Vitamin C

Volume I

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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 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.

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.)

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.
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> 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, …

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.
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> 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.

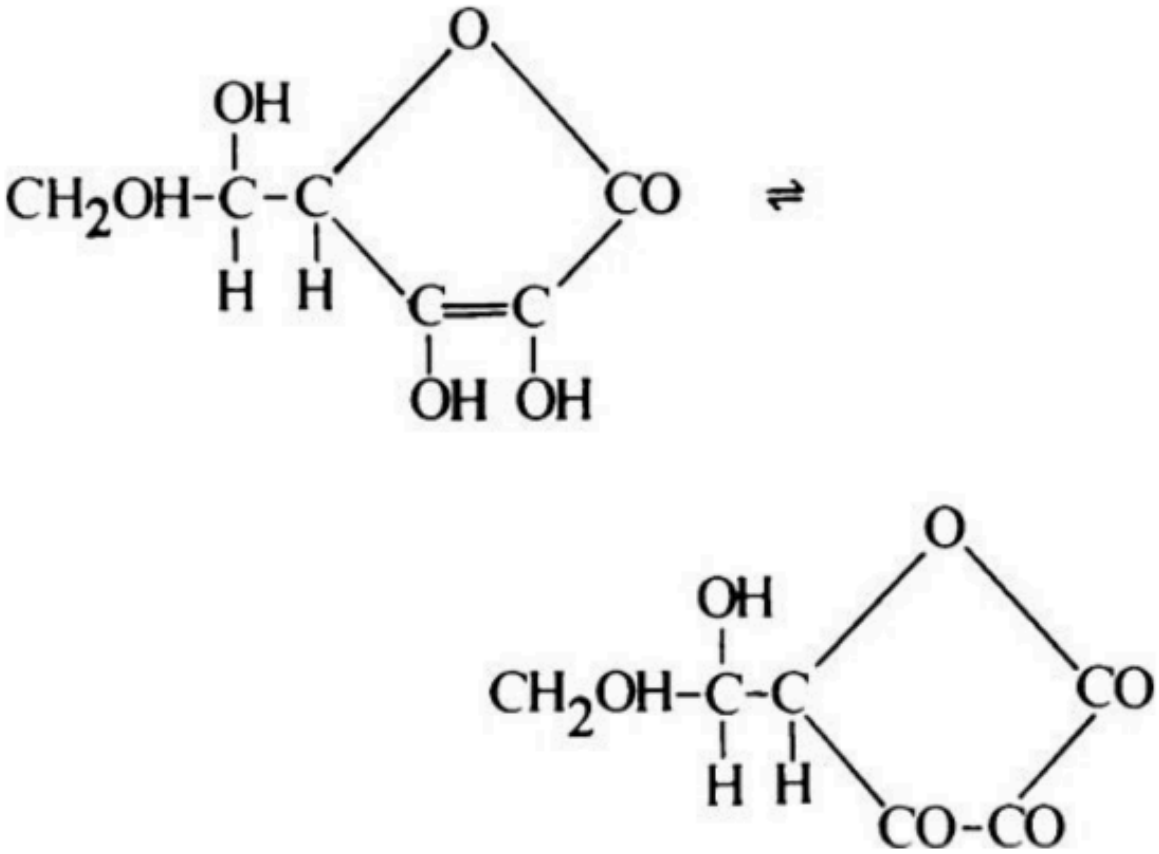

FIGURE 2. The structures of ascorbic acid (above) and its reversible oxidation product dehydroascorbic acid (below). (From Haworth, W. N. [1933], *J. Soc. Chem. Ind.*, 52, 482. With permission.)

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 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.

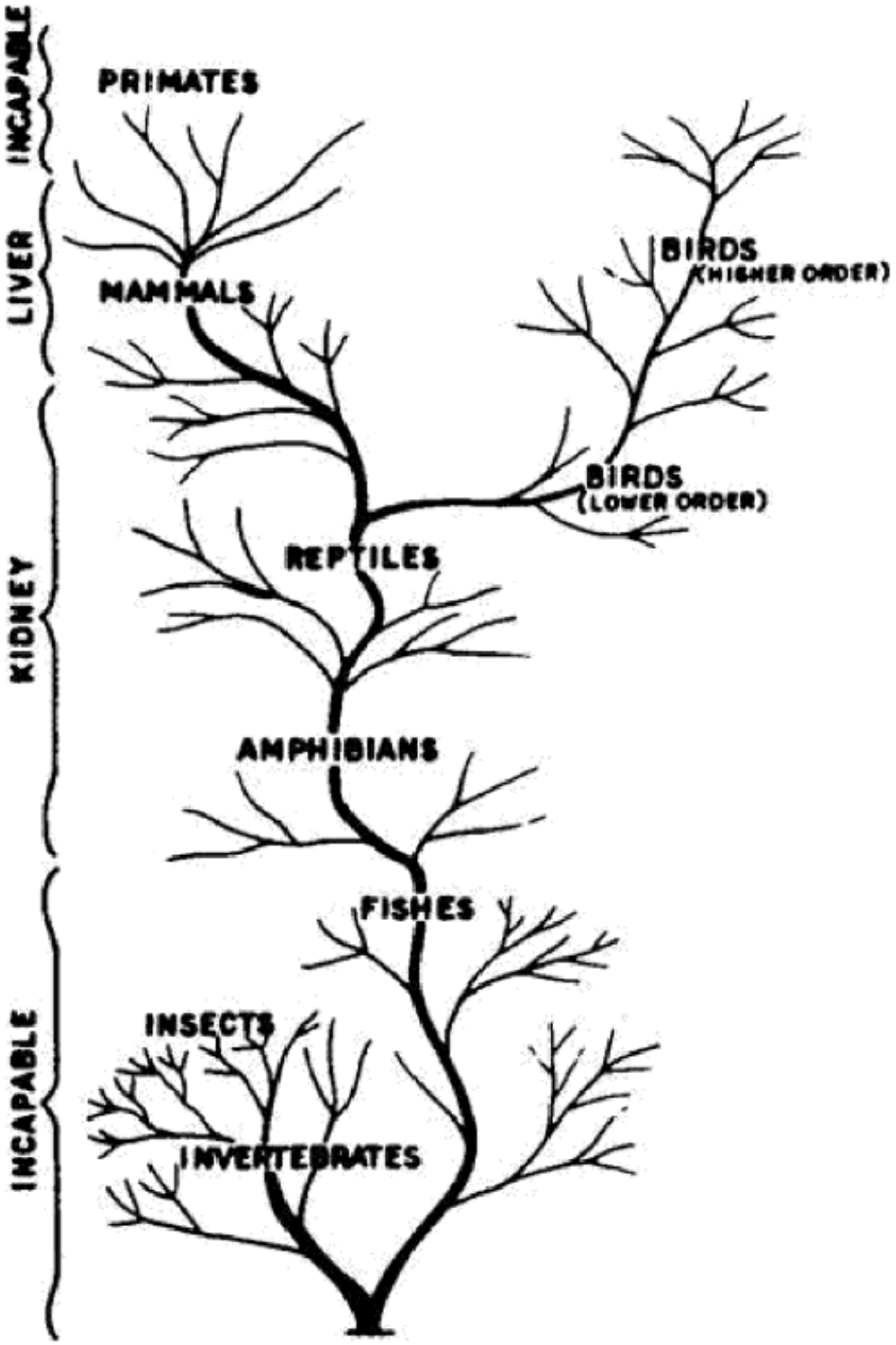

FIGURE 1. Schematic representation of ascorbic acid synthesizing abilities of various species of animals in relation to their phylogeny. (From Chatterjee, I. B. [1973], *Science*, 182, 1271. With permission.)

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 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.

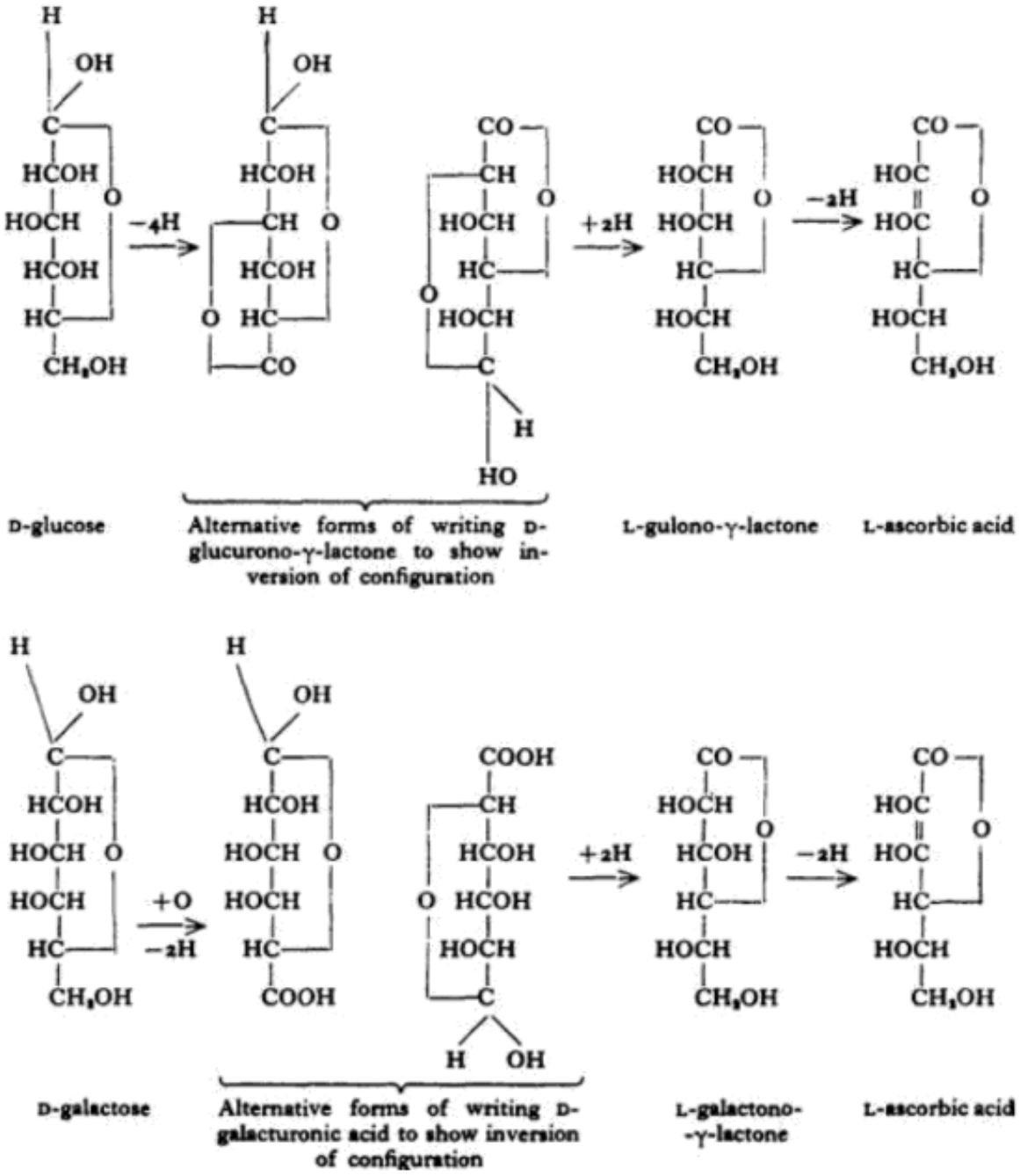

FIGURE 2. Synthesis of L-ascorbic acid in rats. (From Isherwood, F. A. [1953], *Proc. Nutr. Soc.*, 12, 335. With permission.)

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 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.

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 (*Macaco mulatto*)	—	—
Man	—	—

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

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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