic acid concentration in their adrenals and liver fell to low levels (Table 1). Belser et al. (1939) observed that high environmental temperatures decreased the urinary ascorbic acid excretion of human volunteers on a constant ascorbate intake.

One of the effects of physical or psychic stress is the release of epinephrine and norepinephrine from the adrenal medulla, and another is an increased secretion of adrenal cortical hormones, mediated by the release of adrenocorticotropic hormone (ACTH) from the pituitary. The reasons for an increased requirement of adrenal cortical hormones in response to stress are not well understood, but adrenal corticosteroids do somehow reduce the mortality of animals exposed to various forms of injury. Moreover, hypophysectomy causes adrenal atrophy associated with a progressive decrease in the adrenal ascorbic acid concentration, and decreased resistance to cold, as shown by Tyslowitz (1943) in the rat.

It is odd that ACTH deficiency and ACTH administration both cause adrenal ascorbic acid depletion; perhaps adrenal corticosteroids are needed to promote ascorbic acid storage by reducing dehydroascorbic acid (DHAA) to AA*. Thus, we can envisage the ascorbic acid as leaving the adrenal when the corticosteroids leave. This hypothesis has some appeal, for Tyslowitz also found the ascorbic acid levels of the testes, liver, kidneys, and serum of hypophysectomized rats to be reduced to a degree similar to that of the adrenals (i.e., 40% after 8 weeks). However, it is not consistent with the *in vitro* findings of Sharma et al. (1963) who reported that both ACTH and corticosteroids reduced the uptake of ascorbic acid I-^{14}C by slices of guinea pig adrenal cortex.

Certainly it is well established that stress, or the injection of pure ACTH, into rats is followed by a prompt fall in the adrenal ascorbic acid concentration, which may drop to less than half of its initial value, within 1 h, and that there is an equally profound fall in the adrenal cholesterol content, as shown by Sayers et al. (1944, 1946) and many other workers (Figures 1 and 2). Undoubtedly it is the ability of the rat to synthesize ascorbic acid that accounts for the rapid return of its adrenal ascorbic acid to normal 12 h later, and even above normal by 24 h, for the animals received no dietary ascorbic acid during the experiment. The guinea pigs on the other hand, being unable to synthesize ascorbic acid, had to rely on slow replenishment of their adrenal ascorbic acid by withdrawing it from other tissues (Figure 2).

Vogt (1944) assayed adrenal cortical hormones by their ability to increase the survival time of cold-stressed adrenalectomized rats; she observed that epinephrine caused a marked increase in the cortical hormone content of the adrenal vein plasma of dogs and supposed that the epinephrine might be acting directly on the adrenal cortex.

* AA — ascorbic acid, reduced form.

Table 1
COMPARISON OF ADRENAL AND LIVER ASCORBIC ACID CONCENTRATIONS IN NORMAL RATS AND IN RATS FATIGUED BY RUNNING ON A TREADMILL FOR $1^1/_2$ TO 6 h[a]

	Adrenal ascorbic acid (AA) (mg/100 g)				Liver ascorbic acid (AA) (mg/100 g)	
	n		n		n	
Normal rats	8(M)	354 ± 74	10(F)	371 ± 71	8(M)	21 ± 3.3
Fatigued rats	13(M)	156 ± 68	11(F)	146 ± 89	10(M)	13 ± 3.3
Less fatigued rats; after $^3/_4$ h of running	3	202			3	19
12 h after treadmill	2	144			2	5

Note: It is interesting to observe that the adrenal and liver ascorbic acid levels were still severely depleted in two rats studied 12 h after the treadmill.

[a] Summary of data from van Eekelen and Kooy (1933)

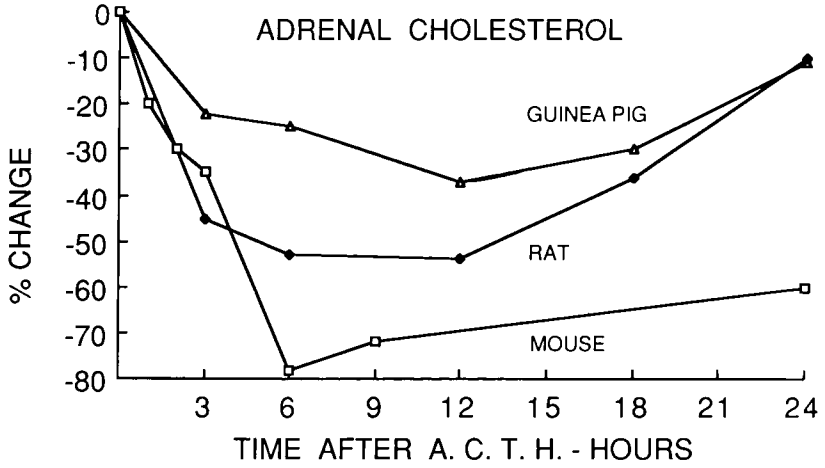

FIGURE 1. The effect of a single injection of ACTH on the adrenal cholesterol of the guinea pig, rat, and mouse. (From Long, C. N. H. [1947], *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 6, 461. With permission.)

Observations by Long and Fry (1945) and Long (1947) revealed that epinephrine injection and also many forms of stress, including cold, pain, hemorrhage, scald, injection of dead *E. coli* or simulated altitudes of 20,000 ft, depleted the adrenal ascorbic acid content and concentration in rats, However, this effect of epinephrine and of these various stressors was found to be blocked by hypophysectomy; they all act by stimulating the release of ACTH from the pituitary.

Dugal and Thérien (1949) observed that ascorbic acid prevented the adrenal hypertrophy and markedly improved the survival rates of rats exposed to the stress of a cold environment. They found that guinea pigs were similarly protected by ascorbic acid against cold-induced adrenal hypertrophy; these authors suggested that ascorbic acid may play a role similar to that of one of the adrenal hormones. In fact, this may be true, for we have seen in Chapter 13 of this volume that cortisone has been reported as increasing the AA/DHAA ratio in the blood plasma. Ascorbic acid can do the same, as shown in Figure 5 of Chapter 12 of this volume, although the effect may be only temporary; thus, it would seem that both ascorbic acid and cortisone can reduce the oxidation-reduction or redox potential of the plasma.

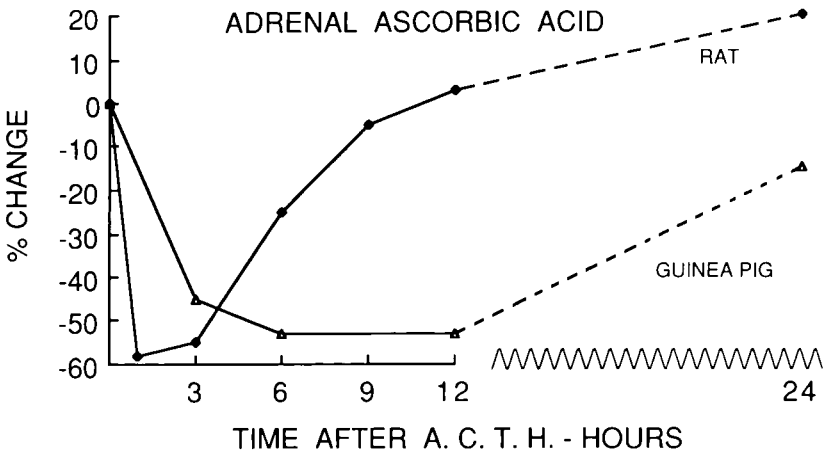

FIGURE 2. The effect of a single injection of ACTH on the adrenal ascorbic acid of the rat and guinea pig. (From Long, C. N. H. [1947], *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 6, 461. With permission.)

Bacchus et al. (1951) reported findings suggesting that the protective action of ascorbic acid in rats may require the presence of intact adrenals. Cortical hormones, on the other hand, exert their protective action even in the absence of the adrenals.

Sayers and Sayers (1947), had observed that pretreatment of animals with cortical hormones prevented the depletion of adrenal ascorbic acid when the animals were subsequently exposed to cold, heat, epinephrine, or histamine stressors, but did not prevent adrenal ascorbic acid depletion in response to ACTH. They concluded that the elaboration or release of ACTH by the pituitary fluctuates according to the requirements of the tissue cells for cortical steroids.

Similarly Bacchus et al. (1951, 1952b) observed that ascorbic acid pretreament of rats and mice prevented the eosinopenia and the adrenal histological changes following the stress of epinephrine injection, but did not block the eosinopenia that follows ACTH administration; so they suggested that the vitamin acted by blocking the pituitary-adrenal system at a pituitary or prepituitary level. It seems that the tissue requirements for cortisone were reduced by ascorbic acid and that this was sensed by the pituitary or by the hypothalamus.

Eisenstein and Boniface (1952) confirmed that ascorbic acid prevents adrenal hypertrophy in cold-stressed rats.

Bacchus et al. (1952a) reported that ascorbic acid alone did not affect the leukocyte pattern of rats, but prolonged the eosinopenia and the lymphopenia produced by cortisone in adrenalectomized rats (Figure 3); so they concluded that the effect was due to a joint action of cortisone and ascorbic acid. They also observed a similar effect of ascorbic acid in prolonging the leukocyte response to ACTH in adrenal demedullated rats (Figure 4).

Katsh et al. (1954) studied prolonged stress in rats by exposing them to a temperature of 2 to 4°C for 28 d and found a decrease in the adrenal ascorbic acid content after 24 h. Thereafter, the adrenal weight and adrenal ascorbic acid content changed in parallel, so that there was little change in the adrenal ascorbic acid concentration during the rest of the experiment. The pituitary ascorbic acid content and concentration had fallen after 24 hr, but rose almost to normal by 10 d and had risen to a level 60% above normal on day 28. The rat, being able to synthesize ascorbic acid, seems to react to stress by increased synthesis of ascorbic acid, but humans, monkeys, and guinea pigs are unable to react in this way.

Booker et al. (1955) reported that both cortisone and ascorbic acid were effective in increasing the survival of intact and adrenalectomized mice subjected to cold stress; their results suggested a common peripheral action of adrenocortical hormones and ascorbic acid. DesMarais (1956) administered ascorbic acid or desoxycorticosterone acetate (DOCA) to

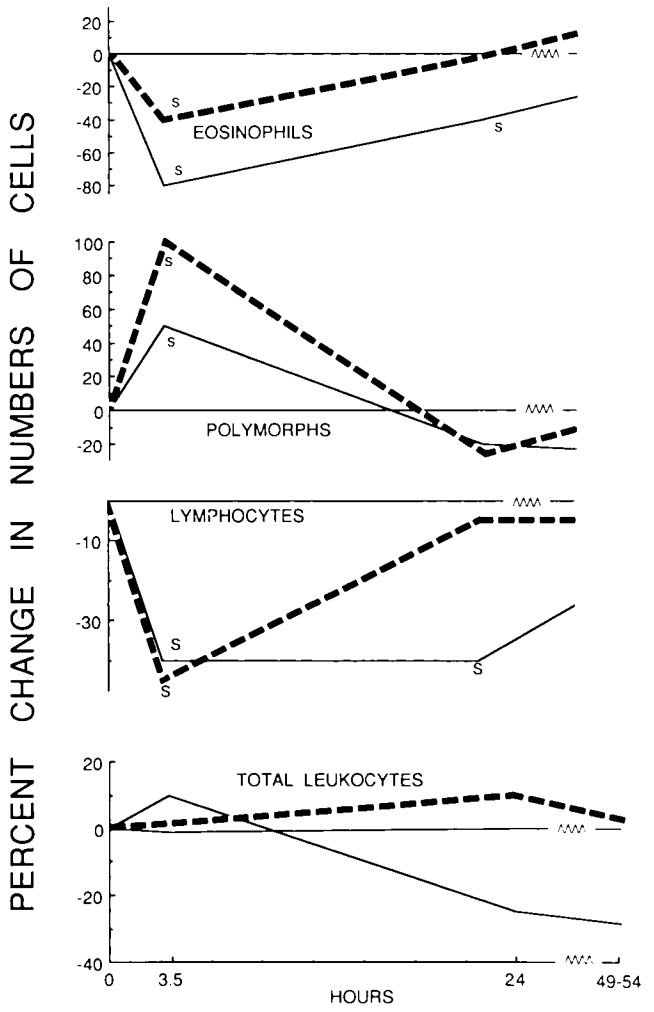

FIGURE 3. Leukocyte response to cortisone in adrenalectomized rats pretreated with ascorbic acid (——) and with saline (---). Statistically significant differences are indicated by S in the figure. Intraperitoneal injection of ascorbic acid accentuated and prolonged the eosinopenic and lymphopenic responses to cortisone. (From Bacchus, H., Altszuler, N., and Heiffer, M. H. [1952a], *Proc. Soc. Exp. Biol.*, 80, 88. With permission.)

adrenalectomized rats treated with large doses of cortisone and subjected to cold exposure. In these experiments either ascorbic acid or DOCA increased survival time, decreased thymolysis, and decreased the activation of the thyroid; Bacchus et al. (1951) had reported a complete loss of action of ascorbic acid in the protection of adrenalectomized rats against cold in the absence of cortical hormones, but DesMarais reported that in adrenalectomized rats receiving no cortisone or DOCA, or low doses of these hormones, ascorbic acid administration still showed beneficial effects in resistance to cold. Thus, DesMarais stated, "This again strongly indicates that ascorbate has some effects in animals exposed to cold which do not depend upon an increase in the secretion of corticoadrenal hormones." In thyroidectomized rats exposed to cold, ascorbate treatment was not effective unless thyroxin was also administered. It was therefore suggested that the role of cortical hormones in resistance to cold might be limited to "a conditioning action on the beneficial effects of

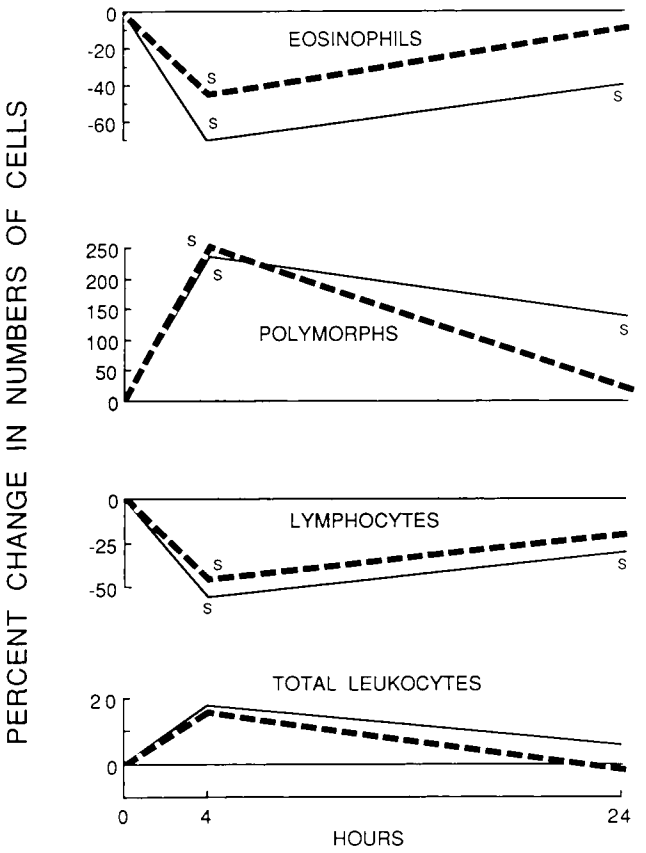

FIGURE 4. Leukocyte response to ACTH in adrenal-demedullated rats treated with ascorbic acid (——) or with saline (---). Statistically significant differences are indicated by S in the figure. Intraperitoneal injection of ascorbic acid accentuated and prolonged the eosinopenic and lymphopenic responses to ACTH. (From Bacchus, H., Altszuler, N., and Heiffer, M. H. [1952a], *Proc. Soc. Exp. Biol.*, 80, 88. With permission.)

ascorbate," and that the favorable effect of ascorbate is most likely coupled with the action of thyroxine.

Costa et al. (1956) confirmed that epinephrine causes adrenal TAA* depletion in rats and observed a similar stress effect following excessive doses of glucagon.

Sayers and Sayers (1949) cited from the literature many substances that have been found to act as stressors. They listed epinephrine, benzene, estrogens, ether, chloroform, insulin, diphtheria toxin, anoxia, infectious diseases, atropine, nicotine, histamine, cold, heat, killed typhoid organisms, hemorrhage, dibenamine, and intraperitoneal administration of glucose as all depleting the ascorbic acid content of the adrenal; presumably their main action is stimulation of the release of ACTH or corticotrophin releasing factor.

This action of histamine is particularly interesting, as a relationship has been shown to exist between the dose of histamine and the absolute drop in the concentration of ascorbic acid in the adrenal, as shown in Figure 5. Moreover, histamine is now known to be present in excess in vitamin C deficiency and most probably accounts for the known hyperactivity of the adrenals in scurvy. Sayers and Sayers demonstrated that corticosterone or 17-hydroxycorticosterone administration to rats, before exposure to various forms of stress can

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

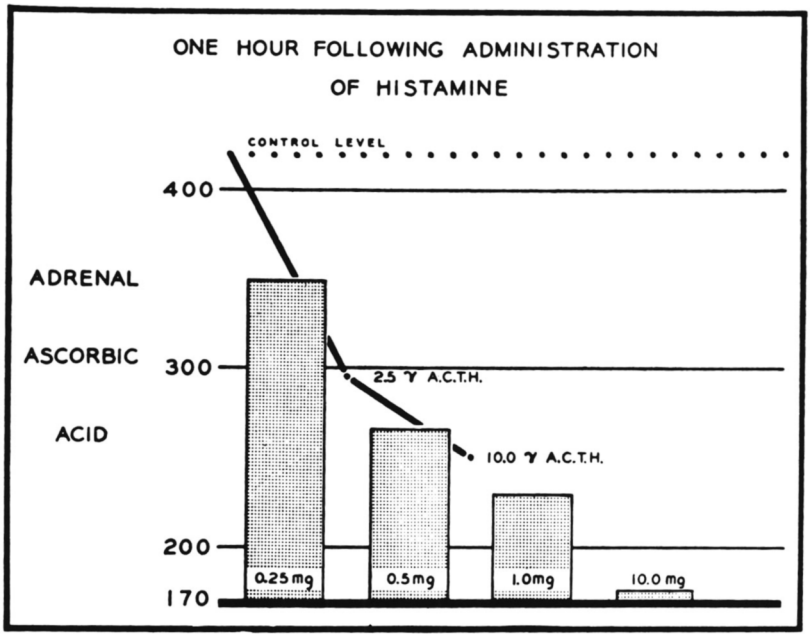

FIGURE 5. The effect of various doses of histamine phosphate upon the concentration of ascorbic acid in the adrenals. The level of the dotted line represents the mean concentration of ascorbic acid in the adrenals of untreated rats. The heights of the columns represent the concentrations of ascorbic acid in the adrenals of the treated rats. The distance the top of a column extends below the dotted line is proportional to the amount of ACTH elaborated by the pituitary. Superimposed on the bar chart is a graph showing the relationship between the dose of ACTH (log) and adrenal ascorbic acid. (From Sayers, G. and Sayers, M. A. [1949], *Ann. N.Y. Acad. Sci.*, 50, 522. With permission.)

completely inhibit their adrenocorticotrophic response. Moreover, the greater the degree of stress to which the animal is subjected, the greater is the amount of cortical hormone required to inhibit pituitary adrenocorticoptrophic activity. "Whereas 20 micrograms of 17-hydroxycorticosterone per 100 gm of body weight completely inhibited the increase in adrenal cortical activity which otherwise follows injection of 0.25 mg of histamine, this dose of steroid produced only partial inhibition in rats treated with 0.5 mg of histamine. The greater pituitary adrenocorticoptrophic activity produced by this larger dose of histamine could be blocked by 100 micrograms of 17-hydroxycorticosterone. In animals treated with 1.0 mg of histamine, a dose of 1.0 mg of 17-hydroxycorticosterone resulted in only partial suppression of pituitary activity."

Thus, histamine is a particularly potent stimulant of adrenocortical activity and unlike other forms of stress it cannot be completely blocked by adrenal corticoid administration.

Comprehensive reviews of this subject by Sayers (1950), Pirani (1952), Meiklejohn (1953), Hayano et al. (1956). Chalopin et al. (1966), and Baker (1967) have attempted to understand the role of such a rich supply of ascorbic acid in the adrenal glands and the meaning of its rapid release from the adrenals in situations of stress or stimulation by ACTH. Certainly ascorbic acid may be present in the adrenal medulla to maintain epinephrine and norepinephrine in the reduced state. Hagen and Welch (1956) point out that ascorbic acid performs this function very well *in vitro*. As regards the function of ascorbic acid in the adrenal cortex, all we seem to find is that ascorbic acid acts as a restraining factor on certain reactions in the sequence involving the conversion of cholesterol to progesterone in the synthesis of cortisol and corticosterone.

Kersten et al. (1958) discovered that adrenal microsomes contain an ascorbate-dependent enzyme that oxidizes DPNH. They believe that "monodehydroascorbic acid" acts as an intermediary electron acceptor in this system, but the precise role of this system has not been elaborated.

Howard and Cater (1959) studied the weight, histology, and mitotic counts of the adrenal cortex of scorbutic, pair-fed, and normal guinea pigs. They found that the enlarged adrenals of scorbutic guinea pigs were histologically similar to those of ACTH-stimulated animals and showed an increased precentage of cells in metaphase, especially in the outer zona fasciculata. ACTH increased the number of mitoses fourfold in guinea pigs with early avitaminosis C and sevenfold in guinea pigs on a normal diet (Table 2). Moreover, cortisone administration to guinea pigs with avitaminosis C caused both the absolute and relative adrenal weights to decrease to values which were not significantly different from controls. Cortisone also caused a profound reduction in the number of mitoses in the adrenals of scorbutic guinea pigs (Table 3). Clearly, the adrenal hypertrophy of scurvy is due to ACTH stimulation and is inhibited by cortisone, so Howard and Cater suggested that it is caused by the "stress" resulting form the scorbutic state.

Another explanation is that the pituitary or the hypothalamus is particularly sensitive to ascorbic acid deficiency (or to histamine excess). Such a system could be the feedback mechanism for controlling the oxidation-reduction potential of the tissues.

Liakakos et al. (1975) studied the plasma cortisol values before and at 4 and 6 h after intramuscular administration of a depot preparation of synthetic corticotrophin in 12 healthy children before, and on the fifth day of continuous ascorbic acid administration (1 g t.i.d. orally). Eight other healthy children were similarly treated with ACTH, but were not given ascorbic acid. On the fifth day of ascorbic acid administration, the mean cortisol values after ACTH were significantly lower than the corresponding values in the control group ($p < 0.02$) or the post-ACTH values in the test group before receiving ascorbic acid. On the other hand, the ascorbic acid supplements had no effect on the fasting plasma cortisol values of the children. Thus, these workers have confirmed in human subjects that ascorbic acid excess exerts an inhibitory effect on cortisol secretion; they concluded that ascorbic acid may therefore be of no benefit in conditions of stress. Certainly their conclusion that AA loading decreases the adrenal response to stress is justified, but there is plenty of evidence that ascorbic acid is beneficial and may even be life saving in cold-stressed animals, so it would seem more logical to think that high plasma ascorbic acid levels may partially substitute for, or reduce the need for, so great an adrenal response to stress. In fact, if both ascorbic acid and cortisone have similar effects in reducing the oxidation-reduction potential of the plasma, this may be their common effect.

It is hard to interpret the role of ascorbic acid in pituitary and adrenal function, but it seems that cortisone and ascorbic acid have some similarities of action and may be involved in the stress reaction in three places: (1) they both decrease ACTH release by the pituitary; ascorbic acid probably has this effect by virtue of decreasing the blood histamine level (Chapter 1, Volume III); (2) they both decrease corticosteroid synthesis (Chapter 8 of Volume III); and (3) ascorbic acid also seems to have a peripheral action similar to, or synergistic with, cortisone; both can increase the ratio of AA to DHAA in the plasma and thus reduce the oxidation-reduction or redox potential of the blood plasma and tissues. One can conceive of a feedback system designed towards the achievement of a low oxidation-reduction potential in the plasma and tissue fluids; that might explain why the adrenal cortex becomes hyperactive in scurvy.

Most human experiments have shown little or no effect of stress on blood or urine total ascorbic acid levels. Kark (1953) summarized the results of three series of investigations in Canada on soldiers exposed to severe cold; one experiment — "The Musk Ox Expedition" — involved 50 men who traveled 3000 mi by snow mobile, through the barren lands in the

Table 2
EFFECT OF ACTH ON THE ADRENALS OF GUINEA PIGS WITH EARLY AVITAMINOSIS C AND NORMAL GUINEA PIGS

Means ± SEM

Group	ACTH (IU/d)	Ascorbic acid (mg/d)	No. of animals	Body wt at death (g)	Adrenal wt (mg)	Adrenal wt (mg/100 g body wt)	Mitoses in adrenal (no./10-mm² section)
1	10	Nil	6	370 ± 19	288 ± 17	79.2 ± 7.5	23.5 ± 4.5
2	Nil	Nil	5	324 ± 16	232 ± 19	73.8 ± 8.0	6.4 ± 1.3
3	10	60	5	411 ± 14	319 ± 18	77.6 ± 2.5	63.0 ± 12.4
4	Nil	60	5	371 ± 21	190 ± 4	51.8 ± 4.1	8.6 ± 2.9
				Values of p^a for Comparison Between Groups			
		Group 1 vs. 2		—	*	—	**
		Group 1 vs. 3		—	—	—	**
		Group 2 vs. 4		*	—	*	—
		Group 3 vs. 4		*	***	***	***

Note: Injections of ACTH significantly increased adrenal weight when given to scorbutic animals and markedly so when given to normal animals. Ascorbate deficiency also increased the adrenal weight of guinea pigs. The adrenals of animals of both scorbutic and normal groups showed a significant increase in mitoses compared with uninjected controls. Ascorbic acid given with ACTH increased the mitotic counts compared to the group given ACTH but no ascorbic acid.

[a] —$p > 0.05$; * $p = 0.05$—0.01; ** $p = 0.01$—0.001; *** $p < 0.001$.

From Howard, A. N. and Carter, D. B. (1959), *J. Endocrinol.*, 18, 175. With permission.

Table 3
EFFECT OF CORTISONE ON THE ADRENALS OF GUINEA PIGS WITH AVITAMINOSIS C AND PAIR-FED CONTROLS

Means ± SEM

	Group	Ascorbic acid (mg/d)	Injection (i.m.)	No. of animals	Body wt at death (g)	Adrenal wt. (mg)	Adrenal Wt. (mg/100 g body wt)	Mitoses in adrenal (no./10-mm² section
1	Scorbutic	Nil	Cortisone (5 mg/d)	5	359 ± 31	265 ± 14	75.6 ± 5.7	2.4 ± 2.4
2	Scorbutic	Nil	Saline	5	346 ± 22	384 ± 22	112 ± 13.6	76.6 ± 20
3	Pair-fed controls (of Group 2)	5	Cortisone (5 mg/d)	5	440 ± 22	269 ± 20	61.8 ± 5.7	5.0 ± 1.8
4	Pair-fed controls (of Group 1)	5	Saline	5	388 ± 29	308 ± 31	80.0 ± 6.8	8.4 ± 3.8

Values of p[a] for Comparison Between Groups

Group 1 vs. 2	—	*	*	**
Group 2 vs. 4	—	—	*	**
Group 2 vs. 3	*	*	**	**
Group 1 vs. 3	—	—	—	—
Group 3 vs. 4	—	—	—	—

Note: Administration of cortisone to scorbutic guinea pigs caused both the absolute and relative adrenal weights to decrease to values which were not significantly different from controls. Cortisone also produced in the controls a decrease in the relative adrenal weight. The high mitotic counts of scorbutic guinea pigs were completely suppressed by the administration of cortisone.

[a] $*p = 0.05—0.01$; $**p = 0.01—0.001$.

From Howard, A. N. and Carter, D. B. (1959), *J. Endocrinol.*, 18, 175. With permission.

Winter of 1945-1946. They showed no change in their plasma ascorbic acid levels between December 1945 at Fort Churchill, Manitoba, and April 1946 at Fort Nelson, British Columbia. He concluded that adrenocortical activity does not increase ascorbic acid requirements to any significant degree.

More recently, a possible explanation for the disparity between the widespread clinical impression that stress plays a role in the onset of scurvy and the nonconfirmatory results of ascorbic acid analyses of blood samples from stressed individuals may have been found. It is new work, presented here for the first time, in Chapter 17 of this volume, under the heading "Lack of Sleep". The observations of Subramanian et al. (1973, 1974), Nandi et al. (1974), and Chatterjee et al. (1975a, b) in guinea pigs and of Clemetson (1980) in human subjects have made it clear that ascorbic acid is essential for the detoxification of histamine and that markedly elevated blood histamine level is an integral part of scurvy. We now know that blood histamine levels are inversely related to plasma ascorbic acid levels (see Chapter 1, Volume III) and are significantly elevated even in healthy human beings when the plasma ascorbic acid (AA) level falls below 0.7 mg/100 ml. Moreover, we know that various forms of stress cause further elevation of the blood histamine level in guinea pigs, and that lack of sleep causes a significant elevation of the blood histamine level in human subjects; also we know that these elevated blood histamine levels rapidly return to normal following ascorbic acid administration. Both ascorbic acid (Schultzer, 1934) and cortisone (Kramer et al., 1956) maintain capillary strength, but histamine weakens the capillaries, causing increased permeability, as shown by Stead and Warren (1944). Thus, it seems probable that histamine intoxication may contribute to the hemorrhagic manifestations of scurvy.

If we think of vitamin C deficiency as causing a series of metabolic defects, such as

1. Decreased hydroxylation of proline and lysine
2. Decreased carbohydrate tolerance
3. Decreased conversion of folic to folinic acid
4. Decreased conversion of histamine to hydantoin-5-acetic acid
5. Defective tryptophan metabolism
6. Defective tyrosine metabolism, etc.

it becomes clear that

1. The defective wound healing of C deficiency will occur sooner if the diet is also deficient in protein.
2. The carbohydrate intolerance of vitamin C deficiency will become evident sooner in a patient on a high carbohydrate diet.
3. Megaloblastic anemia will appear sooner if the patient is folic acid deficient.
4. Similarly we may conjecture that the bleeding gums, the subcutaneous hemorrhages, and joint pains due to periarticular hemorrhages may appear sooner if the blood histamine level is already elevated as a result of any kind of stress.

Analyzed in this way, it is evident that the clinical manifestations of scurvy might appear in one vitamin C-deficient individual under stress, while another equally C-deficient individual might remain free from the symptoms and signs of scurvy for a much longer period. Indeed, this is probably one of the principal differences between clinical scurvy and experimental vitamin C deficiency.

Nevertheless, an attempt is made here to review the enormous literature concerning the effects of stress on ascorbic acid metabolism via the influence of the cerebral cortex on the hypothalamus, the pituitary, and the adrenals.

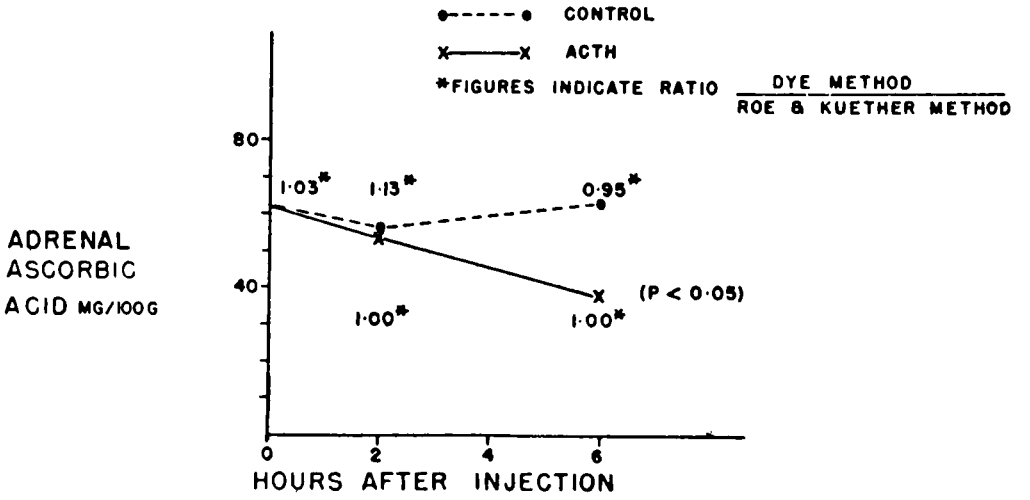

FIGURE 6. The effect of intraperitoneal injection of ACTH (60 units) in guinea pigs on a normal diet, in which vitamin C saturation had been ensured by prior injection of ascorbic acid (200 mg). There was a significant fall in the adrenal ascorbic acid concentration, compared with control animals, but no DHAA was found in these glands. (From Prunty, F. T. G., Clayton, B. E., McSwiney, R. R., and Mills, I. H. [1955], *Ciba Found. Colloq. Endocrinol. (Proc.)*, 8, 324. ©John Wiley & Sons, Ltd. With permission.)

II. ACTH AND ADRENAL ASCORBIC ACID DEPLETION

The administration of ACTH certainly causes corticosteroid secretion and a rapid and profound but temporary decrease in the ascorbic acid content in the adrenals of rats, guinea pigs, and other animals, as shown by Sayers et al. (1944, 1948, 1949) and by Long (1947) (Figure 2). Many have pondered the reason for this association between adrenal cortical activity and ascorbic acid release. Lowenstein and Zwemer (1946) reported isolation of a biologically active adrenal steroid in which the side chain was conjugated with ascorbic acid, conferring water solubility to the compound. If confirmed, such a compound would certainly have explained why adrenal steroids and ascorbic acid both leave the adrenal when it is stimulated by ACTH. However, the work of Lipscomb and Nelson (1960) showed that ascorbic acid (TAA) appears and peaks in the adrenal vein blood before corticosterone in ACTH-stimulated rats (Figure 2, Chapter 18 of this volume), so clearly these two compounds are not secreted in any conjoined form.

Prunty et al. (1955) studied the form in which ascorbic acid is to be found in the adrenals of guinea pigs following ACTH administration. They used an indophenol dye method to measure reduced ascorbic acid (AA) and the method of Roe and Kuether (1943) to measure total ascorbic acid (TAA) which includes AA, as well as its reversibly oxidized form dehydroascorbic acid (DHAA) and any diketogulonic acid (DKG) derived from it by irreversible hydrolysis. While there was a significant drop in the adrenal ascorbic acid concentration, there was no evidence of any oxidized forms of ascorbic acid in the adrenals, as shown in Figure 6.

Slusher and Roberts (1957) studied the fate of ascorbic acid disappearing from the adrenal glands of rats subjected to the stress of laparotomy and in hypophysectomized rats injected intravenously with a large does of ACTH. In both groups of animals the amount of the vitamin lost by the cannulated adrenal gland could be quantitatively recovered in the venous effluent. Similar findings were reported by Briggs and Toepel (1958) following ACTH administration in hypophysectomized rats, but their "stressed" intact rats appeared to lose more from the adrenal than could be found in the adrenal venous blood, presumably because

the stress of anesthesia and surgery started 10 min before cannulation and collection of adrenal vein blood. Lahiri and Lloyd (1962a) reported that approximately 85% of the ascorbic acid lost by the rat adrenal was detected in its venous blood following cortisone, surgical stress, and corticotrophin, and it was almost entirely in the reduced (AA) form.

Lahiri and Lloyd (1963b) reported that laparotomy, hemorrhage, and the administration of corticotrophin all decreased the adrenal ascorbic acid content and increased the levels of ascorbic acid in the blood, liver, spleen, and kidneys of rats (Table 4). The ascorbic acid gains were far greater than could be accounted by the ascorbate lost from the adrenals and occurred even when the adrenals were excluded from the circulation. The highest blood ascorbic acid (TAA) concentrations were found in samples obtained from the vena cava above the diaphragm (Table 5), suggesting that the ascorbate was entering the circulation from the liver. These authors suggested that the increased concentration of ascorbic acid was associated with decreased ascorbate catabolism in that organ, but there must also have been increased ascorbate synthesis.

It is interesting to observe in Table 5A that the percentage of ascorbic acid in the oxidized form (DHAA) in the blood of these rats after the stress of surgery, and in most of their tissues, 1 h after the injection of corticotrophin (Table 6), was decreased, just as Stewart et al. (1953a) had observed in human plasma 1 h after the administration of cortisone. This in spite of the results obtained on analysis of vena cava blood (Table 5B), which suggest that ascorbate leaves the liver as DHAA.

Salomon (1957), utilizing ascorbic acid 1-^{14}C in hypophysectomized rats found that all radioactive material within the adrenal glands could be identified as ascorbic acid, both before and after the administration of ACTH. But the radioactive material appearing in the adrenal vein after ACTH could no longer be identified as ascorbic acid, although systemic plasma from the same animals contained no radioactive substances other than ascorbic acid. They therefore concluded that ascorbate is released from the adrenal in the dehydro (DHAA) form and is rapidly reduced to ascorbic acid (AA) in the peripheral blood.

Harding and Nelson (1963) found all ascorbic acid and glutathione to be in the reduced form in the adrenals from normal, hypophysectomized, or ACTH-stimulated animals. However, adrenal vein blood ascorbate following stress in intact rats, or ACTH stimulation in hypophysectomized rats, was found to be partly oxidized, and calculations from their data (Table 6) reveal that the percentage of ascorbic acid in the oxidized forms increased with time following hypophysectomy.

Recent work by Kipp and Rivers (1987) confirmed that ACTH injection into guinea pigs caused the adrenal ascorbic acid concentration to fall, 4 h later, to 33% of that in saline-injected controls. However, the same authors reported an increased adrenal uptake of radioactive (1-^{14}C)-labeled ascorbic acid (to 172% of saline control) within half an hour, when ACTH was injected immediately after the labeled ascorbic acid. No change in labeled ascorbate was found in any other tissue following ACTH administration. These workers concluded that ACTH causes both an accelerated uptake and release of ascorbic acid by the adrenals.

III. THE EFFECTS OF ACTH AND CORTISONE ON ASCORBIC ACID METABOLISM

Beck et al. (1950) observed a sharp increase in the urinary excretion of ascorbic acid during the first 24 h of the administration of ACTH to patients with chronic disease, but observed no such increase following the administration of cortisone.

Beck et al. (1951) extended these findings and confirmed that injections of ACTH, 100 mg daily, caused a definite peak of urinary ascorbic acid (TAA) excretion in 12 out of 15 patients maintained on a constant ascorbate intake (250 mg daily), as exemplified in Figure

Table 4
EFFECTS OF ACTH ON THE PERCENTAGE OF BLOOD AND TISSUE ASCORBIC ACID IN THE OXIDIZED FORM (RAT)[a]

Organ	TAA[b]	AA[c]	DHAA[d]	Percentage oxidized	TAA	AA	DHAA	Percentage oxidized
	Group I (n = 6)				Group II (n = 9)			
Liver	27.6	23.7	3.9	14.1	35.3	34.9	0.4	1.1
Spleen	36.7	30.0	6.7	18.3	40.0	34.9	5.1	12.8
Lung	29.4	20.0	9.4	32.0	29.6	19.8	9.8	33.1
Brain	43.4	40.0	3.4	7.8	43.9	42.0	1.9	4.3
Kidney	16.5	13.8	2.7	16.4	17.7	17.1	0.6	3.4
Adrenal	386.	356.	30.	7.8	254.	244.	10.	3.9
Pituitary	116.	102.	14.	12.1	131.	116.	15.	11.5
Blood from neck	0.85	0.87	−0.02	0	1.10	1.12	−0.02	0
	Group III (n = 6)				Group IV (n = 8)			
Liver	27.2	21.6	5.6	20.6	24.1	21.7	2.4	10.0
Spleen	34.4	24.5	9.9	28.8	36.4	29.4	7.0	19.2
Lung	32.1	22.9	9.2	28.7	33.8	25.6	8.2	24.3
Brain	42.4	38.6	3.8	9.0	42.6	40.1	2.5	5.9
Kidney	15.2	12.0	3.2	21.1	15.5	13.4	2.1	13.5
Adrenal	346.	326.	20.	5.8	257.	239.	18.	7.0
Pituitary	116.	99.	17.	14.7	113.	100.	13.	11.5
Blood from neck	0.78	0.72	0.06	0	1.51	1.45	0.06	4.0
Blood from carotid after laparotomy	—	—	—	—	0.77	0.81	−0.04	0

Note: The six rats in Group I were anesthetized and decapitated; blood samples were collected from their necks. The nine rats in Group II were anesthetized and given ACTH, 40 IU/kg by i.p. injection; they were killed and blood was collected from the neck 1h later. The six rats in Group III were given normal saline injections instead of corticotrophin, but were otherwise treated as Group II. The eight rats of Group IV were laparotomized after anesthesia and blood equal to 2% of their body weight was allowed to leave through a cannulated carotid artery. They were otherwise treated like the rats of Groups II and

Table 4 (continued)
EFFECTS OF ACTH ON THE PERCENTAGE OF BLOOD AND TISSUE ASCORBIC ACID IN THE OXIDIZED FORM (RAT)[a]

III except that no injection was given. Following ACTH injection, there was a loss of ascorbate from the adrenals and a gain of ascorbate by the liver and spleen, but the most consistent finding was a reduction in the percentage of ascorbate in the oxidized forms, as seen in the liver, spleen, kidney, brain, and adrenal following ACTH (Group II) and in the liver, spleen, kidney, brain, and pituitary after hemorrhage (Group IV).

[a] Derived from the data of Lahiri and Lloyd (1962b).
[b] TAA — ascorbate + dehydroascorbate + diketogulonic acid.
[c] AA — reduced ascorbic acid.
[d] DHAA — dehydroascorbate + diketogulonic acid.

Table 5
EFFECTS OF SURGICAL STRESS ON THE STATE OF OXIDATION OF ASCORBIC ACID IN ARTERIAL AND VENA CAVAL BLOOD (RAT)[a]

Experiment A[b]

Initial				Final			
TAA[c]	AA[d]	DHAA[e]	Percentage oxidized	TAA	AA	DHAA	Percentage oxidized
1.07 ± 0.10	1.02 ± 0.07	0.05* ± 0.026	4.7	1.92 ± 0.12	1.86 ± 0.13	0.06 ± 0.033	3.1

Experiment B[f]

Arterial				Inferior vena cava			
TAA	AA	DHAA	Percentage oxidized	TAA	AA	DHAA	Percentage oxidized
1.70 ± 0.13	1.64 ± 0.15	0.06 ± 0.008	3.5	3.03 ± 0.23	2.72 ± 0.26	0.31** ± 0.059	10.2

Note: Of the estimates of dehydroascorbic acid, only those for the initial arterial blood* (0.054 mg/100 ml; 15 rats; $p < 0.05$) and for the inferior vena cava blood** (0.31 mg/100 ml; 9 rats; $p < 0.01$) were statistically significant.

[a] Data derived from the work of Lahiri and Lloyd (1962b).

[b] Arterial blood immediately after laparotomy and about 1 h later (15 rats).

[c] TAA— total ascorbic acid, estimated the method of Roe and Kuether (1943).

[d] AA— reduced ascorbic acid, estimated by a modification of the method of Mindlin and Butler (1938).

[e] DHAA— obtained from the difference between TAA and AA, DHAA estimates dehydroascorbic acid, the reversibly oxidized form of ascorbic acid, plus any diketogulonic acid derived by hydrolysis of the latter.

[f] Comparison of arterial blood and blood from the vena cava above the diaphragm about 1 h after laparotomy (nine rats).

Table 6
STUDY OF THE FORM IN WHICH ASCORBIC ACID IS RELEASED FROM THE ADRENAL (RAT)[a]

	Intact stressed[b]	Hypox[c] 18 h ACTH[d]	Hypox 24 h ACTH
Rat Adrenal Vein Blood (3 to 30 min Collection)			
n[e]	4	7	5
TAA[f] (mg/100 ml)	1.81	2.66	3.01
AA[g] (mg/100 ml)	1.19	1.42	1.21
DHAA + DKG[h] (mg/100 ml)	0.62	1.24	1.80
Percentage in oxidized forms	34%	47%	60%
Rat Aorta Blood (3 to 30 min Collection)			
n	4	7	5
TAA (mg/100 ml)	1.37	1.59	1.69
AA (mg/100 ml)	1.02	0.79	0.96
DHAA + DKG (mg/100 ml)	0.35	0.80	0.73
Percentage in oxidized forms	26%	50%	43%

Note: It appears that the percentage of adrenal vein blood ascorbic acid which is in the oxidized form increases with time after hypophysectomy.

[a] Data derived from the work of Harding and Nelson (1963).
[b] "Stressed" refers to the stress of surgery and anesthesia.
[c] Hypox = hypophysectomized.
[d] ACTH = adrenocorticotrophic hormone, 0.8 to 1.0 IU by i.p. injection.
[e] n = number of animals.
[f] TAA = total ascorbic acid.
[g] AA = reduced ascorbic acid.
[h] DHAA + DKG = dehydroascorbic acid and diketogulonic acid.

7. However, ACTH did not increase the ascorbic acid excretion in a patient with Addison's disease, so they suggested that the effect was probably mediated by the adrenal. But only one out of nine patients treated with cortisone acetate, 200 mg daily, showed a definite increase in urinary ascorbic acid; they suggested that this failure of cortisone to produce a peak of urinary ascorbic acid excretion was probably due to inadequate dosage. These same workers also studied the effect of ACTH on a 9-month-old infant with scurvy. The child, which had clinical and X-ray evidence of scurvy, was placed on a vitamin C-free diet and received 30 mg of ACTH by injection in 24 h. Urinary ascorbic acid (TAA) initially varying between 0.5 and 1.5 mg/24 h, increased to 2.3 mg in 24 h and was closely followed by an increased excretion of chemically determined urinary corticoids (Figure 8); the blood ascorbic acid (TAA) remained 0 during the period of the study. Following this short course of ACTH, there occurred "definite clinical improvement, corroborated by calcification in the femoral sub-periosteal haematomata." The small increase of urinary ascorbic acid following ACTH administration to the scorbutic infant may have been due to a release of ascorbic acid from the adrenal, for Harris and Ray (1933) reported that the adrenal is not entirely devoid of ascorbic acid in scurvy.

This study of a human infant is analogous to the disputed findings of Schaffenburg et al. (1950), Hyman et al. (1950), and of Herrick et al. (1952), that ACTH and cortisone prolonged the lives of scorbutic guinea pigs. Hyman et al. also observed that cortisone markedly decreases the adrenal hypertrophy of scurvy in guinea pigs, while ACTH increases it. The fact that not only cortisone, but also ACTH delayed death from scurvy suggested, what we

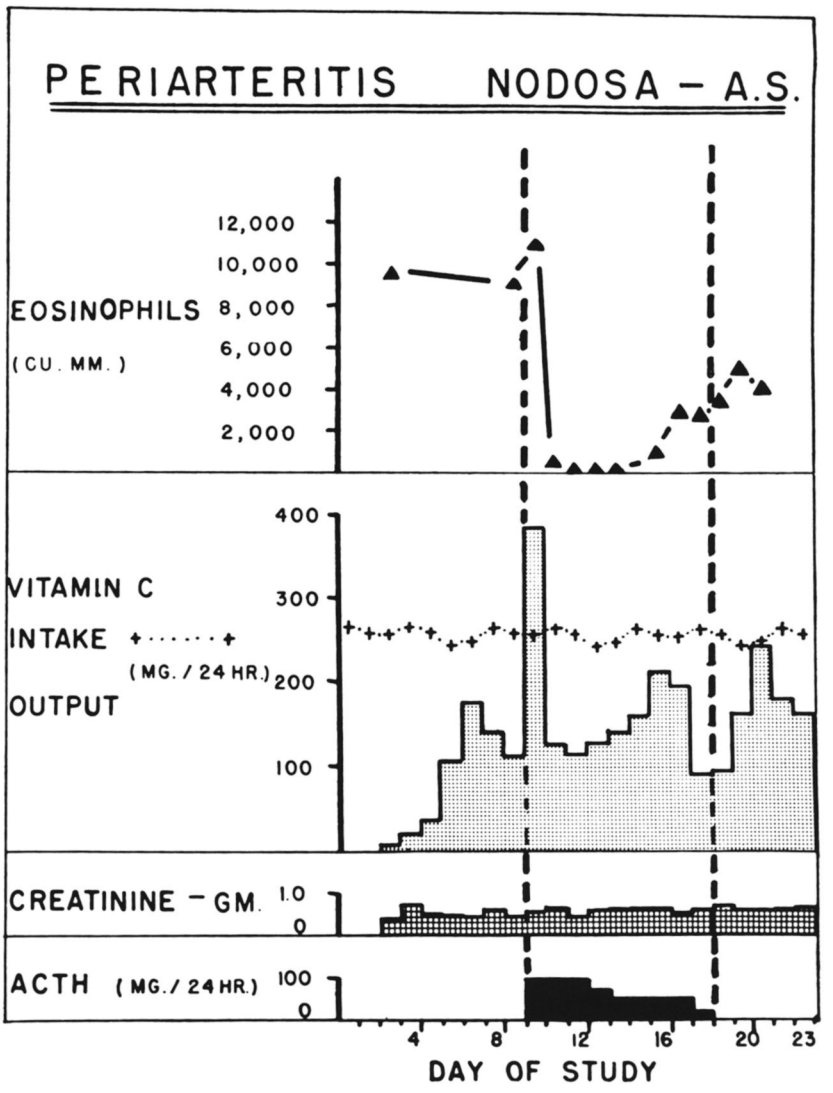

FIGURE 7. Increased urinary ascorbic acid (TAA) excretion after ACTH with a diminution in excretion upon cessation of ACTH administration. (From Beck, J. S., Browne, J. S. L., and MacKensie, K. R. [1951], in *Proc. 2nd Clinical ACTH Conf.*, Vol. 1, Mote, J. R., Ed., Blakiston, Philadelphia, 355. With permission.)

now know, that ascorbic acid deficiency does not prevent the synthesis of corticoids by the adrenal.

Prunty et al. (1955) found that adrenalectomy hastens death from scurvy in guinea pigs, but reported that the administration of corticosteroids, DOCA, and cortisone acetate, does not delay death from scurvy in the intact animal. They observed that the adrenal gland was already hyperactive in scurvy and reported increased urinary excretion of 17-ketogenic and 17-keto steroids in scurvy.

Cornforth and Long (1952) theorized that cortisone might facilitate the oxidation of ascorbic acid (AA) to DHAA. However, Stewart et al. (1953a,b), studying healthy human volunteers, found just the opposite; they observed that ACTH caused a modest increase in the plasma ascorbic acid level and an increase in the AA/DHAA ratio 2 to 4 h after injection, and that cortisone caused a virtual disappearance of DHAA from the plasma within 1 h of

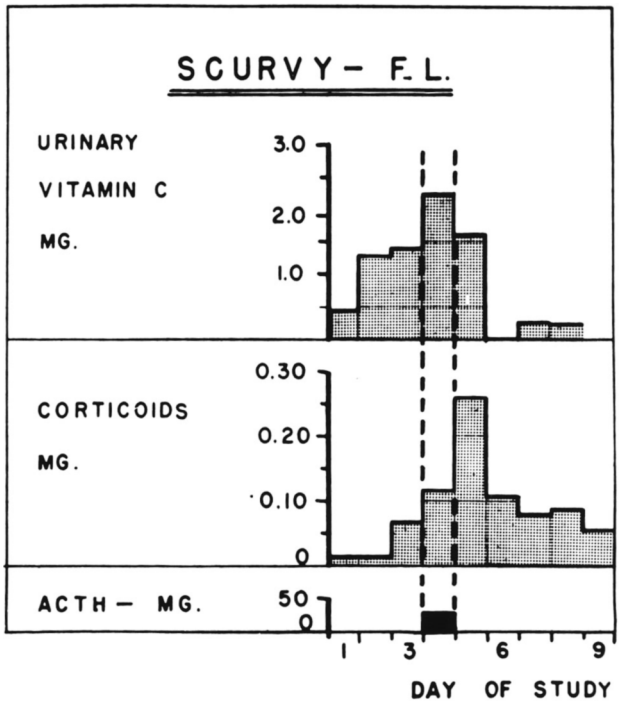

FIGURE 8. Urinary ascorbic acid (TAA) and urinary corticoids in an infant with scurvy receiving ACTH. (From Beck, J. S., Browne, J. S. L., and MacKensie, K. R. [1951], in *Proc. 2nd Clinical ACTH Conf.*, Vol. 1, Mote, J. R., Ed., Blakiston, Philadelphia, 355. With permission.)

its administration, as shown in Figures 1 and 2 of Chapter 13 of this volume. In all probability, a small quantity of ascorbic acid, probably in the form of DHAA, must be present in the scorbutic animal for cortisone or ACTH to have any beneficial effect in scurvy, and then by increasing the ratio of AA to DHAA, so decreasing the oxidation-reduction potential in the tissues.

McSwiney et al. (1954) and Prunty et al. (1955), studying patients with rheumatoid arthritis, observed a temporary increase in the urinary excretion of both ascorbate and dehydroascorbate, leading to a negative ascorbic acid balance in three out of six patients in the first few days of treatment with ACTH. They reported that guinea pigs also showed an increased excretion of ascorbic acid and DHAA while on treatment with ACTH. McSwiney et al. also studied the daily plasma ascorbic acid levels of the same six patients; they found no consistent change in the ratio of AA to DHAA during treatment, but there was a tendency for the oxidation products (DHAA + DKG) to rise at first, and then to fall after a week or so (Figure 9).

The main difference between the work of Stewart et al. and that of McSwiney et al. was that the former group studied AA/DHAA ratios within hours after the administration of cortisone or ACTH, while McSwiney et al. were studying the effects after days and weeks of treatment. Also, McSwiney et al. were studying patients with rheumatoid arthritis who may have had a disturbance of AA metabolism which only slowly responds to cortisone. It would seem that the effect of cortisone in decreasing the oxidation-reduction potential lasts for only a matter of hours, while the ascorbic acid mobilizing effect of ACTH or cortisone lasts for days. McSwiney et al. believe that the increased urinary excretion of ascorbic acid is much more than can be accounted by loss from the adrenals, so they suggest that there

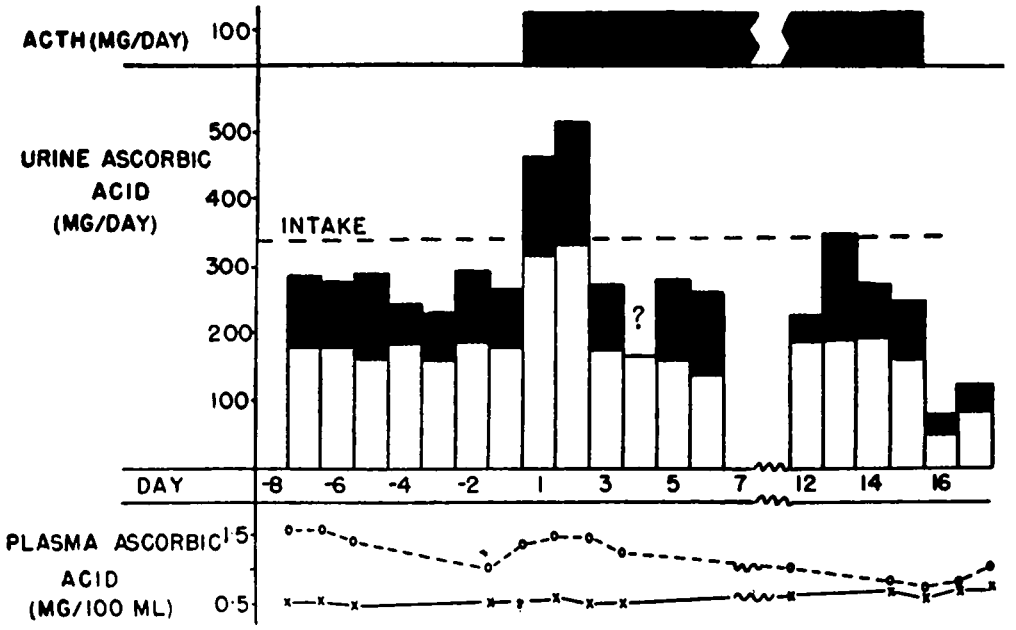

FIGURE 9. The effect of ACTH on a patient with rheumatoid arthritis on a constant dietary ascorbate intake (40 mg daily) who was receiving a constant ascorbic acid supplement (300 mg daily). Both the urinary ascorbic acid (AA, white bars) and the urinary total ascorbic acid (TAA, black plus white bars) increased following the administration of ACTH; the total urinary ascorbic acid loss exceeded the intake for the first 2 d of treatment. At first, the plasma total ascorbic acid concentration (TAA, o-o-o) increased more than the plasma reduced ascorbic acid (AA, ×-×-×) indicating an increase in the oxidized forms (DHAA + DKG) and a decrease in the AA/DHAA ratio, but after 2 weeks of treatment, the AA/DHAA ratio increased and was restored to normal. (Compare Figure 5 of Chapter 10, Volume III, concerning the author's personal observations during cortisone treatment of a patient with rheumatic fever. (From McSwiney, R. R., Clayton, B. E., and Prunty, F. T. G. [1954], *Lancet*, 1, 178. With permission.)

is a shift of ascorbic acid from the cells to the tissue fluids and perhaps an increased renal clearance of AA following ACTH. They state that the temporarily increased levels of AA and DHAA in the urine during ACTH treatment are similar to those found after oral administration of AA to human volunteers.

These apparently contradictory reports may be reconciled if there is a rapid and short-lived effect of ACTH converting DHAA to AA in the plasma, lasting only a few hours, and a much longer effect which results in the mobilization of ascorbic acid from the tissues and increased excretion of AA and DHAA in the urine.

The findings of Stewart et al. are consistent with the observations of Loxton and Le Vay (1963), who observed a fall in the oxidation-reduction potential of the plasma 2 h after giving cortisone by mouth.

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Chapter 17

LACK OF SLEEP

I. INTRODUCTION

In the days of sail, when long ocean voyages took so many months, scurvy was a common hazard for all sailors because they had to depend on food held in storage. The ordinary seamen usually developed the disease before the officers, perhaps because of their less adequate nutritional state before departure, but there was always a suspicion that the "stress" of long hours of arduous duty played a role in the onset of this dreaded disease.

Extensive studies of various forms of stress (Chapter 16 of this volume) have shown only a redistribution of ascorbic acid from the adrenals to the tissues, a reduction of dehydroascorbic acid (DHAA) to ascorbic acid in the blood plasma, and a temporary increase in the urinary excretion of ascorbic acid.

However, it is now known that high blood histamine levels are present, even in mild vitamin C deficiency, and are an integral part of scurvy (Chapter 1, Volume III). Indeed, the lower the ascorbate, the higher the histamine level. Evidence to be presented here clearly shows that the stress of long hours of duty, without sleep, does increase the blood histamine level. Thus, it is evident that "lack of sleep" and probably other forms of "stress" can accelerate the histaminemia of vitamin C deficiency. We may conjecture that this could increase the vascular damage and promote the onset of the bleeding gums, the ecchymoses, and the painful swollen joints so characteristic of clinical scurvy.

II. ORIGIN OF STUDY

The normal blood histamine level is 5 to 15 ng/ml when the plasma ascorbic acid (AA)* level is greater than 1 mg/100 ml, but is doubled when the ascorbate level falls to 0.5 mg/100 ml and is quadrupled when it falls below 0.2 mg/100 ml. The highest blood histamine level found among some 900 people whose blood had been analyzed in our research laboratory at the Methodist Hospital of Brooklyn was 180 (duplicate 180) ng/ml associated with a low plasma ascorbic acid level of 0.14 mg/100 ml. The sample was from a 31-year-old male resident obstetrician (S.G.F.), who was a heavy cigarette smoker and had just completed a day and a night on duty without sleep. He appeared exhausted, with puffy eyelids, but was otherwise in good health; he had no history of allergy and showed no evidence of any allergic phenomenon. When informed of the results of the analysis of his blood sample, he volunteered not to change his diet or his smoking habits for the time being and to let us draw blood from him again after a night of sleep. After sleeping, his plasma ascorbic acid level was still low, 0.20 mg/100 ml, but his whole blood histamine level had fallen to 80 (repeat 84) ng/ml. Nevertheless, this histamine level was still higher than all but one other subject we had ever studied. He was then given six 500-mg ascorbic acid tablets and was requested to take one tablet twice a day for 3 d. Blood was drawn again on the fourth day, in the morning after a night of sleep. His plasma ascorbic acid had then risen to a normal level of 1.15 mg/100 ml and his whole blood histamine level had fallen to 15 (duplicate 18) ng/ml. It was these findings that prompted a study of 12 more resident physicians, in order to find out whether long hours on duty, without sleep, affects blood histamine levels.

* AA — ascorbic acid, reduced form.

III. BLOOD HISTAMINE AND ASCORBIC ACID LEVELS OF RESIDENT PHYSICIANS AFTER NIGHT DUTY*

Twelve physicians volunteered for this study; they knew that we were studying the effects of stress and were asked to give one blood sample after a "heavy" night on duty and one after a night of sleep. They were asked to keep a record of the number of hours they slept or did not sleep and agreed not to make any departure from their usual diets. It should be understood that night duty follows directly on day duty and is not a separate shift, as it would be in most other jobs. Moreover, the next day is also a day on duty, so it would have been better to have studied blood samples drawn at 5 or 6 p.m. on the second evening, after a 35-h stretch on duty, but this was not possible, as our laboratory was closed at night. "Fasting" venous blood samples, 20 ml each, were drawn into dry heparin tubes between 8 and 10 a.m. on week days; these samples were taken directly to the department laboratory for analysis without delay.

Addition of acid for both analyses was always carried out within 1 h of collecting the blood. Avoidance of delay in analysis for ascorbic acid is essential, as reduced ascorbic acid in blood plasma can be lost by oxidation at a rate of 5 to 8% per hour at room temperature, as shown by Clemetson and Andersen (1964).

A. Whole Blood Histamine Analysis

As soon as each 20-ml blood sample arrived in the laboratory, 10 ml was pipetted off and added to 1 ml of 12 *N* perchloric acid and 9 ml of distilled water, as the first step in the analysis for histamine by the method of Shore et al. (1959), using a spectroflourometer (Model 430, Turner Associates, Palo Alto, CA). In this method, histamine is extracted and then condensed with ortho-phthalaldehyde to yield a relatively stable fluorescent compound. Duplicate analyses were obtained for all specimens.

B. Plasma Reduced Ascorbic Acid Analysis

The remainder of the blood was centrifuged to obtain clear plasma with no evidence of hemolysis; 4 ml of the plasma was drawn off using a rubber bulb aspirator with a finger pressure control valve and was added to 6 ml of 5% metaphosphoric acid as the first step in the analysis for ascorbic acid by the 2,6-dichloroindophenol method of Roe (1954). Care was taken to avoid the buffy coat of leukocytes and platelets, as these are rich in ascorbic acid. The tube was then covered by a paraffin wax film (Parafilm M®, American Can Co., Marathon Products, Neenah, WI), shaken to mix the contents and centrifuged to remove the proteins. The supernatant was decanted and filtered twice using the same filter paper (Whatman® No. 50, Whatman Inc., Clifton, NJ) to obtain a clear filtrate for analysis. A grating spectrophotometer with a 25-cm linear-log recorder (Model DBG, Beckman Instruments, Fullerton, CA) was used to obtain a graphic record of all analyses. All specimen filtrates were analyzed in triplicate. Further details of the method of analysis, including preparation of solutions, acid washing of glassware, method for rapid mixing of the dye and test solutions in the spectrophotometer cuvette, correction for fading of the dye, and correction for any traces of turbidity, have been described by Clemetson (1980).

C. Results

The 12 physician volunteers reported an average of 8 h of sleep before giving the "rested" blood sample, and an average of 1 3/4 h sleep during the night before giving the sample following a day and a night on duty. In every instance the whole blood histamine concentration was found to be higher after a day and a night on duty than after a night of sleep,

* A previously unpublished study conducted in 1981 by C. Alan B. Clemetson, George D. K. Kofinas, and Virginia I. Cafaro in the Department of Obstetrics and Gynecology of the Methodist Hospital, Brooklyn.

as shown in Table 1. The difference between the mean "rested" histamine level of 13 ng/ml and the mean level of 29 ng/ml after a day and a night on duty, is highly significant ($p < 0.001$), using Student t test.

Table 1 also shows that there was a slightly lower mean plasma ascorbic acid level of 0.79 mg/100 ml after a day and a night on duty than after a night of sleep, 0.86 mg/100 ml. However, a reduction of the ascorbic acid level was observed in only 9 of the 12 subjects and was not statistically significant; it could have been due to a slight variation of diet, as that was not controlled.

It may be noted in Figure 1 that the mean blood histamine level of the "rested" physicians fell very close to the mean histamine level found in our laboratory for women of similar age (25 to 35 years) and similar plasma ascorbic acid levels (0.8 to 0.89 mg/100 ml). However, the same figure shows that the mean blood histamine level of the physicians after a day and a night on duty was much higher than could have been due to the slightly lower ascorbate level.

IV. DISCUSSION

This study leaves no doubt that the whole blood histamine level is elevated by working long hours; there is also no doubt that the blood histamine level is elevated by ascorbic acid deficiency.

The present study did not show a significant decrease in the plasma ascorbic acid level after long hours without sleep, but did not disprove it either. It is entirely possible that a study of blood samples from resident physicians on the evening of the second day, after 35 h of continuous duty, without sleep (a common occurrence in most busy general hospitals), might show a significant change in plasma ascorbate, if diet were strictly controlled.

However, the increased blood histamine level due to lack of sleep in this experiment was clearly greater than could have been caused by a change in the ascorbate level.

Studies by Puizillout et al. (1979) have shown a decreased 5-hydroxytryptamine (5 HT) release from the cerebral cortex of cats during sleep, and the work of Ogasahara et al. (1980) suggests that this is mainly due to decreased 5 HT release during paradoxical sleep. Moreover, Urba-Holmgren et al. (1979) report that yawning is modulated by 5 HT. It is now fairly well established that 5 HT is produced by brain tissue and is involved in brain function, almost certainly as a neurotransmitter, as reported by Brodie et al. (1955, 1958) and by Weseman (1978). It is therefore interesting to observe that 5 HT stimulates the release of histamine, as shown by Feldberg and Smith (1953) and that brain histamine is increased by stress, as observed by Mazurkiewicz-Kwilecki in a study of rats.

So it would seem that the mental stress of long hours on duty could have been responsible for the elevated blood histamine levels observed after a day and a night on duty in the present study. However, it is also possible that the physical stress of long hours of ambulant activity could have been responsible for the elevated blood histamine levels.

We need to know whether it is harmful to have an elevated blood histamine level and, if so, what kind of harm could result. Recent studies by Owens and Hollis (1979) suggest that histamine plays an important role in subendothelial lipid deposition and atherogenesis. Moreover, De Forrest and Hollis (1980) studied the relationship between low-intensity shear stress, aortic histamine formation, and aortic albumen uptake. They concluded that aortic histamine formation may be important with respect to increased vascular wall permeability involved in the initiation of atherosclerosis. Indeed, antihistamines have been reported by Harman (1962) and Hollander et al. (1974) as possessing antiatherosclerotic activity; so histamine may well be responsible for the intimal damage which predisposes to cholesterol deposition and atherosclerosis.

It has previously been observed (Clemetson, 1979) that nine factors predisposing to

Table 1
PLASMA ASCORBIC ACID AND WHOLE BLOOD HISTAMINE LEVELS OF 12 RESIDENT PHYSICIANS AFTER A NIGHT OF SLEEP, AFTER A DAY AND A NIGHT ON DUTY

					After a night of sleep		After day and night on duty		Hours of sleep	
Department	Seniority	Duty	Sex	Age	Plasma ascorbic acid (mg/100 ml)	Blood histamine (ng/ml)	Plasma ascorbic acid (mg/100 ml)	Blood histamine (ng/ml)	While on duty	Off duty
Medicine	PGY[a]2	CCU[b]	M	33	*0.95[c]	12	0.92	35	1	6
						15		37		
Medicine	PGY 3	ER[d]	M	33	0.93	12	*0.89	25	0	8
						8		25		
OB/GYN[e]	PGY 1	DR[f]	M	27	0.87	12	*0.85	29	3	6 1/2
						11		25		
OB/GYN	PGY 2	ER	M	35	*0.92	8	0.93	23	3 1/2	10
						9		25		
OB/GYN	PGY 1	DR	M	33	*1.09	6	0.90	28	3	8
						6		29		
OB/GYN	PGY 1	DR	M	32	*0.92	12	0.87	25	2	8
						9		29		
Medicine	PGY 1	ER	M	30	0.94	17	*0.76	25	0	8 1/2
						17		32		
Medicine	PGY 1	ER	M	27	*0.73	13	0.69	29	2	7 1/2
						13		34		
Medicine	PGY 1	ER	M	29	0.73	21	*0.73	33	0	13
						20		35		
Medicine	PGY 1	Ward	F	27	*0.63	15	0.49	33	2	7 1/2
						12		30		
OB/GYN	PGY 2	ER	M	30	0.78	15	*0.55	27	0	6
						15		27		
OB/GYN	Subintern	DR	M	28	*0.85	13	0.89	25	5	7
						13		32		
Means				30.3	0.86	12.67	0.79	29.04	1 h 47 min	8 h
SD					(±)0.125	(±)3.886	(±)0.148	(±)4.005		

Note: The blood histamine level of every physician was higher after a day and a night on duty ($p < 0.001$).

[a] PGY — postgraduate year.
[b] CCU — coronary care unit.
[c] Use of an asterisk denotes those samples which were drawn first.
[d] ER — emergency room.
[e] OB/GYN — obstetrics and gynecology.
[f] DR — delivery room.

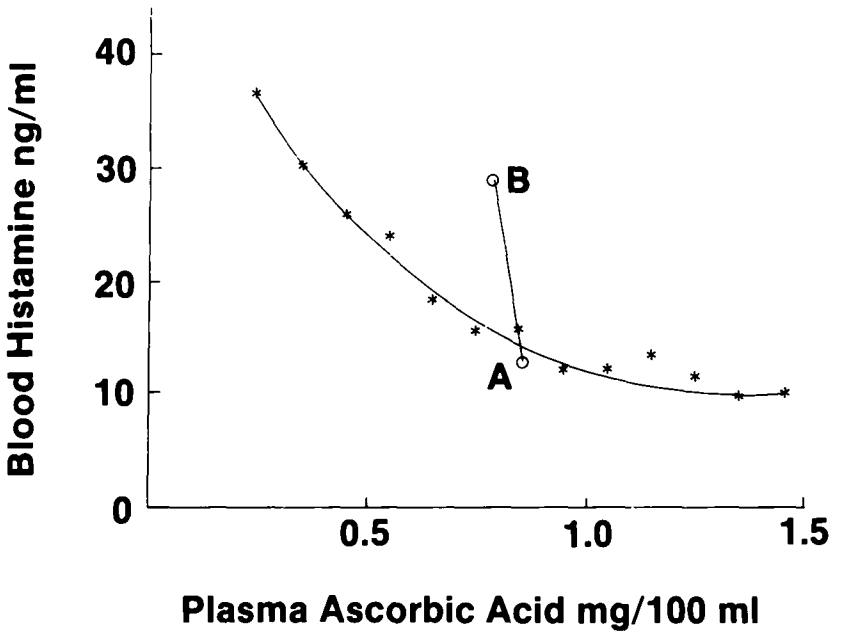

FIGURE 1. Here the mean histamine levels of blood samples from 322 healthy women aged 25 to 35 years (*) are plotted in 0.1-mg/100 ml plasma ascorbic acid groups and are shown for comparison with the results obtained from 12 resident physicians with a mean age of 30.3 years (○) (A) after a night of sleep and (B) after a day and a night on duty. The 322 women were pregnant (not in labor), 6 weeks post partum, or other nonpregnant women, not taking birth control pills or other medications. The mean histamine level of the "rested" blood samples from the resident physicians (A) fell close to the normal histamine level for the ascorbate group, but the mean blood histamine level of the same physicians after a day and a night on duty (B) was higher than could be accounted for by the slightly lower mean ascorbate level of these blood samples.

cardiovascular disease are all associated with a tendency towards low plasma ascorbate levels: smoking, infection, trauma, surgery, soft water, high-dose combined birth control pills, pregnancy, aging, and winter season are all associated with a tendency towards decreased ascorbic acid levels in the blood. If we add the male sex, that makes ten factors. Since ascorbic acid deficiency is invariably associated with a high blood histamine level, and we now know that stress also increases blood histamine, there are 11 factors predisposing to cardiovascular disease, all of which can or do increase blood histamine levels.

In any event, we should pay more attention to the health of resident physicians, and we should perhaps advise ascorbic acid supplements for physicians and others doing long hours of duty.

In conclusion, it would seem that this work provides chemical evidence of the need for sleep, as well as a diet including plenty of fresh fruit and vegetables; the lack of either causes an accumulation of histamine in the blood.

As physicians, we have all been aware of the need for rest and sleep in the healing process; now we have chemical evidence of this need and it should serve to remind us that sleep is necessary, not only for healing, but also for the maintenance of health.

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Chapter 18

TIME OF DAY

I. RELATED CIRCADIAN RHYTHMS

The word circadian, derived from circa, about, and dies, day, is perhaps preferable to diurnal when referring to 24-h cycles, as it is intended to refer to the 24-h day and to include nocturnal as well as diurnal events.

There is evidence of a circadian rhythm in the acorbic acid metabolism of human beings as well as other animals, and there are reasons for believing that this is due to a circadian rhythm in adrenal cortical activity. This seems to be synchronized by a critical cyclic burst of secretion of the adrenocorticotrophic hormone (ACTH) by the pituitary, resulting from the cyclic secretion of releasing factor by the hypothalmus, in turn influenced by the light/dark cycle of the environment and the sleep/wake cycle of the individual.

One might not think of this as having any real clinical importance, but it seems that it does, for there is also a synchronous circadian rhythm in human sensitivity to histamine, and this shows itself as a circadian rhythm in the response of allergic individuals to penicillin and to house dust, etc. Ascorbic acid seems to be involved, for we now know that blood histamine levels are markedly responsive to plasma ascorbic acid levels (Chapter 1, Volume III), and such allergic responses can be modified by ascorbic acid supplements.

A. Circadian Rhythms of Adrenal Ascorbic Acid Content and Adrenal Cortical Function

Even as early as 1933, Van Eekelen et al. observed a rhythmical change in the urinary output of ascorbic acid by human subjects, which was lowest at night. Moreover, these workers noted that this change was independent of the quantity of liquid taken and the volume of the urine output; they also noted that the day-night rhythm persisted when the food had been deprived of vitamin C for some time.

Studies conducted by Rinne and Kytömäki (1961) in June at the University of Turku in Finland, showed a definite circadian rhythm in the ascorbic acid (TAA)* concentration of rat adrenals, as shown in Figure 1. The depletion of ascorbic acid occurred at 1600 h, which is consistent with the time of the highest plasma corticosterone level in the rat according to Guellemin et al. (1959) and McCarthy et al. (1960) and in the mouse, as reported by Halberg et al. (1959). Actually there is a slight time lag between adrenal ascorbic acid release and corticosterone secretion, as shown by Lipscomb and Nelson (1960), following ACTH administration to hypophysectomized rats, but it is only a matter of a few minutes (Figure 2).

There is also a circadian rhythm in the adrenal cortical function of man, but the studies of Orth et al. (1967), Krieger (1970, 1975), and others have shown that human cortisol output is normally greatest at about 0800 h.

Presumably the fact that we are active by day, while rats and mice are nocturnal animals, accounts for this difference.

Wilbur and Walker (1977) estimated plasma cortisol concentrations every 4 h in three groups of male guinea pigs receiving normal, deficient, and excessive ascorbic acid intakes, while on a fixed photoperiod of 12 h of light and 12 h of darkness. They observed a biphasic circadian cycle in plasma cortisol concentration, quite different from that of the rat, having minima at the beginning of the light and dark periods (0800 and 2000 h) and maxima 4 to 8 h later. A deficiency of vitamin C greatly elevated the plasma cortisol throughout the 24-

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

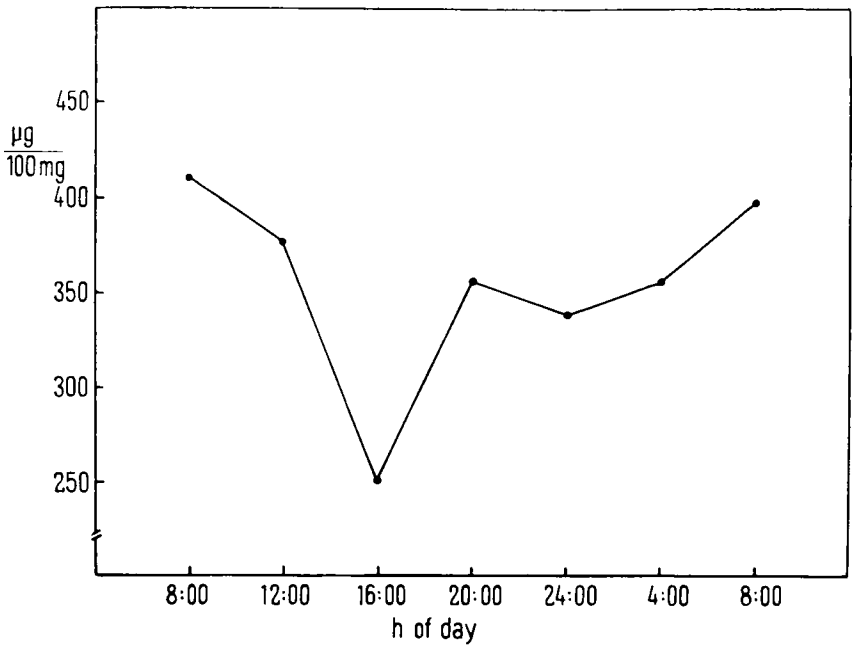

FIGURE 1. Circadian rhythm of adrenal ascorbic acid concentration in rats. Each observation represents the mean value obtained from a study of seven rats. (From Rinne, U. K. and Kytömäki, O. [1961], *Experientia,* 17, 512. With permission.)

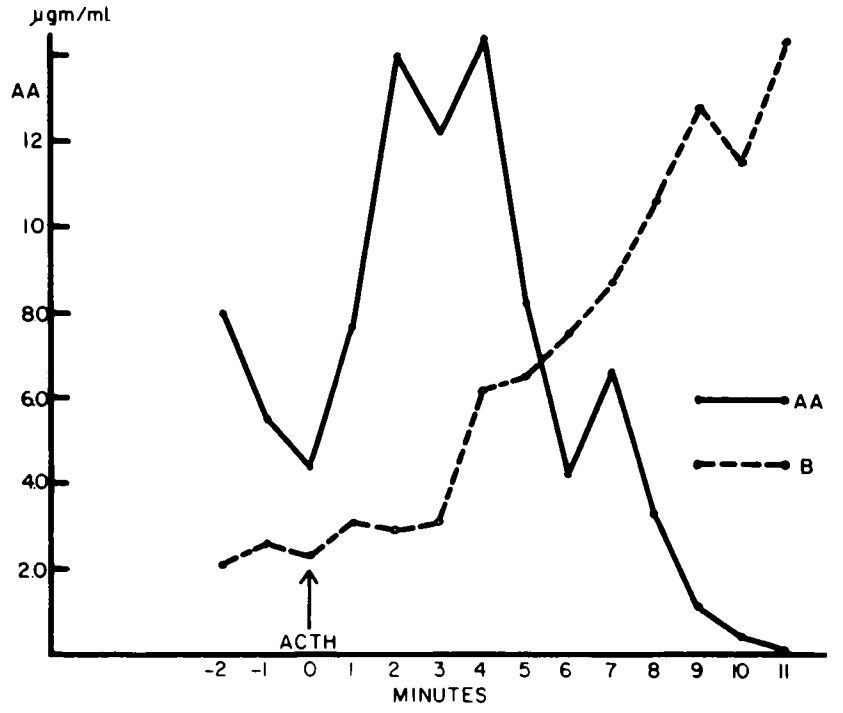

FIGURE 2. Concentrations of total ascorbic acid (AA) and corticosterone (B) in the adrenal vein blood of hypophysectomized rat following ACTH administration. (From Lipscomb, H. S. and Nelson, D. H. [1960], *Endocrinology,* 66, 144. ©The Endocrine Society. With permission.)

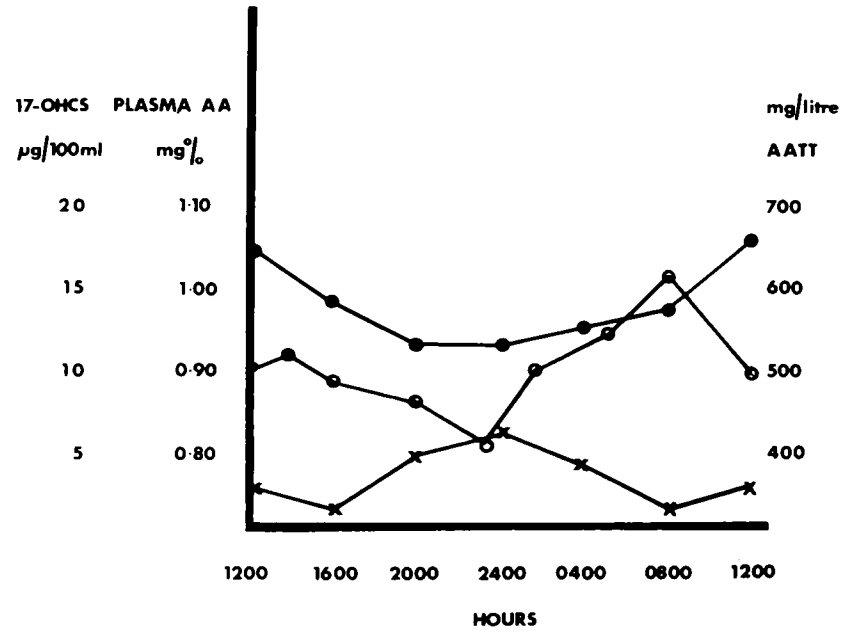

FIGURE 3. Plasma ascorbic acid concentrations (●–●) were estimated and ascorbic acid taste threshold assessments (×–×) were made at four hourly intervals in healthy human volunteers who were accustomed to darkness between 2000 h and 0400 h. The plasma cortisol (○–○) circadian rhythm observed by Krieger (1970) is also shown for comparison. (From Loh, H. S. and Wilson, C. W. M. [1973], *Int. J. Vitam. Nutr. Res.*, 43, 355. With permission.)

h period and exaggerated the amplitude of the plasma cortisol changes, but did not alter the phase or the period of the cycle.

Andrews and Folk (1964), studying the golden hamster, and Andrews et al. (1968), in a study of the brown lemming of Alaska, have observed that isolated adrenal glands retain their circadian cyclic rhythm of corticosteroid secretion even when maintained in tissue culture for as long as 5 d. However, in intact animals there is plenty of evidence that adrenal phase synchronization is controlled by the plasma ACTH rhythm, which continues in the absence of the adrenals. The pituitary rhythm is controlled by neuroendocrine pathways in the brain, which are influenced by the light/dark, sleep/wake, and rest/activity cycles of the individual and his or her environment. A reversal of the human light/dark cycle results in a reversal of the adrenocortical cycle within a period of about 8 d.

B. Circadian Rhythms of Plasma Ascorbic Acid and Plasma Cortisol

Studying 12 Dublin University students in the month of May, on a schedule of light from 0400 to 2000 h and dark from 2000 to 0400 h, Loh and Wilson (1973) made four hourly measurements of the plasma ascorbic acid (TAA) levels. They reported that the plasma ascorbate levels showed a definite circadian rhythm, with a maximum value of 1.04 ± 0.19 mg/100 ml at 1200 h and a trough of 0.92 ± 0.20 mg/100 ml at 2400 h. This was in phase with the rhythm of cortisol values reported by others (Figure 3).

C. Circadian Rhythm of Susceptibility or Resistance

Franz Halberg (1960a, b) demonstrated variations of animal susceptibility to various stimuli. Under standardized conditions the resistance of the total organism changes predictably according to a 24-h cycle. These changes may tip the scale towards death or survival

in the response of mammals to a wide range of potentially harmful chemical, physical, pharmacological, endocrinological, toxicological, bacteriological, or anesthetic agents. Thus, this rhythm of changing sensitivity is of great importance in many respects, especially in the design of biological experiments.

D. Circadian Rhythm of Histamine Sensitivity and Allergic Response

Wilson (1965) demonstrated a circadian rhythm in the urinary excretion of histamine by the rat. The work of De Vries et al. (1962) and Reinberg et al. (1965, 1966, 1969, 1971) extended the study of susceptibility rhythms to human subjects. This led them to discover a circadian rhythm in the response of human subjects to histamine. Reinberg et al. studied adults who were apparently healthy, as well as allergic patients, who were acclimatized for at least a week to a routine of diurnal activity and nocturnal rest (from 2300 to 0700 h).

In healthy subjects a standard dose of histamine (10 μg in 0.1 ml of *N* saline) was injected subcutaneously every 4 h for 24 h; the resulting erythematous area was measured 15 min after each injection. Allergic subjects received appropriate standard injections of diluted house dust extract, or were scratch tested with diluted sodium benzyl penicillin.

Such tests demonstrated significant rhythms of sensitivity to histamine, penicillin, and house dust (Figure 4), all of which showed maximal sensitivity at 2300 h, which precedes the trough of 17-OH steroid, 17-keto steroid, and K^+ excretion, according to Reinberg et al. (1965, 1969). This is to be expected, as the curve for urinary corticosteroid excretion lags slightly behind that for plasma levels. Indeed, the plasma 17-OH corticosteroid trough was observed by Krieger (1970) to occur at 2400 h, which coincides with the plasma total ascorbate trough observed by Loh and Wilson (1973) as seen in Figure 3.

II. ASCORBIC ACID MODIFICATION OF HISTAMINE AND ALLERGIC RESPONSES

Zuskin et al. (1973) observed that ascorbic acid supplementation reduces the airway constriction induced by inhalation of histamine in human adults. Moreover, Valik and Zuskin (1973), at Zagreb University, found that an ascorbic acid supplement, taken before work, reduced the airway constriction of workers exposed to flax dust; 20 women flax workers, 13 with symptoms of byssinosis and 7 without, took part in the study. Their mean age was 35 years and their mean duration of employment in the flax industry was 10 years. They were exposed to a total flax dust concentration of 16.9 mg/m^3 (respirable fraction 3.4 mg/m^3). Measurement of forced expiratory volume ($FEV_{1.0}$) and of the maximum air flow rate at 50% of the control vital capacity (V_{max} 50% VC) were carried out on these workers before and after the work shift on four consecutive Mondays. In the first week, ventilatory capacity measurements were performed without drug treatment. On the following Monday, orciprenaline was administered by inhalation before the shift. On the third Monday, the subjects were given a tablet of antihistamine (diadril) orally before the shift. On the fourth Monday, one tablet of placebo was given before the shift, and then, starting from the following Tuesday, 500 mg of ascorbic acid was given through the remaining days of the week, including the Monday before the shift.

The results are shown in Table 1, where it is evident that ascorbic acid supplements had an effect which was similar to that of the antihistamine or the brochodilator, causing a significant reduction in the bronchoconstrictor effect of the flax dust.

Analyzing the workers' comments on their subjective feelings, it was found that 12 byssinotic workers claimed to have felt much better during dust exposure following the administration of ascorbic acid tablets. After the antihistamine or bronchodilator drug, eight byssinotic subjects said they felt less dyspnea or chest tightness during the following dust exposure.

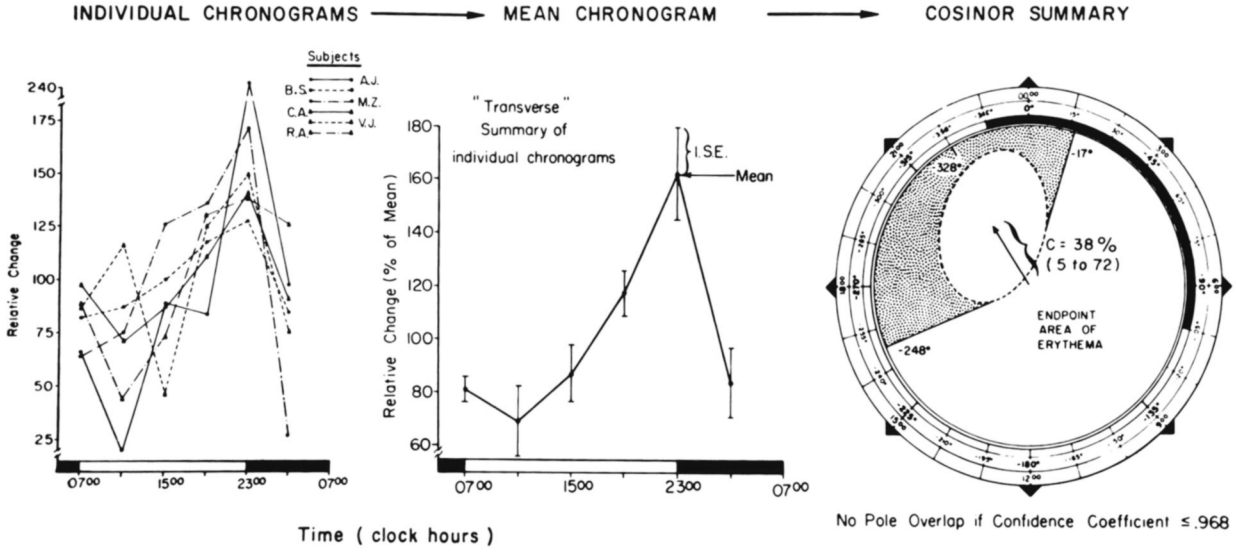

FIGURE 4. Circadian rhythm of susceptibility to house dust: six mature human beings (two women and four men). The direction of the arrow in the cosinor and the adjacent shaded area indicate the span of greater susceptibility; the white area within the shaded area is the error elipse. (From Reinberg, A., Zagulla-Mally, Z., Ghata, J., and Halberg, F. [1969], *J. Allergy*, 44, 292. With permission.)

Table 1
MEAN CHANGES IN $FEV_{1.0}$ AND V_{max} 50% VC IN 13 BYSSINOTIC FLAX WORKERS WITHOUT AND WITH DRUG TREATMENT BEFORE THE WORK SHIFT

	$FEV_{1.0}$(ml)				V_{max} 50% VC (1/s)			
		Mean reductions over shift				Mean reductions over shift		
	Before shift	ml	%	*p*	Before shift	1/s	%	*p*
Without treatment	2598	257	9.9	<0.01	3.72	0.68	18.3	<0.01
Placebo	2590	237	9.2	<0.01	3.70	0.61	16.5	<0.01
Bronchodilator	2578	119	4.6	<0.01	3.79	0.18	4.7	<0.05
Antihistamine	2605	103	3.9	<0.01	3.70	0.16	4.3	NS[a]
Ascorbic acid	2583	127	4.9	<0.01	3.77	0.29	7.7	<0.05

Note: Ascorbic acid supplements, 500 mg daily, taken before each work shift, caused a significant reduction in the bronchoconstrictor effect of the flax dust in byssinotic workers compared with placebo ($p < 0.05$). The forced expiratory volume and maximum air flow rate benefits resulting from the ascorbic acid supplements were similar to those obtained with bronchodilators and with antihistamines.

[a] NS = difference statistically not significant ($p > 0.05$)

From Valik, F. and Žuškin, E. (1973), *Br. J. Ind. Med.*, 30, 381. With permission.

Two byssinotic workers mentioned a somewhat weakened effect of dust after placebo application. Out of seven workers without byssinosis, four felt better after ascorbic acid or bronchodilator, three after antihistamine, and none after placebo.

It does not seem at all likely that the small difference between the maximum total ascorbic acid level of 1.04 and the minimum of 0.92 mg/100 ml observed by Loh and Wilson, could account for the circadian histamine sensitivity cycle. However, the ratio of reduced to oxidized ascorbic acid, which is controlled by ACTH and cortisone, as shown by Stewart et al. (1953a), is associated with a profound change in the oxidation-reduction potential, which certainly could account for an alteration of histamine metabolism.

In this context it is interesting to note that the whole blood histamine levels are elevated in physicians who have been on duty all day and all night without sleep and that their histamine levels can be returned to normal by high doses of ascorbic acid (Chapter 17 of this volume and Chapter 1, Volume III).

Doring and Riecke (1952) observed a circadian rhythm of skin capillary resistance, with a trough (or fragility state) at 2300 h, which was confirmed by Reinberg et al. (1965).

Kramar et al. (1956) have shown that capillary resistance is increased by adrenal cortical hormones, but it is not known whether this is a direct effect or is mediated by the change in ascorbic acid metabolism.

Further evidence that these cyclic phenomena are of more than academic interest is provided by the report of Reinberg et al. (1963) that asthma attacks occur predominantly at night.

III. UNANSWERED QUESTIONS FOR FUTURE RESEARCH

We do not know whether the ratio of reduced to oxidized ascorbic acid in plasma shows a circadian rhythm. Nor do we know whether the blood histamine level exhibits a circadian rhythm in human subjects.

Studies of volunteers in a controlled environment, receiving no ascorbic acid for 36 h,

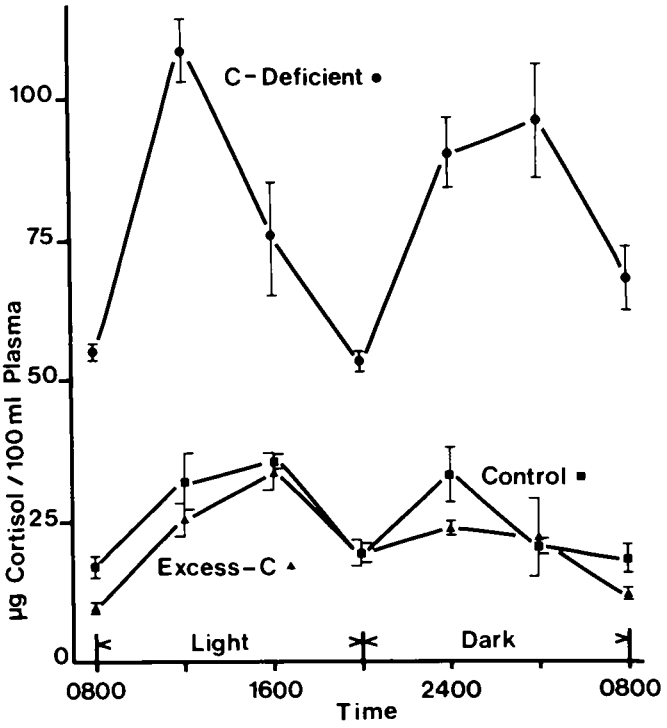

FIGURE 5. Effect of dietary vitamin C on the daily variation of plasma cortisol in the male guinea pig. Ascorbic acid deficiency caused a pronounced increase in the plasma cortisol levels throughout the 24 h and an increase in the amplitude of the cortisol rhythm, but did not alter the phase or the period of the biphasic circadian cycle. (From Wilbur, V. A. and Walker, B. B. [1977], *Nutr. Rep. Int.*, 16, 403. With permission.)

but a steady intravenous pump infusion of other nutrients, might provide valuable information concerning these matters.

Since the ratio of reduced to oxidized ascorbic acid is so difficult to measure, it might be easier to study the oxidation-reduction potential (E_h) of the blood or tissues by the method of Loxton and Le Vay (1953). "A platinum-iridium needle insulated to within a few millimetres of the tip forms the electrode, which is inserted subcutaneously and connected to one arm of an electrometer type of potentiometer. The other arm of the potentiometer is connected to a calomel half-cell which is dipped in a beaker of saturated potassium chloride solution; the patient places one finger into the same beaker to complete the electrical contact with the skin." By this method, Loxton and Le Vay observed that ascorbic acid causes a prompt fall in the oxidation-reduction potential within 2 min after intravenous injection; so do other reducing agents such as reduced glutathione.

Using this technique, these workers demonstrated that intramuscular injection of cortisone causes a definite, 21- to 35-mV fall in the tissue oxidation-reduction potential ($p < 0.001$).

IV. HYPOTHESIS

It seems that control of tissue E_h may be one of the principal functions of cortisone. Moreover, the fact that cortisone output increases so much in ascorbic acid-deficient guinea pigs, as shown by Wilbur and Walker (1977; Figure 5), suggests that cortisone may partially compensate for low tissue ascorbic acid levels. Presumably the high cortisone levels in

ascorbic acid deficiency can maintain a high ratio of reduced ascorbic acid to dehydroascorbic acid and a low E_h in the tissues until ascorbate levels fall so low that the capacity of the ascorbate battery becomes inadequate to hold a charge and compensation fails. At that point frank scurvy develops and only traces of dehydroascorbic acid are to be found in the blood and tissues.

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Chapter 19

SEASON

In the Northerly parts of the Northern Hemisphere, vitamin C deficiency is most common in the late winter and early spring and occurs least in the late summer and fall. Lind (1753) knew this well, for he quoted as follows from the descriptions of scurvy in "Nova Francia", written in 1601 by French explorers and settlers in eastern Canada. "The deadly season is the end of January, the months of February and March; wherein the sick die most commonly, every one in his turn, according to the time they begin to be ill; in such sort, that he who is taken ill in February and March may escape; but those who betake themselves to bed in December and January, are in danger of dying in February, March or the beginning of April. Which time being past there are hopes and assurance of safety."

Blood ascorbic acid levels are still lowest in the first 3 months of the year in the northern U.S., Canada, Britain, Finland, and other northern regions, as we shall see. This is undoubtedly due to diet rather than temperature, for Sadovsky et al. (1940), in Jerusalem (also in the Northern Hemisphere), found that the average blood vitamin C concentration was highest from October through February, which is the citrus season in that part of the world.

Cottingham and Mills (1943) observed that guinea pigs on a scorbutogenic diet lived somewhat longer in a hot room than in a cold room, so cold stress can perhaps contribute to the seasonal effect, but undoubtedly diet is the main seasonal factor affecting human ascorbic acid levels.

Harris and Olliver (1943) calculated the vitamin C content of the food provided at a home for "waifs and strays" in Cambridge (U.K.) during wartime and found a marked seasonal fluctuation from a low of 20 mg/d in March and April to a high of 55 mg/d in August and September. Moreover, this seasonal variation of vitamin C intake was reflected in the results of ascorbic acid saturation tests, showing a good response in all instances after the summer and a poor response in all instances after the winter.

Working at the University of Wisconsin, Lund and Kimble (1943) stated, "In this latitude there are only four months during which the supply of fresh home-grown fruits and vegetables is abundant and generally available. These months are June through September." As the plasma vitamin C values tend to lag slightly behind increased consumption of the vitamin when the body is unsaturated, they divided the year into three periods, March to June, July to October, and November to February. Conducting a study of plasma ascorbic acid (AA)* levels of women during all three trimesters of pregnancy, they extended it over 2 years to produce the results shown in Table 1, and concluded that the plasma vitamin C levels reflected the dietary intake of this substance and that season exerted a marked influence. It may be noted that during the summer months, only 7% of the diets were deficient in vitamin C, but during the remainder of the year, 19 to 20% of the diets were deficient. Crandon et al. (1952), studying the whole blood, buffy coat, and plasma ascorbic acid (TAA)** levels of 20 normal individuals, found the values in the winter months (February to March) to be lower than those in the Fall (October to November), but these same workers, studying 200 seriously ill surgical patients admitted to the Boston City Hospital, observed deficient ascorbate levels at all seasons of the year. Kirk and Chieffi (1953) cited studies by Trier in Denmark and by Hagtvet in Norway during World War II, showing marked seasonal changes in serum ascorbic acid levels which were adequate only in July, August, September, and October.

* AA — ascorbic acid, reduced form.

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

Table 1
THE RELATIONSHIP OF DIET AND SEASON TO PLASMA VITAMIN C[a] VALUES OF PREGNANT WOMEN IN MADISON, WI

Vitamin C in diet	Period A March—June				Period B July—October				Period C November—February				Total			
	Incidence		Vitamin C		Incidence		Vitamin C		Incidence		Vitamin C		Incidence		Vitamin C	
	Number	Percent	Mean mg%	SD	Number	Percent	Mean mg%	SD	Number	Percent	Mean mg%	SD	Number	Percent	Mean mg%	SD
Poor	19	20	0.14	±0.08	7	7	0.26	±0.14	19	19	0.14	±0.08	45	15	0.18	±0.09
Fair	29	31	0.59	±0.27	30	29	0.55	±0.22	44	43	0.45	±0.20	103	34	0.52	±0.22
Adequate	47	49	0.94	±0.24	66	64	0.97	±0.26	39	39	0.92	±0.25	152	51	0.95	±0.25

[a] Reduced ascorbic acid.

From Lund, C. J. and Kimble, M. S. (1943), *Am. J. Obstet. Gynecol.*, 46, 635. With permission.

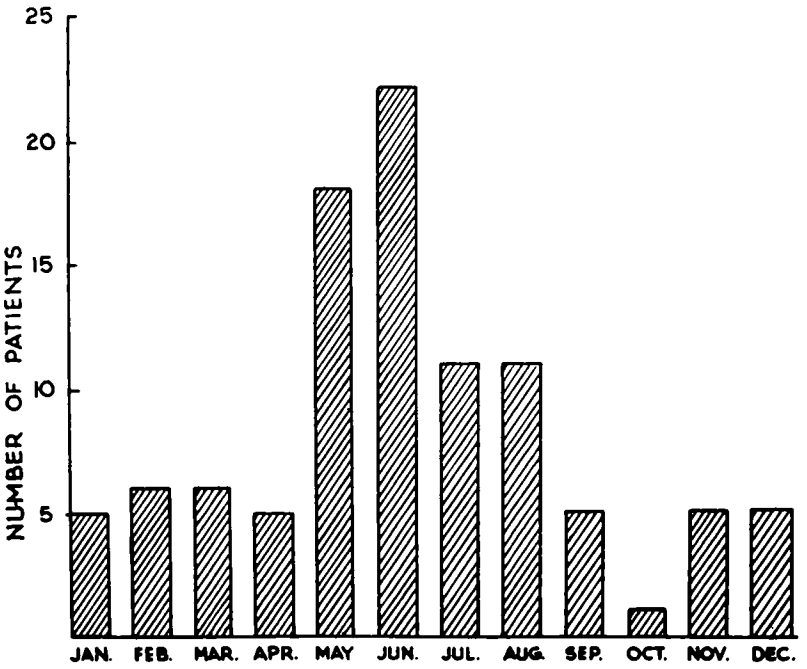

FIGURE 1. Analysis of 100 patients with scurvy admitted to the Stobhill General Hospital over 15 years, by month of admission. (From Thomson, T. J. [1954], *Glasgow Med. J.*, 35, 363. With permission.)

For some strange reason, however, Thomson (1954) found that admissions of patients with scurvy to the Stobhill General Hospital in Glasgow were most frequent in the summer months, from May to August, as shown in Figure 1. Most of their patients were elderly bachelors in the 7th and 8th decades of life and most lived alone or in lodging houses and did their own cooking. One can surmise that these poor, vitamin C-deficient people were already too lethargic or too poor to take advantage of the fruits and vegetables of summer when these became available. In all probability they were dependent on old potatoes even when the new ones were available.

Potatoes constitute a very large part of the vitamin C intake for many individuals in the northern temperate zones. Moreover, potatoes lose 66% of their vitamin C content during storage for 6 to 8 months, and there is an additional loss of 40 to 45% during boiling or frying, or up to 70% if they are kept warm on a hot plate for an hour, as shown by Mareschi et al. (1983).

Pankamaa and Räihä (1957) studied the stillbirth rates at different times of the year at the Women's Clinic of the University of Helsinki in the years between 1924 and 1953, as shown in Table 2. They also compared the monthly intrauterine fetal death rates with the ascorbic acid levels found in fetal brain tissues by Leppo at the same times of the year, as shown in Figure 2. They concluded that the intrauterine death rates are highest when the ascorbic acid levels are lowest. They also cited Sauvage-Nolting who reported a defective development of the brain caused by vitamin C deficiency.

Räihä (1958) provided the data from the State Bureau of Statistics for Finland for 1956. The frequency of stillbirths was highest (2.03%) in December and January, and was lowest (1.5%) in September.

MacKinnon and MacKinnon (1958), working in London, studying the adrenals of 44 women of childbearing age who had died by suicide or misadventure, found a seasonal rhythm in the morphology of these glands. The most marked finding was an increase in the

Table 2
TOTAL NUMBER OF DELIVERIES AND STILLBIRTHS AT THE WOMEN'S CLINIC, UNIVERSITY OF HELSINKI, 1924—1953

Month	Deliveries	Stillbirths		Stillbirths with unknown cause	
		No. of cases	%	No. of cases	%
January	9,855	167	1.69	32	0.32
February	9,327	157	1.68	32	0.34
March	10,320	176	1.71	35	0.34
April	10,206	159	1.56	31	0.30
May	9,996	162	1.62	37	0.35
June	9,915	147	1.48	35	0.36
July	8,889	129	1.45	31	0.35
August	10,009	135	1.35	34	0.34
September	9,185	122	1.33	32	0.35
October	10,209	142	1.39	33	0.32
November	9,346	154	1.65	35	0.37
December	9,535	151	1.58	30	0.31
Sum	116,790	1,801		397	

Note: The total stillbirth rate was highest in January, February, and March and lowest from August to October. Stillbirths of unknown cause did not show any evident seasonal change.

From Pankamaa, P. and Räihä, N. (1957), *Etud. Neo-Natales*, 6, 145. S. Karger AG, Basel. With permission.

size of the inner zona fasciculata in summer, but the significance of this finding is not at all clear.

Andrews et al. (1966) recorded a 5% fall in the mean leukocyte ascorbic acid level of 16 healthy volunteers, aged 22 to 49 years, between October and February in London. None of these subjects was ascorbic acid deficient, but the seasonal change was significant ($p < 0.02$).

Griffiths et al. (1967), reporting whole blood, and Griffiths (1968), reporting leukocyte ascorbic acid levels in elderly patients admitted to the Farnborough Hospital in Kent, found that ascorbic acid deficiency showed a seasonal incidence, being worst from April to June, and least in October and November (Figure 3), which they say is to be expected, as the main source of vitamin C for these elderly people is the potato.

In a nutritional survey of representative households in the U.K., Allen et al. (1968) recorded an average ascorbic acid intake of 55 mg per person per day, but 15% of individuals consumed less than 30 mg/d and 3% received less than 20 mg/d. There were marked seasonal variations; 54% of those surveyed received less than 30 mg/d during the period from February to March. These results were reviewed in an Editorial (1969).

Studies of food consumption by agricultural families in eastern Slovakia by Ginter et al. (1970) revealed the expected seasonal variation of the average ascorbic acid intake, i.e., 40.2, 37.1, 86.4, and 53.4 mg per person per day for the four quarters of the year. From the whole group of 256 persons in the study, the authors selected 50 persons known to have relatively low blood vitamin C and relatively high blood cholesterol levels in December 1967. These subjects were divided into two groups, test and control; at the end of January 1968, the test group commenced a regimen of supplementary ascorbic acid, 100 mg three times a day, which was continued for 7 weeks, while the controls did not know they had been selected for further study until blood was drawn again from both groups in March. The results of this winter study, shown in Table 3, are interpreted by Ginter et al. as indicating

Figure shows the monthly distribution of the cases of intrauterine death as compared to the ascorbic acid content of fetal brain according to LEPPO.

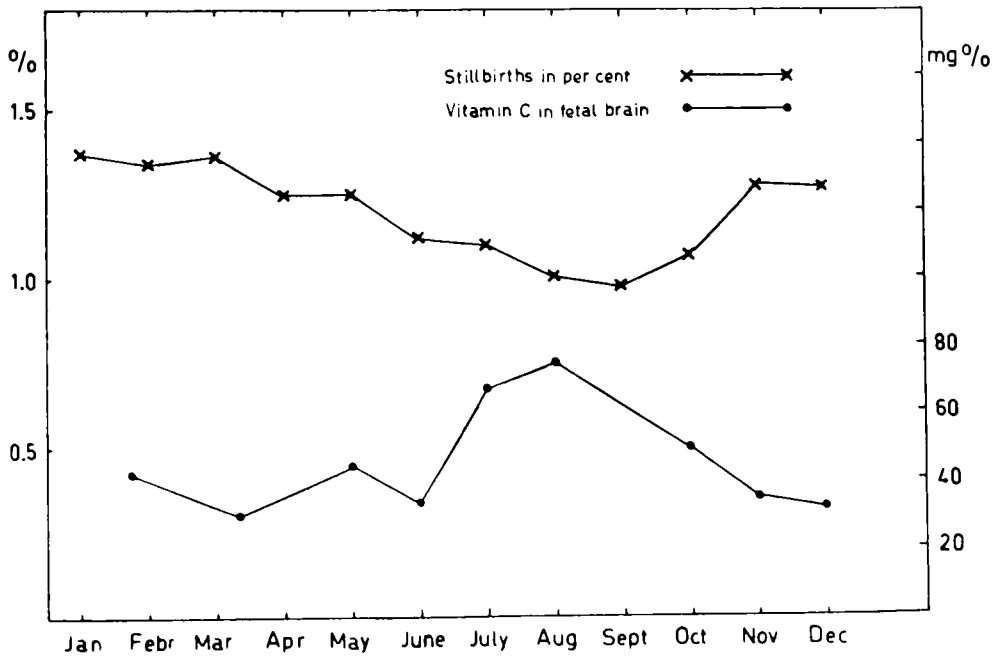

FIGURE 2. Here the stillbirths of unknown cause were subtracted from the total stillbirth rate, presumably to highlight fetal deaths from abruptio placentae, etc. Clearly intrauterine deaths are most frequent during the months when the ascorbic acid levels are lowest. (From Pankamaa, P. and Räihä, N. [1957], *Etud. Neo-Natales*, 6, 145. S. Karger AG, Basel. With permission.)

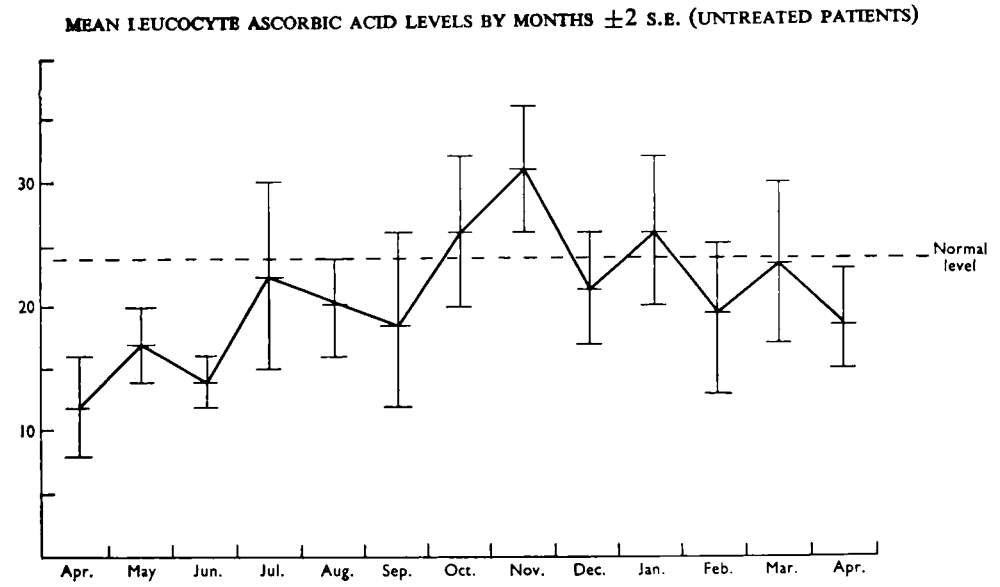

FIGURE 3. Leukocyte ascorbic acid levels of geriatric patients were found to be low on admission to hospital all year round in the Farnborough study, but more so in April and June and least in October and November. (From Griffiths, L. L. [1968], in *Vitamins In The Elderly*, Exton-Smith, A. N. and Scott, D. L., Eds., John Wright, Bristol, 34. With permission.)

Table 3
THE INFLUENCE OF VITAMIN C ON CHOLESTEROLEMIA (mg%)
MEANS ± SE ARE GIVEN

Group	December 1967	March 1968	Statistical significance: Student *t* test	Statistical significance: *t* test of pair sample
Control group	251 ± 3	263 ± 6	$p > 0.1$	$p < 0.1$
Vitamin C-treated group	255 ± 2	238 ± 8	$p < 0.05$	$p < 0.02$
Statistical significance				
Student *t* test (control/treated group)	$p > 0.5$	$p < 0.05$		
t test of pair sample		$p < 0.01$		

Note: This experiment was intentionally conducted in winter, when ascorbic acid levels are lowest, on selected individuals with somewhat elevated blood cholesterol levels. There was a significant decrease in the mean blood cholesterol level of the ascorbic acid-supplemented group.

From Ginter, E., Kajaba, I., and Ninzer, O. (1970), *Nutr. Metab.*, 12, 76. S. Karger AG, Basel. With permission.

Table 4
BLOOD VITAMIN C LEVELS (mg%) IN CONTROL AND VITAMIN C-TREATED GROUPS

Group	December 1967	March 1968
Control group	0.56 ± 0.07	0.40 ± 0.09
Vitamin C-treated group	0.62 ± 0.05	0.67 ± 0.06
Statistical significance	$p > 0.5$	$p < 0.02$

Note: Here the mean blood ascorbic acid (TAA) levels of the test and control hypercholesterolemic subjects are shown for comparison.

From Ginter, E., Kajaba, I., and Ninzer, O. (1970), *Nutr. Metab.*, 12, 76. S. Karger AG, Basel. With permission.

that ascorbic acid supplements do reduce the blood cholesterol levels of hypercholesterolemic subjects who are ascorbate deficient. The mean blood ascorbic acid (TAA) levels of the test and control groups, before and after this study, are shown in Table 4.

Definite winter and spring peaks in the incidence of admissions to hospital for ischemic heart disease in Scotland have been observed by Dunnigan and Harland (1970). They also observed a winter maximum in the total registered Scottish deaths from this disease (Figure 4) and attributed it mainly to the cold weather. However, Spittle (1970), commenting on these findings, suggested that this seasonal incidence in myocardial infarction is most likely due to vitamin C deficiency; this is entirely possible in view of the relationships which ascorbic acid deficiency bears both to atherosclerosis (Chapter 8, Volume II) and to coronary thrombosis (Chapter 20, Volume III). Indeed, the extremely low plasma ascorbic acid levels observed by Roine et al. (1974) in elderly Finnish people, at all seasons, but especially in the winter (Table 5), may well account for the fact that Finland has the highest recorded incidence of coronary heart disease in the world.

Milne et al. (1971) studied leukocyte ascorbic acid levels in random samples of blood from elderly men and women (62 to 94 years of age) living in Edinburgh. The mean value for all women (23.9 $\mu g/10^8$ cells) was significantly higher than that for all men (18.1 $\mu g/10^8$ cells), and the mean values were significantly higher in the period from July to December

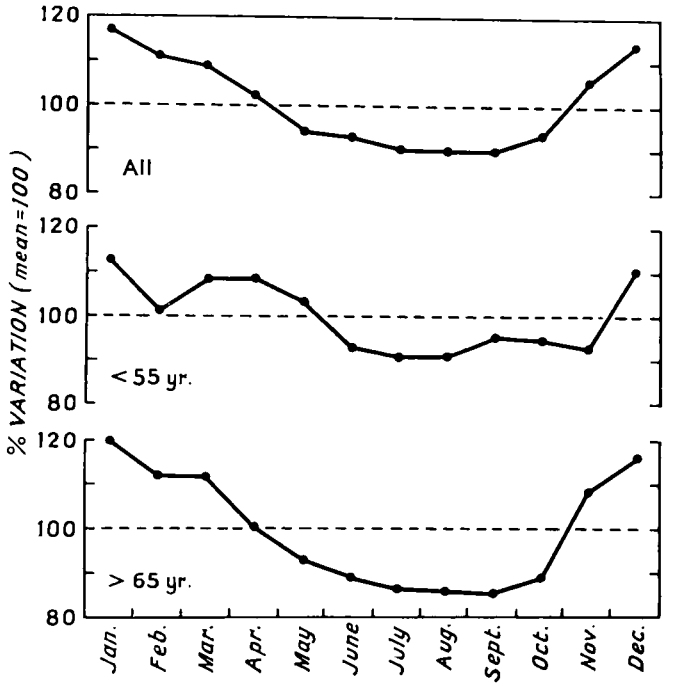

FIGURE 4. Seasonal variation in 65,491 total registered deaths from ischemic heart disease in Scotland (1962 to 1966). (From Dunnigan, M. G. and Harland, W. A. [1970], *Lancet*, October 17, 793. With permission.)

than in the first half of the year, both in men ($p < 0.01$) and in women ($p < 0.05$), as shown below.

	Men	**Women**
January to March	15.4	21.2
April to June	15.2	22.2
July to September	25.7	24.2
October to December	23.4	28.0

These leukocyte ascorbic acid levels lagged behind the calculated vitamin C intakes of these people, which were higher from April through September than from October through March.

Buzina et al. (1973), studying village school children in Yugoslavia, observed that the prevalence of bleeding gums and angular stomatitis both showed marked seasonal variations and were associated with seasonal changes in the dietary intakes of ascorbic acid and of riboflavin. Plasma ascorbic acid levels were highest in October and lowest in April, but were still low in June, when the percentage of children with bleeding gums was at its highest, 18.6, 36.4, and 47.8% at three different schools. There was a definite lag between the time when the diet improved, in the summer, and the season of least gingival bleeding in October. The authors noted that ascorbic acid supplements, 30 mg daily, were effective in raising the plasma ascorbic acid levels, but were not as effective as fresh fruits and vegetables in curing the bleeding gums. These delayed responses may have been due to (1) inadequate ascorbic acid dosage, (2) bacterial endotoxin of established periodontitis denying ascorbic acid access to the gingival tissues, as suggested by Aleo and Padh (1985; see Chapter 12,

Table 5
PLASMA LEVELS OF VITAMIN C IN ELDERLY WOMEN AND MEN

Groups	No. of persons	Dietary intake (mg/d)	Plasma level (mg/100 ml)			
			Winter	Spring	Fall	Mean
A. Women						
Institutions (Helsinki)	21					
Mean		28.9	0.17	0.25	0.44	0.29
Range			0—0.49	0—1.03	0.08—1.24	0.03—0.78
SD		17.1	0.14	0.27	0.35	0.23
Rural communes	19					
Mean		31.0	0.12	0.23	0.61	0.32
Range			0—0.56	0.03—0.95	0.07—1.54	0.04—0.70
SD		12.7	0.14	0.25	0.45	0.20
Alone at home	16					
Mean		49.0	0.44	0.50	0.49	0.48
Range			0.03—1.23	0.09—1.15	0.17—1.06	0.10—0.85
SD		41.9	0.35	0.33	0.22	0.22
B. Men						
Institutions (Helsinki)	11					
Mean		20.0	0.08	0.17	0.33	0.19
Range			0—0.17	0.01—0.97	0.06—1.56	0.06—0.90
SD		6.9	0.07	0.28	0.43	0.25
Rural communes	12					
Mean		32.4	0.07	0.04	0.41	0.18
Range			0.01—0.22	0.01—0.16	0.06—1.49	0.04—0.53
SD		12.6	0.06	0.04	0.41	0.15
Alone at home	3[a]					
Mean		59.0	0.10	0.23	0.14	0.16
Range			0.01—0.22	0.03—0.48	0—0.33	0.01—0.34
SD		68.4	0.11	0.22	0.17	0.17

Note: This study of the plasma ascorbic acid levels of elderly men and women at an old people's home in Helsinki, of others at an old people's home in a rural area, and of other elderly people living at home in Finland, shows low mean levels all year round, but even lower in the winter.

[a] Because of the small number of people, the results are not representative.

From Roine, P., Koivula, L., Pekkarinen, M., and Rissanen, A. (1974), *Int. J. Vitam. Nutr. Res.*, 44, 95. With permission.

Volume II), or (3) to the need for the bioflavonoids, catechins, or tannins of natural fruits and vegetables to promote tissue storage of ascorbic acid (Chapter 11 of this volume).

Schorah et al. (1978), working at the University of Leeds, observed only modest seasonal changes in the mean leukocyte ascorbic acid levels of women in the first trimester of pregnancy, which were 35.8, 30.2, 39.2, and 34.2 $\mu g/10^8$ leukocytes, respectively, in the four quarters of the year: no association was found between low leukocyte ascorbic acid levels in the first trimester and spontaneous abortions, stillbirths, or neonatal deaths in that study, but there was an increased frequency of low values in women who subsequently gave birth to infants weighing less than 3250 g.

However, Solomenko (1975) observed a very pronounced seasonal variation of maternal and newborn plasma ascorbic levels in Vladivostok, U.S.S.R., reaching prescorbutic levels in the maternal blood in the spring, as shown below:

	Mean maternal blood plasma ascorbic acid mg/100 ml	Mean umbilical cord blood plasma ascorbic acid mg/100 ml
Winter	0.31 ± 0.02	1.7 ± 0.2
Spring	0.12 ± 0.01	0.37 ± 0.02
Summer	0.39 ± 0.02	1.14 ± 0.15
Autumn	0.73 ± 0.03	3.02 ± 0.15

This seasonal variation was also reflected in the vitamin C content of the mother's milk.

In a study of geriatric patients, Schorah (1981) recorded a highly significant fall in the mean plasma ascorbic acid levels from 0.50 mg/100 ml in the period from July to September to 0.11 mg/100 ml in the period from March to May ($p < 0.005$).

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Chapter 20

ACHLORHYDRIA

Although ascorbic acid was not isolated until 1932, a considerable body of knowledge concerning the properties of this vitamin had accumulated before that time. Hess and Unger (1919) reported that the alkaline reaction of a commercial "malt soup" was due to added potassium carbonate, and that this had been responsible for the development of scurvy in some human infants. In one experiment they showed that a milk diet containing potassium carbonate led to scurvy, while feeding the same milk diet without the potassium carbonate led to a full recovery (Figure 1). They also showed in guinea pig experiments that the addition of potassium carbonate to a hay and milk diet led to scurvy (Figure 2). Guinea pig feeding experiments by La Mer et al. (1922) confirmed that vitamin C is more stable in acid than in alkaline solutions. Hess (1922) therefore suspected that a lack of hydrochloric acid secretion by the stomach could cause losses of the antiscorbutic vitamin, for he stated

> Nothing is known of the fate of the vitamines within the body. However, the clinical course of scurvy and our knowledge of the characteristics of its vitamine suggest certain deductions. As you all know, the antiscorbutic vitamine is protected from destruction by the acid reaction of food, and, on the other hand undergoes more or less destruction when rendered even slightly alkaline, a result which is intensified by moderate degrees of heat. Experiments clearly demonstrating this behaviour have been carried out for acid antiscorbutic foodstuffs, such as orange juice and tomato. La Mer who has studied this question very carefully, was able by the addition of alkali to tomato juice to increase the destructive effect of heat as much as 22 per cent. It is evident therefore, that gastric juice protects and preserves the antiscorbutic potency of food, whereas a lack of hydrochloric acid tends to destroy it. Have we not here a condition which may prevail in some cases of scurvy which develop in spite of what should be an adequate supply of the specific vitamine? Those who have followed the literature of scurvy must have been impressed by the authenticity of reports of this nature, which have been reiterated for centuries. It would seem a logical explanation of these irregularities that the vitamine has been destroyed within the body by prolonged contact with alkaline digestive juices.

Öhnell (1928), using the Hess test, reported increased capillary fragility in patients with scurvy and also in latent scurvy.

Göthlin (1931) conducted systematic studies to find the earliest stages of scurvy by use of a capillary resistance test; he also reported an association between capillary fragility and achylia gastrica.

A proportion of normal people, increasing from 4 to 20% with increasing age, are found to have no free acid in the stomach; so the finding of Schultzer (1934) that 10 out of 42 adult patients with capillary fragility had achylia gastrica was somewhat in excess of expectation. In some patients the capillary fragility was attributed to infection or other disease states, but 25 of the 42 patients gave dietary histories suggesting vitamin C deficiency as the cause, and this was confirmed when their capillary fragility decreased after consumption of a diet containing more vitamin C. However, the ten achylics were more resistant to treatment; only three of the ten responded to an increased ascorbic acid intake. Schultzer (1934) concluded, "It is possible, however, that this might have been accomplished with larger doses, whatever may have been the cause of the capillary fragility — whether destruction of vitamin C in the gastro-intestinal canal or poor absorption of vitamin C due to changes in the mucous membranes. So it may be that there is a new therapeutic conquest to make in relieving the vitamin C deficiency that may be present in achylics."

Clearly today we have the solution to Schultzer's problem; it is the chelating fiber of food, and includes catechins and tannins as well as the "capillary active" flavonoids, which retard the oxidation of ascorbic acid by inactivating heavy metal catalysts (Chapter 11 of this volume).

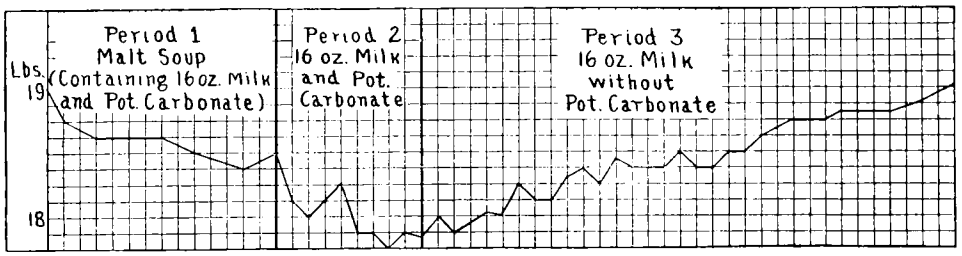

FIGURE 1. Human scurvy: weight curve of a baby that developed scurvy on a diet of malt soup (Period 1). During Period 2, the flour and malt soup were omitted from the diet, the same amount of milk and potassium carbonate being continued. There was no improvement. In Period 3, the potassium carbonate was omitted, and this brought about a gain in weight and complete cure of the scurvy, showing the destructive effect of alkali on the vitamin (The milk was boiled for 5 min in each of the three periods). (From Hess, A. F. and Unger, L. J. [1919], *JAMA*, 73, 1353. ©1919 American Medical Association. With permission.)

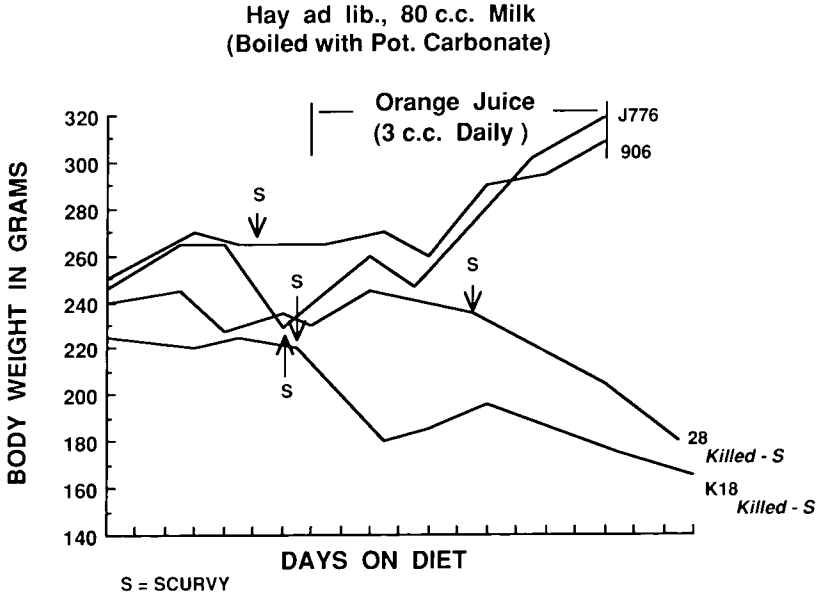

FIGURE 2. Guinea pig scurvy: all four giunea pigs developed scurvy on a daily diet of hay and 80 cc of milk to which had been added the same percentage of potassium carbonate contained in the ordinary malt soup preparation and which had been boiled for 5 min. After the disorder had developed, two of the guinea pigs were given orange juice. They recovered and gained weight rapidly, in spite of the continuation of the alkali in their diet, thus proving that the disorder was scurvy. (From Hess, A. F. and Unger, L. J. [1919], *JAMA*, 73, 1353. ©1919 American Medical Association. With permission.)

Jennings and Glazebrook (1938) reported histamine-fast achlorhydria in a 52-year-old man with scurvy, who presented with painful joints and breathlessness associated with severe megaloblastic anemia. Oral administration of ascorbic acid, 600 mg daily, had to be continued for 3 weeks (13.6 g), and much more vitamin C had to be given as fresh fruit before his urinary ascorbic acid excretion even approached 50% of intake; so clearly there was an impairment of ascorbic acid uptake, most probably due to oxidation of ascorbic acid to dehydroascorbic acid (DHAA) in the achlorhydric stomach and loss of vitamin activity due to hydrolysis of DHAA, which is a very unstable compound.

Kendall and Chinn (1938) showed that ascorbic acid can be destroyed by certain bacteria isolated from the upper gastrointestinal tracts of patients with achlorhydria.

Table 1
ANALYSIS OF GASTRIC SAMPLES FROM 32 SUBJECTS FOLLOWING HISTAMINE STIMULATION

Mean acidity (in parts per 1000)	Ascorbic acid (mg/100 ml)
0. Achylia	0.32
0.1 to 0.99	0.49
1.0 to 1.99	0.69
2.0 to 2.99	0.68
3 and above	0.76

Note: Results show a relationship between low acid secretion and low ascorbic acid concentration in the gastric juice.

From Demole, M. and Issler, A. (1939), *C.R. Soc. Biol.*, 130, 1225. With permission.

Demole and Issler (1939) observed that low gastric acidity is associated with low ascorbic acid levels in the gastric juice, as shown in Table 1.

Alt et al. (1939) observed that a number of studies in the literature had suggested that achlorhydria might be "a factor in the cause of vitamin C deficiency." They noted that the diagnosis of hypovitaminosis C in patients with achlorhydria had been based on (1) symptomatology by Hausmann, 1922, Hoff, 1936, Mahlo, 1936, and Schroeder and Einhauser, 1936; (2) increased capillary fragility by Göthlin, 1931, Ekvall, 1934, Schultzer, 1934, and Schultzer and Griis, 1935; (3) decreased urinary excretion of ascorbic acid following ingestion of the vitamin, by Einhauser, 1936; or (4) a decreased ascorbic acid level in the blood, by Nielsen, 1938.

So, Alt et al. (1939) undertook a thorough study of this question. The subjects consisted of 44 clinic patients at Northwestern University Medical School in Chicago; all had been previously diagnosed as having pernicious anemia, or iron deficiency anemia, associated with histamine fast achlorhydria. Studies were also conducted on 24 clinic patients who exhibited no evidence of systemic disease. The diet of each subject was evaluated as accurately as possible and classified as being adequate or inadequate in ascorbic acid. Less than 30 mg/d was regarded as inadequate. Blood samples were drawn from all of these subjects 3 or 4 h after a breakfast or a lunch that contained no citrus fruit. Plasma ascorbic acid (AA)* levels were estimated by the micromethod of Farmer and Abt. The results of these analyses are shown in Table 2. With diets adequate in vitamin C, the plasma AA was significantly decreased from the normal in patients with pernicious anemia, but not in the iron deficiency anemia group. With diets inadequate in vitamin C, the plasma ascorbic acid was significantly decreased from the controls in both the pernicious and the iron deficiency anemia groups. Discussing the reasons why achlorhydria might be associated with a decreased assimilation of ascorbic acid, Alt et al. considered (1) increased destruction of ascorbic acid in nonacid gastric juice, (2) increased destruction of the vitamin by bacteria present in the small intestine, and (3) decreased absorption of ascorbic acid due to changes in the gastrointestinal tract.

Although the mean plasma AA levels of the achlorhydric patients in Table 2 are not unduly low, they are sufficiently different from normal to demonstrate the existence of a

* AA — ascorbic acid, reduced form.

Table 2
BLOOD PLASMA ASCORBIC ACID (AA) VALUES IN NORMAL INDIVIDUALS AND IN PATIENTS WITH ACHLORHYDRIA[a]

	Normals	Pernicious anemia	Iron deficiency anemia
Adequate Intake of Ascorbic Acid			
Number	15	16	9
plasma AA (mg/100 ml)	0.87	0.57[b]	0.73
Inadequate Intake of Ascorbic Acid			
Number	9	8	9
plasma AA (mg/100 ml)	0.64	0.47[b]	0.45[b]

[a] Summary of results obtained by Alt et al. (1939).
[b] Significantly different from normal.

potential problem. The data of Nielsen (1938), concerning a study conducted in Denmark, showed the blood ascorbic acid level of the achlorhydrics to be 0.20 mg/100 ml, as compared with 0.35 mg/100 ml in normal individuals, so clearly the potential problem could become a real problem in some individuals when the dietary intake of ascorbic acid is inadequate.

Ludden et al. (1941) studied the plasma ascorbic acid levels of 28 patients with "gastritis" investigated at Bellevue Hospital in New York City. Five had no evidence of pathology, five had chronic superficial gastritis, five had chronic hypertrophic gastritis, four had chronic atrophic gastritis, five had gastric ulcers, and four were patients who had undergone gastric surgery. All had low plasma ascorbic acid levels (mean 0.18 mg/100 ml) which were considered prescorbutic. The ascorbic acid levels of the four with achlorhydria and chronic atrophic gastritis were among the lowest (mean 0.16 mg/100 ml), and they "responded somewhat less satisfactorily to oral doses of ascorbic acid than did patients with similar degrees of deficiency but other forms of gastric pathology."

Studies by Hawley et al. (1937) of tissue ascorbic acid levels in guinea pigs given ascorbic acid with acidic (NH_4Cl) or basic ($NaHCO_3$) salts, and studies by Meyer and Hathaway (1944) of "ascorbic acid utilization" in children given ascorbic acid, with or without potassium citrate, gave inconsistent and anachronistic results, probably because of variations in the contamination of analytical reagent grade salts by traces of heavy metal catalysts, which can have a profound effect upon the rate of oxidation of ascorbic acid *in vitro*. Meyer and Hathaway, after studying two groups of four children each for 1 year, in a controlled environment, concluded that some factor other than the potassium citrate was partially responsible for the observed results.

Vilter et al. (1946) gave a summary of the findings in 19 adults who were admitted to the Medical Service of the Cincinnati General Hospital between the years 1935 and 1945 with a primary diagnosis of scurvy. They reported that gastric analyses showed hypochlorhydria or achlorhydria after histamine stimulation, and the plasma vitamin C content was 0.0 mg/100 ml in these patients.

Studies of experimental scurvy in monkeys by May et al. (1949) brought new light to bear on this subject. These workers found that the feeding of an iron-supplemented ascorbic acid-deficient milk diet caused the development of scurvy and megaloblastic anemia after 3 or 4 months and that 10 out of 11 monkeys developed histamine refractory gastric achlorhydria. Similarly, May et al. (1950) observed achlorhydria in human infants in the

late stages of illness with megaloblastic anemia and scurvy due to ascorbic acid-deficient milk diets, with or without infection. Thus, we have to consider the possibility that scurvy can be the cause of gastric achlorhydria, rather than the other way around.

Another possibility is the existence of a vicious cycle, if achlorhydria can lessen ascorbic acid levels and low ascorbic acid levels can impair gastric acid secretion. However, this is hard to comprehend, for low plasma ascorbic acid levels are associated with high blood histamine levels (Chapter 1, Volume III), and histamine stimulates the secretion of acid by the stomach. It would seem that there must be a point in ascorbic acid deficiency at which the oxyntic cells of the stomach cease to respond to histamine. If so, this is very fortunate, for otherwise the histaminemia of scurvy might cause hyperchlorhydria and gastric or duodenal ulceration.

Brown (1951), in Glasgow, found achlorhydria in 3 out of 4 patients with scurvy whom he tested, and Bronte-Stewart (1953), at the Groote Schuur Hospital in Cape Town, reported histamine-fast achlorhydria in 7 and hypochlorhydria in 3 out of 12 scorbutic patients tested.

Evidence that achlorhydrics can benefit from treatment with high-dosage ascorbic acid was provided by Moore (1955) working at the Washington University School of Medicine in St. Louis, who stated "For many years it has been thought that individuals with hypochlorhydria or achlorhydria probably assimilate food iron poorly. In such patients, however, we have not been able to increase absorption of the radioiron by adding 60 cc. of 0.1 N HCl to cooked eggs, or by adding enough 1 N HCl to reduce the pH of the mixture to 1.5 before it was given by stomach tube. On the other hand, ascorbic acid in a dose of 250 to 1,000 mg did increase iron absorption very significantly, even though it had comparatively little effect on gastric acidity."

Neilson (1960) observed classical scurvy and megaloblastic anemia in a 71-year-old retired blacksmith admitted to Stobhill Hospital in Glasgow; the basal gastric juice pH was recorded as 9; it fell to 8 in the fasting state and to 7 after histamine administration. Even though there was a slight increase in the "total acid" content of the gastric juice after histamine, there was clearly no "free acid". Both the scurvy and the megaloblastic anemia responded to treatment with ascorbic acid, 700 mg daily for 21 d, without the need for any folic acid, liver, or vitamin B_{12} therapy (Chapter 4, Volume III).

Histamine-fast achlorhydria was observed by Hyams and Ross (1963) in a 54-year-old housewife admitted to St. Pancras Hospital in London with osteoporosis and megaloblastic anemia due to scurvy. She presented with a complaint of low back pain, loss of height, and cramps in the calves. X-rays of the lumbar spine showed osteoporosis with ballooning of the intervertebral discs of L1, 2, 3, and 4, and almost complete collapse of the vertebral body at L1. The dorsal spine also showed osteoporosis with wedging of the midthoracic vertebrae. Both her scurvy and her anemia responded well to treatment with ascorbic acid alone, 500 mg daily, and the progress of her osteoporosis was arrested.

Goldsmith (1961) observed that patients with peptic ulcer may develop ascorbic acid deficiency as a result of receiving large doses of alkali, presumably due to destruction of the vitamin in the gastrointestinal tract.

Guinea pig studies by Hughes and Lewis (1965) have shown that ascorbic acid can be rapidly absorbed by the stomach and also more slowly by the intestine. By incubating portions of stomach *in vitro,* they found that the reducing agent homocysteine depressed the uptake of ascorbic acid by stomach tissue at pH 7.4. At this pH there would be spontaneous oxidation of AA to DHAA, but that reaction is reversed by homocysteine. They stated, "It would therefore appear that in the absence of free acid, vitamin C is at least in part absorbed as DHAA."

The problem in achlorhydrics arises from the fact that DHAA is an unstable compound, with a very short half-life, being hydrolyzed to diketogulonic acid almost as fast as it is formed at pH 7.4 (Figure 2, Chapter 1 of this volume), so there can be considerable losses of the vitamin, even before much of the food comes in contact with the stomach wall.

Further studies by Grimble and Hughes (1967) have shown the existence of a high concentration of "dehydroascorbic acid reductase" in the wall of the guinea pig stomach; this enzyme reduces DHAA to AA in the presence of reduced glutathione (GSH). They have also reported that orally administered DHAA is more effectively reduced to AA in the guinea pig than is DHAA administered by the intraperitoneal route.

Umanskii (1970) reported hypovitaminosis C and achlorhydria or hypochlorhydria to be common during pregnancy and childbirth.

As we have seen, ascorbic acid is unstable in neutral or alkaline solutions, so the acid in the stomach provides a protection against losses of ascorbic acid by oxidation to DHAA and subsequent hydrolysis to diketogulonic acid with loss of vitamin activity. Compare the 90% loss of total ascorbic acid (AA + DHAA) in 30 min at pH 7.4 (Figure $2B_1$, Chapter 11 of this volume), with the less than 20% loss in 30 min at pH 5.0 (Figure 6A, Chapter 11 of this volume). This comparison is not strictly justified, as pH 7.4 was achieved by the use of a phosphate buffer, and pH 5.0 was achieved by the use of an acetate buffer. The heavy metal contaminants in the analytical reagent (A.R.) grade salts comprising the two buffers were therefore different, but the effect of pH is genuine; the oxidation of ascorbic acid is slowed in all acid media.

Thus, we may anticipate that larger doses of ascorbic acid may be needed in achlorhydrics, unless one provides acid with the vitamin or, better still, provides chelating flavonoids such as rutin or catechin (Chapter 11 of this volume) or protein (Chapter 12 of this volume) to inactivate the heavy metal catalysts and protect the vitamin.

Citrus fruits contain citric acid, as well as some chelating flavonoids like eriodictyol in orange juice, so that the vitamin remains stable, even in the stomach of an achlorhydric. Similarly, raw fish and raw meat, which are the only traditional sources of ascorbic acid for the Eskimos, contain plenty of protein to bind heavy metal catalysts.

So the potential problem exists in people and guinea pigs on high-cereal diets, as noted by Chatterjee et al. (1971, 1975a), or in people taking vitamin C tablets with tap water, as they may suffer losses of ascorbic acid in the stomach, if they have achlorhydria, as well as in the alkaline medium of the jejunum if the vitamin has not already been absorbed. Moreover, more seriously there may be toxic effects due to release of both dehydroascorbate and "ascorbate-free-radical", during the oxidation of ascorbic acid. Indeed, Chatterjee et al. (1975a) have observed increased blood sugar levels in guinea pigs and in human volunteers on high-cereal diets following the feeding of large doses of ascorbic acid (Chapter 12 of this volume).

Also, Stich et al. (1976) have shown that ascorbic acid is mutagenic in the presence of copper and oxygen, undoubtedly due to ascorbate-free-radical release during its oxidation, so it is very important that anyone taking ascorbic acid by mouth, whether they have achlorhydria or not, should take the vitamin with citrus fruit juice or as catechin-coated pills, and not as ascorbic acid tablets with tap water alone.

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Chapter 21

IONIZING RADIATION

I. THEORY

There are several theories concerning the manner in which ionizing radiation damages living tissues; all of them have to take into account the facts that (1) radiation damage is increased by oxygen and decreased by hypoxia; (2) radiation damage increases exponentially with the copper concentration of the tissue and is decreased by heavy metal chelating agents; and (3) that radiation damage is decreased by several antioxidants and by many, but not all, reducing agents.

Weiss (1947) developed the mathematics for a free radical theory of radiation. Patt's theory suggested that water is not only ionized, but oxidized to H_2O_2 and also to a very short-lived potent oxidant HO_2, the peroxyl radical, which is capable of causing great nuclear and cytoplasmic cell damage. This is still believed to be true, but a multitude of additional reactions have now been described by Schaich (1980) and by other workers in the field of radiation biology.

It would be useful in radiation therapy if we could protect normal tissues against damage by use of a reducing agent, like cysteine, or a reducing and chelating agent, like dimethyl cysteine (penicillamine), but unfortunately such treatment affords protection to the malignant cells as well as the normal tissues and thus defeats our purpose.

One cannot avoid noticing the fact that radiation damage, being increased by copper and by oxygen and being decreased by sulfhydryls, chelating flavonoids, and some reducing agents, is influenced by the same factors which control the tissue oxidation-reduction state and the ascorbate:dehydroascorbate equilibrium.

Radiation damage is in many ways like an accelerated form of aging. It is therefore interesting to note the degree to which the oxidation of ascorbic acid is involved in radiation effects.

II. *IN VITRO* EFFECTS OF RADIATION

Anderson and Harrison (1944) showed that X-irradiation of ascorbic acid in aqueous solutions resulted in a loss of from 1.7 to 2.4 μmol/1000 rad; this loss occurred even in fresh blood plasma. However, the loss of ascorbic acid from samples of rat muscle exposed to radiation *in vitro* was less than one third that in plasma.

During the course of investigations of the electron spin resonance (ESR) signals of free radicals induced by X-ray irradiation, Kenny and Commoner (1969) found that relatively radiosensitive tissues, such as testis, exhibited a sharp signal which decayed rapidly after radiation ceased and was absent from the response of a relatively radiation-insensitive tissue, such as liver. Subsequently, Vaughan et al. (1973) demonstrated that a solution of ascorbic acid during irradiation emits an ESR signal which is identical in g-value and in separation between the hyperfine peaks to the signal observed in irradiated rat testis, thymus, and lung tissue (Figure 1). Moreover, they showed that the signal was present during irradiation of normal guinea pig spleen and testis, but absent during irradiation of spleen and testis from scorbutic guinea pigs (Figure 2). Thus, they concluded that the ESR signal emitted by radiosensitive tissues is due to ascorbate free radical (semidehydroascorbate). Further studies by Floyd et al. (1973) confirmed and elaborated these findings. Figure 3 presents some properties of the ESR doublet observed during X-irradiation of rat testis. Figure 3A shows that this signal is absent before irradiation of the testis homogenate, but becomes quite

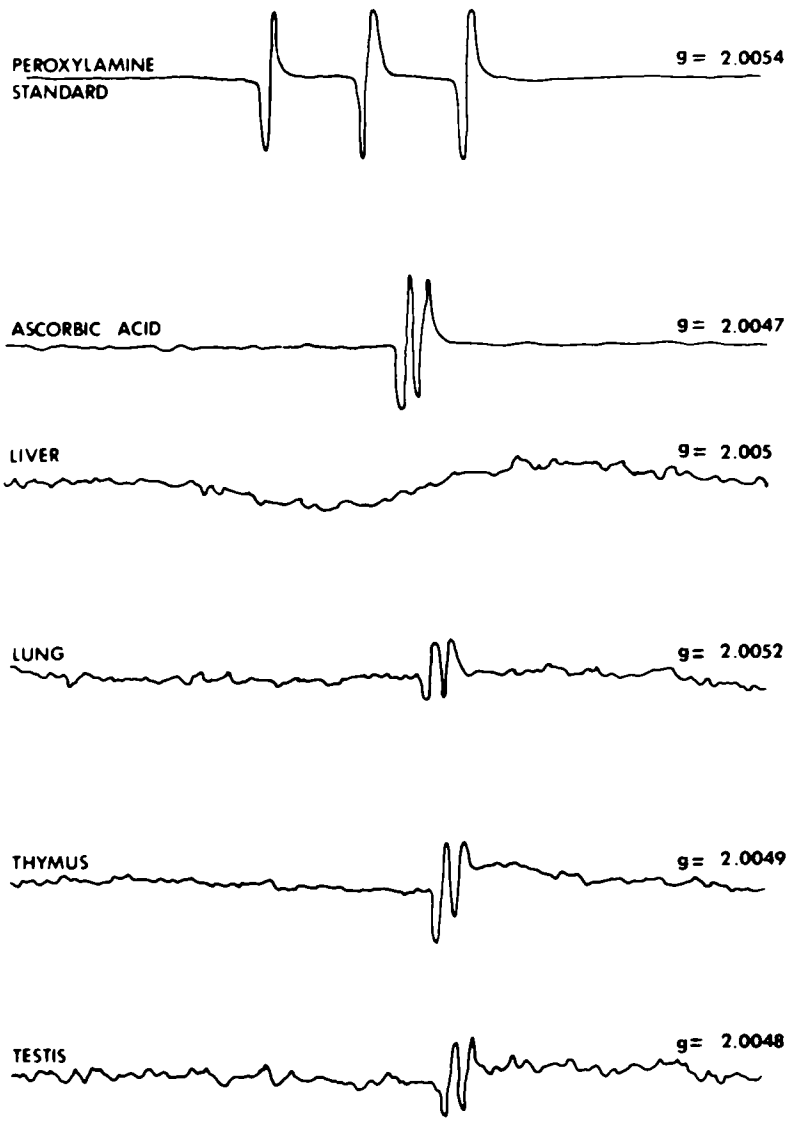

FIGURE 1. Electron spin resonance (ESR) signals from minced rat tissue samples and from two standard solutions during X-irradiation. The radiosensitive tissues, lung, thymus, and testis showed ESR couplets similar to those of the ascorbic acid solution, while liver tissue emitted no such signal. (From Vaughan, W. N., Henry, J. I., and Commoner, B. [1973], *Biochim. Biophys. Acta*, 329, 159. With permission.)

apparent during X-irradiation and decays to 60% of the original value after 10 min of radiation. When irradiation ceases, the signal decays immediately.

The ESR signal observed in an X-irradiated 10-m*M* ascorbate solution is also shown in Figure 3A. The similarity between the ascorbate doublet and that observed in testis is evident. In another experiment, ascorbate (to a concentration of 20 m*M*) was added to the testis preparation. Figure 3B shows that prior to X-irradiation a small doublet is present. This becomes very prominent during irradiation, and a small doublet is still observable after irradiation ceases. Unlike that observed in the absence of added ascorbate (Figure 3A), the intensity does not decline during irradiation. Irradiation of X-ray-sensitive hepatic tumors, induced in rats by feeding an aminazo dye, incited the typical ESR couplet, while normal liver tissue did not show any such signal.

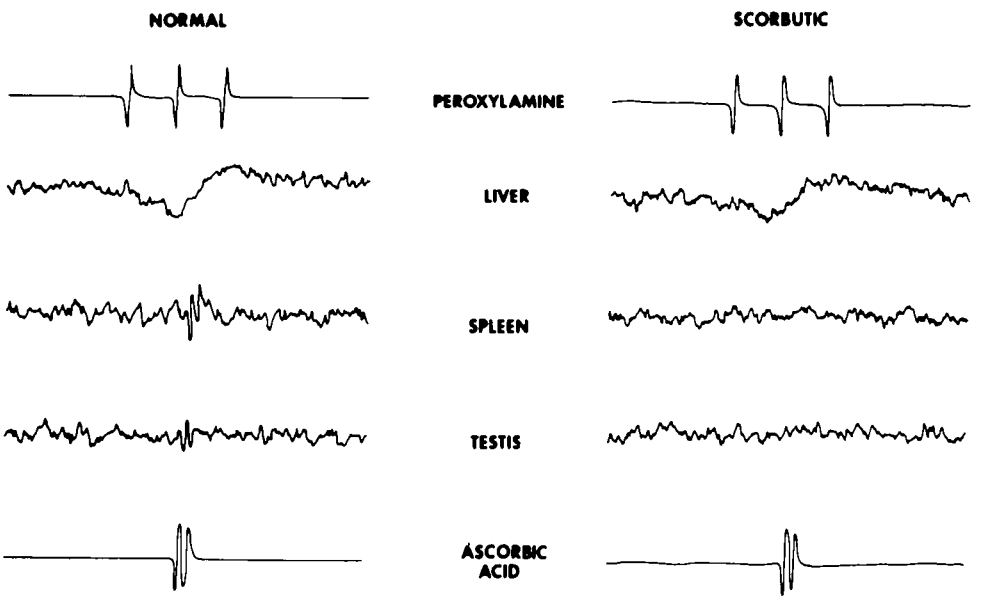

FIGURE 2. Comparison of electron spin resonance signals from normal and scorbutic guinea pig tissues. The spleen and testis of the normal guinea pigs emitted the usual couplet signal, but the scorbutic tissues did not. (From Vaughan, W. N., Henry, J. I., and Commoner, B. [1973], *Biochim. Biophys. Acta*, 329, 159. With permission.)

O'Connor et al. (1977) observed a paradox in that ascorbic acid, 30 mg/100 ml, slowed the growth of Chinese hamster ovary cells in tissue culture, but the same concentration of ascorbic acid provided a ($\times 7$) radioprotective effect for these cells. This *in vitro* radioprotective effect of ascorbic acid has been discussed by Gregory (1978), by Mothersill (1978), and by Baverstock (1979); the possibilities of electron scavenging, superoxide scavenging, free radical scavenging, oxygen sharing, and potentiation of the repair of DNA damage have all been discussed.

Ascorbic acid is known to be toxic to cultured human fibroblasts in the presence of free cupric ions *in vitro*, as shown by Stich et al. (1976), but presumably not *in vivo*, because *in vivo* the cupric ions are chelated by proteins and amino acids and ascorbic acid is protected by reducing agents such as reduced glutathione.

Van der Schans (1978) has shown that single- and double-strand breaks in DNA can be produced either by gamma radiation or by the action of ascorbic acid in the presence of cupric ions and oxygen. Radiation damage is likewise potentiated by copper; clearly the *in vitro* protective effect of ascorbic acid may represent some form of competition, but it does not necessarily have a parallel *in vivo*.

Goldman (1982) has pointed out that the endothelial cells of the microvascular system are particularly sensitive to ionizing radiation. Damage to these cells undoubtedly causes the hemorrhagic phenomena so characteristic of radiation sickness. It is understandable that oxidation of ascorbic acid and release of ascorbate free radical would seriously affect the most ascorbate-dependent tissue.

III. THE PITUITARY-ADRENAL "STRESS" EFFECT

Patt et al. (1947) found that total-body X-radiation (650 rad) induced the following changes in the adrenal cortex of rats: an initial short phase of adrenal cholesterol depletion to 50% at 3 to 6 h, followed by a period of increased cholesterol accumulation for several days; the third and last phase involved a marked hypertrophy of the adrenal glands, reduction of

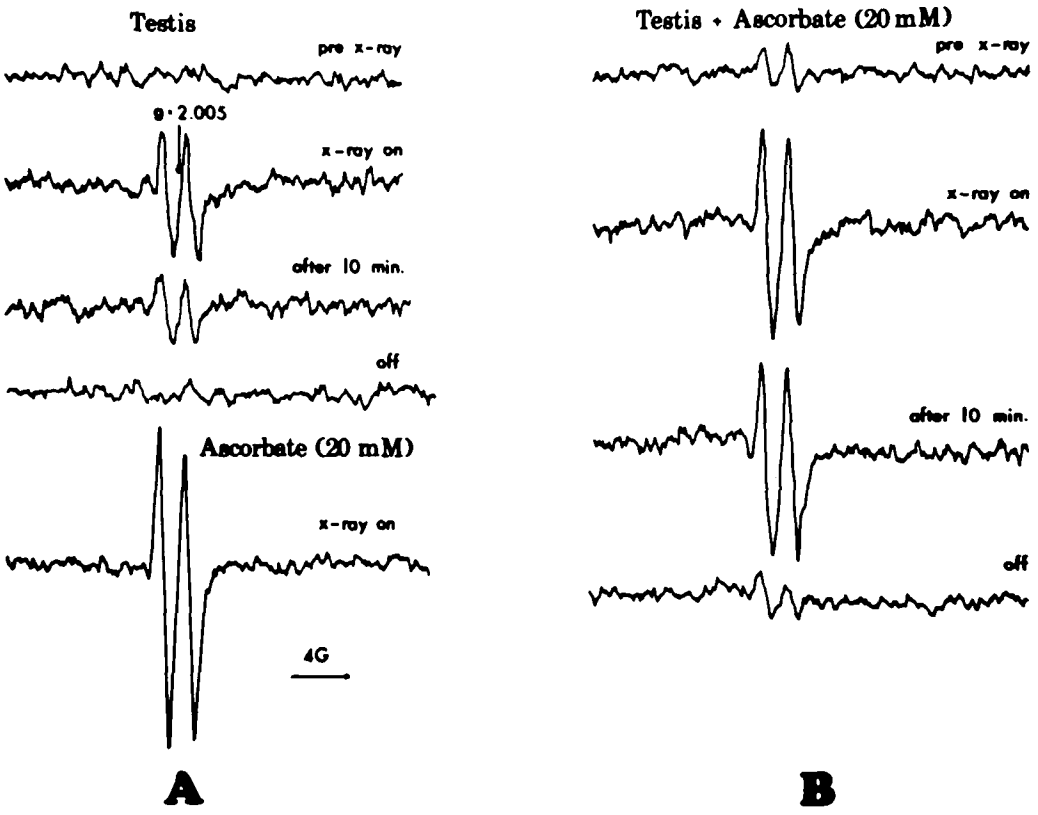

FIGURE 3. Electron spin resonance (ESR) spectra of rat testis tissue taken during X-irradiation. (A) Minced testis tissue, (B) minced testis tissue with added ascorbic acid. The four upper tracings from the top down show the ESR spectra before irradiation, immediately after the start of radiation, after 10 min of X-irradiation, and then after X-rays were switched off. The lowest A trace shows the ESR spectrum during X-irradiation of 10 m*M* ascorbate in 0.5 *M* Tris buffer, pH 7.5. (From Floyd, R. A., Brondson, A., and Commoner, B. [1973], *Ann. N.Y. Acad. Sci.*, 222, 1077. With permission.)

accumulated cholesterol, and death in some of the animals. Subsequently, Patt et al. (1948) obtained evidence that the initial adrenal cholesterol depletion at 3 h following X-radiation is a pituitary-adrenal "stress" effect, as it was abolished by the simultaneous administration of adrenal cortical extract. Moreover, the adrenal cholesterol changes did not occur in hypophysectomized rats; thymic and splenic involution was not altered by hypophysectomy; X-ray mortality was increased.

North and Nims (1949) confirmed that total body irradiation has a depleting effect on the cholesterol content of the adrenals and also observed a decrease in the ascorbic acid content of the adrenals in fasting albino rats subjected to X-irradiation in doses ranging from 500 to 2000 rad.

Venters and Painter (1951) studied the ascorbic acid (TAA)* levels in the pituitary and adrenal glands of rats at 1, 4, 18, and 72 h after 770 rad total-body irradiation. They observed a 34% decrease in the adrenal ascorbic acid during the first hour. Following this there was a gradual increase to normal levels by 18 h. Weight changes at 18 h indicated adrenal hypertrophy, which was still increasing at 72 h. In the pituitary there was an increase of 2.5% during the first hour and 13.5% by 72 h after radiation. However, the pituitary weight decreased by 23.5% during this period. These results are clearly suggestive of pituitary stimulation and adrenocorticotropic hormone (ACTH) release.

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

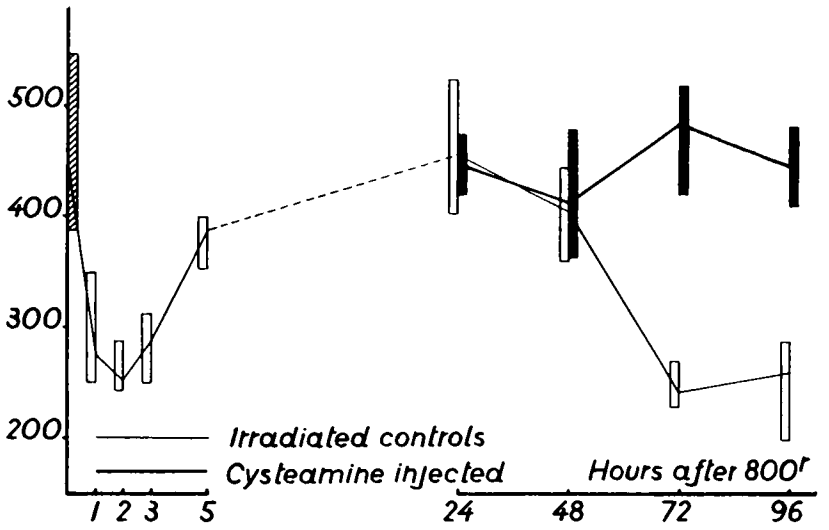

FIGURE 4. Changes in the ascorbic acid content of the adrenals of rats after X-irradiation (800 rad) with and without protection by cysteamine. (From Bacq, Z. M. and Fischer, P. [1957], *Radiat. Res.*, 7, 365. With permission.)

Wexler et al. (1952) reported that the adrenal ascorbic acid depletion following total-body X-irradiation (625 rad) of Long-Evans rats was more marked in the males than in the female animals; they suggested that intact ovaries may offer some protection against radiation.

Hochman and Bloch-Frankenthal (1953) studied the effects of low and high X-ray dosage on the adrenal ascorbic acid content of albino rats. With a dose of 25 rad total-body irradiation, a 6.9% decrease of the ascorbic acid concentration was observed. With a dose of 50 rad the decrease of ascorbic acid reached a mean value of 20.2%; 100 rad brought about a decrease of 26.1%. Further increase of the doses — even to the lethal range of 600 and 1000 rad — did not further increase the adrenal ascorbic acid depletion which remained at 23.3% for the 600-rad dose and at 26.7% for the 1000-rad dose. Thus, a lowering in the ascorbic acid concentration of the adrenals of rats was observed, not only in the lethal and sublethal range of 1000, 600, and 400 rad, but also in the high and low therapeutic ranges of 200, 100, and 50 rad. Since the effect did not increase significantly with doses exceeding 50 rad, they suggested that it was a stress effect and that both low and high doses of X-rays produce a total mobilization of cortical hormones. These authors therefore suggested that some of the therapeutic effects of X-rays on ailments such as rheumatoid arthritis might be due to adrenal corticosteroid release.

Bacq and Fischer (1957) found that the ascorbic acid content of the adrenal glands of rats undergoes a very significant decrease, even within an hour after whole-body X-irradiation (800 rad); this is the "first reaction" (Figure 4). A gradual increase restores the ascorbic acid content of the gland to normal after 24 h, but a second drop of the ascorbic acid level begins after 48 h. This "second reaction" reaches a very low level on the fourth day before the animals die (mortality 100%). These authors found that morphine and barbiturates completely blocked the "first reaction", but did not affect the "second reaction" and did not decrease mortality. On the other hand, cysteamine β-mercaptoethylamine) did not affect the "first reaction", but blocked the "second reaction" and allowed 95% of the animals to survive. Clearly, the "first reaction" is a hypothalamopituitary-adrenal cortical stress effect which is blocked when narcotics inhibit the hypothalamic response to stress. The "second reaction" is "radiation sickness" due to the oxidant effects of radiation and is blocked by the reducing action of –SH compounds injected just before irradiation.

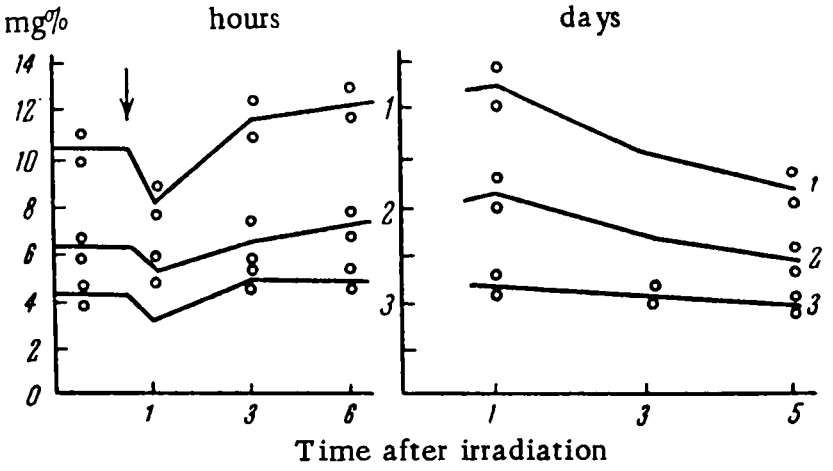

FIGURE 5. Content of ascorbic acid (AA) and dehydroascorbic acid (DHAA) (in mg/100 g) in aorta tissues of rabbits in acute radiation sickness. (1) AA + DHAA, (2) AA, (3) DHAA. Dots show variation from average; arrow indicates moment of irradiation. (From Polikarpova, L. I. [1960], *Biochemistry (U.S.S.R.)*, 25, 356. With permission.)

IV. BLOOD VESSELS — THE HEMORRHAGIC DIATHESIS

Barnes and Furth (1943) studied the indirect general effects of radiation by exposing one of a pair of parabiotic twin mice to X-rays. The nonirradiated mouse consistently showed pathological changes, nuclear pyknosis, and cellular fragmentation in the lymph nodes, spleen, and thymus within 7 h after its twin had received a large (6000 rad), whole-body dose of radiation. They concluded that nonspecific factors resulting from tissue injury pass by the blood stream from the irradiated to the nonirradiated animal.

Some such toxic substance or substances must be responsible for the indirect general effects of radiation, and clearly the endothelium of the blood vessels throughout the body of the animal will be exposed to this toxin, even when the radiation is local.

The endothelium of the blood vessels, like any other tissue, is subject to the direct effects of radiation and to the indirect local effects, but it is also particularly exposed to the indirect general effects of radiation. If the direct effect includes damage by the peroxyl radical, the indirect local effect may include oxidation and loss of ascorbic acid by the endothelium, and the indirect general effect may include histaminemia.

Griffith et al. (1947a) produced capillary fragility by exposure of the peritoneal cavity of the rat to excessive alpha irradiation from radon ointment; they used this as a model for the study of capillary fragility.

Being unable to obtain specimens of the small blood vessels for analysis, Polikarpova (1960) studied the effects of radiation on the ascorbic acid and dehydroascorbic acid (DHAA) concentrations in the aortas of rabbits and guinea pigs. Acute radiation sickness was produced by a single whole-body external irradiation with gamma rays of ^{60}Co (500 rad/min) in a dose of 1000 rad for rabbits and 800 rad for guinea pigs. The results were different in rabbits, which can synthesize ascorbic acid, from those in guinea pigs, which do not, as shown in Figures 5 and 6. In guinea pigs the concentration of ascorbic acid in the aortic wall decreased 3 to 5 d after irradiation, and there was a sharp increase in the concentration of DHAA on the third day. In rabbits the concentration of ascorbic acid decreased at 1 h and then increased during the first day; it then fell back to normal in 3 to 5 d. While the ratio of dehydroascorbate to ascorbate (AA)* was little changed in rabbits, the ratio of

* AA — ascorbic acid, reduced form.

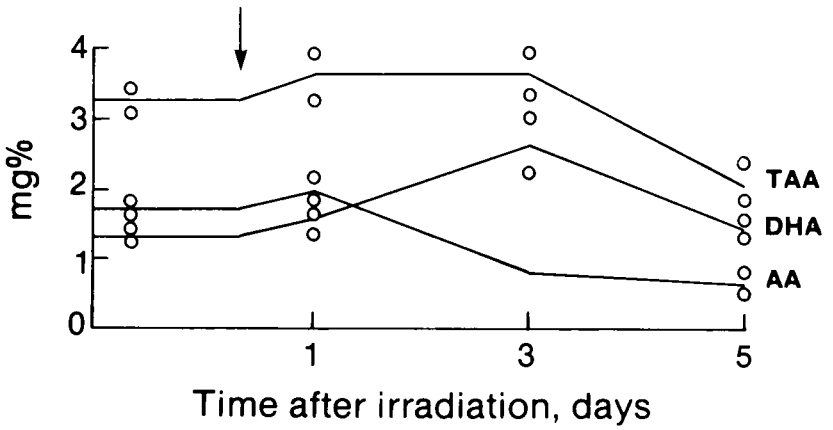

FIGURE 6. Content of ascorbic acid (AA) and dehydroascorbic acid (DHA) (in mg/100 g) in aorta tissues of guinea pig in acute radiation sickness. TAA = AA + DHA. Dots show variation from average; arrow indicates moment of irradiation. (From Polikarpova, L. I. [1960], *Biochemistry (U.S.S.R.)*, 25, 356. With permission.)

Table 1

Species of animal	Normal	After irradiation					
		1 h	3 h	6 h	1 d	3 d	5 d
Rabbit	67 ± 5	62 ± 2	80 ± 6	70 ± 7	52 ± 3	68 ± 5	80 ± 7
Guinea pig	78 ± 8	—	—	—	79 ± 7	326 ± 70	412 ± 18

Note: Dehydroascorbic acid expressed as a percentage of reduced ascorbic acid in the aortic tissues of rabbits and guinea pigs during acute radiation sickness.

From Polikarpova, L. I. (1960), *Biochemistry (U.S.S.R.)*, 25, 356. With permission.

DHAA to AA in the wall of the guinea pig aorta rose from 0.78:1 to 4.12:1 5 days after irradiation (Table 1). These data of Polikarpova suggest that a profound disturbance of ascorbic acid metabolism may be responsible for the vascular damage and purpura associated with radiation sickness.

V. BLOOD AND TISSUE ASCORBATE LEVELS

After many tests on experimental animals, and after many studies of patients undergoing radiation therapy, Carrié (1938) reached the conclusion that a pronounced vitamin C deficiency occurs as a result of exposure to X-rays. He therefore recommended that vitamin C replacement therapy should commence at the beginning of X-ray treatment. Moreover, he suggested that the physician and other personnel treating the patient should also receive supplementary ascorbic acid; the patients treated with vitamin C seemed to tolerate the radiation better and the effect on the tumor was thought to be greater.

Kretzschmar and Ellis (1947) studied the serum ascorbic acid (AA) levels of 35 outpatients attending the London Hospital before, during, and after X-ray therapy for various, mostly neoplastic conditions. Patients who developed X-ray sickness were excluded, as they could have introduced a source of error. Nevertheless, 32 of the patients showed a decrease and only 3 an increase in their serum ascorbic acid levels after treatment.

These workers also studied rabbits following high-dose (1500 to 2000 rad) X-ray irradiation of partial body fields and observed a profound reduction of their serum ascorbate levels

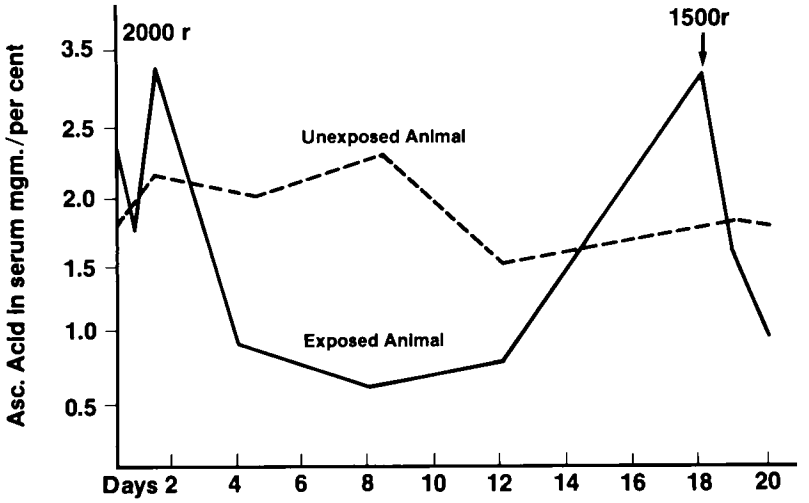

FIGURE 7. Serum ascorbic acid levels in a control and in an exposed rabbit following 2000 rad X-irradiation to the whole left side of the body. Following a second exposure to 1500 rad 17 d later, another fall in the serum ascorbic acid level was observed, this time without the sharp rise which was seen 24 h after the first exposure. (From Kretzschmar, C. H. and Ellis, F. [1947], *Br. J. Radiol.*, 20, 94. With permission.)

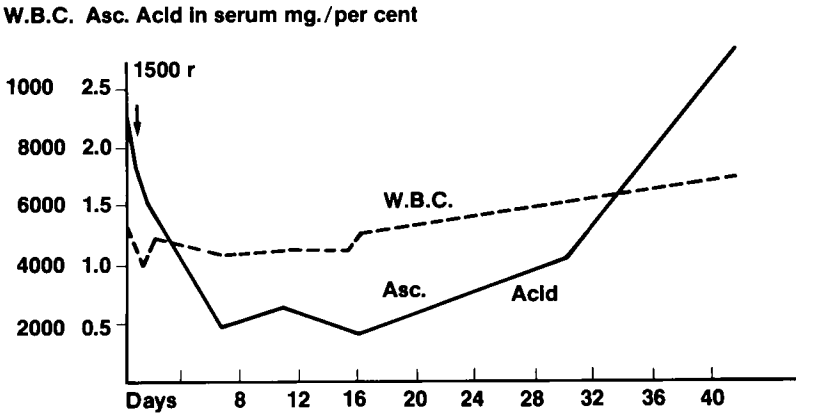

FIGURE 8. Leukocyte (WBC) counts and serum ascorbic acid levels following exposure of the left side of the thorax of a rabbit to 1500 rad X-irradiation. (From Kretzschmar, C. H. and Ellis, F. [1947], *Br. J. Radiol.*, 20, 94. With permission.)

which persisted for 1 or 2 weeks (Figures 7 and 8). Also studying kidney and muscle tissues of rats exposed to X-rays *in vivo* they found the ascorbate levels to be lower in the X-ray exposed than in the unexposed organs.

The work of Oster et al. (1953) demonstrated different tissue ascorbate responses by two different strains of rats. Whole-body irradiation of Long-Evans rats with a midlethal dose of X-rays (710 rad) resulted in a statistically significant reduction in the ascorbic acid (TAA) content of the adrenals (from 300 to 260 mg/100 g), muscle (3.2 to 2.3 mg/100 ml), and blood (0.81 to 0.57 mg/100 ml) immediately after irradiation, and less marked decreases in the thymus, liver, and spleen. Whole-body irradiation of Wistar rats (710 to 1000 rad) showed an even more marked immediate reduction in the ascorbic acid content of the adrenal (minus 150 mg/100 g), but the initial response in the blood, thymus, spleen, liver, kidney, and muscle was a modest increase in the total ascorbate of the thymus, spleen, and muscle

at 24 h. It was suggested that these immediate postirradiation increases might have been due to synthesis of ascorbic acid following X-ray stimulaton, but adrenal ascorbate release would seem to be a more likely cause, especially as regards the total ascorbate content of the blood.

Dolgova (1962) observed that acute radiation injury caused ascorbic acid deficiency in a number of organs in guinea pigs, as shown in Table 2. Single dose, whole-body irradiation by Roentgen rays in a 600-rad dose caused a significant decrease in the ascorbic acid (AA) concentration in the tissues of the brain, adrenals, testes, spleen, and small intestine. The greatest changes were observed in the brain, the endocrine glands, and the blood. The fall in the ascorbic acid concentration of the blood was late, on the third and seventh days after irradiation.

Schreurs et al. (1985) have reported a significant reduction in the mean blood vitamin C level of women undergoing radiation treatment for carcinoma of the cervix or the body of the uterus ($p < 0.01$), from 53.9 μmol/l before treatment to 38.5, 42.4, and 41.9 μmol/l at weeks 4, 7, and 19, respectively, following the initiation of therapy. There was also a significant reduction in the level of vitamin E at 4 weeks and in vitamin B_{12} levels at weeks 4, 7, 13, and 19. Clearly it behooves us to provide nutritional supplements for our patients following radiation therapy.

VI. URINARY ASCORBATE LOSS

Monier and Weiss (1952) observed a highly significant increase in the excretion of DHAA and diketogulonic acid by rats after total body X-ray irradiation of 800 rad in air. DHAA excretion increased by 150%, from 34 to 88 μg/24 h, and diketogulonic acid by 230%, from 19 to 63 μg/24 h. The mean ascorbic acid (AA) excretion also showed an increase from 347 to 477 μg/24 h after radiation, but this change was not statistically significant.

VII. COPPER AND RADIATION DAMAGE

Anbar (1963) discussed the potentiating effect of copper on radiation damage and suggested that the effect is most probably due to the oxidation of cupric ions, when in the complexed form, by HO_2 or O_2 radicals, to their trivalent state. The trivalent copper is then supposed to oxidize its organic ligand. It stands to reason that diethyldithiocarbamate, being a strong copper-binding agent, provides definite radiation protection.

VIII. RADIOPROTECTION BY SULFHYDRYLS AND DISULFIDES

Studies by Patt et al. (1950) clearly demonstrated that the amino acid cysteine (–SH) provided protection against X-ray irradiation damage and reduced radiation mortality following 800 rad whole-body exposure, provided it was injected before X-ray exposure. Cysteine (–SS–), on the other hand, had no such protective effect; it was therefore speculated that cysteine acts by protecting certain cellular elements from oxidation by the products of irradiated water.

Many other sulfhydryl compounds, including homocysteine, dimethylcysteine, reduced glutathione, and cysteamine, when injected into animals a few minutes before exposure to radiation, provide excellent radiation protection and may even reduce the mortality from radiation sickness from 100 to 5%, as already mentioned in the work of Bacq and Fischer (1957), using cysteamine in rats. It is particularly interesting in this respect to note that Gheorghe et al. (1978) reported markedly increased excretion of taurine, an amino acid which results from the oxidation and subsequent decarboxylation of cysteine, in the urine of rats following X-ray irradiation. In fact, the quantity of taurine in the urine is said to be proportional to the amount of radiation exposure both in man and in animals.

Table 2
ASCORBIC ACID CONTENT (mg%) IN THE ORGANS AND WHOLE BLOOD OF GUINEA PIGS AFTER IRRADIATION

Experimental material	Number of observations	Control	After irradiation					
			1st day		3rd day		7th day	
		M[a] ± m[b]	M ± m	T[c]	M ± m	T	M ± m	T
Brain	10	17.63 ± 0.79	13.56 ± 0.88	3.4	11.92 ± 1.07	4.3	11.50 ± 1.07	4.6
Adrenals	10	123.45 ± 7.98	71.13 ± 13.18	3.4	75.91 ± 8.70	4.0	56.75 ± 9.63	5.3
Testes	10	24.50 ± 1.22	13.75 ± 1.76	5.0	16.44 ± 1.85	3.6	13.57 ± 1.42	5.9
Spleen	10	39.0 ± 2.20	21.53 ± 2.85	4.85	24.58 ± 2.61	4.2	19.94 ± 2.75	5.4
Intestines	10	24.84 ± 2.30	14.19 ± 6.18	1.61	18.72 ± 1.86	2.07	12.55 ± 1.81	4.2
Liver	10	17.69 ± 1.14	14.30 ± 2.82	1.11	12.49 ± 1.46	2.8	12.03 ± 1.96	2.5
Blood	12	1.25 ± 0.098	1.39 ± 0.109	1.34	0.81 ± 0.090	3.3	0.87 ± 0.066	3.2

[a] M — arithmetic mean.
[b] m — standard deviation.
[c] T — significance of difference (difference significant if T >3).

From Dolgova, Z. Ya. (1962), *Fed. Proc. Fed. Am. Soc. Exp. Biol.*, 22, 130, 1963. With permission.

IX. RADIATION PROTECTION OR ENHANCEMENT BY ASCORBIC ACID

Patt et al. (1950) reported that ascorbic acid (500 mg/kg), injected intravenously 5 min before whole-body X-ray exposure (800 rad), failed to alter the survival of irradiated rats.

Stone (1972) cited Yusipov (1959), who reported that ascorbic acid given to rats and rabbits before irradiation exerted an unfavorable effect, but when given afterwards was beneficial. This seems to have been a very astute observation and may well represent the truth for humans, too.

Shapiro and Kollman (1965, 1967) demonstrated excellent protective action by ascorbic acid against gamma radiation damage to certain enzymes (lysozyme and aldolase) *in vitro*; DHAA did not provide any protection. They found that radiation accelerated the oxidation of ascorbic acid to DHAA which was rapidly hydrolyzed to diketogulonic acid. It was suggested that ascorbic acid exerted its protective action by scavenging free radicals produced by the radiation of water. Thus, the ascorbate, being more readily oxidized, underwent oxidation instead of the enzymes. However, we must remember that ascorbic acid itself liberates ascorbate free radical during rapid oxidation by X-rays, and this can damage tissues. Nevertheless, Kalnins (1953), studying lesions produced by X-ray irradiation (1200 to 4200 rad) in the mandibles and the incisor teeth of young guinea pigs, observed that supplementary ascorbic acid provided definite protection. They studied two groups of animals: one receiving 1 mg of ascorbic acid s.c. on alternate days and one receiving 50 mg s.c. on alternate days, for 2 weeks before and also during repeated X-ray exposures. The reduction of bone deposition and the osteoradiolysis were less pronounced in the animals receiving the large doses of ascorbic acid. However, in this series of experiments it is not clear whether vitamin C protected the guinea pigs against radiation injury or whether the vitamin C protected the guinea pigs against vitamin C deficiency which was induced by radiation in the guinea pigs with borderline hypovitaminosis C. Either way, the vitamin supplement was beneficial.

Bacq (1965), in his book entitled *Chemical Protection Against Ionizing Radiation*, states that he has tried many times to repeat some earlier favorable reports of radiation protection by ascorbic acid, but failed regularly. Moreover, reviewing the literature, he found the results of other workers to be overwhelmingly negative.

However, Ala-Ketola et al. (1974) observed that supplementary ascorbic acid given to rats before and after exposure to 900 rad from a cobalt machine, provided considerable protection and reduced the mortality from 36 to 6%.

Tewfik et al. (1982), studying X-irradiated mice, found no such protection; in fact, the administration of 0.1% ascorbic acid in the drinking water of the mice for 1 week prior to radiation was found to increase the weight loss and the mortality following whole-body radiation.

X. RADIOPROTECTION BY CHELATING FLAVONOIDS

Griffith et al. (1947b) reported that subcutaneous injections of the natural plant polyphenol rutin appeared to hasten recovery following X-ray irradiation of one leg in rats. Griffith also reported that rutin offered protection against increased capillary fragility following the administration of radon ointment in rats. However, he indicated that rutin probably had no beneficial effect in total-body irradiation of rats. On the other hand, Rekers and Field (1948) found that rutin, 50 mg, given three times a day orally, decreased the mortality of dogs when given for 1 week before and also after total-body irradiation (350 rad); 16 out of 25 (64%) of the untreated dogs died in 13 to 30 d after X-irradiation, whereas only 3 out of 25 (12%) of the rutin-treated dogs died 16, 28, and 31 d after radiation. Although the characteristic widespread hemorrhage was seen in 2 out of 3 rutin-treated dogs that died, the remaining 22 rutin-treated dogs remained relatively free from the petechiae and ecchymoses which affected all of the 16 control dogs that died.

Likewise, Clark et al. (1948) observed that guinea pigs receiving 0.2% ascorbic acid in their drinking water fared much better following X-irradiation if their drinking water also contained 0.2% calcium flavonate for 1 week before and also following total-body X-irradiation (225 rad). The mortality rate was decreased from 67 to 35%. Moreover, the hemorrhagic signs (petechial hemorrhages, ecchymoses, generalized purpura) in the flavonoid-treated animals were considerably less marked than in the controls.

Field and Rekers (1949) also administered test substances for 1 week before and during irradiation. After exposure to 350 rad single-dose, whole-body X-irradiation, 59% (22 of 37) untreated control dogs died with a prominent hemorrhagic syndrome. Five flavonoid substances appeared to be of roughly equal activity in reducing the hemorrhagic signs of the irradiation disease when administered continuously pre- and postirradiation. When rutin was fed, 11% (3 of 27) of the dogs died; with hesperidin, 17% (1 of 6); with epimerized *d*-catechin, 10% (1 of 10); and with homoeriodictyol, none of 5, and with morin, none of 6 irradiated dogs died. Ascorbic acid alone failed to influence the course of irradiation disease, and 50% (6 of 12) of treated dogs died. However, when ascorbic acid was given simultaneously with quercetin, which by itself was ineffective (50% mortality), only 10% (one of ten) dogs died. It was observed that the severe thrombocytopenia which was present in all the radiated dogs was not significantly altered by the administration of any of these bioflavonoids, so the reduction of hemorrhagic signs would seem to be an effect upon the vascular system itself.

Sokoloff et al. (1950a, b, c) reported a similar reduction of radiation mortality in rats, from 80 to 10%, as a result of feeding citrus bioflavonoid compounds for 1 week before and 3 weeks after radiation.

Griffith and Couch (1951) observed that rutin administered subcutaneously or by implantation can protect the rat against capillary fragility due to irradiation with alpha particles. This is particularly interesting because rutin has been found by the writer to be, among the bioflavonoids, the most active as an indirect antioxidant for ascorbic acid by virtue of its potent heavy metal chelating action (Chapter 11 of this volume).

Dauer and Coon (1952) reported no radiation protection by any of the bioflavonoids that they tested. However, it seems that they started treatment after irradiation and they "solubilized" rutin and other flavonoids by addition of an aqueous solution of piperazine hexahydrate.

Arons et al. (1954a) observed that oral administration of citrus bioflavonoids, 600 mg daily, protected the capillaries of the human nail bed against radiation injury when administered for 10 d before exposure to irradiation, but gave no protection when administered after irradiation. Likewise, Arons et al. (1954b) reported that oral administration of citrus bioflavonoids, 600 mg daily, for 6 to 7 d before irradiation, caused a markedly increased tolerance of radiation in 403 patients receiving X-ray therapy for cancer, compared with 613 patients radiated for cancer who had not received bioflavonoids. This stands in contrast to ascorbic acid therapy, which seems to be the most beneficial when given following radiation.

Much controversy has existed concerning the efficacy of bioflavonoids in radiation protection, but it is now evident that these insoluble, heavy metal-chelating compounds were effective only when given by mouth for some time before exposure to radiation. In all probability they acted by withdrawing heavy metals, especially copper, from the body into the lumen of the bowel for excretion, for radiation damage is proportional to the copper content of the tissues.

XI. ASCORBIC ACID IN THE TREATMENT OF RADIATION INJURY

Favorable clinical effects of ascorbic acid on leukopenia due to X-ray therapy were reported by Carrié (1938) and by Ellis (1942). A sufficiently large dose of ascorbic acid was reported

to prevent or minimize the reduction of the leukocyte count following X-ray exposure. Carrié reported that the best results were obtained if ascorbic acid was given at an early stage of radiotherapy, or preferably before it began, and continued during treatment; this also caused a considerable improvement in the general condition of the patient, reducing or abolishing X-ray sickness.

Clausen (1942) also reported success in ten cases in checking a pronounced Roentgen leukopenia which had appeared in connection with protracted Roentgen treatment of cancer of the stomach by means of large intravenous doses of vitamin C.

Wallace (1941) reported that the administration of vitamins B_1 and C almost entirely eliminated the severe nausea and vomiting that follow radiation therapy for carcinoma of the cervix, but did not reduce the incidence of diarrhea. Others have reported similar benefits from the use of nicotinic acid for radiation sickness. Wallace reported that the characteristic ileal changes that follow heavy pelvic irradiation were not prevented by the vitamin supplements.

XII. RADIATION AND TISSUE HISTAMINE LEVELS

Cramer (1928) remarked on the similarities between the effects of histamine intoxication and radiation damage, in that both cause capillary dilation, nausea, headache, bronchiolar spasm, vomiting, and hypotension. He suggested that the damage to the capillary walls and the shock following Roentgen irradiation must be due to the release of a histamine-like substance. Subsequently, Ellis (1942) not only demonstrated that ascorbic acid improved the leukocyte counts of patients following X-ray therapy, but also obtained uniform success from the intramuscular administration of histaminase as a remedy for radiation sickness. These findings are most interesting, as they were reported so many years before there was any knowledge of the histaminolytic property of ascorbic acid.

Weber and Steggerda (1949) observed a marked increase in the plasma histamine concentration of rats during the first 2 h and again on about the fifth day following midlethal X-ray irradiation (600 rad). They observed a close correlation between the times at which the plasma histamine levels were increased and the periods of decreased blood pressure; these occurred at 2 h and again on the fourth to sixth day after radiation, when the animals showed a critical fall in blood pressure with loss of weight and appetite. Clearly, the plasma histamine levels were highest during both the "first reaction" and the "second reaction" of Bacq and Fischer (Figure 4), when the adrenal ascorbate levels are lowest. The inverse relationship between blood histamine and ascorbate levels is discussed in Chapter 1, Volume III. Venters and Painter (1950) reported that dogs and rabbits were more sensitive to histamine at 2 to $2^1/_2$ h following irradiation; also, Leitch and Haley (1955) reported a twofold increase in the urinary excretion of histamine by rats following irradiation. In contrast, Feldberg and Loeser (1954) observed a reduced level of histamine in skin that had been radiated several years previously in two human subjects; they suggested that this may be a permanent change. Eisen et al. (1956), also using atropinized guinea pig ileum for biological assays, observed a gradual decrease in the histamine concentration in the skin of rats, falling to 65% of normal between the third and fifth days following X-irradiation and persisting for more than 2 weeks. They attributed this to degeneration of subcutaneous mast cells, but could find no such explanation for the even greater depletion in the jejunum, where the histamine level fell to 6.5% of normal by day 5 after irradiation. Eisen and Wilson (1957) observed similar skin changes following beta radiation, when a strontium-90 plaque was applied to the shaved abdominal skin of rats; within 5 d of radiation, the skin histamine level fell to 47% of normal and remained at that level until the tenth day. They suggested that the fall in the skin histamine level was attributable to the concurrent mast cell destruction and histamine release. They concluded that radiation ulcers are caused by a mechanism that is independent of intrinsic skin histamine.

XIII. CONCLUSIONS

Radiation protection is not always desirable, because it will most likely protect the cancer cells as well as the normal tissues in radiation therapy. When, however, radiation effects are undesirable, as at Chernobyl, then it would seem that an antioxidant like vitamin E, a reducing agent like cysteine, and a nontoxic, nonmutagenic chelating food fiber component like D-catechin should be given — preferably for some time before, during, and after exposure to irradiation. However, an ascorbic acid supplement given before irradiation could be counterproductive, as it would most likely increase the quantity of ascorbate free radical released during irradiation (Figure 3).

Treatment of radiation sickness following exposure, on the other hand, requires ascorbic acid therapy. Ascorbic acid depletion and histaminemia form part of the picture in radiation sickness. People who have been radiated should receive additional supplies of ascorbic acid, either with D-catechin or, better, as catechin-coated ascorbic acid tablets to prevent any further release of ascorbate free radical resulting from contact between ascorbic acid and any copper that may be present in the drinking water.

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Chapter 22

ASPIRIN AND SALICYLATES

There are ways in which aspirin and ascorbic acid seem to have similar beneficial actions, and yet other ways in which aspirin is antagonistic to ascorbic acid. Both compounds have been advocated for use in prophylaxis against coronary thrombosis and venous thrombosis, in spite of their differing actions on platelet function. Both compounds increase capillary strength or resistance and decrease capillary fragility, perhaps by polymerizing the mucopolysaccharides of the subendothelial connective tissue. Ascorbic acid and aspirin share this capillary-strengthening capacity with some other acidic hydroxyl-bearing compounds such as low-dose estrogens and bioflavonoids. However, like high-dose estrogens, aspirin can interfere with ascorbic acid storage, and this must be kept in mind.

Daniels and Everson (1936) discovered that the urinary excretion of ascorbic acid is increased by aspirin. They suggested that this might explain the frequent association between rheumatoid arthritis and vitamin C deficiency, as reported by Rinehart et al. (1936), as many of the patients were treated with salicylates. They also reported the finding of Giardina and Ets (1936) that sodium salicylate will precipitate scurvy and death in guinea pigs receiving diets containing an insufficient amount of antiscorbutic vitamin, in a shorter time than in pigs on similar diets with no sodium salicylate.

Daniels and Everson gave a dose of only 2.5 gr (162 mg) of acetylsalicylic acid orally to children between 4 and 6 years of age, while each was receiving a constant weighed diet including 64 to 100 mg of ascorbic acid a day in different experiments. The urinary ascorbic acid content rose in all of seven experiments, from a control 24-h mean of 36 mg to a mean of 57 mg during the 24 h after receiving one 2.5-gr tablet of aspirin. This is only a small dose of aspirin, yet it had a profound effect on the ascorbic acid excretion of these children.

Van Eekelen (1937), studying the urinary ascorbic acid of an adult following 1, 1.5, and 3 g of aspirin, and Youmans et al. (1937), giving 1.3 g of aspirin twice in one day to healthy young adults on a constant ascorbic acid intake (50 mg daily), were unable to confirm the findings of Daniels and Everson. Both the studies of Keith and Hickmans (1938), giving sodium salicylate (50 gr) with sodium bicarbonate (100 gr) and of Parsons (1938), giving 50 to 75 gr of aspirin with 100 to 150 gr of sodium bicarbonate, demonstrated a subsequent increase in ascorbic acid excretion in children. However, neither of these was an adequate test of the full effect of salicylate, as Hawley et al. (1936) had already shown that sodium bicarbonate, in large doses, depressed urinary ascorbic acid output of men.

Longenecker et al. (1939) observed that aspirin caused a modest increase in the urinary ascorbic acid excretion by rats, but this could have been due to stimulation of increased synthesis of the vitamin by the rat liver.

Studies by Samuels et al. (1940) showed that both sodium salicylate and acetylsalicylic acid caused a pronounced increase in total and reduced ascorbic acid excretion in the urine of rats and guinea pigs. Following two 30-mg doses of aspirin in one day, rats on an ascorbic acid-free diet showed a doubled urinary ascorbic acid excretion for 24 h, and then a compensatory decrease to half-normal excretion on the third day before returning to normal. Guinea pigs, too, showed a markedly increased urinary ascorbic acid excretion for 5 d following two 40-mg doses of aspirin when on a diet containing 30 mg of ascorbic acid per 100 g body weight per day, but a much less marked effect when fed a diet containing 20 mg ascorbic acid per 100 g per day.

These data could have been interpreted as suggesting an effect of aspirin on renal tubular reabsorption, which might only become apparent when the ascorbate load for reabsorption is high. However, subsequent studies by Ritz et al. (1940) provided evidence that the effect

of salicylates is probably due to decreased tissue storage of ascorbic acid. These workers found that rats receiving sodium salicylate, or acetylsalicylic acid, lose ascorbic acid from the brain and the liver soon after administration of the drug. On continued administration, the concentration in other tissues also decreased. The decrease in the ascorbic acid concentration of the brain was found to begin within 18 h after administration of salicylates, even in nephrectomized rats where the blood level of ascorbic acid was increased. Clearly, this brain ascorbic acid loss could not be due to decreased ascorbate synthesis, nor could it be due to renal ascorbate loss; it seems to represent a decreased ascorbate storage capacity by the brain.

One wonders what may happen to the ascorbic acid content of the liver and the brain of a malnourished child who develops a fever and is treated with aspirin. Could local ascorbate deficiency be one of the factors responsible for the development of Reye's syndrome, in which children with a fever, especially when treated with aspirin, may develop fatty degeneration of the liver and permanent brain damage? Do they develop amyloidosis of the brain or is it some other form of encephalopathy?

In this context it is worthy of note that Pelner (1942) observed complete relief of all symptoms of salicylate toxicity by the simultaneous administration of vitamin C. The patient was a 16-year-old boy who was seriously ill with rheumatic fever and was under treatment with sodium salicylate, 1 g every 4 h orally, and 4 g daily per rectum. On the third day of illness and salicylate therapy, the patient developed a nosebleed and severe ringing in the ears. During the next 2 d, the tinnitus continued severely, in spite of reduction of salicylate medication to 1 g daily by mouth. The nose bled profusely at this time. A tourniquet test showed 12 petechiae in a 2.5-cm diameter circle after 15 min of positive pressure. The plasma ascorbic acid (AA)* level was 0.4 mg/100 ml. Ascorbic acid (100 mg) was then administered three times a day. Within 48 h the nosebleed, as well as the ringing in the ears, had ceased. The salicylates were then stepped up to former doses without the development of tinnitus or indeed any other signs of intolerance.

Spitzer and Shapiro (1948) reported finding that the effect of salicylates in increasing urinary ascorbic acid excretion was somewhat variable; it was more commonly seen in children than in adults, and especially in children with virus infections.

Coste et al. (1953) studied the effects of subcutaneous injections of sodium salicylate (500 mg/kg) on the adrenal ascorbic acid concentrations of rats, using the Sayers technique. They found both a significant, direct adrenal ascorbic acid-depleting effect of salicylate (-18%), which was observed in hypophysectomized rats, and also a greater adrenal ascorbic acid-depleting effect (-36%) in intact rats due to the general pituitary-adrenal stress effect.

This no doubt accounts for the finding of Stewart et al. (1953) that large doses of sodium salicylate (7 g) produce a rapid effect on the plasma ascorbic acid of human subjects similar to that of adrenocorticotropic hormone (ACTH) — an increase in the "total" ascorbic acid combined with a reduction and virtual disappearance of dehydroascorbic acid (DHAA); some degree of eosinopenia was also noted. DHAA reappeared in the plasma while the salicylate concentration was still high, so this would seem to represent a nonspecific "stress" effect, causing release of ACTH and cortisone. Such an adrenal cortical stimulation may be responsible for the capillary-strengthening effect of salicylates such as carbazochrome salicylate, which is marketed for intravenous use in the arrest of capillary hemorrhage. However, this is only a short-term effect, and the longer term adverse effect of salicylates on ascorbic acid metabolism must be remembered.

Albanese et al. (1955), studying plasma and urinary total ascorbic acid levels in children with rheumatic fever, concluded that, "the adrenocorticotropic action of salicylates . . . does not cause catabolic effects of vitamin C or nitrogen stores in the human body." However,

* AA — ascorbic acid, reduced form.

blood and urine studies do not allow us to study internal redistribution of ascorbic acid, as in the liver and brain ascorbate depletion observed in rats.

Douthwaite and Lintott (1938) were the first to show that aspirin may precipitate bleeding from the gastric mucosa. Subsequent work by Hurst and Lintott (1939) and by Hurst (1943) suggested that aspirin was the principal cause of bleeding in at least half of 58 patients with gastrointestinal hemorrhage; in these patients, all other causes of bleeding had been excluded. At first the bleeding was thought to result from a direct action of aspirin on the gastric mucosa. However, Grossman et al. (1961) showed that aspirin can cause gastrointestinal bleeding in ulcer patients, even when it is given intravenously. Moreover, Brodie and Chase (1967) showed in rats that a similar incidence of gastric irritation was produced by aspirin whether administered orally or parenterally.

Parry and Wood (1967) found that 69% of their patients admitted to hospital with acute gastrointestinal hemorrhage had taken aspirin during the week before admission, whereas 32% of patients without hemorrhage had taken aspirin during the preceding week. Investigating 60 patients admitted to the hospital with gastrointestinal hemorrhage, Russel et al. (1968) found them to have a mean leukocyte ascorbic acid level of 14.2 $\mu g/10^8$ cells, compared with 17.6 μg in a peptic ulcer control group and 23.7 $\mu g/10^8$ cells in a healthy control group. Also, 40 of the 60 patients with gastrointestinal hemorrhage had taken aspirin, alcohol, or both in the week before admission to the hospital. The mean leukocyte ascorbic acid level was significantly lower in patients who had taken aspirin or alcohol than in those with no precipitating factor; this difference was more significant in patients over the age of 45 (see Tables 3 and 4, Chapter 17, Volume III).

Further work by Russel and Goldberg (1968) showed that guinea pigs on a scorbutogenic diet bled from the gastric mucosa significantly more often than those on a normal diet. The addition of aspirin to a scorbutogenic diet significantly increased the likelihood of gastric mucosal bleeding. The possible causes of bleeding after aspirin ingestion were discussed by Croft (1968) and in Editorials in the *British Medical Journal* (1969, 1970). Stewart (1974) has suggested that subclinical scurvy could be responsible for hematemesis following aspirin.

There is evidence that aspirin impairs platelet function and also some evidence that those who have achlorhydria bleed more easily after aspirin. Could it be that aspirin substitutes itself for some of the ascorbic acid in the platelets? Studies by Sahud suggest that this may indeed be the case. Sahud (1970) demonstrated *in vitro* that platelets take up DHAA, but not ascorbic acid, and that aspirin in high concentration impairs the uptake of DHAA by platelets.

Furthermore, Sahud and Cohen (1971) studied plasma and platelet ascorbic acid levels in 48 normal subjects and in 34 patients with confirmed rheumatoid arthritis. Plasma ascorbic acid levels were abnormally low in all rheumatoid subjects except those taking supplemental vitamin C. However, significantly low platelet levels of ascorbic acid were found only in those rheumatoid patients receiving high doses of aspirin, i.e., 12 or more tablets a day (Table 1).

Born and Payling Wright (1967) reported that platelet adhesiveness was significantly impaired in guinea pigs with scurvy. Wilson and Douglas (1967) also found decreased platelet adhesiveness in human scurvy. However, Harrison and Honour (1967) reported that despite the presence of a defect in platelet stickiness in scorbutic guinea pigs, their capacity for producing firm hemostatic plugs following injury was not impaired.

O'Brien (1968), MacMillan (1968), and Barkhan and Youssef (1968) studied the effect of salicylates on platelet function and found that aspirin reduces platelet aggregation or clumping.

Clearly, aspirin is a poor substitute for ascorbic acid in the platelets.

Studies by Loh et al. (1973) demonstrated that aspirin substitutes itself for ascorbic acid in human leukocytes and blocks their uptake of ascorbic acid. These workers incubated

Table 1
PLATELET AND PLASMA ASCORBIC ACID CONCENTRATIONS IN RHEUMATOID ARTHRITIS PATIENTS DIVIDED INTO FOUR GROUPS ACCORDING TO THERAPEUTIC REGIMEN

Group	Treatment category	No. of patients	Platelet ascorbic acid concentration ($\mu g/10^{10}$ platelets) mean ± SD	Plasma ascorbic acid concentration ($\mu g/100$ ml) mean ± SD
Normal subjects		48	68.60 ± 15.28	102.77 ± 35.01
IA	High aspirin dosage	7	30.86 ± 18.04	52.29 ± 36.34
IB	High aspirin dosage + corticosteroids	7	35.00 ± 10.88	49.43 ± 27.09
II	Low or no aspirin dosage	10	53.40 ± 19.82	59.60 ± 35.89
III	High aspirin dosage + vitamin C	8	55.38 ± 13.84	108.63 ± 81.45
IV	Indomethacin	2	24.00 ± 8.49	31.50 ± 12.02

Note: Plasma ascorbic acid levels were low in all patients with rheumatoid arthritis (Groups I, II, III, and IV). However, significantly low platelet levels of ascorbic acid were found only in those rheumatoid patients receiving high doses of aspirin (12 or more tablets a day).

From Sahud, M. A. and Cohen, R. J. (1971), *Lancet*, May 8, 937. With permission.

human leukocytes at 37°C in a buffered medium containing ascorbic acid (3 mg/100 ml), with and without aspirin (3.5 mg/100 ml). While the leukocyte ascorbic acid concentration rose from 23.2 to 52.0 $\mu g/10^8$ cells in 2 h with ascorbic acid alone, it rose to only 25.7 $\mu g/10^8$ cells with aspirin and ascorbic acid. In another test it was found that aspirin alone did not cause any loss of ascorbic acid from the leukocytes *in vitro*. Administration of ascorbic acid (500 mg) to normal adults caused a significant increase in plasma and leukocyte ascorbic acid during the following 2 h. Simultaneous administration of two aspirin tablets (600 mg) further increased plasma ascorbic acid concentrations, but completely arrested uptake of ascorbic acid by the leukocytes of normal adult male volunteers (Figure 1). Administration of ascorbic acid every 6 h increased urinary excretion of ascorbic acid, and there was a concomitant increase in leukocyte ascorbic acid. Simultaneous administration of aspirin with ascorbic acid resulted in a further significant increase in excretion of ascorbic acid and a simultaneous fall in leukocyte ascorbic acid. Administration of aspirin (600 mg) every 6 h for 7 d caused a modest reduction of the plasma ascorbic acid and a profound reduction in the leukocyte ascorbic acid concentration to 10 $\mu g/10^8$ cells in normal adult human volunteers (Figure 2).

In further studies, Loh and Wilson (1975) confirmed that two aspirin tablets (600 mg acetylsalicylic acid) given with 500 mg of ascorbic acid completely inhibited the uptake of ascorbic acid by the leukocytes of normal adult male volunteers. Moreover, increasing the dose of ascorbic acid given with two aspirins from 500 mg to 1000 mg, 1500 mg, or 2000 mg did not overcome this inhibition of ascorbic acid uptake by the leukocytes (Table 2). Increasing doses up to 1500 mg resulted in increased plasma ascorbic acid levels, after which renal excretion prevented further increase. Increasing the dose of aspirin in the presence of a constant dose of ascorbic acid (500 mg) progressively reduced the uptake of ascorbic acid into the leukocytes and, ultimately, at a dose of three aspirins, was associated with loss of ascorbic acid from the leukocytes *in vivo* (Table 3). Absorption of ascorbic acid from the gastrointestinal tract was not found to be impaired by aspirin, but urinary ascorbic acid excretion was increased.

Wilson (1975) made the unexpected observation that the effect of aspirin on the uptake

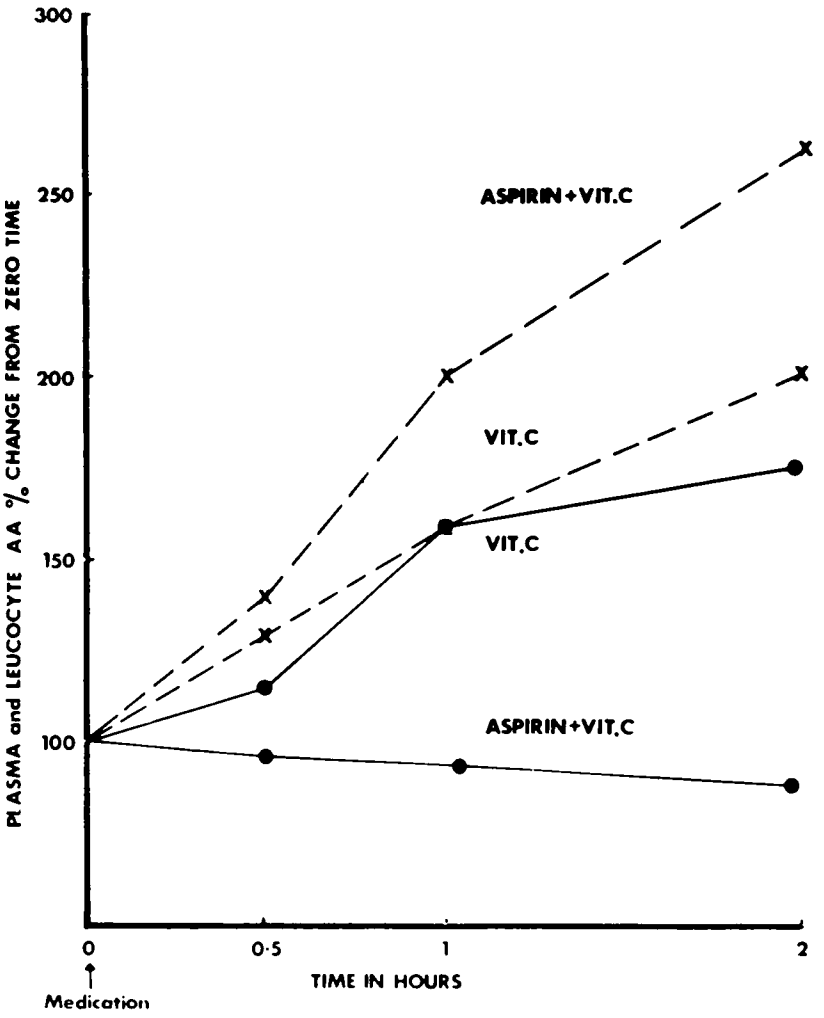

FIGURE 1. Percentage change in plasma (× ------ ×) and leukocyte (●——●) ascorbic acid concentration during a 2-h period after oral administration of 500 mg of Vitamin C, and of 500 mg vitamin C with 600 mg aspirin. Aspirin completely blocked the uptake of ascorbic acid by the leukocytes. (From Loh, H. S., Watters, K., and Wilson, C. W. M., [1973], *J. Clin. Pharmacol.*, 13, 480. With permission.)

of ascorbic acid by leukocytes is reversed on the third day of the common cold. Ascorbate levels are markedly depleted during corysa, but aspirin administered with ascorbic acid was found to increase, rather than decrease, ascorbic acid uptake by human leukocytes at this time, both *in vivo* and *in vitro*; 3 weeks later the effect of aspirin on leukocyte ascorbic acid uptake had reverted to its usual inhibitory effect (Figure 3). Wilson drew an analogy with the observation that aspirin has no antipyretic effect in healthy subjects, but does exert an antipyretic effect during immunological response to a bacterial or viral infection.

Molloy and Wilson (1980) have demonstrated that aspirin competes with and displaces ascorbic acid from both primary and secondary binding sites on bovine serum albumen.

Interference with protein binding of ascorbic acid could account for the decreased storage of ascorbic acid, which has been reported in the liver, the brain, the leukocytes, and the blood platelets, as well as the increase of free ascorbic acid in the plasma and the increased renal excretion of ascorbic acid following aspirin treatment.

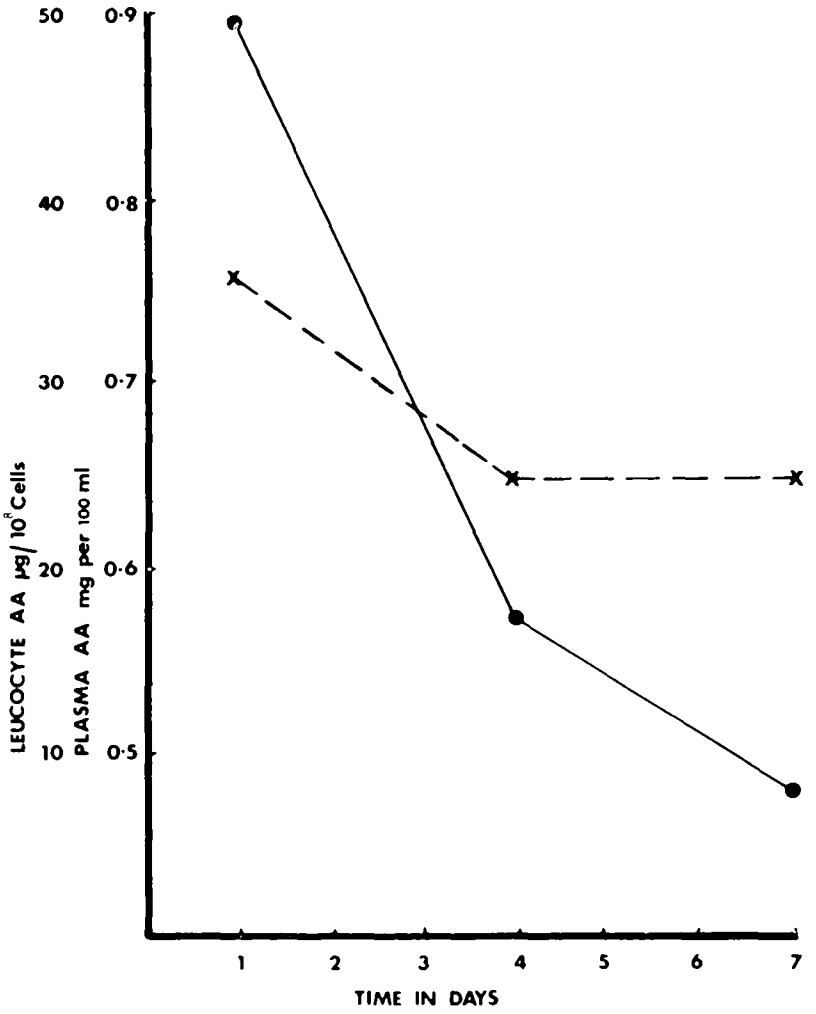

FIGURE 2. Leukocyte (●——●) and plasma (× --- ×) ascorbic acid concentrations on days 1, 4, and 7 while aspirin was being administered every 6 h during 7 d. (From Loh, H. S., Watters, K., and Wilson, C. W. M. [1973], *J. Clin. Pharmacol.*, 13, 480. With permission.)

In contrast to the findings of Loh and Wilson (1975), Basu (1981, 1982) has reported that the gastrointestinal absorption of ascorbic acid is impaired by aspirin. Basu summarized his work as follows:

> The effect of soluble aspirin on the availability of vitamin C has been studied in guinea-pigs and human subjects. In the human study, the concentrations of vitamin C in plasma, leucocytes and urine were found to be markedly elevated at various intervals following administration of a single oral dose of 500 mg of the vitamin. The vitamin C-associated increases, however, appeared to be blocked when the vitamin was given simultaneously with aspirin (900 mg). Similar findings were observed in guinea-pigs, where in addition faecal excretion of vitamin C was found to be significantly increased when the vitamin was administered together with aspirin. These results suggest that aspirin may impede gastrointestinal absorption of vitamin C. This hypothesis has been strengthened with *in vitro* studies using everted gut sac preparations where both the serosal/mucosal concentration gradient and the uptake of vitamin C per unit weight of intestine were markedly lowered by acetylsalicylate. Such an interaction is relevant to the population where vitamin C intake is borderline.

Johansson and Åkesson (1985), at the University of Lund, studied four healthy adults who received a low-ascorbic acid diet (4 to 5 mg/d) for 2 weeks and were given acetylsalicylic

Table 2
THE PLASMA (mg/100 ml) AND LEUKOCYTE (μg/10^8 CELLS) ASCORBIC ACID CONCENTRATIONS $^1/_2$, 1, AND 2 h AFTER A FIXED DOSE OF ASPIRIN (600 mg) AND WITH INCREASING DOSES OF ASCORBIC ACID (500, 1000, 1500, AND 2000 mg)

Means and Standard Deviations

Medication	Plasma blood concentrations				Leukocyte blood concentrations			
	0 h[a]	$^1/_2$ h	1 h	2 h	0 h[a]	$^1/_2$ h	1 h	2 h
500 mg AA[b]	1.12 ± 0.28	1.57 ± 0.59	1.86 ± 0.44	2.16 ± 0.30	47.92 ± 14.39	54.89 ± 16.74	71.53 ± 15.70	79.83 ± 10.81
p value from 0 h	—	>0.05	<0.01	<0.01	—	>0.05	<0.05	<0.05
600 mg ASA[c], 500 mg AA	1.04 ± 0.31	1.44 ± 0.47	1.94 ± 0.55	2.63 ± 0.53	63.08 ± 13.28	60.88 ± 13.41	60.87 ± 13.71	60.16 ± 14.00
p value from 0 h	—	>0.05	<0.01	<0.01	—	>0.05	>0.05	>0.05
600 mg ASA, 1000 mgAA	0.63 ± 0.16	1.13 ± 0.25	1.55 ± 0.45	2.17 ± 0.68	43.48 ± 14.14	44.96 ± 11.75	45.07 ± 9.00	43.85 ± 7.21
p value from 0 h	—	<0.05	<0.01	<0.01	—	>0.05	>0.05	>0.05
600 mg ASA, 1500 mg AA	0.66 ± 0.14	1.36 ± 0.12	1.90 ± 0.10	2.60 ± 0.35	43.41 ± 11.62	46.61 ± 11.53	43.33 ± 10.90	41.13 ± 11.34
p value from 0 h	—	<0.05	<0.01	<0.01	—	>0.05	>0.05	>0.05
600 mg ASA, 2000 mg AA	0.65 ± 0.18	1.30 ± 0.23	1.82 ± 0.32	2.66 ± 0.61	42.75 ± 11.74	45.95 ± 11.03	43.34 ± 9.86	37.84 ± 11.45
p value from 0 h	—	<0.05	<0.01	<0.01	—	>0.05	>0.05	>0.05

Note: Administration of ascorbic acid (500 mg) alone to healthy adult male volunteers caused an increase in both plasma and leukocyte ascorbic acid levels, but simultaneous administration of two aspirin tablets (600 mg) completely inhibited the uptake of ascorbic acid by the leukocytes. Increasing the dose of ascorbic acid, given with the two aspirins, did not overcome this inhibition.

[a] Plasma and leukocyte ascorbic acid concentrations at 0 h were determined before medication was given.
[b] AA — ascorbic acid.
[c] ASA — acetylsalicylic acid.

From Loh, H. S. and Wilson, C. W. M. (1975), *J. Clin. Pharmacol.*, 15, 36. With permission.

Table 3
PLASMA (mg/100 ml) AND LEUKOCYTE (μg/10^8 CELLS) ASCORBIC ACID CONCENTRATIONS $^1/_2$, 1, AND 2 h AFTER A FIXED DOSE OF ASCORBIC ACID (500 mg) AND WITH INCREASING DOSES OF ASPIRIN (300, 600, AND 900 mg)

Means and Standard Deviations

Medication	Plasma concentrations				Leukocyte concentrations			
	0 h[a]	$^1/_2$ h	1 h	2 h	0 h[a]	$^1/_2$ h	1 h	2 h
500 mg AA	1.12 ± 0.28	1.57 ± 0.59	1.86 ± 0.44	2.16 ± 0.30	47.92 ± 14.39	54.89 ± 16.74	71.53 ± 15.70	79.83 ± 10.81
p value from 0 h	—	>0.05	<0.01	<0.01	—	>0.05	<0.05	<0.05
500 mg AA, 300 mg ASA	1.25 ± 0.71	1.64 ± 0.94	2.30 ± 0.76	2.83 ± 0.68	63.74 ± 11.17	67.19 ± 9.05	71.25 ± 9.62	77.97 ± 10.80
p value from 0 h	—	>0.05	<0.05	<0.05	—	>0.05	>0.05	<0.05
500 mg AA, 600 mg ASA	1.04 ± 0.31	1.44 ± 0.47	1.94 ± 0.55	2.63 ± 0.53	63.08 ± 13.28	60.88 ± 13.41	60.87 ± 13.71	60.16 ± 14.00
p value from 0 h	—	>0.05	<0.01	<0.01	—	>0.05	>0.05	>0.05
500 mg AA, 900 mg ASA	0.97 ± 0.51	1.36 ± 0.43	1.69 ± 0.48	2.43 ± 0.68	70.46 ± 18.08	67.39 ± 13.69	66.72 ± 17.10	60.11 ± 16.15
p value from 0 h	—	>0.05	<0.05	<0.05	—	>0.05	>0.05	>0.05

Note: Study of healthy adult male volunteers given ascorbic acid (AA) (500 mg) orally, alone or with acetylsalicylic acid (ASA). Increasing the dose of aspirin in the presence of a constant dose of ascorbic acid progressively reduced the uptake of ascorbic acid by the leukocytes and, ultimately, at a dose of three aspirins (900 mg),was associated with a loss of ascorbic acid from the leukocytes.

[a] Plasma and leukocyte ascorbic acid concentrations at 0 h were determined before any medication was given.

From Loh, H. S. and Wilson, C. W. M. (1975), *J. Clin. Pharmacol.*, 15, 36. With permission.

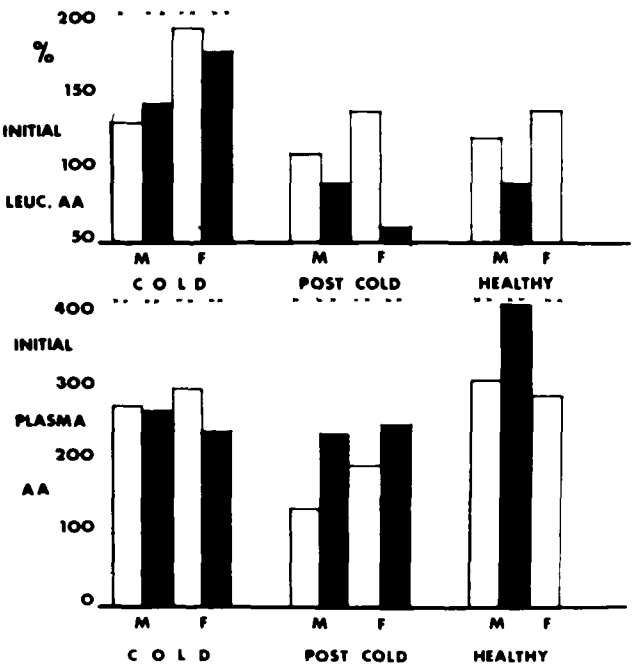

FIGURE 3. Values for leukocyte and plasma ascorbic acid as a percentage of the concentrations at zero time, 4 h after administration of a loading dose of 2000 mg vitamin C (white bars), or 2000 mg vitamin C with 600 mg aspirin (black bars). The loading doses were administered to men and women during and 3 weeks after corysa. These values are compared with values in healthy subjects without recent history of respiratory symptoms. (From Wilson, C. W. M. [1975], *Ann. N.Y. Acad. Sci.*, 258, 355. With permission.)

acid, 1 g three times a day, throughout the second week. Also, following a rest period on a free diet, these same volunteers were given a dietary supplement of ascorbic acid, 500 mg twice a day for 2 weeks, and received acetylsalicylic acid 1 g three times a day for the duration of the second week. "At low ascorbic acid intake, acetylsalicylic acid increased urinary ascorbic acid, but at high ascorbic acid intake, acetylsalicylic acid instead decreased urinary ascorbic acid. The latter effect was probably due to an inhibited intestinal absorption of ascorbic acid, and the former may reflect decreased protein binding and tissue uptake of ascorbic acid caused by acetylsalicylic acid."

It has been suggested that frank scurvy does not appear during salicylate therapy because renal tubular reabsorption of ascorbic acid becomes more efficient as the glomerular filtration rate decreases. However, very low tissue stores undoubtedly do occur and should make us pause for thought — especially concerning the etiology of Reye's syndrome in children, because

- Children with low ascorbic acid levels are more susceptible to infection (Chapter 12, Volume II).
- Infection reduces ascorbic acid levels even more (Chapter 8 of this volume).
- Aspirin causes decreased brain and liver ascorbic acid levels in rats (Ritz et al., 1940).
- Salicylates block the entry of ascorbic acid into the rabbit brain (Spector and Lorenzo, 1973).
- Ascorbic acid deficiency causes amyloid degeneration in the liver, spleen, and adrenals of guinea pigs (Pirani and Bly, cited in Chapter 10, Volume II).

- Amyloid degeneration of the brain causes presenile dementia in humans (Schwartz, cited in Chapter 10, Volume II).
- Aspirin given to children with fevers increases the risk of Reye's syndrome ($p < 0.01$) (Starko et al., 1980); this has been questioned by Eichenwald (1983).
- Ascorbic acid reduces the toxicity of salicylates (Pelner, 1942).
- Reye's syndrome involves fatty degeneration of the liver and serious residual brain damage in children who survive (Reye et al., 1963; Partin et al., 1975, 1978; Brunner et al., 1979).

The writer has corresponded with Dr. John Partin to find out whether he had observed amyloid in the brains of children dying from Reye's syndrome. In a letter dated June 2, 1982, Dr. Partin stated that he had not seen any amyloid, but he had not, however, stained any section with Congo Red.

A computer search of the literature also failed to find any cross reference between Reye's syndrome and amyloid, but the matter will not be settled until Congo Red birefringence studies are reported.

The strongest argument against ascorbic acid deficiency playing a role in Reye's syndrome is the consistent finding of hypoglycemia or even 0 mg/100 ml blood sugar levels in this disease; one usually finds hyperglycemia in ascorbic acid deficiency.

If indeed the reducing agent ascorbic acid is deficient in the liver and the brain in Reye's syndrome, then the concomitant absence of another reducing agent — glucose — could cause a very marked increase in the oxidation-reduction potential, which might be the cause of the severe hepatic and cerebral damage.

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Chapter 23

ALCOHOL

Wortis et al. (1938) studied the ascorbic acid content of the blood and the cerebrospinal fluid of a large group of normal individuals and of 103 alcoholics admitted to Bellevue Hospital from the streets of New York City. The results of their study, which are summarized in Table 1, indicated that chronic alcoholics without peripheral neuritis or psychosis had relatively normal ascorbic acid levels, but those with peripheral neuritis and various forms of alcoholic psychosis tended to have subnormal ascorbic acid levels in the blood and in the cerebrospinal fluid. While they acknowledged that this vitamin C deficiency was usually associated with general malnutrition and other vitamin deficiencies, they suggested that, "vitamin C likely plays a role in the metabolism of nervous tissue". This prediction was ahead of its time, and has since been shown to be true, for we now know that ascorbic acid is needed for the synthesis of neurotransmitters, being involved in the conversion of tyrosine to dopamine, norepinephrine, and epinephrine (Chapter 6, Volume III), in the conversion of tryptophan to serotonin (Chapter 7, Volume III), and in the detoxification of histamine (Chapter 1, Volume III). Wortis et al. did not suggest that alcoholic neuritis and alcoholic psychosis were due to ascorbate deficiency; they thought that the B vitamins and vitamin A were more important in this respect. Nevertheless, they could not escape the conclusion that ascorbic acid deficiency contributed in some way to the neuritic and psychotic disorders of chronic alcoholics. When asked his thoughts about the etiology of Wernicke's encephalopathy, with its proliferation of small blood vessels and small hemorrhages in the midbrain, Herman Wortis expressed the opinion that it could be produced in guinea pigs either by ascorbic acid deficiency or by starvation, so one could not be sure that it was truly due to vitamin C deficiency. Of course, we now know that it is cerebral beriberi which is due to vitamin B_1 deficiency, but it is usually complicated by ascorbic acid deficiency. Moreover, it is now evident that ascorbic acid plays an important role in the metabolism of thiamine when thiamine intake is low; for Bánhidi (1960) has shown that the thiamine disulfide of food cannot be utilized unless it is activated by the reducing action of ascorbic acid or cysteine. Thus, a dietary deficiency of protein and ascorbic acid can convert a borderline thiamine deficiency into a manifest deficiency.

Alexander et al. (1938) reported one chronic alcoholic patient with Wernicke's encephalopathy who had clinical and pathological evidence of frank scurvy, with subperiosteal, intramuscular, subcutaneous, and perifollicular hemorrhages. In a survey of five alcoholic patients admitted to hospital with delirium tremens, four with Korsakoff's psychosis and one with acute halucinosis, these same workers found an average plasma ascorbic acid level of 0.37 mg/100 ml, compared with 0.85 mg/100 ml in ten control patients. They suggested that subclinical scurvy, along with other vitamin deficiencies, might predispose to Wernicke's encephalitis hemorrhagica and to subdural hematomas which they felt were not always due to trauma in chronic alcoholics.

Joliffe and Goodhart (1948) observed that the polyneuropathy of the alcohol addict is apt to be more severe and less responsive to vitamin B_1 therapy than the nutritional polyneuropathy generally observed in nonalcoholics; we may conjecture that this may in part be due to other associated vitamin deficiencies.

Smith (1950) found clinical and biochemical similarities between delirium tremens and Addisonian crisis and reported that both conditions responded well to the same treatment, namely intravenous injections of cortisone and ascorbic acid. Describing his results with this treatment in profoundly agitated patients, he said, "His craving for alcohol disappears, a sense of well-being is induced promptly, appetite is restored, the nervous tension and jitteriness disappear, the patient becomes calm and usually sleeps without sedation."

Table 1
BLOOD AND CEREBROSPINAL FLUID ASCORBIC ACID (AA) CONCENTRATIONS FOUND IN NORMAL INDIVIDUALS, AND IN A STUDY OF 103 ALCOHOLIC PATIENTS ON THE PSYCHIATRIC AND NEUROLOGICAL WARDS OF BELLEVUE HOSPITAL IN NEW YORK CITY[a]

	Blood ascorbic acid (mg/100 ml)		Spinal fluid ascorbic acid (mg/100 ml)	
Ages	25—35	36—59	20—35	36—59
Normal individuals	0.6—1.3		2.64	2.54
Chronic alcoholism without psychosis or peripheral neuritis	(4)[b] 0.57	(2) 0.87	(4) 2.78	(2) 2.86
Chronic alcoholism with peripheral neuritis	(1) 0.49	(7) 0.35	(1) 1.10	(7) 0.82
Alcoholic halucinosis with mild peripheral neuritis	(3) 0.23	(6) 0.32	(3) 0.61	(5) 0.88
Alcoholic halucinosis	(7) 0.29	(25) 0.31	(8) 0.80	(21) 0.67
Alcoholic halucinosis following recovery	(1) 0.30	(4) 0.40	(2) 1.26	(4) 1.69
Delirium tremens	(5) 0.28	(7) 0.30	(4) 0.96	(5) 0.77
Korsakow's psychosis		(4) 0.33		(4) 1.07
Alcoholic encephalopathy	(2) 0.30	(3) 0.30	(2) 0.66	(2) 0.91

Note: The results obtained in patients under 20 years and over 59 years of age have been omitted because of insufficient numbers.

[a] Data shown are the reported work of Wortis et al. (1932).
[b] Numbers in parentheses indicate the number of subjects in each group.

It would be interesting to study blood histamine levels of patients during such treatment; we might expect a dramatic fall in the blood histamine level as a result of ascorbic acid and cortisone administration, and also as a result of sleep in someone who had not slept for days.

Fishbach et al. (1952) reported their lack of success in the treatment of delirium tremens by the use of paraldehyde sedation and large doses of B_1 and B complex vitamins, with fluid therapy, in a 42-year-old alcoholic; he died 78 h after admission to hospital. They contrasted this with the successful use of ascorbic acid, 1000 mg, corticotropin, and fluids in a 50-year-old alcoholic with delirium tremens who became free from hallucinations and completely recovered on the fifth hospital day. This treatment is particularly interesting, as cortisone is beneficial in the metabolism of ascorbic acid, increasing the ascorbate/dehydroascorbate ratio in the blood, as noted in Chapter 13 of this volume, and also decreasing capillary fragility, as shown by Kramar et al. (1956).

In a study of the tetraethylthiuram disulfide (antabuse)-alcohol reaction, Niblo et al. (1951) observed that ascorbic acid is very valuable in overcoming the adverse symptoms. Markham and Hoff (1953), in a further study of this reaction, observed that electrocardiographic changes occurred in 91% of 44 patients. The principal changes were lowering, flattening, or inversion of the T waves, ST segment depression in one or more leads, and prolongation of the QT interval. The electrocardiogram was reported as being favorably affected by ascorbic acid and tended to return to normal more quickly when this vitamin was administered.

Taverna (1954) found significantly low vitamin C levels in patients with alcoholic psychoses and in other chronic alcoholics.

Smith et al. (1954) reported that methylene blue was of no benefit in the treatment of alcohol-antabuse reactions, but Wörner (1955) obtained results from the use of ascorbic acid in such reactions.

Armstrong and Gould (1955) called for high doses of ascorbic acid and B vitamins in the

treatment of delirium tremens. They recommended ascorbic acid (1500 mg), aneurin hydrochloride (1000 mg), nicotinamide (100 to 400 mg), and pyridoxin (200 mg); this was repeated in 4 to 8 h if necessary, followed by half doses at intervals of 4 to 8 h as indicated clinically.

Writing on alcohol poisoning, Imrie (1955) asserted that intravenous administration of vitamins B_1 (1000 mg), B_6 (200 mg), and C (1500 mg) is particularly suitable in the treatment of delirium tremens.

Studying 16 alcoholics with cirrhosis of the liver, Jandl and Lear (1956) found that ascorbic acid was completely absent from the buffy coat (leukocytes and platelets) of the peripheral blood of 11 of them on admission to hospital; 1 had clinical signs of frank scurvy; all 16 had normal serum vitamin B_{12} levels; 3 of the 11 with chemical scurvy had severe megaloblastic anemia which responded to folic acid therapy, but the remainder had milder hemolytic anemias with mild to moderate macrocytosis which did not respond to folic acid alone.

Using the percentage urinary excretion of oral test doses of ascorbic acid to assess vitamin C saturation, Lester et al. (1960) reported ascorbate deficiency in 85% of 85 alcoholic patients on admission to hospital and in 39% cent of 23 nonalcoholic subjects. Their results indicated that a 250-mg daily oral dose of ascorbic acid was insufficient to correct the initial deficiency of the alcoholics in 1 week and that a daily dose of at least 500 mg daily is necessary.

Pawan (1968) studied the effects of vitamins and various sugars on alcohol metabolism in four men and reported that 600 mg of ascorbic acid had no effect on the rate of ethanol clearance. However, several subsequent reports (*vide infra*) have shown a significant increase in the rate of ethanol clearance as a result of ascorbic acid administration both in man and in animals, and a highly significant increase in survival following ethanol poisoning in mice.

Russell et al. (1968), studying the leukocyte ascorbic acid levels of 60 patients with gastrointestinal hemorrhage, noted that the mean level of 14.2 $\mu g/10^8$ WBC was significantly lower than that found in a matched peptic ulcer control group (17.6) and in a healthy group (23.7). They also noted that the levels were significantly lower in those in whom aspirin or alcohol might have precipitated the bleeding than in those in whom no precipitating factor was evident (see Table 4, Chapter 17, Volume III).

Leevy et al. (1970) assessed serum or blood vitamin levels as being low when they were 20% below the normal range. They found low circulating levels of vitamins in each of 140 patients with Laennec's cirrhosis, in 80% of 10 patients with chronic viral hepatitis and in 75% of 12 patients with biliary cirrhosis. The most common vitamin deficiency in Laennec's cirrhosis was folic acid, followed in frequency by low levels of B_6, thiamine, nicotinic acid, B_{12}, ascorbic acid, and pantothenic acid, as shown in Figure 1. The normal range for serum ascorbic acid was given as 0.4 to 1.5 mg/100 ml, it is therefore evident from Figure 1 that 25% of those admitted to hospital with cirrhosis of the liver had serum ascorbate levels below 0.32 mg/100 ml.

A study of the ascorbate status of alcoholics in Glasgow was reported by O'Keane et al. (1972); the mean leukocyte ascorbic acid (AA)* level of 50 chronic alcoholics (18.18 ± 11.01 $\mu g/10^8$ WBC) was found to be significantly lower than that of control subjects (27.41 ± 7.59 $\mu g/10^8$ WBC) who were matched for age and sex ($p < 0.0005$), as shown in Table 2. None of these patients showed any clinical signs of scurvy, but the results clearly indicate that alcoholics, as a group, are in a state of borderline ascorbate deficiency. Dietary histories indicated that the ascorbate deficiency in most of these alcoholics was related to an inadequate intake of vitamin C-containing food (Figure 2), but the vitamin deficiency of a few may have resulted from other factors such as impaired absorption or abnormal metabolism.

* AA — ascorbic acid, reduced form.

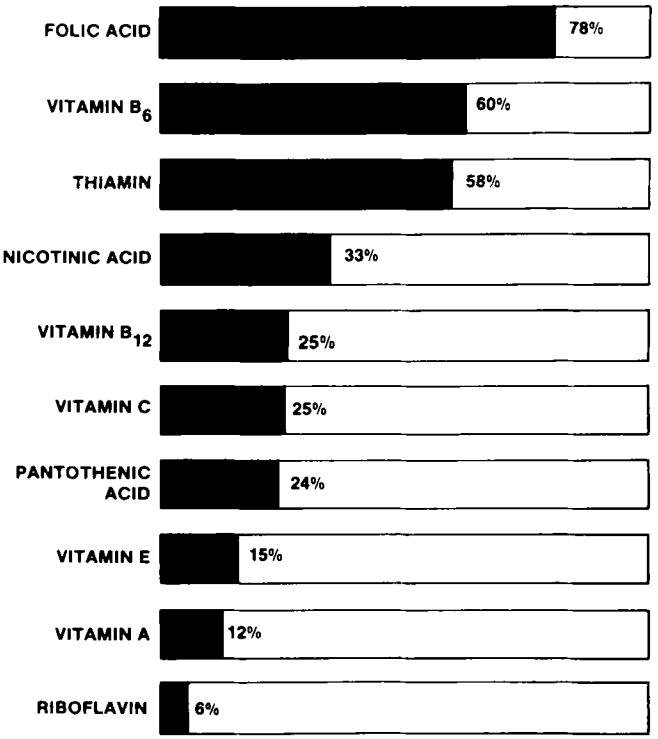

FIGURE 1. Incidence of low circulating levels of vitamins in 140 patients with Laennec's cirrhosis. (From Leevy, C. M., Thompson, A., and Baker, H., [1970], *Am. J. Clin. Nutr.*, 23, 493. ©American Society for Clinical Nutrition. With permission.)

Table 2
LEUKOCYTE ASCORBIC ACID LEVELS IN ALCOHOLIC PATIENTS AND NORMAL SUBJECTS IN DIFFERENT AGE GROUPS

Age group	Alcoholic L-AA levels ($\mu g/10^8$ WBC) mean ± SD	Normal L-AA levels ($\mu g/10^8$ WBC) Mean ± SD	*p*
Over 45 years (25)	17.2 ± 11.9	25.1 ± 7.7	<0.005
Under 45 years (25)	19.4 ± 10.0	27.7 ± 7.9	<0.005
Under 30 years (9)	16.8 ± 10.6	27.7 ± 9.4	<0.025

From O'Keane, M., Russell, R. I., and Goldberg, A. (1972), *J. Alcohol.*, 7, 6. ©1972 Pergamon Press, Ltd. With permission.

Lemoine et al. (1972) made a statistical analysis of alcohol consumption, dietary history, and 10 symptoms of alcoholism, as well as blood levels of thiamine, riboflavin, and ascorbic acid in 605 subjects, hospital patients and healthy volunteers; 302 did not drink alcohol; 123 drank less than 70 g/d; 98 drank 70 to 139 g/d; and 70 drank more than 140 g of alcohol a day. There was a clear relationship between alcohol consumption and the number of symptoms.

The relationship between the number of symptoms and erythrocyte glutathione reductase activity (EGR), which was used to estimate riboflavin, tended towards significance, but did not reach it. On the other hand, as regards thiamine and the number of symptoms there was

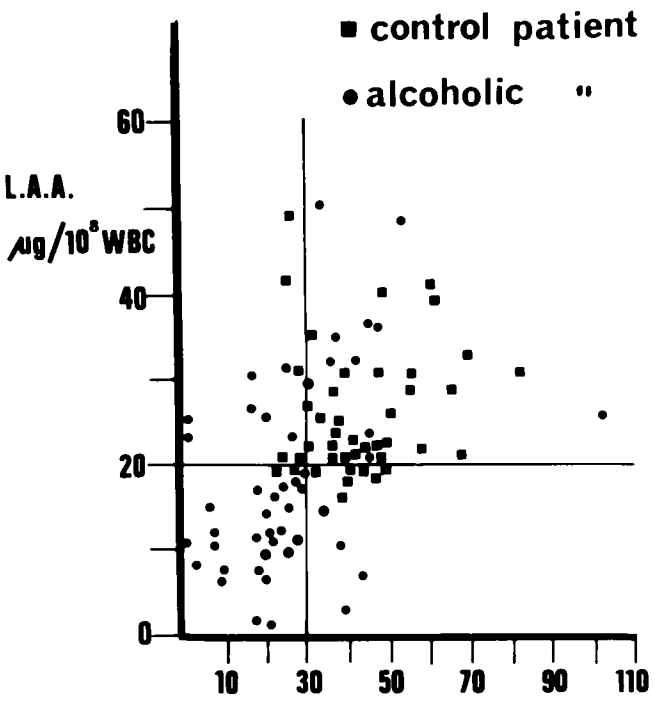

FIGURE 2. Correlation of leukocyte ascorbic acid levels and estimated dietary intake of vitamin C (milligrams per day) in 50 alcoholic and 50 control subjects matched for age and sex. (From O'Keane, M., Russell, R. I., and Goldberg, A., [1972], *J. Alcohol.*, 7, 6. ©1972 Pergamon Press, Ltd. With permission.)

a significant difference between the group in which the erythrocyte transketolase activation coefficient ($^{\alpha}$ETK) was equal to or less than 1.18 and that in which it was equal to or greater than 1.23 ($p < 0.005$). More symptoms were also found in patients with plasma ascorbic acid levels below 0.2 mg/100 ml than in those with levels greater than 0.4 mg/100 ml, and this was even more highly significant ($p < 0.0005$).

Krasner et al. (1974) have demonstrated that the clearance of ethanol from the blood is proportional to the leukocyte ascorbic acid concentration. A highly significant linear correlation ($r = .88$, $p < 0.001$) was found between the hepatic alcohol dehydrogenase activity and leukocyte ascorbic acid levels in 12 patients with nonalcoholic liver disease. In 11 healthy men, the clearance of alcohol was measured before and after 1 g of ascorbic acid daily for 2 weeks. In 9 of the 11, the leukocyte ascorbic acid levels rose and the rate of alcohol disappearance increased significantly, with a correlation coefficient of $r = .6$ ($p < 0.005$).

Dow et al. (1975) found a close correlation between the leukocyte ascorbic acid levels and the hepatic alcohol dehydrogenase activity in patients with various forms of alcoholic liver disease, but no such relationship in control patients with duodenal ulcers who were free from liver disease.

However, acetaldehyde arising from the metabolism of ethyl alcohol and from the vapor of cigarettes is 10 to 30 times as toxic as ethanol, according to Sprince et al. (1974), so it is very pertinent that prior oral administration of L-ascorbic acid (2 m*M*/kg) afforded marked protection against the lethality of an LD_{90} dose of acetaldehyde (18 m*M*/kg) in rats, according to Sprince et al. (1975). Moreover, a combination of L-ascorbic acid with L-cysteine and thiamine hydrochloride gave virtually complete protection.

Dow and Goldberg (1975) studied alcohol clearance from the blood plasma of guinea pigs and found, in contrast to the observations on human subjects by Krasner et al. (1974), that it was unchanged in scurvy. They did find that ascorbic acid deficiency decreased the microsomal aniline hydroxylase, NADPH oxidase, and cytochrome P-450 levels in the liver, but there was no change in the rate of ethanol clearance in these animals after 14 d on an ascorbic acid-deficient diet.

Beattie and Sherlock (1976) observed significantly reduced leukocyte total ascorbic acid (TAA)* levels in 37 patients with alcoholic liver disease, 124 n$M/10^8$ cells, compared with 162 n$M/10^8$ in 28 healthy control subjects ($p < 0.01$). Of the 37 patients with alcoholic liver disease, 21 had leukocyte TAA levels of less than 113 n$M/10^8$ cells; this is two standard deviations below the control mean and may be considered the lower limit of normal. Drug metabolism was studied in 20 patients with liver disease; the mean antipyrine half-lives of 4 patients with leukocyte TAA levels below 100 n$M/10^8$ was 28.3 h, while that of 16 patients above that level was 18.6 h.

Studying 80 patients with nonalcoholic liver disease of unknown cause (60 with cirrhosis and 20 with chronic aggressive hepatitis) Morgan et al. (1976) found low leukocyte ascorbic acid levels in 35%, low plasma vitamin E levels in 38%, low plasma vitamin A levels in 42%, and low plasma carotene levels in 26%. The authors thought that inadequate dietary intake was not a major cause of these deficiencies; malabsorption (steatorrhea) was found in 44% of these patients, so one must consider the possibility that the vitamin deficiencies may have contributed to the development of the liver disease, just as Ginter has shown the development of cirrhosis of the liver in guinea pigs with chronic ascorbic acid deficiency (Chapter 13, Volume II).

It is therefore very pertinent that Krasner et al. (1976) not only found low leukocyte ascorbic acid and low serum folate levels in five out of ten alcoholics, but also demonstrated a highly significant impairment in the absorption of *d*-xylose, water, Na^+, and Cl^- by the small intestine in alcoholics. The mean rate of absorption of water in the alcoholic subjects (50.0 ± 2.3 ml/h) was significantly lower ($p < 0.001$) than the mean value in 14 healthy control subjects (205 ± 15.9 ml/h). Indeed, diarrhea, which is so common in alcoholics, may contribute to ascorbic acid deficiency (Chapter 24 of this volume).

However, Lieber (1976) has pointed out that, "Overconsumption of alcohol can cause cirrhosis and death not only because alcoholism promotes malnutrition but also because alcohol and its products disturb liver metabolism and damage the liver cells."

The data provided by Frank et al. (1976) showed that a single intoxicating dose of alcohol caused a profound shift of ascorbic acid from the cytosol into the heavy mitochondrial fraction of the brain tissue of the rat. This is an interesting observation, but such delicate fractionation needs confirmation.

In addition to the natural desirability of replacing a vitamin deficiency when present, the work of Krasner et al. (1977) has confirmed that vitamin C supplementation increases ethanol clearance rates ($p < 0.005$), even in normal subjects. Moreover, these workers reaffirmed the finding of a significant correlation between low leukocyte ascorbic acid (TAA) levels and low alcohol dehydrogenase activities in liver biopsies both from alcoholics ($p < 0.001$) and from nonalcoholic patients with liver disease ($p < 0.001$). Addition of ascorbic acid *in vitro*, however, was found not to have any effect on the alcohol dehydrogenase activity. It may be that ascorbic acid plays a role in the synthesis of the enzyme alcohol dehydrogenase or an essential coenzyme. It is also possible that ascorbic acid may have some influence on alternative pathways of ethanol metabolism.

Studies of human volunteers by Rawat (1977) confirmed the finding that fructose administration hastens the rate of clearance of alcohol from the blood, while glucose, sucrose,

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

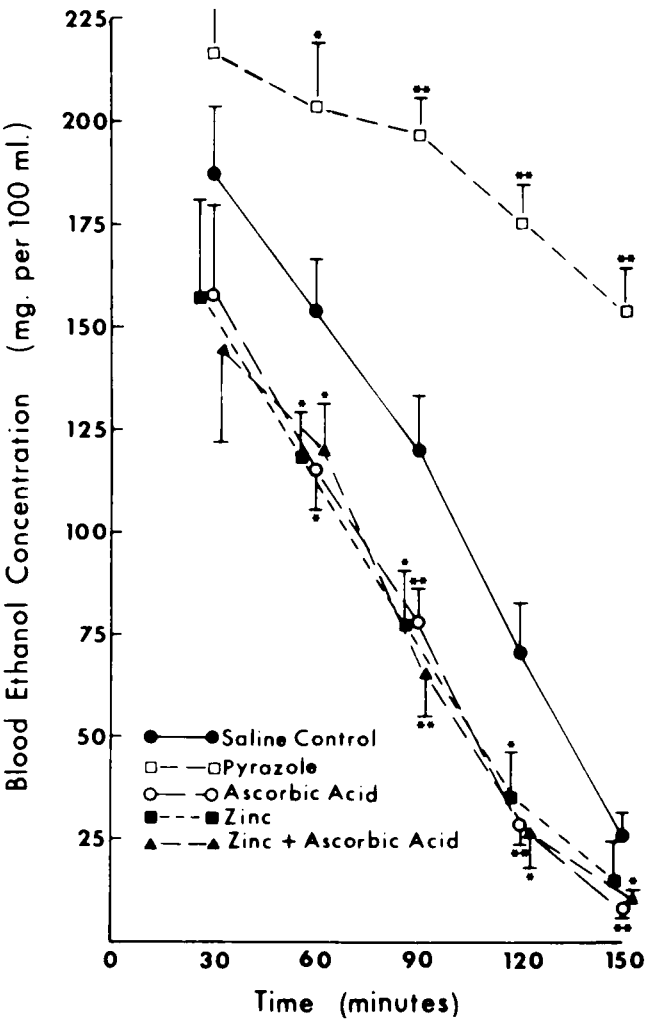

FIGURE 3. Ethanol clearance rates following i.p. injection of ethanol in saline-injected control rats and in rats pretreated with pyrazole, ascorbic acid, zinc sulfate, or zinc sulfate and ascorbic acid. Each point represents the mean ± SE for seven rats. Differences from control did not become statistically significant ($p < 0.05$) until 60 min after injection. * $p < 0.05$, ** $p < 0.01$, when treated groups are compared with control. (From Yunice, A. A. and Lindeman, R. D. [1977], *Proc. Soc. Exp. Biol. Med.*, 154, 146. With permission.)

and alanine had no such effect. Moreover, Yunice and Lindeman (1977) demonstrated that zinc (the metal component of alcohol dehydrogenase), given as zinc sulfate, and also ascorbic acid accelerated the rate of clearance of alcohol from the blood of mice (Figure 3) and markedly decreased their mortality from ethanol poisoning.

Sprince et al. (1977) have pointed out that many heavy drinkers are also heavy smokers, and that acetaldehyde is a major toxic substance which arises both from heavy drinking and from heavy smoking. They pointed out that high oral doses of ascorbic acid provide considerable protection against at least seven toxicants associated with heavy drinking and heavy smoking. These toxicants are ethanol, acetaldehyde, nicotine, carbon monoxide, *N*-nitroso compounds, cadmium, and polynuclear hydrocarbons. Moreover, they noted that combined therapy with L-cysteine or L-cysteine and thiamine with ascorbic acid provides more effective

Table 3
PROTECTION AGAINST ACETALDEHYDE TOXICITY IN THE RAT BY DIFFERENT DOSES OF ASCORBIC ACID

Compounds tested (oral dose)	% anesthetized after 3—10 min	% survivors after		
		3 h	24 h	72 h
Saline control	91 (85/93)	38 (35/93)	13 (12/93)	11 (10/93)
L-Ascorbic acid (AA)				
3.0 m*M*/kg	14 (3/21)	91 (19/21)	91 (19/21)	91 (19/21)
2.0 m*M*/kg	45 (9/20)	75 (15/20)	75 (15/20)	75 (15/20)
1.0 m*M*/kg	79 (33/42)	50 (21/42)	43 (18/42)	41 (17/42)

From Sprince, H., Parker, C. M., and Smith, G. G. (1978), *Nutr. Rep. Int.*, 17, 441. With permission.

protection or antitoxic activity than does ascorbic acid alone. Four toxicants from drinking and smoking, namely ethanol, acetaldehyde, nicotine, and cadmium are known to increase the tissue release and urinary exertion of catecholamines and corticosteroids, so it is interesting to note that these workers found the antitoxic action of ascorbic acid to be enhanced by certain antiadrenergic drugs such as reserpine, phenoxybenzamine, and propranolol, although its precise role in this respect is not fully understood.

While both alpha-adrenergic and beta-adrenergic blocking agents protect rats from the lethal effects of catecholamines released by acetaldehyde administration in rats, Sprince et al. (1978) found that high oral doses of L-ascorbic acid protect well against both the anesthesia and the lethality of acetaldehyde (Table 3).

Roussouw et al. (1978) reported low plasma ascorbic acid levels in 54% of alcoholic and 31% nonalcoholic patients with decompensated chronic liver disease; these patients were also deficient in thiamine, B_6, and niacin. It was suggested that liver disease may bear more responsibility for the vitamin deficiencies than either the poor diet or the alcohol.

Brissot et al. (1978) observed that the mean leukocyte ascorbic acid (TAA) level of 37 patients with alcoholic cirrhosis (22.0 $\mu g/10^8$ WBC) was significantly lower than that of 31 normal control subjects (34.4 $\mu g/10^8$ WBC; $p < 0.001$). Sixty seven patients with idiopathic hemochromatosis were also found to have significantly reduced leukocyte ascorbic acid levels (19.5 $\mu g/10^8$ WBC) before ascorbate and desferrioxamine treatment. This raised the question as to what part excessive iron or copper storage in the liver might play in the ascorbate deficiency of alcoholics. However, excessive iron storage does not seem to have been responsible in these alcoholics, for their serum iron levels were normal and the mean hepatic iron concentrations were normal in 13 out of 14 and elevated in only one.

Bonjour (1979), in a review of the literature, noted that the mean plasma, leukocyte, and urinary ascorbic acid levels are lower than normal in alcoholics and concluded that this is partly due to an insufficient dietary intake of vitamin C and partly due to other effects of alcohol that are not yet fully understood.

Leung and Guze (1981) saw two elderly alcoholics with scurvy in the emergency department of a hospital in Los Angeles and reported them in the *Annals of Emergency Medicine* to make emergency physicians aware of this symptom complex.

Majumdar et al. (1981), measuring the leukocyte ascorbic acid (TAA) levels of chronic alcoholics in Greenwich, found that 24 out of 25 (96%) had deficient levels (mean 68.6 nmol/10^8 WBC), compared with the normal range for a control population (120 to 300 nmol/10^8 WBC). The leukocyte ascorbate levels of the alcoholics were significantly improved after receiving ascorbic acid (500 mg daily) with dextrose and B vitamins by intravenous infusion for 5 d (mean 108.3 nmol/10^8 WBC), but even so, the levels did not return to normal in 16 patients out of 25 (64%). They suggested that conventional detoxification

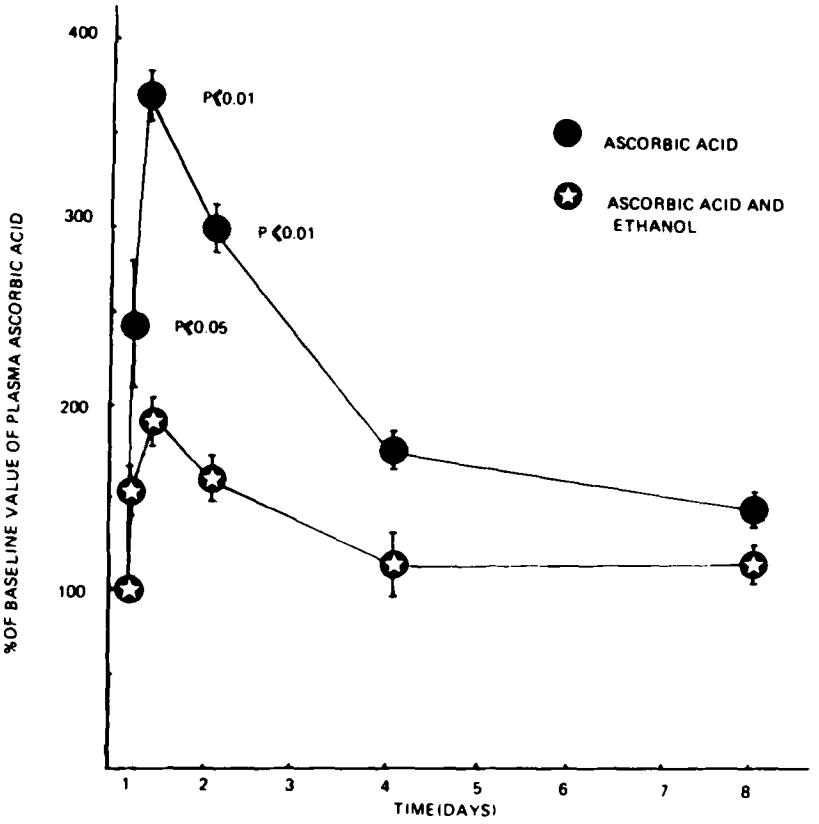

FIGURE 4. The response to a 2-g dose of ascorbic acid at breakfast, with and without 35 g ethanol in five healthy subjects. The plasma ascorbic acid responses were significantly different at 2 h ($p < 0.05$), 6, and 24 h ($p < 0.01$) after the ascorbic acid dose, strongly suggesting that alcohol impairs ascorbic acid absorption. (From Fazio, V., Flint, D. M., and Wahlqvist, M. L. [1981], *Am. J. Clin. Nutr.*, 34, 2394. ©American Society for Clinical Nutrition. With permission.)

therapy for chronic alcoholics should include prolonged polyvitamin and high-dose ascorbic acid therapy.

Studies by Fazio et al. (1981) have provided evidence that ethyl alcohol may predispose to ascorbic acid deficiency by decreasing the availability of ascorbic acid ingested in food. The effects of alcohol on ascorbic acid absorption were assessed in three men and two women whose ages ranged from 21 to 36 years. After ingestion of 2 g of ascorbic acid with a standard breakfast, the plasma ascorbic acid rose from a fasting level of 0.75 mg/100 ml at 9 a.m. to a peak of 2.69 mg/100 ml 6 h later. When the same amount of ascorbic acid was ingested with 35 g of alcohol at breakfast, the mean plasma ascorbic acid level rise from 0.99 to 1.89 mg/100 ml, 6 h later, was significantly less (Figure 4).

Baines (1982) found a mean leukocyte ascorbic acid (TAA) level of 65.6 nmol/10^8 cells in 43 alcoholic patients, which was much lower than the normal range for that laboratory (115 to 340 nmol/10^8 cells) and significantly lower than the mean value found in 21 control subjects, 100.0 nmol/10^8 cells ($p < 0.01$). Ascorbic acid deficiency was common in alcoholics even before they developed cirrhosis of the liver. In fact, 91% of the alcoholics were found to be ascorbic acid deficient, 53% were vitamin B_1 deficient, and 23% showed a deficiency in vitamin B_2.

Majumdar et al. (1983) reported that 17 out of 19 patients with alcoholic liver disease had leukocyte total ascorbic acid levels below the normal range, which they gave as 120 to

300 nmol/10^8 cells. These authors suggested deficient dietary intake, intestinal malabsorption, hepatic hypofunction, and increased requirement as possible causes of this vitamin deficiency.

While earlier animal studies had been conducted on rats and mice, which can synthesize ascorbic acid, Yunice et al. (1984) conducted experiments on the guinea pig and confirmed that (1) ethanol infusion caused a decrease in the body stores of ascorbic acid, as demonstrated by markedly decreased ascorbic acid (TAA) levels in the liver, kidneys, and adrenals; (2) ethanol infusion caused an increase in urinary ascorbic acid excretion; (3) supplementary ascorbic acid caused an increase in the rate of ethanol clearance from the blood at 3 h after ethanol infusion ($p < 0.01$), as demonstrated by mean blood ethanol concentrations of 270 and 125 mg/100 ml in two groups of animals with dietary ascorbic acid intakes of 200 and 2000 ppm, respectively; and (4) high-dosage ascorbic acid seemed to cause a reduction of fatty infiltration in the liver following ethanol infusion, but there were insufficient data for proof of this. These authors therefore suggest that a diet which is nutritionally adequate may no longer be so in the presence of a high ethanol intake, and that supplemental vitamin C ingestion may afford protection against ethanol toxicity.

Alcoholism and a poor diet accounted for one of the three cases of scurvy reported by Reuler et al. (1985).

Piatkowski et al. (1986) confirmed the necessity for ascorbic acid supplementation in chronic alcoholics; both patients with "delirium tremens" and also those classified as "alcohol withdrawal syndrome" were found to have low serum ascorbic acid levels and were given ascorbic acid (500 to 1000 mg daily) either intravenously or orally for 7 d as an essential part of their treatment.

Clearly alcoholics need extra vitamins B_1, B_2, B_6, folic acid, and alpha tocopherol; this has been recognized for many years, but it is now quite evident that high-dosage vitamin C is also necessary for those who continue to drink. Even higher doses of ascorbic acid, as well as sucrose and B vitamins, are needed for alcoholics admitted to hospital with delerium tremens or alcoholic psychosis.

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Chapter 24

OTHER FACTORS AFFECTING ASCORBIC ACID NEEDS

I. INTRODUCTION

Many factors including unaccustomed exercise, high environmental temperatures, and diarrhea have been suspected of interfering with ascorbic acid metabolism (Belser et al., 1939). Moreover, many chemicals and pharmaceutical agents have been found to influence ascorbic acid needs.

II. ATROPINE

During tissue ascorbate saturation studies involving constant ascorbic acid intake by subjects on a controlled diet, Belser et al. (1939) reported that one woman volunteer received atropine as a medication for 1 week. The daily urinary excretion of ascorbic acid was greater when atropine was taken, as compared with similar periods preceding and following the use of this medication. Moreover, the response to a test dose of ascorbic acid was much lower, indicating that the subject was less well saturated. They cited I. S. Wright as having made similar observations.

III. AVIATION

Krasno et al. (1943) studied the effects of exposure of human subjects to a simulated altitude of 18,000 ft in a decompression chamber for 1 h without oxygen, every second or third day. The immediate effect was a decrease in the blood plasma and urinary ascorbic acid levels, but the long-term effect seemed to be an increased urinary ascorbic acid loss.

IV. CALCITONIN

Studies by Basu et al. (1978) and Smethurst et al. (1981) have shown that the bone pain of Paget's disease may be completely relieved in some patients by the administration of ascorbic acid alone (3 g daily) and in others by the use of calcitonin alone (160 MRC units per day). Ascorbic acid caused an increase in the urinary excretion of hydroxyproline and ascorbic acid, while calcitonin caused a reduction in the excretion of ascorbic acid and hydroxyproline. A combined therapy of ascorbic acid and calcitonin is therefore recommended and has been found to be effective without causing any evident change in the urinary hydroxyproline excretion.

V. CHOLESTYRAMINE

Beattie and Sherlock (1976) studied 25 patients with primary biliary cirrhosis and found their mean leukocyte total ascorbic acid (TAA)* level of 130 n$M/10^8$ cells to be significantly lower than the 162 n$M/10^8$ cells found in 28 healthy control subjects ($p < 0.05$); 9 of the 25 patients were receiving cholestyramine treatment in a dose ranging from 1 to 4 sachets daily. The mean leukocyte TAA level in the group receiving cholestyramine was 106 nM, which was significantly less than the level of 143 n$M/10^8$ cells found in the patients not receiving this medication ($p < 0.05$). These workers found no evidence that cholestyramine binds ascorbic acid *in vitro*, so the mechanism of its effect of ascorbic acid metabolism remains unknown.

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

VI. COUMARINS

Studies by Overman et al. (1942), Sullivan et al. (1943), and Dayton and Weiner (1961) have shown that pretreatment with ascorbic acid antagonizes the action of dicoumarol on the prothrombin times of rabbits, guinea pigs, and human subjects. However, ascorbic acid does not itself directly affect the prothrombin level. The explanation seems to be that ascorbic acid deficiency decreases the synthesis of cytochrome P-450 which is needed for the oxidative detoxification of coumarin compounds, as noted in Chapter 13, Volume II.

Even rabbits, which can and do synthesize ascorbic acid in the liver, can benefit from supplementary ascorbic acid, for the writer has demonstrated ascorbic acid-responsive histaminemia in rabbits kept on an ascorbic acid-deficient diet (i.e., even rabbits need fresh greens).

It is therefore wise to give supplementary ascorbic acid with bioflavonoids or catechins to all patients receiving coumarin anticoagulant therapy. Brambel (1961) reported a 5% incidence of bleeding complications of varying severity among 600 patients on long-term coumarin therapy with weekly clotting studies; 1% required interruption of therapy and vitamin K_1 administration. Subsequently, 200 patients received the same coumarin therapy, with ascorbic acid (50 mg) and hesperidin (50 mg) three times a day and showed no hemorrhagic phenomena.

Rosenthal (1971) confirmed the interaction between ascorbic acid and coumarins in his report concerning a 52-year-old woman he treated for deep-vein thrombosis and pulmonary embolism. Following heparin treatment in hospital, she was sent home on warfarin (coumadin sodium), 7.5 mg/d; her prothrombin time remained in the range of 23 s with a control of 12. The prothrombin time, done weekly, remained in that range until 4 weeks later, and then dropped to 19, 17, and 14 s when she started taking high-dose ascorbic acid to ward off a cold. Clearly, a change from high to low ascorbic acid intake would have the opposite effect and cause coumadin overdosage. Moreover, a sudden drop in the serum ascorbate level due to trauma, surgery, or infection could cause a dangerous increase in the prothrombin time of a patient receiving a constant dose of coumadin, and may therefore be responsible for many of the hemorrhagic fatalities which are so well known in people on coumarins.

VII. CROHN'S DISEASE (REGIONAL ILEITIS)

Linaker (1979) observed the occurrence of scurvy in a 28-year-old woman with small bowel and colonic Crohn's disease, proven by radiological and histological evidence. This woman had not undergone bowel resection and was not taking any medication. She probably developed scurvy as a result of a self-imposed low-residue diet, which she took because of colicky abdominal pains. Linaker cited other authors who had recorded low ascorbic acid levels and other nutritional deficiencies in patients with Crohn's disease. Nine more patients were investigated by Linaker; even though the mean ascorbic acid intake of the patients with Crohn's disease was not significantly different from that of healthy matched control subjects, the mean leukocyte ascorbic acid level of the patients (92.9 nmol/10^8 cells) was found to be significantly lower than that of the control subjects (225.5 nmol/10^8 cells, $p < 0.001$).

Clearly defective ascorbic acid absorption by the diseased ileum would seem to be the most likely cause of these findings, but Linaker suggested the possibility that there may be an increased demand for ascorbic acid in Crohn's disease.

VIII. DIARRHEA

Meyer and Robinson (1939), studying infants with diarrhea in Tel-Aviv, found low plasma ascorbic acid and decreased urinary ascorbic acid levels, even when large amounts of ascorbic

acid were given daily by mouth. These findings were confirmed by Abt et al. (1940) who also found that large amounts of orally administered ascorbic acid were excreted in the stools of infants with diarrhea. This is of particular interest because Hayem (1871) reported diarrhea as being a common antecedent to the onset of scurvy during the siege of Paris.

IX. ETHER

The work of Zilva (1935) and Bowman and Muntwyler (1936) showed that administration of the anesthetic diethyl ether to guinea pigs markedly increased their urinary excretion of ascorbic acid. However, this increased urinary ascorbate loss was of short duration and did not affect tissue ascorbic acid levels.

X. EXERCISE

Fishbaine and Butterfield (1984) reported the serum ascorbic acid (TAA) levels to be significantly higher in four athletes running 10 mi/d (1.50 mg/dl) than in the same individuals running 5 mi/d (1.39 mg/dl). These levels were also significantly higher than those found in seven sedentary individuals (1.08 mg/dl) who ate the same diet containing 89 mg of ascorbic acid per 1000 kcal. The runners were also found to have significantly reduced 24-h urinary ascorbic acid excretion, but neither this nor the greater dietary ascorbate intake of the runners was enough to account for their higher serum levels; there may be a shift of ascorbic acid from the tissues to the serum as a result of exercise, but this needs further investigation.

XI. FENFLURAMINE

Experimental findings in guinea pigs by Odumosu and Wilson (1973) suggest that fenfluramine ("Ponderax") exerts its antiobesity action by promoting tissue ascorbate desaturation (Figure 1). Moreover, Wilson (1974) has reported investigations in human subjects indicating that the action of fenfluramine may be potentiated by a vitamin C-deficient diet (Figure 2), and that its administration is associated with a loss of ascorbic acid. Wilson has suggested that, "if fenfluramine is used to accelerate weight loss, catabolic loss of weight may be delayed until tissue ascorbic acid concentrations begin to fall."

XII. GOLD

Zwemer and Elftman (1946) observed that intraperitoneal injections of gold chloride in doses of 10 mg/kg daily for 7 d caused a marked reduction in the plasma ascorbic acid levels of both rats and guinea pigs. They suggested that the gold salt may oxidize ascorbic acid directly, as it does *in vitro*. The vitamin loss would then be due to accelerated hydrolysis of dehydroascorbic acid to diketogulonic acid.

Alternatively, they suggested that the ascorbate depletion may be part of a general toxic reaction, rather than a specific effect of gold.

XIII. HEMODIALYSIS

Lasker et al. studied the problem of water-soluble vitamin losses during hemodialysis and peritoneal dialysis. They observed no consistent change in the blood levels of folic acid, vitamin B_{12}, thiamine, biotin, pantothenic acid, or nicotinic acid measured before and after peritoneal lavage or hemodialysis. However, they did find that patients maintained by intermittent dialysis demonstrated low folic acid and nicotinic acid levels; moreover, one man developed "burning feet" when his nicotinic acid level was very low. These workers made no measurements of ascorbic acid levels.

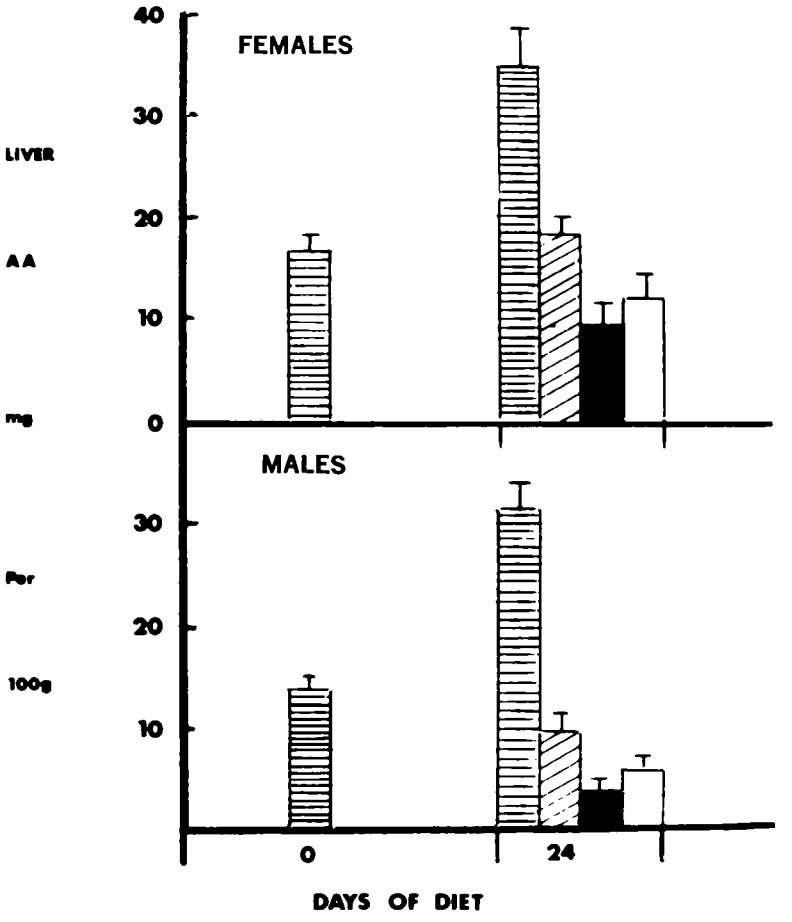

FIGURE 1. Hepatic ascorbic acid concentrations of male and female guinea pigs before (horizontal-lined bar) and after 24 d of four different treatments. The liver ascorbic acid concentrations on day 24 are shown for those receiving a scorbutogenic diet (open bar), those on the same diet with a daily dietary supplement of ascorbic acid (30 mg/kg; horizontal-lined bar), those receiving the supplement with fenfluramine, (15 mg/kg daily; diagonal-lined bar), and those receiving fenfluramine, but no supplement (solid bar). Six guinea pigs per group. Means and standard deviations. (From Wilson, C. W. M. [1975], *Ann. N.Y. Acad. Sci.*, 258, 355. With permission.)

Sullivan and Eisenstein (1970) did measure ascorbic acid losses during hemodialysis in 11 patients and observed a mean decline of 40% in the plasma ascorbate level, associated with the loss of 70 to 100 mg of ascorbic acid during a single dialysis. The plasma total ascorbic acid levels of five patients dropped to critical values between 0.05 and 0.14 mg/100 ml on long-term dialysis, even though they were receiving approximately 34 mg of ascorbic acid daily in their diet. Two of them developed clinical signs of scurvy, one with swelling of the parotid and submandibular glands, as in Sjögren's syndrome (Chapter 18, Volume III), but recovered quickly on treatment with ascorbic acid. A daily dietary supplement of 150 to 200 mg of ascorbic acid was recommended for all patients on long-term hemodialysis.

Sullivan and Eisenstein (1972) and Sullivan et al. (1972) also demonstrated a 26% decline in the mean leukocyte ascorbic acid level of 16 patients during a single dialysis. They showed that this vitamin loss could be prevented by the administration of ascorbic acid either orally,

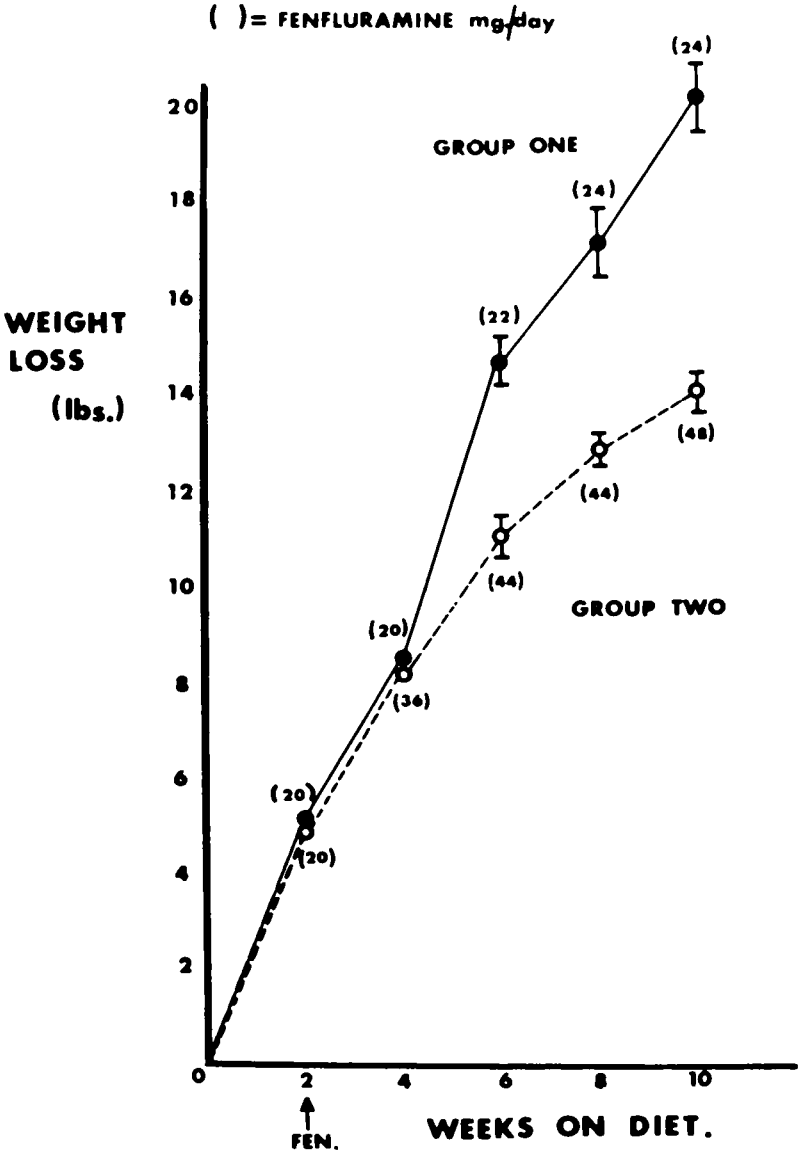

FIGURE 2. Mean weight loss in obese patients on a 1000-cal diet from day 0. At the end of the second week, fenfluramine was administered in doses progressively increased to attain maximal weight loss in individual subjects. Group 2 received 40 mg vitamin C daily in their diet. Group 1 was on a vitamin C-deficient diet. Mean daily dose of fenfluramine shown. (From Wilson, C. W. M. [1975], *Ann. N.Y. Acad. Sci.*, 258, 355. With permission.)

or by adding 1 gm of ascorbic acid to the dialysate at the beginning, and repeating this when the bath is changed at the midpoint of dialysis.

One patient had a decline in plasma ascorbate concentration of 81% during a single dialysis. Oral ascorbic acid supplements must be continued in patients on long-term dialysis as low-potassium diets limit their fruit intake.

Bradley et al. (1973) found an average reduction of 41% in the plasma ascorbic acid and 18% in the whole blood ascorbic acid (TAA) levels of 18 patients during one dialysis. However, an oral supplement of 100 to 200 mg of ascorbic acid daily maintained the

predialysis whole blood total ascorbic acid level in the normal range, between 0.5 and 1.5 mg/100 ml in all but 1 of their 18 patients.

Kelleher et al. (1983) reported that the leukocyte ascorbic acid levels of patients undergoing chronic hemodialysis did not differ significantly from normal, but 5 out of 18 patients on dialysis had low ascorbic acid levels, near 10.6 μg/10^8 leukocytes, which rose to 54.6 μg/10^8 cells within 1 week of starting an oral supplement of ascorbic acid (400 mg daily). Moreover, some other patients showed a slow but progressive fall in their leukocyte ascorbic acid and riboflavin levels. These workers therefore recommend vitamin C and B_2 supplements for all patients on hemodialysis.

De Bari et al. (1984) studied the water-soluble vitamin status of six men and six women patients undergoing chronic hemodialysis, all receiving 1 mg/d of folic acid as a routine dietary supplement. The mean plasma ascorbic acid level of the patients (0.48 mg/100 ml) was significantly lower than that of normal control subjects (0.93 mg/100 ml; $p < 0.001$). Likewise, the ascorbic acid content of the polymorphonuclear leukocytes of the patients (0.8 μg/mg of protein) was significantly lower than normal (2.9 μg/mg protein; $p < 0.01$). Nicotinate was also significantly reduced in the granulocyte fraction ($p < 0.05$), but no other water-soluble vitamin deficiency was found. These workers recommend dietary supplements of ascorbic acid, nicotinic acid, and folic acid for their dialysis patients in the hope that the ascorbic acid will improve the granulocyte function and decrease the infection rate in these patients (Chapter 12, Volume II).

Many factors, including the degree of hemolysis resulting from hemodialysis (Chapter 15 of this volume) and the presence or absence of traces of heavy metals in the dialysis fluids (Chapter 10 of this volume) will affect the extent of ascorbate losses resulting from dialysis. Thus, the differing results of workers in this field may well be due to differences in equipment and technique.

XIV. LEPTAZOL

Studies by Odumosu and Wilson (1974) have shown that intraperitoneal injection of leptazol (60 mg/kg) in guinea pigs causes clonic and tonic convulsions in 100% of the animals. At the time of death, the brain ascorbic acid was found to be significantly reduced to 56 to 70% of normal, and plasma ascorbic acid was elevated to 142% of normal.

Administration of ascorbic acid (200 mg/kg i.p.) 1 h before the leptazol injection resulted in a lower incidence of tonic convulsions after a prolonged latent period, but the brain ascorbic acid level was found to have decreased to the same level as in the unsupplemented animals.

XV. NARCOTICS

Observations by Longenecker et al. (1940) showed that barbiturates markedly increase the ascorbic acid output in the urine of rats.

Ghosh (1942) observed decreased ascorbic acid levels not only in the adrenals, but also in the liver and kidneys of guinea pigs that had been narcotized with chloretone, phenobarbital, paraldehyde, amidopyrine, or bromobenzene (Table 1), most probably as a result of increased urinary excretion of the vitamin. Narcotized rats, on the other hand, showed both increased urinary excretion and increased tissue ascorbic acid levels in the liver and kidneys, undoubtedly due to increased synthesis of this vitamin in response to chemical stress.

XVI. OXYGEN

Many workers have observed that hyperbaric oxygen causes pulmonary edema and lung

Table 1
A STUDY OF THE CONCENTRATIONS OF ASCORBIC ACID PRESENT IN THE ADRENALS, LIVER, AND KIDNEYS OF GUINEA PIGS, AND THE BRAIN, LIVER, AND KIDNEY OF RATS BEFORE AND DURING NARCOSIS WITH VARIOUS AGENTS

No.	Nature of substance administered	Ascorbic acid (mg/g of the fresh tissue)		
		Adrenal	Liver	Kidney
	A. Tissue Ascorbic Acid in the Guinea Pig			
1.	Chloretone (20 mg)	0.176	0.073	0.047
2.	Phenobarbital (20 mg)	0.151	0.081	0.047
3.	Paraldehyde (20 mg)	0.174	0.069	0.043
4.	Amidopyrine (20 mg)	0.318	0.088	0.047
5.	Urethane (100 mg)	0.219	0.104	0.169
6.	Bromobenzene (0.1 cc)	0.127	0.067	0.046
7.	Normal controls	0.326	0.197	0.088
	B. Tissue Ascorbic Acid in the Rat			
		Brain	**Liver**	**Kidney**
1.	Chloretone (20 mg)	0.338	0.279	0.252
2.	Paraldehyde (0.1 cc)	0.310	0.251	0.173
3.	Phenobarbital (20 mg)	0.259	0.224	0.181
4.	Amidopyrine (20 mg)	0.285	0.283	0.243
5.	Normal control	0.316	0.185	0.164

Note: A general tissue ascorbic acid depletion was observed in guinea pigs, but rats showed increased tissue ascorbate levels due to stimulation of liver ascorbate synthesis.

From Ghosh, B. (1942), *Ann. Biochem. Exp. Med.*, II, 221. With permission.

damage in animals. Jamieson et al. (1963) observed that exposure of rats to oxygen at 5 atm pressure for 45 min caused a highly significant decrease in the sulfhydryl groups and a highly significant increase in the disulfide groups of the lungs, as measured by polarography, so that the –SH/–SS– ratio was halved. Moreover Jamieson and Van den Brenk (1964), studying the modification of oxygen toxicity by antioxidants and reducing agents, found that ascorbic acid had a very marked protective effect against the development of pulmonary edema. They gave massive doses (1.5 g/kg) of ascorbic acid to rats before subjecting them to oxygen at 5 atm for 1 h. The mean lung wet weight did not differ from that of unexposed untreated rats and was half that of untreated animals similarly exposed to oxygen. Ascorbic acid was, however, less effective in protecting mice against the development of pulmonary edema, and it had no effect on the preconvulsive period or the survival times of mice exposed to hyperbaric oxygen.

The work of Shanklin et al. (1967) showed that exposure to 100% oxygen at normal atmospheric pressure for 10 d caused a progressive reduction in the ascorbic acid (TAA) concentrations in the lungs, blood, and adrenals of guinea pigs. Moreover, the large areas of lung showing hepatization as a result of oxygen treatment had lower ascorbate levels than did the clear areas of lung.

The data of Willis and Kratzing (1972) showed that hyperbaric oxygen treatment of rats

at 3.5 atm pressure for 1 h caused reductions in the ascorbic acid (AA)* concentrations in the lungs, liver, and adrenals of 43% ($p < 0.001$), 8% ($p < 0.05$) and 10% (not significant), respectively.

Exposure of rats to hyperbaric air at the same pressure, for the same length of time, showed less effect on the ascorbic acid concentration of the lungs — 19% ($p < 0.05$), but more effect on that of the liver — 22% ($p < 0.02$); the adrenals showed only a 12% (not significant) reduction of ascorbate concentration following hyperbaric air.

Part of the fall in the ascorbate concentration in the lungs following hyperbaric oxygen was due to pulmonary edema, but part was due to oxidation of ascorbic acid (AA) to dehydroascorbic acid (DHAA), for the lung DHAA increased from 2.45 ± 0.35 to 3.80 ± 0.26 mg/100 ml. The decreased liver ascorbic acid concentrations almost certainly represented genuine losses of ascorbic acid due to oxidation and subsequent hydrolysis of the vitamin. It is possible that the thickened alveolar membrane resulting from pulmonary edema due to hyperbaric oxygen may have resulted in a lower oxygen transfer to blood than with hyperbaric air. This would explain the greater effect of hyperbaric air than hyperbaric oxygen on the liver ascorbate concentration.

Mettler et al. (1984) discussed the importance of ascorbic acid and of glutathione as antioxidants to reduce lipid peroxidation in the lung. Their experiments revealed that isolated rabbit pulmonary type II epithelial cells showed improved attachment to culture plates in the presence of ascorbic acid (5.6 m*M*) in a medium containing cysteine, glutamine, and glycine, the constituent amino acids of glutathione.

XVII. OZONE

Ozone is a highly toxic oxidant gas. Lung damage can be detected in experimental animals that have inhaled it for only a few hours at a concentration of 1 ppm or less.

Acute exposure of mice to high concentrations of ozone is known to cause tracheobronchial irritation, pulmonary edema, and death, as shown by Matzen (1957). Chronic exposure of mice to low levels of this gas causes premalignant changes in the lungs, as shown by Penha et al. (1972, 1974). Nevertheless, the sale of ozonizers for use in homes, shops, offices, factories, and even in hospitals has not yet been discontinued. Low levels of ozone in the vicinity of electrical equipment may be unavoidable, but the levels in aircraft cockpits can be reduced, and the sale of ozonizers for "purifying" air should be prohibited.

Studies at the Occupational Health Field Headquarters of the Occupational Health Program of the Public Health Service in Cincinnati, Ohio, by Matzer (1957) showed that rats and mice receiving intraperitoneal injections of ascorbic acid $^1/_2$ h before exposure to ozone had a lower mortality than control animals. This in spite of the known ability of rats and mice to synthesize ascorbic acid; they still benefitted from the additional ascorbic acid when exposed to this potent oxidizing agent.

Exposure of 100 mice to ozone (5.2 ppm) for 4 h following control injections 0.5 ml of normal saline resulted in 44 dying in 2 weeks. In contrast, 20 mg of ascorbic acid in 0.5 ml of *N* saline given by intraperitoneal injection to 100 mice before exposure to the same concentration of ozone, for the same length of time, reduced the mortality to 10%. Pretreatment with ascorbic acid was also noted to decrease pulmonary edema in the mice, as measured by the percentage water content of the lungs by weight in animals killed for analysis. Vitamin C was reported to have no effect on the mortality of mice when given after exposure to ozone.

High doses of hydrocortisone hemisuccinate (Solu-Cortef® [Upjohn], 1 mg/mouse) injected intraperitoneally just before exposure to ozone decreased mortality, but lower doses

* AA — ascorbic acid, reduced form.

Table 2
CHANGES IN LUNG ASCORBIC ACID CONTENT AND LUNG TO BODY WEIGHT RATIO AFTER EXPOSURE TO OZONE[a]

Treatment	Ascorbic acid content (μg/mg DNA)	Lung to body weight ratio $\left(\frac{\text{lung wt} \times 10^{-3}}{\text{body wt}}\right)$
Control	41.3 ± 1.6 (17)^{ABC}	4.3 ± 0.2 (17)^{DEF}
20 ppm ozone	30.9 ± 1.0 (9)^{A}	5.2 ± 0.3 (9)^{D}
40 ppm ozone	23.7 ± 0.8 (9)^{B}	5.6 ± 0.4 (9)^{E}
200 ppm ozone	19.8 ± 1.0 (14)^{C}	6.9 ± 0.6 (11)^{F}

Note: Exposure of mice to ozone (200 ppm) for 30 min caused not only pulmonary edema, shown as an increase in the lung to body weight ratio, but also a real loss of ascorbic acid from the lung measured as micrograms of ascorbic acid per milligram of DNA.

[a] Values are means ± SE with the number of animals shown in parentheses. Means with like superscripts differ significantly using Student *t* test with $p < 0.05$.

From Kratzing, C. C. and Willis, R. J. (1980), *Chem. Biol. Interact.*, 30, 53. With permission.

in the pharmacological range (0.125 or 0.250 mg/mouse) seemed, if anything, to increase mortality.

The work of Kratzing and Willis (1980) (Table 2) showed that exposure of mice to ozone (200 ppm) for 30 min caused not only a 50% increase in lung weight due to edema, but also a real loss of ascorbic acid from the lung, as measured in micrograms per milligram of DNA. Their findings suggested that most of the ascorbate loss was from extracellular fluid and especially from the fluid lining the air spaces, where ascorbic acid seems to act as an expendable extracellular antioxidant, protecting the lung from oxidation.

XVIII. PENTOBARBITAL

The work of Kinsey (1940) suggested and the observations of Richards et al. (1941) demonstrated that vitamin C-depleted guinea pigs develop an increased sensitivity to pentobarbitone (nembutal), as indicated by a markedly prolonged sleeping time. No such change occurred with barbital or pentothal; pentobarbital is largely metabolized in the liver before it is excreted, while barbital and pentothal are largely excreted unchanged. This increased sensitivity to pentobarbitone is most probably due to the depression of hepatic microsomal enzyme activities, which have been observed by Zannoni and Sato (1975) in vitamin C-deficient guinea pigs. However, addition of ascorbic acid *in vitro* has little effect on some liver enzyme activities. Several days of ascorbic acid replenishment may be required to restore the full hepatic detoxifying activity of scorbutic guinea pigs to normal.

Confirmation of this increased sensitivity to nembutal was provided by Degkwitz and Staudinger (1974) who stated that they had to reduce the amount of this drug administered in their experiments on ascorbic acid-deficient guinea pigs to about 60% of the amount given to normal guinea pigs, since the deficient animals did not otherwise survive.

Windsor et al. (1972) found no evidence of any change in the leukocyte ascorbic acid levels of three elderly men whom they studied before, during, and after receiving phenobarbitone, 60 mg three times a day for 5d.

Nevertheless, it is wise to give ascorbic acid supplements to all patients before administration of barbiturates to avoid the risk of hypersensitivity due to ascorbic acid deficiency.

XIX. PHENYLEPHRINE

Studies by Willis and Kratzing (1974) showed that large doses of phenylephrine, norepinephrine, or epinephrine given intravenously to rats under pentobarbitone anesthesia caused 26, 33, and 40% reductions in the lung ascorbic acid concentration ($p < 0.001$ for each), and 18, 16, and 16% reductions in lung ascorbic acid content within 15 min, compared with pentobarbitone-treated rats receiving normal saline injections. Clearly much of this effect was due to pulmonary edema, but part was an actual loss of ascorbic acid from the lung. Both the pulmonary edema and the lung ascorbate loss could be blocked by prior injection of phenoxybenzamine or reduced by propranolol. It was suggested that the pulmonary edema and pulmonary ascorbate loss were due to alpha and beta receptor stimulation.

Clearly, these are high-dose effects and do not necessarily have any human clinical relevance.

XX. PHENYTOIN, DIPHENYL HYDANTOIN, DILANTIN®

Gruhzit (1939) reported that administration of sodium diphenylhydantoinate in daily doses of 50 mg/kg did not cause any signs of vitamin C deficiency in guinea pigs on a normal diet and did not alter the course of the disease in guinea pigs on a scurvy diet.

However, Kimball (1939) studied 152 epileptic persons in a special school for epileptics in Detroit and found that more than half the patients on Dilantin® had gingival hyperplasia which was associated with low serum ascorbic acid levels. Moreover, the serum ascorbate levels were lowest in those with the greatest degree of Dilantin® hyperplasia, as shown below.

Degree of Dilantin® hyperplasia	Serum ascorbic acid level mg/100 ml
0	1.14
+	0.58
+ +	0.55
+ + +	0.47

Clearly, these were not scurvy levels, and the patients did not show any of the other signs of scurvy, such as purpura, painful joints, or extreme weakness, but there was evidence of a disturbance of ascorbic acid metabolism which was resistant to treatment. One 15-year-old youth had a serum ascorbic acid level of 0.1 mg/100 ml while on a diet high in vitamin C and required an ascorbic acid supplement of 300 mg/d to raise his serum level to 1.8 mg/100 ml; this was associated with some improvement in his gingival hyperplasia, but did not cure it. We may conjecture that Dilantin® impairs the uptake of ascorbic acid by alveolar tissue.

In a subsequent report, Kimball and Horan (1939) noted that some patients, after being on Dilantin® treatment for 2 months or more, developed epigastric pain with nausea, vomiting, anorexia, and sometimes gastrointestinal hemorrhage, and that they all had gingival hyperplasia. It was suspected that these cases of hemorrhagic gastritis were in some way related to deficiency of vitamin C (c.f., Chapter 17, Volume III). Moreover, it was found possible to cure them and to prevent this epigastric distress in most other children receiving Dilantin® by giving supplementary ascorbic acid and a diet rich in citrus fruits. Also worthy of note is the observation by Kimball and Horan that severe gingival hyperplasia and an associated ascorbic acid deficiency (serum ascorbic acid 0.12 mg/100 ml) in a 14-year-old girl on Dilantin® were both restored to normal within 3 months after withdrawal of the drug; her gums returned to normal and her serum ascorbic acid level rose to 0.85 mg/100 ml on a normal home diet, without any supplementary ascorbic acid.

Drake et al. (1941) showed that sodium diphenylhydantoinate given to rats in a dose of 500 mg/kg caused an increase in the urinary excretion of ascorbic acid and reduced the ascorbic acid levels in the adrenal glands, brain, and skeletal muscle of these animals.

Moreover, Drake et al. (1941) demonstrated that a much lower dose of 13 mg/kg produced a rapid and progressive fall in the blood vitamin C levels of guinea pigs on a constant ascorbate intake of 5 mg/d; these levels returned to normal three weeks after withdrawal of the drug.

Nevertheless, Merrit and Foster (1940) wrote that sodium diphenylhydantoin therapy did not affect the plasma vitamin C levels of their patients, which varied between 0.0 and 1.6 mg/100 ml and averaged 0.45 mg/100 ml. They observed that the administration of foods rich in vitamin C content to patients with low values caused a fivefold increase in the levels of the vitamin, but they felt that neither the hypertrophic gingivitis nor the gastritis were in any way related to vitamin C deficiency.

Stambaugh et al. (1973), on the other hand, treated a 54-year-old man with left hemiplegia, due to a cerebrovascular accident; he not only showed Dilantin® hyperplasia of the gingival tissues associated with a very low plasma ascorbic acid level of 0.08 mg/100 ml, he also complained of mild chronic pain in his elbow and knee joints, as with scurvy. After 6 d of oral ascorbic acid treatment, 1000 mg/d, it was noted that the patient's appetite was better and his attitude was much improved. The patient's gingival condition was seen to improve somewhat earlier. On the fifth day of treatment, a marked reduction of gingival edema and ease of bleeding was noted. The patient's gingival condition on day 13 was markedly improved even though he had received no local treatment of the gums. Remission of the oral lesions following a rise in the serum levels of vitamin C leaves little doubt of the diagnosis. This man had gingival hyperplasia and borderline scurvy due to a combination of Dilantin® treatment and an ascorbic acid deficient diet. Even his cerebrovascular accident may have been precipitated by the ascorbic acid deficiency (Chapter 19, Volume III), his poor attitude, which improved with vitamin C is typical of the mental depression associated with scurvy (Chapter 9, Volume II).

Dawson and Duncan (1975) found many low leukocyte ascorbic acid levels in 29 epileptic children on anticonvulsant treatment in Lanarkshire (mean 16.8 $\mu g/10^8$ cells), but the levels found in these children were not significantly lower than those of 19 untreated children in a home for the mentally deficient (mean 18.95 $\mu g/10^8$ cells). Of them, 17 had leukocyte TAA levels between 7.3 and 10 $\mu g/10^8$ cells and 3 were below 10 $\mu g/10^8$ cells, even though all blood samples were drawn in August and September when their diet was calculated to supply 73 to 133 mg of vitamin C a day. It would seem that many of the children, both in the epileptic group and in the mentally deficient group, were ascorbic acid deficient. Unfortunately, these workers did not supply data for different anticonvulsant drugs, probably because of changes in treatment.

Wilson (1975) cited evidence that the disturbance of folate metabolism which occurs as a side effect of the administration of the anticonvulsant diphenylhydantoin is primarily dependent on an initial alteration of ascorbic acid metabolism which impairs hepatic microsomal activity. Both ascorbic acid and folic acid supplements should therefore be given with Dilantin® in the treatment of epilepsy.

Krause et al. (1982) observed significant reductions in the folate, biotin, and vitamin D levels of 146 epileptic men and women being treated with various combinations of 12 anticonvulsants in Heidelberg. They also reported indications of decreased riboflavin levels in women and nicotinic acid levels in men, but no significant difference between the vitamin B_1, B_{12}, A, C, or E levels of epileptics and normal subjects. These workers did not report separate figures for patients under treatment with Dilantin®. However, their data do suggest the need of multivitamin supplements for epileptics, and a review of the literature suggests that vitamin C and folic acid supplements are especially important for those receiving Dilantin®.

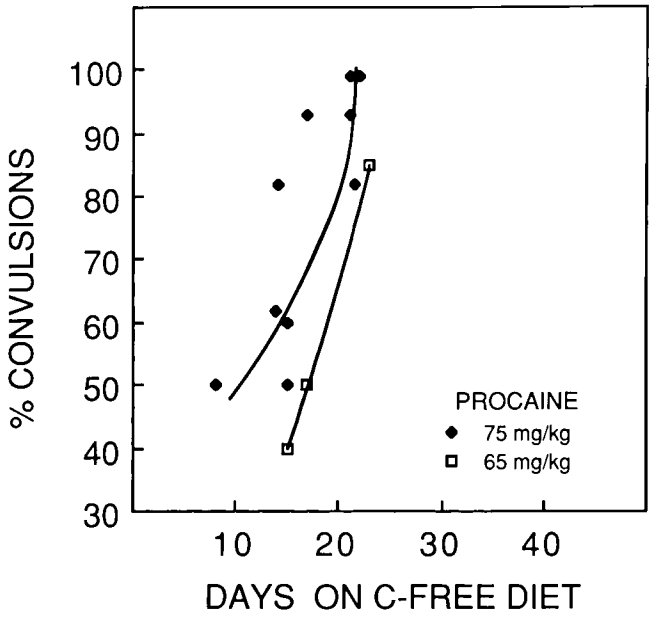

FIGURE 3. Effect of vitamin C depletion upon the convulsant action of procaine in guinea pigs. (From Richards, R. K. [1947], *Curr. Res. Anesth. Analg.*, 26, 22. With permission.)

XXI. PROCAINE

Studying the toxicity of procaine in guinea pigs on different dietary intakes of vitamin C, Richards (1947) observed that the incidence of convulsions following intramuscular injection of procaine (75 mg/kg) rose from 20% on a high vitamin C intake to 60% after 2 weeks on an ascorbic acid-deficient diet and to 100% in scurvy (Figure 3).

However, 48 h starvation also increased the sensitivity to procaine, and the sensitivity could be decreased by pretreatment with glucoronic acid, ascorbic acid, or larger amounts of dextrose, but not by gluconic acid. Moreover, 250 mg of ascorbic acid offered protection to ascorbic acid-deficient guinea pigs when given by intraperitoneal injection 15 min before the injection of procaine, so the protection would seem to be a nonspecific redox-potential effect.

XXII. SULFONAMIDES

Holmes (1943) gave sulfathiazole, 30 gr daily for 4 d, to ten healthy college students and observed a two- to threefold increase in the urinary excretion of ascorbic acid by these volunteers, reaching 100 mg/d on the second and third days. Holmes recommended ascorbic acid supplements for anyone treated with sulphonamides, not only because of this ascorbate-depleting effect of sulfonamides, but also because of the ascorbate loss known to be associated with infection.

This is of interest because Rodney et al. (1947) observed decreased tyrosine oxidation by liver suspensions from rats with sulfasuccidine-induced pteroylglutamic acid (PGA) deficiency. Moreover, Johnson and Dana (1948) showed that the leukopenia of rats made PGA deficient by administration of a 2% sulfasuccidine diet was effectively treated with ascorbic acid. The PGA deficiency of the rats persisted, but their weight gain was renewed and their death was delayed by ascorbic acid administration.

Table 3
OXYGEN CONSUMPTION OF ASCORBIC ACID IN PRESENCE OF SIX ANTIBIOTICS

Antibiotic added	Oxygen consumed (µl)	% activation(+) or inhibition(−)
Water (control)	25.0	—
Aureomycin hydrochloride	24.2	− 3.2
Chloramphenicol	25.7	+ 2.8
Neomycin sulfate	21.5	− 14.0
Dihydrostreptomycin sulfate	30.0	+ 20.0
Penicillin G	18.6	− 25.6
Terramycin® hydrochloride	168.4	+ 573.6

Note: *In vitro* oxidation of ascorbic acid in phosphate buffer (pH 7.0) in the presence of various antibiotics. Terramycin® had a specific catalytic effect on the oxidation of ascorbic acid. Penicillin G, on the other hand, showed an inhibitory effect. We may conjecture that this protective effect of penicillin may have been due to its partial degradation to dimethylcysteine (penicillamine) which is both a reducing agent and a chelating agent.

From Dudani, A. T. and Krishnamurti, C. R. (1954), *Biochim. Biophys. Acta*, 13, 505. With permission.

Jones et al. (1943), however, found that the sprinkling of sulfanilamide powder into the wounds of guinea pigs on different diets did not affect the ascorbic acid content or the tensile strength of the wounds. "The tensile strength of healing wounds varied with the intake of ascorbic acid and the resulting ascorbic acid content of the scars."

Rawall et al. (1974) reported *in vitro* studies showing that ascorbic acid potentiated the action of sulfamethoxazole, ampicillin, erythromycin, polymyxin E, and chloramphenicol on *Pseudomonas aeruginosa*. Indeed, ascorbic acid acted synergistically with agents which on their own have no action on this organism. They confirmed this synergism in the treatment of *P. aeruginosa* infections in mice and reported very encouraging results in the treatment of *P. aeruginosa* infection in five patients with cystic fibrosis, using a combination tablet of trimethoprim, 80 mg, and sulfamethoxazole, 400 mg (Bactrim®, Roche), two tablets, and ascorbic acid, 2.0 g, twice daily for 4 weeks.

XXIII. TETRACYCLINE

Using conventional Warburg manometric techniques, Dudani and Krishnamurti (1954) showed that Terramycin® (oxytetracycline) acts as a specific catalyst for the oxidative degradation of ascorbic acid *in vitro* (Table 3).

Shah et al. (1968) studied the effects of tetracycline, 250 mg four times a day per os for 4 d on ten healthy volunteers and observed a significant reduction in both their plasma and their leukocyte ascorbic acid levels. They suggested that the leukocyte ascorbate depletion might be the cause of the observed inhibition of phagocytic activity during treatment with tetracycline. It was possible to prevent the ascorbate depletion and to increase both plasma and leukocyte ascorbic acid levels by giving tetracycline (250 mg) with ascorbic acid (250 mg) four times a day to five volunteers.

Windsor et al. (1972) confirmed the findings of Shah et al. (Figure 4) and also observed an increased urinary excretion of ascorbic acid during tetracycline administration, which they suggested may be due to blocking of renal tubular reabsorption of ascorbic acid by the antibiotic.

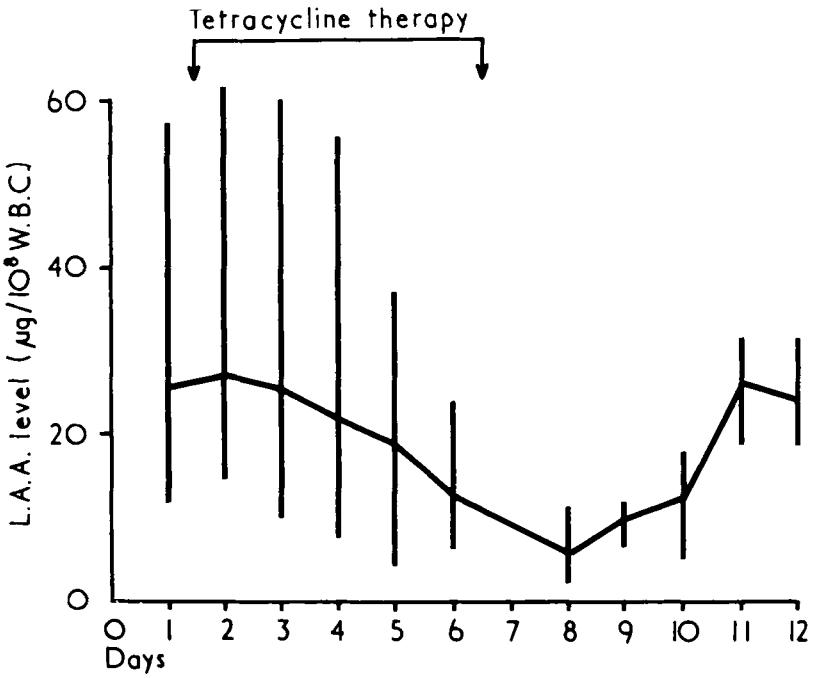

FIGURE 4. Effect of tetracycline on mean and range of leukocyte ascorbic acid levels in 14 men (age 73 to 94 years). (From Windsor, A. C. M., Hobbs, C. B., Treby, D. A., and Astley Cowper, R. [1972], *Br. Med. J.*, 1, 214. With permission.)

Since the leukocyte ascorbic acid concentration fell to a very low level, and since tetracycline has also been reported to cause a reduction in serum B_{12}, B_6, and pantothenic acid levels, it would seem that vitamin supplements may be needed to guard against the fatty degeneration of the liver which sometimes occurs as a result of tetracycline therapy.

XXIV. VITAMIN B COMPLEX

Farmer (1944) studied 12 young men, aged 20 to 30 years, on a vitamin C-deficient diet (0 to 10 mg daily) containing minimal quantities of vitamin B complex. Five subjects remained on this diet, five received adequate daily supplements of B complex, and two, serving as controls, received daily supplements of B complex and 75 mg of ascorbic acid, which was later raised to 150 mg. Calories, protein, minerals, and other vitamins were supplied in adequate amounts. The experiment lasted for 7 months.

The average time required for the plasma ascorbic acid level to fall to zero was 70 d. The white cell platelet ascorbic acid level dropped more rapidly in subjects deficient in both vitamins B complex and C than in those deficient in vitamin C alone; one can readily understand the need for certain B complex vitamins in the storage of ascorbic acid. Riboflavin is needed as a source of flavin adenine dinucleotide and pyridoxine is needed as a source of pyridoxal phosphate for the full activity of glutathione reductase (GR) which converts oxidized glutathione (GSSG) to its reduced form (GSH) not only in the erythrocytes, as outlined by Tonkin (1984), but also in other cells and tissues. Likewise, nicotinic acid is presumably needed by NADPH-dependent GR. Ascorbic acid passes through cell membranes and enters cells in the form of DHAA and is converted into ascorbic acid (AA) for storage within each cell by the action of GSH.

Thus, dietary deficiency, or a disturbance in the metabolism of riboflavin, pyridoxine, or nicotinic acid could impair tissue storage of ascorbic acid.

XXV. ZOXAZOLAMINE

Studies by Conney et al. (1961) demonstrated that vitamin C-deficient guinea pigs are more sensitive to the muscle-relaxing drug zoxazolamine than are normal guinea pigs. They found that guinea pigs receiving ascorbic acid (10 mg/d) were paralyzed for 156 min after intraperitoneal injection of zoxazolamine (100 mg/kg). In contrast, guinea pigs maintained on a scorbutogenic diet for 10 to 14 d were paralyzed for 309 min by the same dose of this drug. This increased sensitivity of vitamin C-deficient guinea pigs was paralleled by decreased activity of the liver microsomal enzyme system that metabolizes zoxazolamine. Under comparable conditions *in vitro*, 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 in the same time.

There is no evidence that this drug increases ascorbate utilization, but it does increase the urgency of our need for ascorbic acid, as the decreased activity of the zoxazolamine-metabolizing enzyme system occurs at an early stage in vitamin C deficiency.

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